Response of maize growth and soil biological characteristics to planting density under fertigation in a semi-arid region

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Abstract: Increasing the[5 planting density can exacerbate crop competition for water, nutrients and space which results in a decline in the crop yields. However, the effect of increasing planting density on crop growth and soil biological characteristics in barren sandy land in the semi-arid regions are still unclear. In this study, we investigated the effects of six planting densities (5.4×10^4, 6.45×10^4, 7.95×10^4, 9.5×10^4, 9.75×10^4 and 10.5×10^4 plants/hm^2) on maize growth, photosynthesis characteristics, yield and soil biological characteristics in barren sandy soil in the semi-arid region of Ningxia, China. The results indicated that the stem diameter and spike length decreased linearly with increasing planting density. The plant height, spike weight, grain weight and 100-grain weight decreased with increasing plating density. Moreover, the root length increased with increasing planting density. The diameter, volume and activity increased and then decreased with increasing planting density. There was no significant difference ($p>0.05$) in the effect of planting density on transpiration rate intercellular CO_2 concentration. As well, the soil microbial biomass carbon and microbial biomass nitrogen decreased with increasing planting density. The soil catalase activities increased and then decreased with increasing planting density. The alkaline phosphatase activity, the amounts of soil bacteria and actinomycetes increased with increasing planting density. Generally, a moderately increasing planting density can improve maize yield when water and nutrients are sufficient. The optimal planting density was 8.29×10^4 plants/hm^2 and the highest yield was 15.84 t/hm^2 in barren sandy soil in semi-arid region of Ningxia, China. This study provides a theoretical basis for high yield and high efficiency of maize.

Keywords: maize growth, planting density, yield, fertigation, soil enzyme activity
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1 Introduction

Maize (*Zea mays* L.) is a thermophilic C_4 short-day plant with high light efficiency and sensitivity to light duration. As a high-yield crop for both food and feed, its cultivation is of great significance to food security in the world[1]. The practice has shown that increasing planting density can improve the maize yield, while excessively dense planting has a negative effect on growth, yield and quality[2]. Lu et al.[3] confirmed that grain yield was highest for a maize planting density of 6.9×10^4 plants/hm^2 and biological yield was highest at 9.0×10^4 plants/hm^2 under flood irrigation conditions[3]. The fresh matter and dry matter yield of maize increased significantly with the increase in planting density[4]. Another study showed a quadratic relationship between planting density and silage maize yield[5]. Resource utilization is insufficient under low-density planting, while the disease and insect pest problems are intensified and lodging is prone to occur which reduces production under high-density planting[5]. In 2016, the highest dry yield record of 22.50 t/hm^2 in China was set for the cultivated density of maize in Xinjiang Province of 12.0×10^4 to 13.5×10^4 plants/hm^2[6].

There is an interaction between planting density and soil characteristics, especially for rhizosphere exudates and soil microorganisms[7]. Cha et al.[8] studied the changes of enzyme activity in the rhizosphere soil of winter wheat under different planting densities, and showed that enzyme activity was higher under medium-density treatment. Ma et al.[9] found that with the increase of planting density, total wheat rhizosphere microbes and enzyme activity showed rising trends at low density, but this trend changed to be a downward trend after reaching a specific density; the yield showed a similar performance, indirectly showed that an increase in microorganism numbers may increase crop yield. There are few reports on the response mechanism of rhizosphere soil, especially rhizosphere microbial characteristics to maize planting density.

The semi-arid zone in the middle part of Ningxia is an interlacing zone of agriculture and pasture. However, maize yield here is only 10.5 t/hm^2 for a density of 6.0×10^4 plants/hm^2. On the basis of satisfying the limiting factors such as water and fertilizer, it is of great significance to increase the unit yield of maize through reasonable dense planting in order to ensure food security. Based on this, under fertigation conditions, this experiment investigated the effects of the planting density on the growth and development, physiology, yield of maize and the soil biological properties. This study provided a theoretical basis for determining a high-yield

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population effect.

2 Materials and methods

2.1 Study site and soil properties

The experiment was carried out in 2022 in Maerzhuang Village, Fengjigou Town, Yanchi County, Ningxia, China (106.8498°E, 37.6760°N), located in the central and western parts of Yanchi County. It has a semi-arid climate in the mid-temperate zone, with an annual average temperature of 8.4°C, annual average rainfall of 265 mm, annual average evaporation of 2000-3000 mm, a frost-free period of 151 d, ≥10°C accumulated temperature of 2949.9°C and annual sunshine hour of about 2800 h. The study area was high-standard basic farmland with flat and water-saving irrigation facilities. The tested variety was Tianci 19, the main maize variety in this area. The soil types are arid soil class, normal arid soil subclass, calcium accumulation normal arid soil type (commonly known as calcareous soil), and sandy calcareous soil subclass. The whole texture was sandy loam, with weak structure and poor water and fertility retention. The basic physical and chemical properties of the experimental soil are listed in Tables 1 and 2.

Table 1 Basic physical properties of the experimental soil

Soil depth/cm	Mechanical composition/%			Bulk density/g·cm^{-3}	Field water capacity/%	Total porosity/%
	Sand (2.000-0.020 mm)	Silt (0.020-0.002 mm)	Clay (<0.002 mm)			
0-20	60.21	30.28	9.51	1.37	21.87	52.32
20-40	58.26	33.49	8.25	1.40	23.21	46.39

Table 2 Basic chemical properties of the experimental soil

Soil depth/cm	pH	TS/g·kg^{-1}	TN/g·kg^{-1}	TP/g·kg^{-1}	OM/g·kg^{-1}	AN/mg·kg^{-1}	AP/mg·kg^{-1}	AK/mg·kg^{-1}
0-20	8.52	0.45	0.35	0.31	6.28	34.26	24.13	135.00
20-40	8.56	0.39	0.32	0.28	4.45	31.25	20.13	124.00

Note: TS, TN, TP, OM, AN, AP, and AK represent soil total salt, total nitrogen, total phosphorus, organic matter, available nitrogen, available phosphorus and available potassium, respectively.

2.2 Experimental design

A single-factor multi-level randomized block design was used to set up six different planting densities: 1) 5.4×10^4; 2) 6.45×10^4; 3) 7.95×10^4; 4) 9.5×10^4; 5) 9.75×10^4 and 6) 10.5×10^4 plants/hm^2. Because the planting distance by the planter was not well adjusted, planting density was not completely set according to the equal spacing. Beidou satellite navigation (FJ-NS300, Fengjiang, China) was used to sow seeds in 70 cm wide and 40 cm narrow rows. A drip irrigation belt was laid in the middle of the narrow rows. Each plot was 92.4 m^2 (4.4 m×21.0 m), and each treatment was repeated three times, with a total of 18 plots. The whole growth period is the integration of water and fertilizer. Fertigation included 14 water applications and eight fertilizer applications. Each application was 225-375 m^3/hm^2; over the whole maize growth period, 3150 m^3/hm^2 of water from the Yellow River was applied. Fertilization was carried out with water at eight physiological stages: two leaves per bud, four leaves per bud, six leaves per bud, jointing, trumpet mouth, tasseling, silking and filling stages. The application amount of water-soluble fertilizer (Ningxia Runhefeng Biotechnology Co., Ltd.) was 930 kg/hm^2, and the total nutrient of water-soluble fertilizer accounted for 50% (among them, N accounted for 30%, P_2O_5 accounted for 8%, K_2O accounted for 12%). and contents of medium and trace elements of 3% and 0.61%, respectively. Sowing was on April 25, 2020, seedling on May 19 and harvest on September 28, with the whole growth period of 153 d.

2.3 Sampling and measurement

2.3.1 Determination of basic physical and chemical properties of soils

Soil samples were collected before maize planting (April 10) to determine related indexes, and the ring knife method was used to determine soil bulk density and field water capacity. The ground was shoveled 3-5 cm flat before sampling. The ring knife was driven vertically into the soil, soil was removed with the ring, both ends were cut flat and then covered and packed with self-sealing bag. Then, the soil samples of plough layer (0-20 cm) were collected by dutch drill(JC-802D, China), mixed and bagged back to the laboratory. After returning to the laboratory, the bottom tail cover of the ring knife was removed and this exposed soil face was put on a suction tank wrapped with absorbent paper in a ceramic basin, and then water was added to soak the top cover of the ring knife. After about 48 h, the ring knife together with the upper and lower covers was weighed and W_1 was recorded. Then, the water-saturated soil sample in the ring knife was taken out and baked in an oven at 105°C to a constant weight, cooled to room temperature, weighed and W_2 was recorded.

$$\rho = \frac{W_2}{V}$$

$$FC = \frac{W_1 - W_2}{W_2} \times 100$$

where, ρ is Bulk density, g/cm^3; V is the volume of ring cutter (100 cm^3); FC is Field water capacity. The topsoil samples were ground and sieved after air-dried for the determination of conventional physical and chemical indicators. Among them, soil pH value was determined by a pH meter (PHS-2F, Leici, China) in a water and soil ratio of 5:1. Soil electrical conductivity and water salinity were determined by a conductivity meter (DDS-11, Leici, China) and the total salt content was deduced from the relationship between conductivity and salt concentration. The contents of soil organic matter, total nitrogen, total phosphorus, alkali-hydrolyzable nitrogen, available phosphorus and available potassium were determined by conventional detection methods. Among them, organic matter content was determined by a potassium dichromate volumetric method, a semi-trace Kelvin method was used for total nitrogen, and a sulfuric acid–perchloric acid digestion method was used for total phosphorus. Alkali-hydrolyzed nitrogen content was determined by an alkali-hydrolyzed diffusion method. The available phosphorus content was determined by 0.5 mol/L sodium bicarbonate extraction and a molybdenum-antimony resistance colorimetry method. The content of available potassium was determined by 1 mol/L ammonium acetate solution extraction and flame photometry[10].

2.3.2 Test of soil biological properties

At the milk ripening stage of maize filling, the plant rhizosphere soil was collected by five-point sampling method in each treatment plot according to the "S" pattern (a brush was used to collect the soil stuck to roots by shaking off method) and the mixed soil samples were stored at low temperature and sent back to the laboratory for determination of enzyme activity and microbial numbers. Urease activity was determined by indophenol blue colorimetry, alkaline phosphatase activity was determined by a benzene disodium phosphate colorimetric method. sucrase activity was determined by a 3.5-dinitrosalicylic acid colorimetric method and catalase activity was determined by potassium permanganate titration[11]. The number of soil microorganisms was counted by the dilution plate method, and bacteria were cultured in beef peptone agar medium. For Actinomycetes, the modified Gauss no. 1 medium was used. Martin-Bengal Red medium was used for fungi[12]. Soil microbial biomass carbon (MBC) and soil microbial biomass nitrogen (MBN) were determined by a chloroform fumigation–K_2SO_4 leaching method. Fresh soil was fumigated with chloroform and then extracted with potassium sulfate; MBC was measured by an automatic organic carbon analyzer and MBN by an automatic Kjeldahl apparatus[13,14].

2.3.3 Determination of maize growth index, yield and yield components

At the beginning of maize filling, an LI-6400P portable photosynthesis meter (LI-COR, USA) was used to measure the photosynthetic characteristics of different treatments. At the milk-ripe stage of maize filling, 20 plants were randomly selected from each plot to measure the root characteristics using root scanner (LD-WinRHIZO, China) analyzer. Plant height and stem diameter were measured using a measuring tape and Vernier caliper, respectively. At the wax-ripening stage, the maize yield was measured by the actual harvest in the plot. First, the total number of plants and panicles in each plot was calculated, and the actual number of plants and panicles per hm^2 was calculated. One panicle was collected for every five panicles in each plot, and a total of 20 panicles were harvested. Spike length, spike diameter, number of rows per spike and number of grains per row were measured, and the number of grains per spike was calculated. Kernel weight per spike, 100 kernel weight and grain water content were determined following threshing, and the yield was converted by 14% water content before entering the warehouse.

2.4 Data analysis

Origin 2021 (OriginLab Inc., USA) was used to organize the data and graphs; SPSS 21.0 software (SPSS Inc., Chicago, IL, USA) was used for the analysis of variance, and the LSD method ($p<0.05$) was used for multiple comparisons. The data in the tables are means±standard errors.

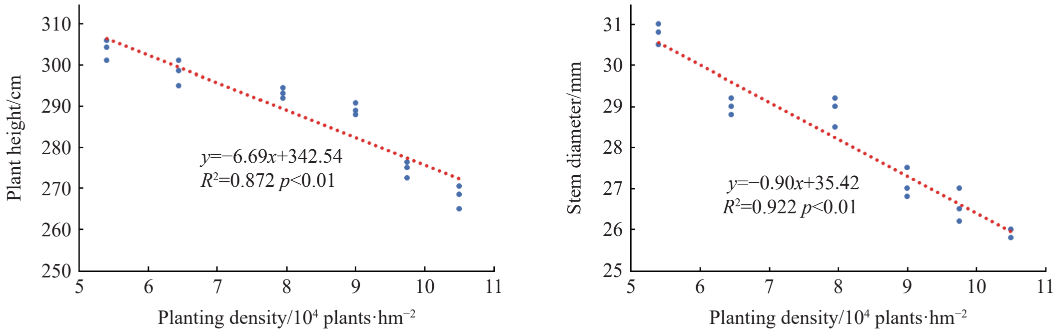
3 Results

3.1 Effects of planting density on maize growth and development

The sensitivity of plant height, stem diameter and other basic growth indicators of maize to planting density are different (Figure 1). With the increase of density, plant height decreased in a quadratic relationship. Plant height was greatest at a planting density of 5.40×10^4 plants/hm^2, which was 305 cm (Figure 1a). Plant height decreased linearly with increasing planting density (R^2=0.872, $p<0.01$) (Figure 1a). With the increase of planting density, plant height decreased slowly, by only 1.80%-4.40%. However, when the planting density was more than 9.00×10^4 plants/hm^2, the plant height decreased rapidly, with a decline rate of 9.17%-11.80%. With the increased density, stem diameter decreased significantly in a quadratic relationship (R^2=0.922, $p<0.01$) (Figure 1b). When the density was 5.40×10^4 to 9.00×10^4 plants/hm^2, stem diameter reached its highest value of 30.8 mm. The response of stem diameter to planting density was linear: stem diameter decreased linearly when the density increased from 5.40×10^4 to 6.45×10^4 plants/hm^2; in the range of 6.45×10^4 to 7.95×10^4 plants/hm^2, stem diameter remained essentially flat and decreased by 6.50%; in the range of 7.95×10^4 to 9.0×10^4 plants/hm^2, stem diameter decreased linearly again, decreasing by 12.90%; and when density exceeded 10.50×10^4 plants/hm^2, the stem diameter decreased linearly again by 16.13%.

3.2 Effects of planting density on photosynthetic characteristics of maize

With the increase of planting density, the photosynthetic characteristics of maize for increasing plant density initially increased and then decreased (Table 3). There was a significant difference in the net photosynthetic rate of maize for densities of 5.40×10^4 and 7.95×10^4 plants/hm^2, but there was no significant difference among other treatments. The stomatal conductance of maize significantly differed for different densities. The maximum stomatal conductance of 630.80 mmol $H_2O/(m^2\cdot s)$ occurred for a density of 6.45×10^4 plants/hm^2. There was no significant difference in transpiration rate and intercellular CO_2 concentration with plant density.



a. Plant height b. Stem diameter

Figure 1 Effects of planting density on plant height and stem diameter

Table 3 Effects of planting density on photosynthetic characteristics

PD/10^4 plants·hm^{-2}	NPR/μmol $CO_2\cdot m^{-2}\cdot s^{-1}$	TR/mmol $H_2O\cdot m^{-2}\cdot s^{-1}$	Int CO_2/μmol·mol^{-1}	SC/mmol $H_2O\cdot m^{-2}\cdot s^{-1}$
5.40	30.12±2.11^{b}	6.62±0.43^{a}	203.05±13.6^{a}	348.23±53.50^{c}
6.45	34.51±3.69^{ab}	7.64±0.74^{a}	183.63±14.7^{a}	630.80±268.90^{a}
7.95	38.03±1.74^{a}	7.91±0.50^{a}	162.66±13.0^{a}	518.22±115.20^{ab}
9.00	36.75±1.58^{ab}	7.57±0.33^{a}	156.65±6.12^{a}	440.82±43.70^{bc}
9.75	34.92±2.51^{ab}	7.60±0.41^{a}	174.65±6.92^{a}	479.24±75.10^{b}
10.50	36.56±2.02^{ab}	7.71±0.29^{a}	166.69±7.45^{a}	468.42±52.80^{b}

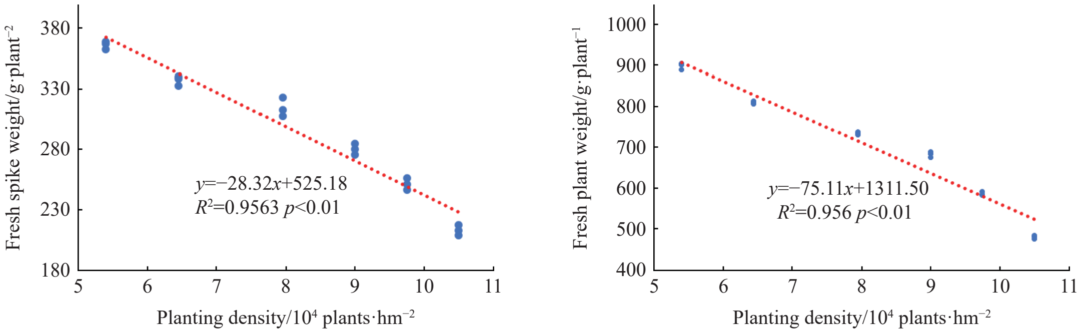
Note: abbreviations: PD, planting density; NPR, net photosynthetic rate; TR, transpiration rate; IntCO_2, Intercellular CO_2 concentration; SC, Stomatal conductance. different letters in the same column indicate a significant difference ($p<0.05$); this is also applied in the following tables.

3.3 Effects of planting density on spike development and yield components

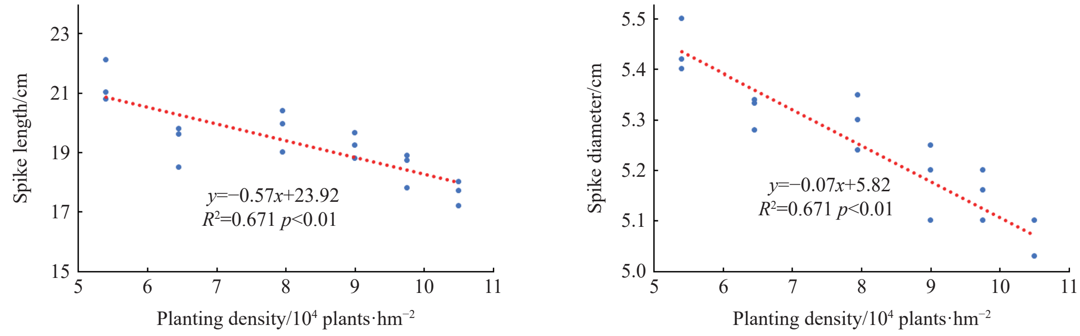
The fresh weight per plant and fresh panicle weight of maize decreased linearly with increasing planting density. As density increased from 5.4×10^4 to 10.5×10^4 plants/hm^2, the fresh panicle weight decreased by 7.92%, 14.84%, 23.64%, 31.65% and 42.13%, respectively (Figure 2a). Correspondingly, fresh plant weight decreased by 10.11%, 18.53%, 24.09%, 35.17% and 46.30% (Figure 2b). Obviously, the decrease of fresh plant weight was higher than that of fresh panicle weight, indicating that with the increase of population density, the contradiction between vegetative growth and reproductive growth intensified, and the inhibition of vegetative growth became more prominent.

The response of maize spike length to density showed a linear pattern. With the increase of density from 5.40×10^4 to 6.45×10^4 plants/hm^2, spike length decreased rapidly by 5.85%, then plateaued, but rapidly decreased again for density up to 7.95×10^4 plants/hm^2. When density was further increased to 9.00×10^4 plants/hm^2, the contradiction between fertilizer and water and physiology was prominent, and spike length decreased rapidly again by 15.83% (Figure 2c).

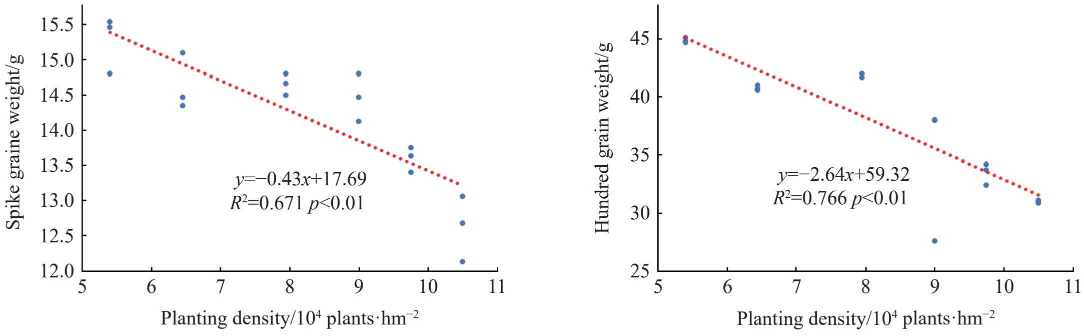
The response of spike diameter to density showed an approximate linear decreasing pattern. With the increase of density from 5.40×10^4 to 10.50×10^4 plants/hm^2, spike diameter decreased slowly by 3.03%, 3.64%, 5.45%, 7.27% and 8.55%, respectively (Figure 2d).



a. Fresh spike weight b. Fresh plant weight



c. Spike length d. Spike diameter



e. Spike graine weight f. Hundred grain weight

Figure 2 Effects of planting density on yield components

With the increase of planting density from 5.40×10^4 to 10.50×10^4 plants/hm^2, affected by competition for fertilizer and water, grain weight per panicle decreased rapidly by 6.88%, 15.44%, 26.32%, 32.64% and 45.82%, respectively (Figure 2e). With the increase of density from 5.4×10^4 to 10.50×10^4 plants/hm^2, the 100-kernel weight of maize decreased gradually from 44.46 to 31.10 g/100 grains. The decrease rates were 8.91%, 5.98%, 15.08%, 24.45% and 30.36% respectively (Figure 2f).

The linear relationship between planting density and each index showed that with the increase of planting density, fresh ear weight, fresh plant weight, ear length, ear diameter, ear grain weight and 100-grain weight all showed a decreasing trend, and there were significant differences in each index under different planting densities.

3.4 Effects of planting density on maize yield

The relationship between planting density and yield was parabolic. When planting density increased from 5.40×10^4 to 7.95×10^4 plants/hm^2, the standard economic yield (14% water content) of maize increased from 13.47 to 15.82 t/hm^2. However, when density exceeded 9.00×10^4 plants/hm^2, the contradiction between water and fertilizer became acute, and it was difficult to meet both vegetative and reproductive growth, and so economic yield decreased rapidly. When density was 10.50×10^4 plants/hm^2, the yield was a minimum, which was 9.25% significantly lower than that for 7.95×10^4 plants/hm^2. The quadratic fitting equation was established by planting density and yield. Based on the equation dY/dx=0, it was found that the maximum economic yield was 15.84 t/hm^2 at the density of 8.29×10^4 plants/hm^2 (Figure 3).

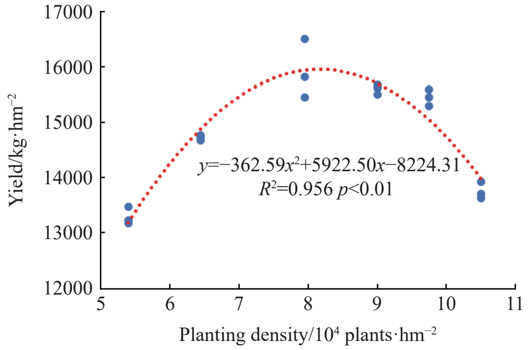


Figure 3 Effects of planting density on economic yield

3.5 Effects of planting density on maize root growth and development

With the increasing planting density, the length of the maize root system increased (Table 4). The main reason for this was that in order to absorb enough water and nutrients in the process of competition and meet the needs of its own growth, maize changed root areas to achieve this purpose. Root surface area, average root diameter, root activity and root volume increased first and then decreased with the increase of planting density. When the density was 7.95×10^4 plants/hm^2, the root surface area, average diameter and volume were the largest, which increased by 55.13%, 28.57% and 98.19% respectively compared with the density of 5.4×10^4 plants/hm^2. When the density was 9.00×10^4 plants/hm^2, the root activity was the greatest. The results indicated that the appropriate planting density could effectively avoid competition between maize plants, so that each maize plant could obtain sufficient water and nutrients, which was conducive to the development of the root system. Overall, maintaining a planting density within a certain range effectively improved the morphology of the maize root system, improved the maize growth state and increased yield.

Table 4 Influence of planting density on the root characteristics of single maize plants

PD/10^4 plants·hm^{-2}	RL/ m	RSA/ cm^2	ARD /mm	RV/cm^3	RA/ $\mu g\cdot g^{-1}\cdot d^{-1}$
5.40	24.49 ± 0.67^{b}	432.91 ± 6.12^{c}	0.56 ± 0.01^{b}	6.08 ± 0.01^{d}	83.95 ± 3.49^{c}
6.45	25.39 ± 1.28^{b}	537.65 ± 22.3^{b}	0.68 ± 0.06^{a}	9.21 ± 1.21^{bc}	159.42 ± 3.49^{b}
7.95	29.76 ± 1.46^{ab}	671.33 ± 28.9^{a}	0.72 ± 0.01^{a}	12.05 ± 0.46^{a}	184.61 ± 8.78^{a}
9.00	29.79 ± 1.82^{ab}	651.52 ± 27.0^{a}	0.70 ± 0.04^{a}	11.43 ± 1.05^{ab}	187.63 ± 8.78^{a}
9.75	30.19 ± 1.61^{ab}	588.51 ± 25.5^{ab}	0.62 ± 0.01^{ab}	9.13 ± 0.30^{bc}	151.42 ± 4.38^{b}
10.50	34.10 ± 2.93^{a}	599.36 ± 45.5^{ab}	0.56 ± 0.02^{b}	8.39 ± 0.62^{c}	78.92 ± 12.6^{c}

Note: abbreviations: PD, planting density; RL, root length; RSA, root surface area; ARD, average root diameter; RV, root volume; RA, root activity.

3.6 Effects of planting density on soil microbial quantity and MBC and MBN

The number of bacteria increased with the increase of planting density, and reached a maximum density of 9.75×10^4 plant/hm^2, which was higher than for the other planting densities, with increases of 2.91%-186.91%. The number of actinomycetes was consistent with the trend for bacteria. The number of actinomycetes for a density of 9.75×10^4 plants/hm^2 was 2.63 times higher than that for 5.40×10^4 plants/hm^2. The planting density had little effect on the number of fungi, with no significant differences among the treatments. The MBC content decreased with increasing planting density, especially when it exceeded 9.00×10^4 plants/hm^2, the content of MBC was reduced to about 140 mg/(kg·d). The MBN content also gradually decreased with the increase of planting density, with decreases ranging within 8.99%-59.89% when density exceeded 5.40×10^4 plants/hm^2 (Table 5).

Table 5 Effect of planting density on soil microbial quantities, soil microbial biomass carbon (MBC) content and soil microbial biomass nitrogen (MBN) content

PD/10^4 plants·hm^{-2}	Bacteria/10^6 cfu·g^{-1}	Actinomycetes/10^4 cfu·g^{-1}	Fungi/10^2 cfu·g^{-1}	MBC/mg·$kg^{-1}\cdot d^{-1}$	MBN/mg·$kg^{-1}\cdot d^{-1}$
5.40	8.25 ± 0.06^{c}	45.33 ± 4.06^{b}	29.00 ± 3.51^{a}	292.10 ± 12.98^{a}	8.90 ± 0.12^{a}
6.45	12.33 ± 2.33^{bc}	62.67 ± 15.68^{ab}	34.67 ± 3.84^{a}	236.30 ± 0.12^{ab}	7.68 ± 0.48^{b}
7.95	14.67 ± 1.20^{b}	70.67 ± 18.85^{ab}	34.00 ± 2.65^{a}	233.50 ± 1.40^{ab}	8.10 ± 0.03^{b}
9.00	14.00 ± 1.15^{b}	76.67 ± 32.83^{ab}	30.00 ± 4.04^{a}	246.40 ± 5.69^{ab}	6.99 ± 0.21^{b}
9.75	23.67 ± 3.96^{a}	119.00 ± 15.52^{a}	25.67 ± 4.67^{a}	143.30 ± 11.82^{b}	3.57 ± 0.31^{c}
10.50	23.00 ± 2.08^{a}	101.00 ± 6.08^{ab}	30.67 ± 1.86^{a}	140.10 ± 11.55^{b}	5.17 ± 1.22^{c}

3.7 Effects of planting density on soil enzyme activities

With the increase of planting density, soil enzyme activity generally initially increased and then decreased (Table 6). Urease activity was at a maximum density of 7.95×10^4 plants/hm^2, and significantly decreased when density exceeded 7.95×10^4 plants·hm^2. The decrease of urease activity inhibited the decomposition of organic nitrogen by rhizosphere microorganisms and reduced nitrogen supply level. Alkaline phosphatase activity reached a maximum at the density of 9.00×10^4 plants/hm^2 and then decreased, meaning that the soil phosphorus supply decreased. Sucrase activity also reached a maximum at the density of 9.00×10^4 plants/hm^2 and then decreased basically consistent with the decrease of MBC. Catalase activity increased in the density range of 5.40×10^4 to 9.0×10^4 plants/hm^2, and decreased slightly when the planting density exceeded 9.00×10^4 plants/hm^2 (Table 6). This is consistent with other results[14].

Table 6 Effects of planting density on soil enzyme activities

PD/ 10^4 plants·hm^{-2}	Urease/ $\mu g\cdot g\cdot d^{-1}$	Alkaline phosphatase/ $\mu g\cdot g\cdot d^{-1}$	Sucrase/ $\mu g\cdot g\cdot d^{-1}$	Catalase/ $\mu g\cdot g\cdot 20\ min^{-1}$
5.40	4.85 ± 0.18^{bc}	0.76 ± 0.03^{c}	29.32 ± 1.45^{b}	1.19 ± 0.00^{b}
6.45	5.27 ± 0.25^{ab}	0.79 ± 0.01^{c}	31.30 ± 0.31^{ab}	1.33 ± 0.01^{ab}
7.95	5.87 ± 0.39^{a}	0.76 ± 0.01^{c}	24.46 ± 0.31^{c}	1.46 ± 0.04^{ab}
9.00	4.75 ± 0.16^{bc}	1.39 ± 0.01^{a}	34.01 ± 2.91^{a}	1.62 ± 0.14^{a}
9.75	5.02 ± 0.17^{b}	1.32 ± 0.03^{a}	21.04 ± 0.21^{c}	1.61 ± 0.13^{a}
10.50	3.97 ± 0.44^{c}	1.04 ± 0.18^{b}	15.81 ± 0.10^{d}	1.49 ± 0.06^{ab}

4 Discussion

Plant height plays a decisive role in light energy interception and light energy utilization of the maize canopy. Maize height is usually tightly linked with its aboveground dry matter and grain yield[15], and can even be used as a single factor to measure vegetative growth and potential yield[16]. Maize height is an important part of the maize plant structure and plays an important role in increases in grain yield[17]. Many studies have shown that maize plant height decreases with increasing planting density due to the competition between nutrients and water. The experiment also showed that plant height decreased slowly as density increased from 5.40×10^4 to 9.00×10^4 plants/hm^2, and decreased sharply for density above 9.00×10^4 plants/hm^2. The latter scenario was obviously a result of sunlight, water and fertilizer competition. Increased density led to lower solar radiation interception and a decrease in plant and spike height[18,19].

Most important plant components like lignin, hemicellulose, α-cellulose and ash are contained in the stem. Thus, biomass inside the stem represents a source of raw material for energy, paper, fiber and chemical production[18]. The increase of photosynthate promotes the enrichment of stem content and the increase in stem diameter. The stem diameter decreased linearly in the range of 5.40×10^4 to 6.45×10^4 plants/hm^2; for the range of 6.45×10^4 to 7.95×10^4 plants/hm^2, stem diameter remained largely unchanged; and above 7.95×10^4 plants/hm^2 it again decreased linearly, basically consistent with the study of Zhang et al.[20] The experiment also showed this: when planting density increased from 5.40×10^4 to 7.95×10^4 plants/hm^2, the economic yield of maize began to increase; when density exceeded 9.0×10^4 plants/hm^2, the contradiction between water and fertilizer was obvious, it increased competition for water, fertilizer and space for maize which reduces economic yields. Accordingly, the biological fresh yield of maize increased gradually with the increase of density from 5.40×10^4 to 9.00×10^4 plants/hm^2 and decreased rapidly above 9.75×10^4 plants/hm^2.

In terms of yield components, this experiment demonstrated that fresh plant weight, fresh panicle weight and panicle grain weight were significantly positively correlated with planting density, while panicle length, panicle diameter and hundred grain weight were significantly negatively correlated with planting density, which was basically consistent with previous research results[21-25]. Under the condition of local recommended fertilization, fresh panicles, spike grain and fresh 100-grain weight decreased in different patterns with the increase of planting density, and fresh plant weight decline synchronously with fresh spike weight, mainly because increasing population density will intensify contradiction in coordinating vegetative growth and reproductive growth, and at this stage, the vegetative growth inhibition is greater.

In general, the growth and development of aboveground parts are closely related to root growth and development. The ability of plants to absorb water and minerals from the soil is attributed primarily to the extensive root system, which determines the impact of agricultural practices on soil, shoot function and crop yield[26,27]. Of root measures, only the length of the maize root system increased with increasing planting density (Table 4). It is clear that increased density creates competition for water and fertilizer, and the only way to absorb enough water and nutrients is by increasing root length. However, the root surface area, mean root diameter, root activity and root volume behaved differently. Those four indices initially increased as planting density increased from 5.40×10^4 to 7.95×10^4 plants/hm^2, the root surface area, root mean diameter and root volume reached maxima at 7.95×10^4 plants/hm^2, with 55.13%, 28.57% and 98.19% increases compared with the control, respectively. The root activity was the highest for density of 9.00×10^4 plants/hm^2. It is suggested that appropriate planting density can effectively avoid competition among plants, and allow each maize plant to obtain sufficient water and nutrients, which is beneficial to root absorption and aboveground part development. This is consistent with the conclusions of many researchers[28-30]. Overall, a density of 7.95×10^4 to 9.00×10^4 plants/hm^2 effectively improved maize root morphology and promoted maize growth and yield increase in the experimental area. However, further increases in density of cultivation impaired root function, resulting in poor growth and reduced yield (Figure 3).

The most active region of root executive function is the rhizosphere, which is a specific zone surrounding the roots, which influences, due to its exudates, the activity of soil microorganisms[30,31]. While absorbing water and nutrients, roots also release secretions and protons, thus stimulating microbial activity and accelerating the circulation and transformation of nutrients such as carbon and nitrogen. Ricardo found that soil from the maize rhizosphere stimulated increases in soil micro-biomass and enzyme activity and contributed to subsequent improvement of cowpea growth[32]. It was found that numbers of bacteria and actinomycetes were much more sensitive than those of fungi in the soil (Table 5). The number of bacteria and actinomycetes increased with increased planting density, reaching a maximum density of 9.75×10^4 plants/hm^2, which was significantly higher than other planting density, with increases of 2.91%-186.91%. This is consistent with the trend in Table 4, that is, with increased planting density, the root length increases, which leads to an increase of root exudates, which can stimulate microbial activity. The number of actinomycetes was consistent with the trend of bacterial expression. The number of actinomycetes at 9.75×10^4 plants/hm^2 was 2.63 times higher than that at 5.40×10^4 plants/hm^2. The planting density had little effect on the number of fungi, and there were no significant differences among the treatments. The MBC and MBN contents represent the highest fraction of soil biodiversity and act on several soil functions that are important for environmental sustainability, such as the dynamics of organic matter and nutrient cycling[33,34]. In this study, both MBC and MBN decreased with the increasing in planting density, especially above 9.00×10^4 plants/hm^2. Apparently, the increase in density increased the length of roots, which secretes more material, stimulates the growth of bacteria and actinomycetes, and accelerates the consumption of more organic carbon and nitrogen to meet the needs of high-density survival competition (Table 5).

Soil enzymes are the secreted products of soil microorganisms and plant roots, and are important components of soil. In fact, more soil microbial biomass releases more enzymes which will promote biogeochemical cycles and contribute nutrients for plant and microbial activity[35,36]. Therefore, monitoring soil enzyme activities under different planting densities can help to clarify the competition between soil nutrient release and maize nutrient uptake, which helps explain the close relationship between plant density and maize growth and yield. The enzyme activity trend at different densities is consistent with those of root surface area, mean root diameter, root activity and root volume, as well as the numbers of bacteria and actinomycetes, and ultimately economic yields show the same results.

5 Conclusions

Stem diameter and panicle length exhibited a linear response to planting density. Plant height, intercellular CO_2 concentration, spike weight per plant, grain weight per spike, 100-grain weight, fresh grain weight per plant, MBC and MBN decreased with the increase in planting density. The root length, total amount of soil bacteria, actinomycetes and alkaline phosphatase activity increased with the increase in planting density. However, root surface area, average root diameter, root activity, root volume, net photosynthetic rate, stomatal conductance, soil urease, sucrase, catalase activities and finally maize yield increased at low density and decreased at high density in the range of 5.40×10^4 to 10.40×10^4 plants/hm^2. The study indicated that moderately increasing planting density increased yields. The optimal planting density of the local maize variety Tianci 19 was 82 900 plants/hm^2 and provided yield up to 15.84 t/hm^2.

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