

Article

Responses of Maize (*Zea mays* L.) Roots to Nitrogen Heterogeneity and Intraspecific Competition: Evidence from a Pot Experiment Using the ‘Root Splitting’ Approach

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Abstract: A heterogeneous distribution of nutrients in the soil and the root interactions of crops coexist in agroecosystems. A ‘root splitting’ approach, i.e., splitting the root system into different nutrient compartments, was used to study nutrient heterogeneity distributions and root interactions. We evaluated root foraging behavior, individual growth, and root development mechanisms in response to soil nitrogen heterogeneity, and intraspecific competition in maize. A heterogeneous distribution of nitrogen increased the foraging precision of roots at flowering and mature stages (Foraging precision > 1), and significantly increased root biomass and surface area on the intraspecific competition and no competition on the high nitrogen side. The heterogeneous distribution had no effect on yield, total root biomass, specific root length, total surface area, or average diameter. Intraspecific competition increased inter-crop competition (RII < 0) and reduced total root biomass, total plant biomass, and the root/shoot ratio. These results inform root trait development studies and provide valid evidence for optimizing and managing fertilizer application in agroecosystems, helping to maximize crop yield and nutrient use efficiency.

Keywords: nitrogen heterogeneity; root growth; intraspecific competition; crop development



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1. Introduction

Soil nutrients are typically heterogeneous and occur in patches. These patches are usually spatially (nutrient patch distribution, size, quantity) and temporally (time of patch appearance, duration, and frequency of patch appearance) heterogeneous [1]. The heterogeneous nutrient distribution is mainly caused by the heterogeneous physicochemical properties of the soil [2], the heterogeneous distribution of plant litter and animal excreta, differences in the physical and chemical properties of different organic litter [3,4], and the activities of different microorganisms. The main reason for nutrient imbalances in production is non-homogeneous fertilizer application [5].

Soil nutrient heterogeneity influences crop plant growth [6], community development, and environmental changes [7]. Plants are influenced by the heterogeneity of their surroundings and exhibit morphological and physiological adaptations to minimize the adverse effects of environmental factors. The adaptations include the proliferation of more roots in patches with high nutrient content, including the elongation of existing roots and the production of new roots [8]. When plants are without competition (including the above- and the underground roots), plants in heterogeneous environments are often better adapted and have higher biomass than those in homogeneous soils [9]. Optimization models suggest that plants growing in patchy soil nutrient environments will spatially allocate their root systems to balance the marginal benefits of each nutrient patch, regardless of the size of the plant and the nature of the nutrient patch [10,11]. This may be due to the proliferation of root systems increasing the uptake potential and increasing the limited binding capacity per unit of soil volume.

With the intensity of modern agriculture, the communication between crop roots has become complex. The plant root system configuration is the result of evolution which favors their successful growth and reproduction in variable natural environments [10]. Nitrogen (N) is an essential element required by plants for growth. Nitrogen limits crop yield and quality due to its high mobility in the soil and its variable distribution over time and space [12]. N heterogeneity can have many effects on plant root growth [13], but information on the relationship between intraspecific competition and nitrogen heterogeneity is scarce. Such effects are mainly specific to intercropping plant populations [14], clonal populations [15], and plant sex [16]. Homogeneous habitats with a homogeneous distribution of resources and growth space are more favorable for plant growth and reproduction. This is probably because homogeneity facilitates access to resources and growth space. However, this response varies among different species [17]. In addition, the density of nutrient patches may influence the root foraging responses of plants, which can either proliferate equally in each patch or allocate more resources to one or several patches [18]. When there is no competition, roots proliferate more strongly in patches closer to the roots. For example, in heterogeneous soils, the roots of *Abutilon theophrasti* Medicus populations proliferate extensively into nutrient-rich patches, but the total above-ground biomass and total root biomass of the populations are not strongly influenced by heterogeneity in the populations [19]. However, these studies did not consider the overall influence of individual root segments. Given the generally heterogeneous distribution of nitrogen in the soil, it is necessary to consider the distribution of nitrogen heterogeneity on individual root development. This is essential for improving nutrient uptake efficiency and yield, and for investigating how roots respond to soil nitrogen heterogeneity and intraspecific competition. This information will increase our understanding of plant-plant coexistence and their evolutionary relationships within ecosystems.

2. Materials and Methods

2.1. Experiment Site

The experiments were conducted from March to August 2022 in a greenhouse at the Daheqiao Experiment Station, Yunnan Agricultural University, Xundian county, Yunnan Province (103°16'41" E, 25°31'07" N). This location is at an altitude of 1860 m, with a mean annual frost-free period of 231 days. The experiment soil was a silty clay loam with maize as the immediately previous crop. The soil (0–20 cm) pH was 6.82, with total N, P, and K concentrations of 1.10 g, 0.68 g, and 10.58 g kg⁻¹, respectively, and available N, P, and K concentrations were 48.37 mg, 21.26 mg, and 59.63 mg kg⁻¹, respectively. The soil organic matter content was 17.22 g kg⁻¹. The soil was air dried, screened by 6 mm mesh, and placed into pots.

2.2. Experiment Design

Two nitrogen distribution patterns (Homogeneous distribution (HO), Heterogeneous distribution (HE)) with or without intraspecific competition (no competition (NC), intraspecific competition (IC)) were established in the experiment. To set up nitrogen heterogeneity and to prevent soil nutrient diffusion, two plastic bottles measuring 18 cm × 17 cm × 41 cm (length × width × height) were tied together, and a groove of a 5 cm height was cut from the middle interface to facilitate the transplanting of seedlings (Figure 1). Replicates were arranged randomly in the greenhouse to reduce the influence of nonhomogeneous light. The intraspecific competition nutrient content (N, P₂O₅ and K₂O) was twice that of the no competition cultivation. A total of 2.5 g of calcium superphosphate (P₂O₅ ≥ 16%) and 4 g of potassium sulphate (K₂O ≥ 52%) per plant were mixed with sieved dry soil for a single application to two conjoined pots. Urea (N = 46.2%) was applied within one week after transplanting and at the flare opening stage at 50%:50% of the total fertilizer application rate (Table 1).

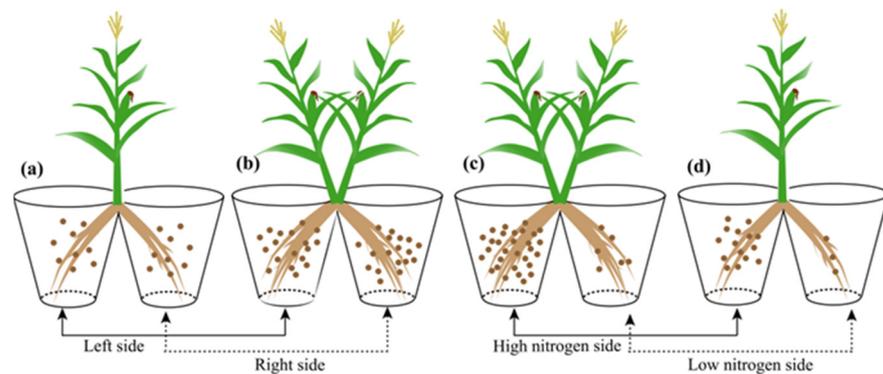


Figure 1. Schematic representation of nutrient distribution patterns and crop cropping patterns. (a) Homogeneous with no competition (NCHO); (b) Homogeneous with intraspecific competition (ICHO); (c) Heterogeneous with intraspecific competition (ICHE); (d) Heterogeneous with no competition (NCHE). The brown dots in the pots represent the amount of nitrogen applied.

Table 1. Nitrogen application amounts of the different treatments (g/pot).

	Homogeneous (HO)		Heterogeneous (HE)	
	Left Side	Right Side	High Nitrogen Side	Low Nitrogen Side
Intraspecific Competition (IC) (2 plants)	12	12	20	4
No competition (NC) (1 plant)	6	6	10	2

2.3. Seedlings Preparation and Planting

The maize seeds (Qiaodan no.6) were soaked in warm water (45 °C) for 24 h and then placed on a moist filter paper seedbed to germinate. After 4 d, the embryonic roots emerging from the seeds were removed from the roots with sterilized scissors. Seeds were then germinated in nutrient cups (8 cm × 10 cm, cylindrical) with a 1:1 mix of dry soil and vermiculite by volume, and seedlings of uniform size were selected for transplanting on 29 March 2022, with a 9:1 mix of dry soil and vermiculite (2–4 mm) by volume (weight about 9.5–9.6 kg/pot). The seedlings were selected by moistening the nutrient cup sufficiently to minimize damage during root extraction. The selected seedlings were rinsed with distilled water to clean the soil and vermiculite adhering to the root surface; then, four lateral roots were selected from each plant for root splitting (the difference in length between each lateral root was less than 3 mm), with two roots on each side of each conjoined pot. After planting, seedlings that died or developed serious diseases within 7 d were replaced with new plants, and watered every 3 d. The watering intensity was controlled to ensure that no leakage occurred at the base. The above-ground parts were properly bound down during the maize trumpeting period to separate the above-ground parts effectively [20].

2.4. Samples and Measurements

In the final sampling, we selected 12 repeats for each treatment, with 6 repeats at the flowering stage (28 June 2022) and 6 repeats at mature stage (12 August 2022). The stems, leaves, and ears were separated, and the leaf area was determined using maximum leaf length × maximum leaf width × 0.75. Then, the leaf was dried at 70 °C for 30 min in an oven at 105 °C to a constant weight. For better separation of soil and roots during sampling, no watering was applied for 3 d prior to sampling. The nutrient pots were gently shaken before sampling; then, the culture pots were cut from all sides with scissors, and the soil samples were rinsed with running water until the roots were separated from the soil. Roots were passed through a root scanner (Shanghai Zhongjing Technology Co., Ltd., China, Shanghai, China, ScanMaker i800 Plus) and then analyzed with WinRHIZO 2019b (Regent Instruments Canada Inc., Québec City, QB, Canada), dried to a constant weight at 70 °C, and recorded as dry matter. This was used to calculate the root/shoot ratio (root dry

weight/above ground dry weight), specific root length (root length/root biomass), root average diameter, and root surface area. If a part of the roots was translucent at sampling, it was stained with bright cresol blue before scanning [21].

2.5. Data Analysis

We used the relative interaction index (RII) to assess the intensity of competition between nitrogen distribution patterns: $RII = (B_W - B_O)/(B_W + B_O)$ [22], where B_O is the above-ground biomass of the target plant when grown alone (no competition), and B_W is the above-ground biomass of the target plant when grown with intraspecific competition. RII ranges from -1 to 1 , with negative values indicating competition; smaller values indicate stronger competition; positive values indicate facilitation; and $RII = 0$ indicate that crop growth was not influenced by neighboring plants.

Root foraging precision analysis: Increased root growth in high nutrient patches is considered to be an adaptive response to heterogeneous nutrients. We used the ratio of root biomass in high to low nitrogen patches as a measure of foraging precision (foraging precision = high nitrogen of root biomass/low nitrogen of root biomass). The higher the ratio, the greater the precision of root foraging.

Before data analysis, the roots could not be completely separated due to the root interaction of the two maize roots crossing each other. During the analysis, the relevant root metrics were averaged into two parts so that the data were at the same level. Two-way ANOVA using SPSS 26.0 (SPSS Inc., Chicago, IL, USA) was used to analyze intraspecific competition and nitrogen distribution patterns on the plant height, stem thickness, leaf area, yield, harvest coefficient, total biomass, total root biomass, average root diameter, surface area, specific root length, and root/shoot ratio. The analyses were followed by multiple comparisons (LSD method) of the indicators. Student's *t*-test was used to analyze the specific root length, surface area, diameter, and biomass in nitrogen homogeneous (left and right pots) and heterogeneous (high and low nitrogen side pots), and root foraging precision, with and without a root interaction. Significance was indicated when $p \leq 0.05$.

3. Results

3.1. Analysis of Variance (ANOVA) Table of Intraspecific Competition and Nitrogen Distribution Patterns

At the flowering and mature stages, the main traits of maize were strongly influenced by intraspecific competition, especially the total biomass, root/shoot ratio, surface area, root biomass, and average diameter which reached significant levels, while the nitrogen distribution pattern mainly influenced the specific root length at the flowering stage and had a significant interaction effect with intraspecific competition. The overall pattern of nitrogen distribution had no significant effect on the above-ground phenotype of maize. This could also be the result of some physiological 'integration' of the plants.

3.2. Influence of Intraspecific Competition and Nitrogen Distribution Patterns on Relative Interaction Index and Root Foraging Precision

The relative interaction index of nitrogen distribution patterns at the flowering (Figure 2a) and mature stage (Figure 2b) did not differ significantly but showed a higher homogeneous nitrogen competitiveness than heterogeneous nitrogen at the flowering stage. The opposite was true at the mature stage, probably because the maximum growth potential of the crop during the reproductive period is reflected at the flowering stage, when the intraspecific competition response is stronger, and its root surface area is higher than heterogeneous. The crop also absorbed relatively high levels of nutrients, and the roots were maximized to meet later growth. Regardless of the pattern of nitrogen distribution after the flowering stage, the crop absorbed nutrients mainly towards the kernel, and its competitiveness was relatively reduced.

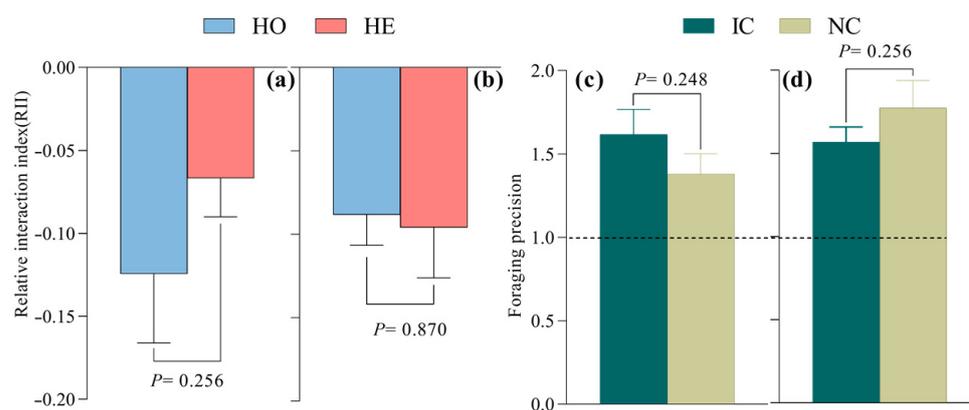


Figure 2. Effect of nitrogen distribution pattern on the relative interaction index and root foraging precision of maize. (a) Relative interaction index at flowering stage. (b) Relative interaction index at mature stage. (c) Foraging precision at flowering stage. (d) Foraging precision at mature stage, HO: Nitrogen homogenization supply, HE: Nitrogen heterogeneous supply, IC: Intraspecific competition, NC: No competition, the same as below.

Nitrogen heterogeneity generally increased root foraging precision with intraspecific competition and no competition at the flowering stage. This suggested that the root interaction stimulated root foraging behavior (Figure 2c), while intraspecific competition was less than no competition at the mature stage (Figure 2d). This result was probably due to the high nitrogen demand of maize at the flowering stage where intraspecific competition increased root competition. In the mature stage, the nitrogen consumption in the soil was exhausted, and the intraspecific competition increased the nitrogen consumption rate, while the nitrogen consumption was always in a ‘moderate’ state when there was no competition.

3.3. Effects of Intraspecific Competition and Nitrogen Distribution Patterns on Maize Growth and Development

Plant height can reflect the production potential of the crop [23]. Under the homogeneous nitrogen distribution pattern at the flowering stage, the height of intraspecific competition plants was significantly higher than that of no competition, and the difference in heterogeneous nitrogen was not significant (Figure 3a). The nitrogen distribution pattern had no significant effect on the height of intraspecific competition and no competition plants, but there was a significant interaction between the two (Table 2). Neither intraspecific competition nor the nitrogen distribution pattern had a significant effect at the flowering and mature stages (Figure 3b) but showed that homogeneous was higher than heterogeneous (Figure 3c). The stem diameter, which reflects the crop ability to resist lodging, was not significantly affected at flowering (Figure 3c) and was significantly higher at the mature stage without competition than between roots (Figure 3d), while the difference between nitrogen distribution patterns was not significant. The leaf area, an indicator of the photosynthetic intensity of the crop, did not differ significantly at flowering, regardless of the nitrogen distribution pattern and with or without intraspecific competition (Figure 3e, Table 2). In summary, the nitrogen distribution pattern did not have a significant effect on apparent traits such as maize plant height and leaf area, and these data suggest an integration of the above-ground parts of the plant in response to the process of nitrogen heterogeneity to reduce the adverse or beneficial effects resulting from the below-ground roots.

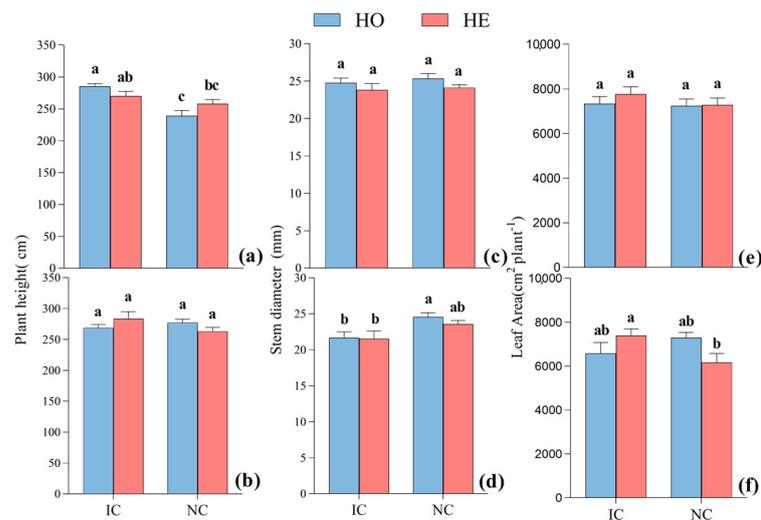


Figure 3. Effect of nitrogen distribution pattern and intraspecific competition on maize plant height, stalk thickness, and leaf area. (a) Plant height at flowering stage. (b) Plant height at mature stage. (c) Stem diameter at flowering stage. (d) Stem diameter at mature stage. (e) Leaf area at flowering stage. (f) Leaf area at mature stage. Values are means \pm standard error of the mean. Treatments with different lowercase letters are significantly different at $p < 0.05$, the same as below.

Table 2. Analysis of variance for maize yield, growth parameters, and root traits in flowering stage and mature stage (F value).

Parameters	Flowering Stage			Mature Stage		
	ND	RI	ND \times RI	ND	RI	ND \times RI
Plant height	0.08	17.70 **	6.09 *	0.01	0.56	3.47
Stem diameter	2.69	0.45	0.03	0.51	9.78 **	0.32
Leaf area	0.52	0.80	0.37	0.16	0.47	6.59 *
Total biomass	0.02	7.09 *	0.58	0.53	17.09 **	<0.01
Root/shoot ratio	0.32	10.09 **	4.12	0.03	12.24 **	1.57
Yield	-	-	-	0.18	2.31	3.43
Harvest coefficient	-	-	-	0.40	4.54 *	8.27 **
Specific root length	29.35 **	4.22	10.76 **	2.30	2.30	5.53 *
Total root surface area	2.06	7.14 *	0.05	0.51	15.76 **	0.09
Total root biomass	0.12	15.43 *	0.31	0.06	22.35 **	0.95
Average root diameter	3.58	9.59 **	0.36	1.12	5.12 *	4.28

ND: nitrogen distribution pattern, RI: with or without intraspecific competition, ND \times RI: interaction effect. * and ** represent p values < 0.05 and < 0.01, respectively.

3.4. Effect of Intraspecific Competition and Nitrogen Distribution Patterns on Total Biomass, Root/Shoot Ratio, Yield, and Harvest Coefficient

The differences between nitrogen distribution patterns for biomass were not significant (Figure 4a,b) but all showed higher no competition treatments than intraspecific competition, especially at the mature stage (Figure 4b, Table 2). The root/shoot ratio reflected the correlation between above-ground and below-ground parts of the crop, and overall, the no competition root/shoot ratio was significantly higher than intraspecific competition. Nitrogen distribution patterns did not significantly affect the root/shoot ratio at the flowering and the mature stage (Figure 4c,d, Table 2). At the flowering stage of intraspecific competition, the root/shoot ratios were higher in homogenous conditions, and it had a higher root/shoot ratio in the heterogeneous condition of no competition, but the opposite was true at the mature stage. There was no significant difference in yield between different nitrogen distribution patterns, but homogeneous nitrogen was 10.20% higher than heterogeneous nitrogen in intraspecific competition, and heterogeneous nitrogen was

6.08% higher than homogeneous nitrogen in no competition (Figure 4e). The harvest coefficients were the opposite of yield, with heterogeneous nitrogen significantly higher than homogeneous nitrogen at intraspecific competition (Figure 4f, Table 2), and non-significant differences at no competition. In agroecosystems, when basal fertilizer is applied heavily, it helps to build fertilizer homogeneity, and, when fertilizer follow-up is applied heavily, it helps to build nutrient heterogeneity [24]. Based on our results, especially for yield differences, we recommend that basal fertilizer should be applied heavily when intraspecific competition is present, and follow-up fertilizer should be applied appropriately when there is no competition.

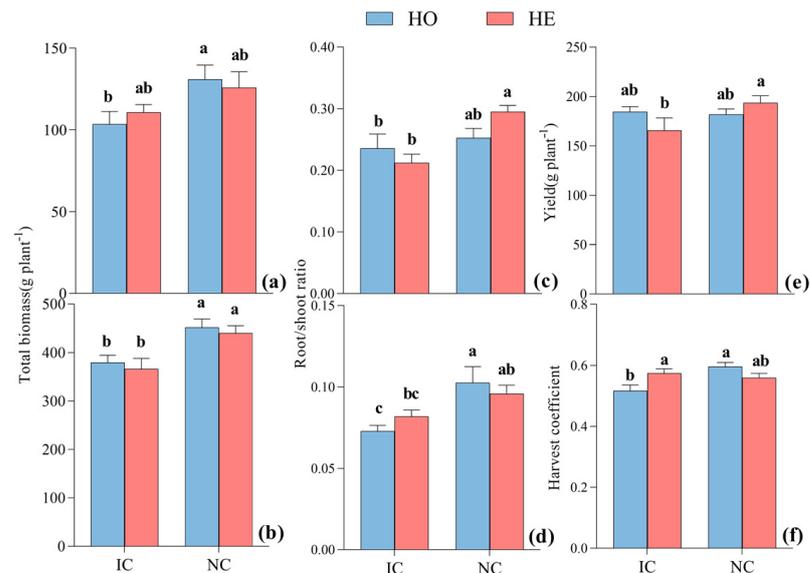


Figure 4. Effect of nitrogen distribution pattern and intraspecific competition on total biomass, root/shoot ratio, yield, and harvest coefficient of maize. (a) Total biomass at flowering stage. (b) Total biomass at mature stage. (c) Root/shoot ratio at flowering stage. (d) Root/shoot ratio at mature stage. (e) Yield. (f) Harvest coefficient.

3.5. Effect of Intraspecific Competition and Nitrogen Distribution Patterns on Root Traits of Maize

The specific root length is a key root trait that determines the root ability to absorb water and nutrients, and is also closely related to root respiration and plasticity. There was no significant difference in the effect of the nitrogen distribution pattern on the specific root length of intraspecific competition at both flowering (Figure 5a) and mature stages (Figure 5b). There was a significant interaction between homogeneous nitrogen and heterogeneous nitrogen in no competition of the flowering stage, and the opposite at mature, where heterogeneous nitrogen was significantly higher than homogeneous nitrogen (Table 2). Student's *t*-test of the specific root length of roots on both sides of intraspecific competition and no competition showed that there were no significant differences in the homogeneous nitrogen distribution (left and right side) and heterogeneous nitrogen distribution (high and low nitrogen side) in the specific root length at the flowering stage (Figure 5c,e) and the maturity stage (Figure 5d,f). The nitrogen heterogeneity distribution increased the specific root length of no competition at the mature stage, although it did not increase at the high nitrogen side, indicating that the roots were consistent in coordinating nutrient uptake and growth.

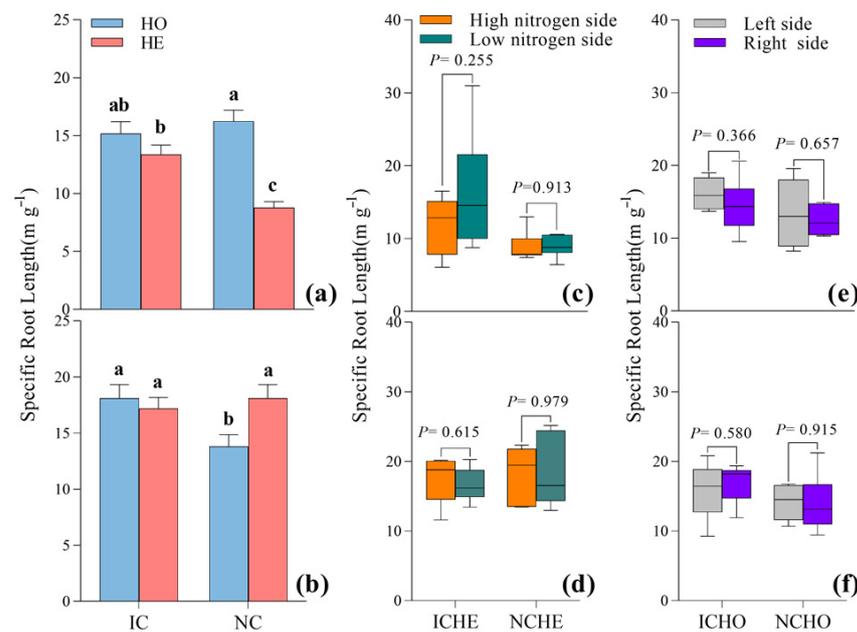


Figure 5. Effect of nitrogen distribution pattern and intraspecific competition on specific root length. (a) Specific root length at flowering stage. (b) Specific root length at mature stage. (c) Under heterogeneous nitrogen distribution conditions, specific root length at flowering stage with intraspecific competition. (d) Under heterogeneous nitrogen distribution conditions, specific root length at mature stage with intraspecific competition. (e) Under homogeneous nitrogen distribution conditions, specific root length at flowering stage with no competition. (f) Under homogeneous nitrogen distribution conditions, specific root length at mature with no competition.

In both intraspecific competition and no competition, the nitrogen distribution patterns did not differ significantly on the root average diameter at the flowering stage (Figure 6a). All showed a heterogeneous nitrogen distribution over a homogeneous nitrogen distribution, and, at the mature stage, the differences were not significant for intraspecific competition and significantly higher for no competition homogeneous nitrogen than for the heterogeneous nitrogen distribution. The differences in the root average diameter between the high and low nitrogen side pots of the heterogeneous nitrogen distribution were not significant at the flowering (Figure 6c) and mature stages (Figure 6d), nor were the differences in the root average diameter between the left- and right-side pots of the homogeneous nitrogen distribution significant at the flowering (Figure 6e) and mature stage (Figure 6f).

Root biomass was used as the most direct indicator of the response to the nitrogen distribution and intraspecific competition. The differences in root biomass between the nitrogen distribution were not significant at the flowering and mature stages (Figure 7a,b), but no competition was significantly higher than intraspecific competition (Table 2). Student's *t*-test for intraspecific competition and no competition for high and low nitrogen pots demonstrated that root biomass reached significant levels at both the flowering (Figure 7c) and the mature stage (Figure 7d). These results showed that the high nitrogen basin was significantly greater than the low nitrogen basin, but there was no significant difference in root biomass between the left and right basins at the flowering stage (Figure 7e) and the mature stage (Figure 7f) under the homogeneous nitrogen distribution. In short, the high nitrogen side roots are thicker, while the low nitrogen side roots are finer, which may be that when the low nitrogen side roots obtain more nutrients, more fine roots are developed to increase the contact area with the soil.

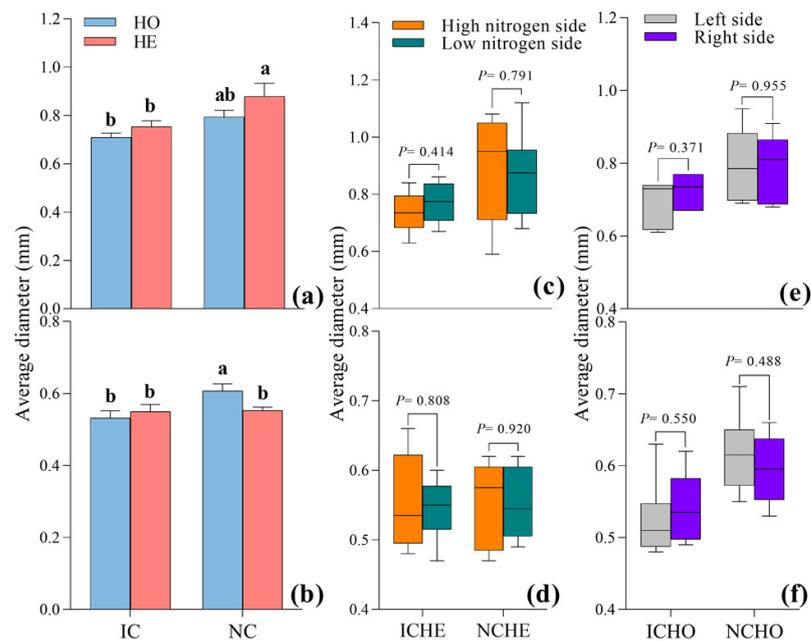


Figure 6. The effect of nitrogen distribution pattern and intraspecific competition on root average diameter. (a) Average diameter at flowering stage. (b) Average diameter at mature stage. (c) Average diameter with heterogeneous distribution of nitrogen at flowering stage. (d) Average diameter with heterogeneous distribution of nitrogen at mature stage. (e) Average diameter with homogeneous distribution of nitrogen at flowering stage. (f) Average diameter with homogeneous distribution of nitrogen at mature stage.

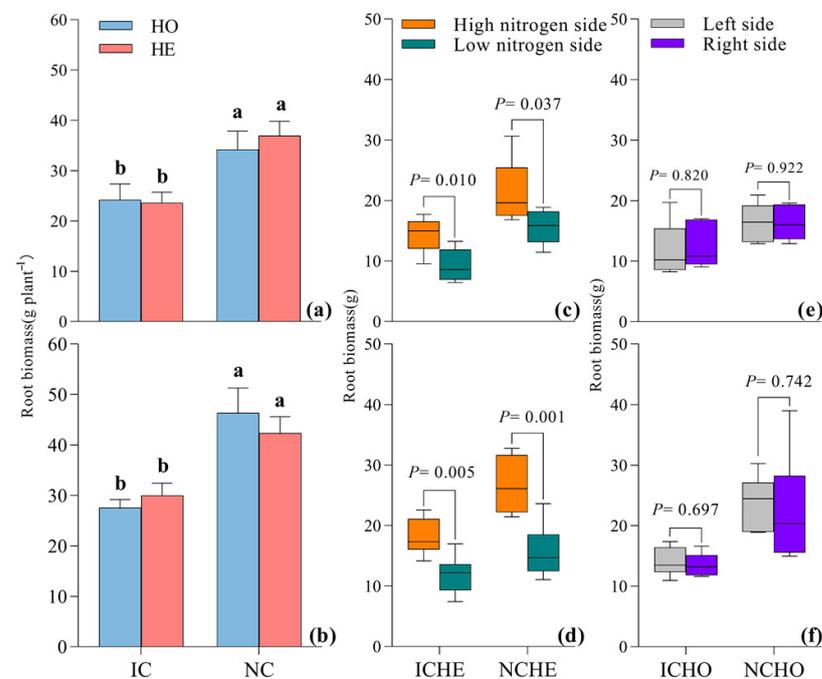


Figure 7. Effect of nitrogen distribution pattern and intraspecific competition on root biomass. (a) Total root biomass at flowering stage. (b) Total root biomass at mature stage. (c) Root biomass with heterogeneous distribution of nitrogen at flowering stage. (d) Root biomass with heterogeneous distribution of nitrogen at mature stage. (e) Root biomass with homogeneous distribution of nitrogen at flowering stage. (f) Root biomass with homogeneous distribution of nitrogen at mature stage.

Plants mainly absorb water and nutrients in soil through roots, and surface area is a commonly used index to evaluate the absorption function of roots [25]. The larger the index value, the greater the probability of roots contacting nutrients, the greater the vigor, and the stronger the absorption capacity. Nitrogen distribution patterns and no competition did not significantly affect total root surface area at the flowering and mature stage (Figure 8a,b). However, no competition was higher than intraspecific competition and reached a significant level at the mature stage (Table 2). Heterogeneous nutrients' distribution at intraspecific competition revealed that the surface area of high-nutrient pots was significantly higher than low-nutrient pots at the flowering (Figure 8c) and the mature stage (Figure 8d), while the differences between the left and right pots were not significant at the flowering (Figure 8e) or mature stage (Figure 8f) in homogeneous nutrients.

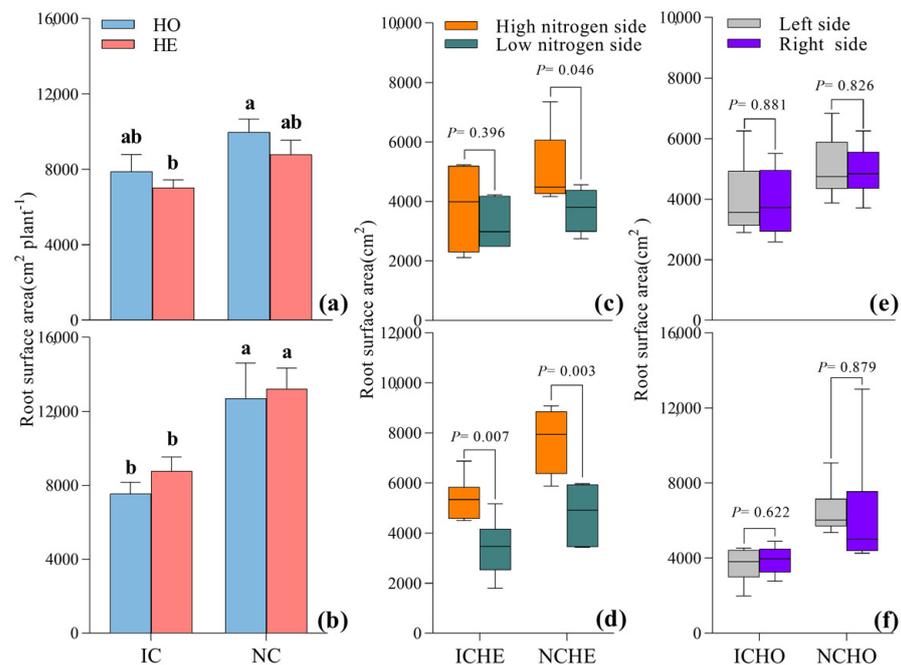


Figure 8. Effect of nitrogen distribution pattern on root surface area. (a) Root surface area at flowering stage. (b) Root surface area at mature stage. (c) Root surface area at flowering with heterogeneous distribution of nitrogen. (d) Root surface area at mature with heterogeneous distribution of nitrogen. (e) Root surface area at flowering with homogeneous distribution of nitrogen. (f) Root surface area at mature with homogeneous distribution of nitrogen.

This study, based on the correlation between maize yield and root foraging precision, showed that intraspecific competition had a negative correlation at both the flowering and mature stages (Figure 9a). The relationship reached significant levels at the flowering stage, while no competition showed a negative correlation at flowering, while showing a positive correlation at the mature stage (Figure 9b). These results suggest that although intraspecific competition nitrogen heterogeneity increases the precision of roots, the behavior of the roots in obtaining nutrients does not seem to result in yield improvement.

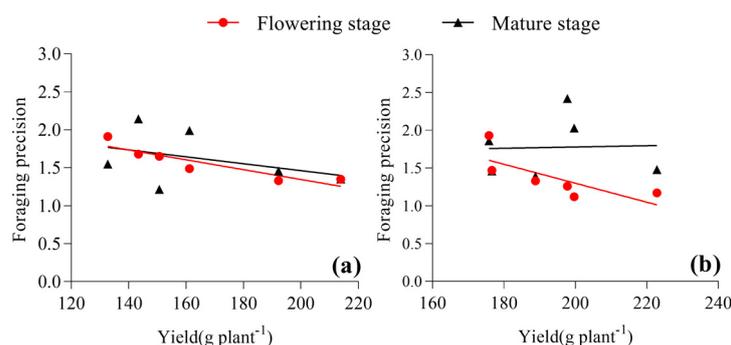


Figure 9. Relationship between maize yield and (a) Intraspecific competition (Flowering stage: $r = -0.909$, $p = 0.011$; Mature stage: $r = -0.385$, $p = 0.450$), (b) No competition (Flowering stage: $r = -0.742$, $p = 0.091$; Mature stage: $r = 0.036$, $p = 0.945$).

4. Discussion

Plants exhibit a high degree of plasticity in the morphological and physiological responses of their roots to access heterogeneous soil nutrients. The morphological plasticity of the roots provides the means for capturing heterogeneous nutrient resources. In the present study, the heterogeneous distribution of nitrogen increased root biomass on the high nitrogen side relative to the homogeneous distribution of nitrogen. However, the difference in total biomass was not significant, and inter-intraspecific competition significantly reduced the total root biomass (Figure 7a). This is similar to other studies; the heterogeneous distribution of nitrogen can significantly promote root proliferation in nitrogen-enriched areas [26], including increased root length and biomass. For example, wheat exhibited increased biomass in nitrate- and phosphorus-enriched areas [27], a finding that was also observed in forest trees [28] and weeds [29]. Perhaps grasses such as maize can make better use of nutrient patches without increasing root biomass and the root/shoot ratio, as it has a relatively high root mass fraction and a small root diameter. However, this inter-rooting is based on a combination of roots with the same structure, and this combination can lead to more intense competition [30], which is detrimental because it wastes the limited resources available for root foraging. Intraspecific competition similarly increases root length (Figure 5b), and the optimal allocation of nitrogen to roots in the presence of competitors is more complex than in the absence of competition. It generally increases with the ability of competitors to produce root length. This capacity also reduces the ability of competitors to obtain the nitrogen supply and thereby increases the net resource balance of neighboring plants. However, this increased allocation comes at the expense of neighboring plants [31,32]. In the present study, distribution of nitrogen did not change the specific root length of the roots on either side, either by intraspecific competition or no competition, but the heterogeneous distribution of nitrogen changed the total specific root length of the no competition roots (Figure 5a,b). This was especially obvious at the mature stage, where heterogeneous nitrogen significantly increased the specific root length of the roots. This phenomenon was not reflected in intraspecific competition (at the flowering and mature stages) and may have occurred because root foraging was less important in the no competition cultivation than in intraspecific competition. In single-species plots, individual plants propagated in rich patches may not gain biomass because their neighbors adopt the same strategy, thus counteracting a possible advantage, while roots of neighboring plants growing in a heterogeneous distribution of nitrogen reduce nutrient availability in the patch [26,33].

The morphology and spatial distribution of crop roots determine the root configuration and influence the ability of the roots to obtain water and nutrient resources from the soil. The root surface area directly or indirectly responds to the ability of the crop roots to absorb nutrients [34]. In the present study, intraspecific competition significantly reduced the total root surface area, especially at the mature stage. This may be because plants produce more roots in higher nutrient patches while also increasing the root competition in

higher nutrient patches, thus creating a so-called tragedy of the commons (which results in plants grown with neighbors over-proliferating their roots at the expense of above-ground biomass) [32,35]. This means that root proliferation in heterogeneous soils is essential for plants to gain growth benefits and thus exhibit optimal growth strategies. In contrast, the root surface area was significantly higher on the high nitrogen side of the interaction and no competition heterogeneous nitrogen distribution than on the low nitrogen side, a result that is consistent with root biomass, yet the nitrogen distribution pattern did not change the total surface area of the interaction and no competition roots. Since root proliferation depends on a limited number of binding sites on the roots and soil particles, thereby increasing nutrient availability [36,37], the proportion of total nutrient uptake from the soil by the roots increases more rapidly in no competition situations than in intraspecific competition.

Increased foraging precision in crops is usually a sign of adaptation, helping to promote nutrient uptake and enhance competitive advantage [38]. However, we found that although foraging precision increased at both the flowering and mature stages under intraspecific competition compared to no competition (Figure 2c,d), in combination with total biomass of maize (Figure 4a,b), there was a significant reduction in intraspecific competition. This suggests that increased foraging precision did not necessarily significantly enhance interplant competition. In addition, the degree of nitrogen heterogeneity distributed in our study was 5:1, which may also result in maize roots influencing this degree of heterogeneity in a manner that is not favorable. It is possible that an excessive degree of heterogeneity may increase the excessive energy consumption of root foraging processes, and this seems to be detrimental to the long-term evolutionary selection of plants. We therefore suggest that there is some 'behavior' of the roots that reduces nutrient acquisition efficiency during foraging, as foraging itself is an energy-consuming process [39,40]. Although the intraspecific competitions appeared to be twice as uncompetitive in terms of nitrogen inputs, they were not equally nutrient-depleting for the intraspecific competitions. This was because, at the beginning, the two individual roots of the intraspecific competitions were close to each other, and the intraspecific competitions became increasingly complex over time. During flowering, competition for the homogeneous nitrogen distribution was stronger (Figure 2a), because homogeneous nutrients resemble many small nutrient patches. The maize roots were able to respond quickly to nutrient patches and proliferate roots, thus gaining a competitive advantage by occupying more nutrient patches, a situation that becomes more asymmetrical over time [41,42]. In addition, in our study, nitrogen distribution patterns did not change the overall specific root length of intraspecific competition. In no competition, nitrogen distribution patterns had opposite effects on root length at flowering and mature stages, which may be a balancing and coordinating effect of plants in order to adapt to the interaction between nutrients and roots [43,44], and this competitive process deserves further study.

Changes in root foraging behavior and the foraging capacity of plants affect their individual growth and alter intraspecific interactions [45]. Nutrient patches can significantly contribute to above-ground biomass in graminoid and non-nitrogen-fixing dicot species [46,47]; however, this effect is generally observed in the early stages of crop growth, as plants respond to a heterogeneous nitrogen distribution over a short period (4 weeks) of time [48]. In addition, such studies are based on monocultures, and, in agroecosystems, there are always interrelationships between or within species. For example, plants with fast growing roots within nutrient patches or plants that are exposed to nutrient patches first are able to take up or deplete most of the nutrients within the nutrient patches in a short period of time before the roots of other plants can reach them. This short-term exploitation advantage may be maintained in the natural ecosystem to provide a long-term growth advantage [49]. In the present study, the heterogeneous distribution of nitrogen in the intraspecific competitions was consistent with this pattern (in terms of total biomass). The two plants in the flowering period were not equally absorbing nutrients, so there was asymmetric growth [50]. This response mechanism allows plants to occupy nutrient

patches by increasing root growth even in the absence of nutrient deficiency, representing an ecological strategy for plant adaptation to nutrient heterogeneity [51].

In the present study, roots were only sampled at the flowering and the mature stages. Although flowering is the most active period of crop development, it is unclear when nitrogen heterogeneity begins to act on root development, and how nitrogen heterogeneity regulates inter-intraspecific competition. Molecular biology studies have shed light on the regulation of root development, such as the phenotypic response of root system architecture to heterogeneous nitrogen availability and the importance of phenotypic plasticity for nitrogen acquisition [52,53]. In addition, our study was based on the exclusion of competition for light from the above-ground part of the plant, but, in real agroecosystems, competition for light from the above-ground part of the plant cannot be ignored and becomes more intense as planting density increases [1]. Competition for light and nutrient heterogeneity between above- and below-ground plant parts remains a challenge in agroecosystem research.

5. Conclusions

Over the entire period of maize growth, the heterogeneous distribution of nitrogen had no significant effect on the indicators related to agronomic traits in the above-ground parts of maize, regardless of intraspecific competition or no competition. Intraspecific competition in the nitrogen heterogeneous distribution increased inter-root competition, although we provided double the nutrients, but it was detrimental to the yield increase. The no competition, heterogeneous nitrogen distribution slightly increased yield. Overall, the heterogeneous distribution of nitrogen increased the root foraging precision, but this increase was negatively correlated with yield. Although it increased the biomass of the roots on the high nitrogen side, the effect on total root biomass and surface area was not significant. When there is intraspecific competition, we recommend that basal fertilizer should be applied heavily to reduce the heterogeneity of the soil nutrient distribution. This will reduce the energy consumed by root foraging behavior and reduce the impact on yield. When there is no competition, heavy application of topdressing fertilizer will increase heterogeneity in the soil nutrient distribution and improve crop root foraging behavior.

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