Architectural and anatomical responses of maize roots to agronomic practices in a semi-arid environment

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Abstract

Root architecture and anatomy are important determinants of nitrogen (N) and water acquisition, but they are also environmentally plastic to adapt to N and water availability. Therefore, understanding the relationship between root traits and environmental factors is essential for improving N and water acquisition. A field experiment was conducted in the semi-arid region of the Loess Plateau in northwestern China to quantify the architectural and anatomical root traits of maize (*Zea mays* L.) in response to plastic film mulching and N fertilization. We compared four treatments: non-mulching with and without N supply as well as plastic film mulching with and without N supply. Variation existed for all root architecture and anatomy traits within maize root crowns. Crown and brace root angles to the soil line decreased in response to film mulching and N fertilization. Crown roots under plastic film mulching showed a significantly decreased distance to branching, reduced lateral root length, and overall increased root diameter. Similarly, N application significantly decreased the distance to branching, yet induced more compact and denser crown roots, and increased the root diameter. Brace roots exhibited an increased distance to branching, greater lateral root length and density, as well as a larger root diameter in response to plastic film mulching and N fertilization. Additionally, the accumulated number of nodal roots increased greatly under plastic film mulching and N treatments. At the anatomical level, N application reduced the proportion of the root cortical aerenchyma area. In contrast, aerenchyma area, cortex cell size, and late metaxylem vessel diameter were increased as a result of plastic film mulching. These results demonstrate root architectural and anatomical traits respond to mulching practices and N fertilization.

Key words: N application / plastic film mulching / root anatomy / root architecture / water

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1 Introduction

Root architecture encompasses the spatial configurations of a root system that influence plant fitness by regulating access to soil resources (*Lynch*, 1995). Root anatomy has been related to differences in functional efficiency and stress responses in several crop species (*Uga* et al., 2008; *Peña-Valdivia* et al., 2010; *Saengwilai* et al., 2018). Root architecture and anatomy play a central role in plant growth, resource allocation, and acquisition of soil resources (*Lynch* and *Brown*, 2012). However, architectural and anatomical root traits are environmentally plastic to adapt to water and nutrient availability. Understanding the relationship between root traits and environmental factors is therefore essential for improving water and nutrient acquisition.

Maize (*Zea mays* L.) is the main crop grown in the semi-arid region of the Loess Plateau, located in the northwest of China. Groundwater resources on the Loess Plateau are scarce and deep, so most of the farmland relies solely on rainfall ranging from 150 to 300 mm y^{-1} in the north to 500–700 mm y^{-1} in the south (*Wang* et al., 2012). However, declining trends have been recorded between 1961 and 2010 (*Li* and *Xiao*, 1992). Over the past decades many agronomic practices have been tested and implemented on the Loess Plateau to mitigate the effects of limited water availability (*Wang* et al., 2011; *Gan* et al., 2013; *Zhao* et al., 2014). One of the most effective agronomic techniques is plastic film mulching. With this mulching system, soil water evaporation is reduced and soil temperature is increased to improve water-use efficiency and grain yields in rain-fed regions (*Gan* et al., 2013). Therefore, plastic film mulching has been extensively used for dryland agriculture in arid and semi-arid areas of China (*Luo*, 1982; *Dong* et al., 2009). It was estimated that

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approximately 1.4 teragrams of plastic film was applied to 18.1 million hectares of cropland in 2014 (equivalent to 11% of China's arable farmland), resulting in an estimated increase of maize yield by 39–137% (*Liu* et al., 2014a, 2014b; *Ma*, 2015; *Zhang*, 2015). Enhanced soil water status promotes root growth and development, which in turn improves the absorption of water and nutrients from the soil (*Fang* et al., 2011; *Wasson* et al., 2012). The effects of plastic film mulching on maize root development include increased root weight, root length, crown diameter, and volume (*Gao* et al., 2014). *Wang* et al. (2018) reported significantly greater root dry weight and density compared to the unmulched control environments. *Jia* et al. (2018) found that plastic film mulching promotes root distribution in the topsoil in arid environments.

Soil fertility is another main factor contributing to the expression of architectural and anatomical root traits. In particular, soil nitrogen (N) content is an important indicator of soil fertility and productivity (*Reeves*, 1997). Compared to non-limiting conditions, brace and crown root angles from the horizontal increased by 18° under N-deficient conditions (*Trachsel* et al., 2013). The proportion of root aerenchyma increased under N-deficient conditions (*Drew* et al., 1989; *Saengwilai* et al., 2014). In barley, lateral root growth was promoted by an increase in the N supply (*Drew*, 1975).

Numerous studies have assessed the effects of mulching and N application on root architectural and anatomical traits (*Gaudin* et al., 2011; *Rose* et al., 2009; *Luo* et al., 2015). However, none has characterized the root architectural and anatomical traits of maize plants under plastic film mulching cultivation systems and their response to N application. We thus evaluated the architectural and anatomical root traits of maize plants under different mulching practices used in semi-arid regions and their response to N fertilization. The identification and understanding of root traits and their utility for soil resource acquisition is an important step in phene-based or ideotype breeding critical for crop improvement (*Lynch*, 2013).

2 Material and methods

2.1 Site description

The study was conducted in 2015 and 2016 at the Changwu Agricultural and Ecological Experiment Station (35.28°N, 107.88°E; 1200 m elevation) which is located in a typical dryland farming area on the Loess Plateau of northwestern China. The area is rain-fed and allows one harvest per year. The amount of rainfall was 520 mm in 2015 and 489 mm in 2016, with 325 mm and 374 mm falling during the maize growth season (April–September), respectively. The soils at this site are Cumuli-Ustic Isohumosols (*Gong* et al., 2007). The top 20 cm of the soil had a pH of 8.4, a bulk density of 1.3 g cm^{-3}, and contained 14.1 g kg^{-1} soil organic matter, 0.90 g kg^{-1} total N, 10.5 mg kg^{-1} available phosphorus (P, Olsen-P), 136.7 mg kg^{-1} available potassium (K, as NH_4OAc-K), and 10.5 mg kg^{-1} mineral N.

2.2 Experimental design

The field experiment was set up in a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Two N-fertilization regimes were investigated: no N applied (N0) and 250 kg N ha^{-1} applied (N250). Both fertilization regimes were tested under two mulching practices: (1) NM treatment, which was a flat field without mulching treatment, and (2) FM treatment, which consisted of alternating broad and narrow ridges and a furrow covered by a transparent polyethylene plastic film (colorless and transparent, 0.008 mm thick and 1200 mm wide). The broad ridges were 60 cm wide and 10 cm high; the narrow ridges were 40 cm wide and 15 cm high (Fig. 1). Both ridges were manually

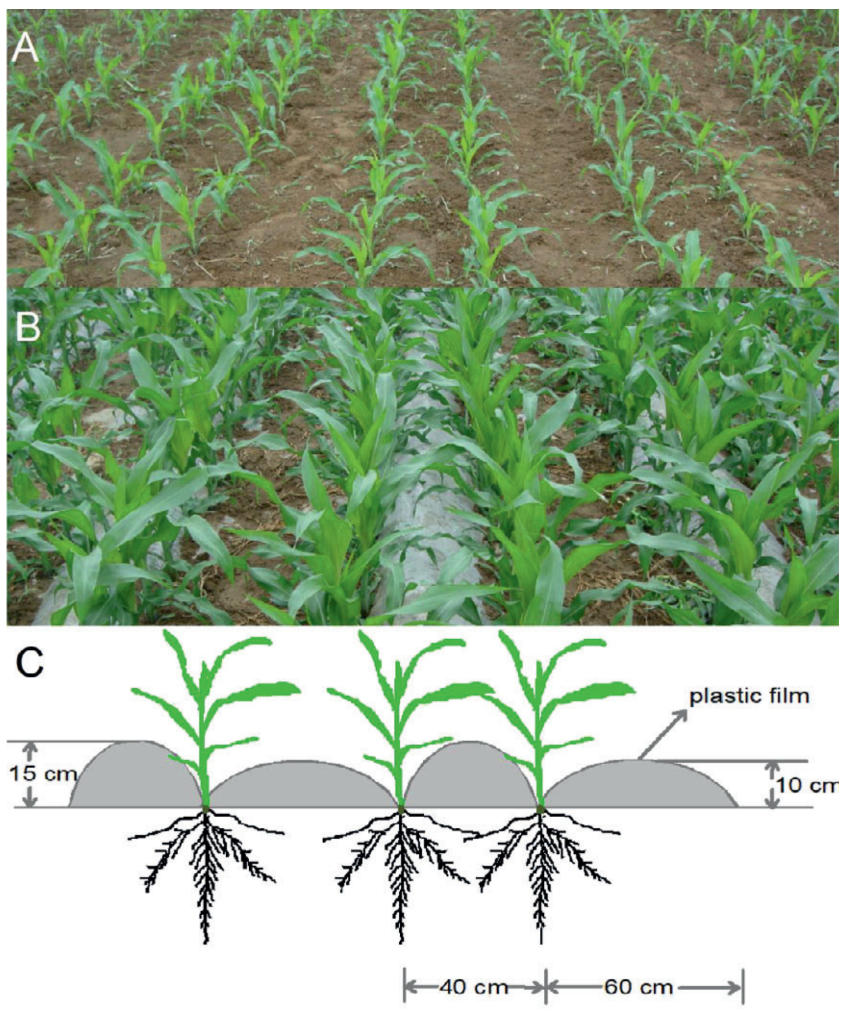

Figure 1: Photographs showing a bare plot without mulching (A), the plastic film mulching treatment (B) at the 6th leaf stage of maize, and the schematic diagram illustrating the two ridges and furrow arrangement with film mulching (C).

mulched with the plastic film. Two pieces of plastic film were joint at the midline of the broad ridge by covering the ends of both plastic film pieces with soil. Perforations (1 cm in diameter every 25 cm to match plant spacing of the row) were drilled through the film using a handheld device, which allowed rainwater to diffuse into the root zone. The plastic film was used throughout the maize growth season, and removed at harvest and re-mulched before planting in the following year.

Each treatment was repeated three times with a plot size of 28 m^2 (4 m × 7 m). N fertilizer was applied three times for the N250 treatment as urea (N 46%); 40% was manually broadcast as basal dressing over the soil surface before sowing and then plowed into the subsurface. The remaining N fertilizer was applied at the 10-leaf stage (V10) and silking stage (R1) each with 30% by using a hole-sowing machine following precipitation. In addition, all treatment plots received 40 kg P ha^{-1} as calcium superphosphate and 80 kg K ha^{-1} as potassium sulfate before sowing and then plowed into the subsurface. Hybrid maize (*Zea mays* L. cv. Pioneer 335) was planted at a density of 80,000 plants ha^{-1}. The maize seeds were sown to a depth of 5 cm using a hand-powered hole-drilling machine on April 25 and 24, 2015 and 2016. Natural rainfall was the only water source.

2.3 Root sampling and harvest

Shoots and roots were evaluated at the silking (R1) stage. Three representative, adjacent plants in the same row were randomly selected per replicate. The shoots of the selected plants were oven-dried to constant weight at 80°C and ground for chemical analysis. After that, roots were excavated and processed using the shovelomics protocol (*Trachsel* et al., 2011), with the shovel inserted approximately 40 cm horizontally and 25 cm vertically from the base of the plants. The excavated root crowns were cleaned by vigorous rinsing with low-pressure water. One of the three root crowns was selected for architectural measurements. A second root crown was selected for anatomical sampling. All nodal roots emerging above-ground were classified as brace roots and nodal roots emerging below-ground were classified as crown roots. At the maturity stage (R6), 10 m^2 (four rows, each 5 m long) in the middle of each plot was manually harvested to determine the yield. Plant samples were analyzed for N concentration (Nc, micro-Kjeldahl), and N content was calculated by multiplying the Nc by the oven-dry weight.

In 2016, soil cores were collected at the R1 stage to determine the distribution of roots in the soil. We used a soil-coring tube with a diameter of 9 cm and a length of 70 cm to sample the soil profile. The core was taken within a planting row midway between two plants. Each soil core was subdivided into 10 cm segments and roots were extracted from each segment manually. Extracted root samples were scanned using a flatbed scanner (Perfection V700 Photo; Epson America) and analyzed using the image-processing software WinRhizo Pro (Regent Instruments). The gravimetric soil water content was determined for each plot at the silking (R1) stage by taking one soil core sample at which plant samples were collected. Core samples were divided into 20 cm intervals to a depth of 100 cm. The samples were oven-dried at 105°C to constant weight to determine soil water content.

2.4 Imaging of the crown root system and architectural traits

Root crowns were imaged using a digital camera (Nikon COOLPIX100). An additional image was taken from the outermost layer of nodal roots by excising all whorls of brace roots. A representative nodal root of the whorl was excised and placed to the side of the root crown when imaged. Image analysis was conducted using ImageJ software (http://rsb.info.nih.gov/ij/; *Schneider* et al., 2012). The nodal root angle from the horizontal, number, and diameter of nodal roots, distance to branching (along with the distance from where the representative root was excised from the shoot to where the first lateral roots emerged), lateral root branching density, and lateral root length were measured according to *York* et al. (2015). In brief, the nodal root angle from the horizontal was calculated using trigonometric equations that account for culm width, maximum root crown width, and the height between culm width and root crown width. The number of nodal roots in each whorl was counted. The diameter of the representative nodal root was measured at its base. Distance to branching is the length from the excision point to the first lateral root along the centerline of the nodal root. The length of five representative lateral roots was measured and averaged to obtain the trait value. Lateral root branching density was counted along a measured length of a representative nodal root.

2.5 Imaging of anatomical traits

For each plant, three 5-cm root segments at 5–10 cm distance from the base of a representative second whorl crown root were collected for anatomical analysis as described previously (*Burton* et al., 2013). Root segment samples were stored in 75% ethanol at 4°C until processing and analysis. The segments were placed in a drop of water over dental wax and cut using double-edged stainless steel blades under a dissecting microscope. Three random sections were selected for imaging. Images of each section were obtained using an Olympus BX51 microscope and analyzed using Image-J. "ObjectJ" plug-in (https://sils.fnwi.uva.nl/bcb/objectj/download/current/), an image analysis tool, was installed in Image-J to measure root anatomy. The total cross-section area, aerenchyma lacunae, cortical cell size, the stele area, and late xylem vessel number and diameter were measured by counting the total number of pixels constituting the respective anatomical part. The total aerenchyma area was calculated as:

$$\begin{aligned} \textit{Total aerenchyma area} = {} & \textit{Whole cross} - \textit{section area} \\ & - \textit{Stele area} \\ & - \textit{Cortical cell area}. \end{aligned} \quad (1)$$

The proportion of the aerenchyma area in the whole cortical area was calculated. Cortical cell file number was counted manually (CCFN). The diameters of the root section (DX) and stele (DS) were measured separately by counting the total number of pixels. Each measurement was repeated five times for each root section. The average cortical cell diameter was calculated as the fraction of (DX – DS)/CCFN. The mean cortical cell size was estimated under the assumption that each cortical cell is perfectly circular as $\pi(CD/2)^2$. Total late xylem vessel number (LXVN) was counted manually. The late xylem vessel diameter (LXVD) was calculated as the average of two independent measurements for each late xylem vessel. The mean late xylem vessel diameter of one root section was calculated as the sum of all LXVD divided by LXVN.

2.6 Statistical analysis

Data were subjected to an analysis of variance (ANOVA) and Tukey's honestly significant difference test ($P \leq 5\%$) using SAS 8.0 (SAS Institute, Cary, NC).

3 Results

3.1 N uptake and shoot growth

We observed a significantly elevated N uptake and higher shoot biomass at the silking (R1) and maturity (R6) stages in 2015 and 2016 under the plastic film mulching (FM) and N250 treatments (Tab. 1). N uptake at the R1 and R6 stages under FM increased by 16% and 11% in 2015, and by 112% and 63% in 2016, respectively, compared to the non-mulching (NM) treatment. Additionally, increased N uptake was observed under N250 at the R1 and R6 stages compared to the N0 treatment with respective increases of 251% and 282% in 2015, and 264% and 437% in 2016.

Shoot biomass was significantly affected by the interactions of mulching practices (M) × N application (N), M × year (Y), and N × Y at the R1 stage, and N × Y and M × N × Y at the R6 stage (Tab. 1). Compared to NM-N0, shoot biomass at the R1 stage under NM-N250, FM-N0, and FM-N250 was 42%, 14%, and 90% higher in 2015, and 206%, 215%, and 548% higher in 2016 (Tab. 1). At the R6 stage, shoot biomass under NM-N250, FM-N0, and FM-N250 was 326%, 66%, and 362% higher in 2015, and 335%, 67%, and 585% higher in 2016, compared to NM-N0 (Tab. 1).

Both N supply and mulching practices significantly affected grain yield in both years (Tab. 1). Grain yields increased in 2015 by approximately 30% and in 2016 by approximately 60% under NM treatment compared to FM treatment. Increased yield was even more dramatic if N0 and N250 were compared. N250 treatment resulted in a 351% increase for 2015 and a 656% increase for 2016 compared to N0 treatment.

Table 1: Maize N uptake, shoot biomass, and grain yield (kg ha^{-1}) as influenced by mulching practices (NM, non-mulching; FM, plastic film mulching) and N rate (N0, without N supply; N250, 250 kg N ha^{-1} supply) at the silking (R1) and maturity (R6) stages in 2015 and 2016.

Year	Treatment	N uptake (kg ha^{-1})		Shoot biomass (t ha^{-1})		Grain yield (t ha^{-1})
		R1	R6	R1	R6	
2015	NM-N0	37.29c[a]	38.2b	6.29c	5.63c	1.92d
	NM-N250	129.00b	193.37a	8.92b	23.99a	11.95b
	FM-N0	42.30c	63.31b	7.17c	9.35b	3.87c
	FM-N250	150.16a	194.19a	11.95a	26.00a	14.15a
2016	NM-N0	18.80c	27.30c	2.20c	3.06c	0.81c
	NM-N250	88.71b	133.21b	6.73b	13.30b	7.45b
	FM-N0	53.44c	38.91c	6.94b	5.11c	1.70c
	FM-N250	174.31a	222.04a	14.26a	20.97a	11.52a
	Summery of ANOVA					
	Mulching practice (M)	35.51***[b]	9.83**	93.38***	20.78***	22.04***
	Nitrogen (N)	252.43***	203.59***	132.23***	324.93***	358.54***
	Year (Y)	0.91NS	2.81NS	6.29*	44.21***	28.70**
	M×N	7.46*	1.72NS	8.67**	1.34NS	3.10NS
	M×Y	14.66***	3.42NS	24.93***	1.41NS	0.17NS
	N×Y	0.13NS	0.01NS	7.02*	6.92*	3.93NS
	M×N×Y	2.00NS	6.34*	0.148NS	4.69*	2.27NS

[a]Different letters within each column represent significant differences ($P \leq 5\%$) among the four treatments within the same year.
[b]F Values and the Tukey's honestly significant difference at $P \leq 5\%$. NS, not significant, *$P \leq 5\%$, **$P \leq 1\%$, and ***$P \leq 0.1\%$.

3.2 Soil water content

Soil water content between the soil line and 100 cm depth was measured at the R1 stage (Fig. 2). Across all treatments, the soil water content initially declined with soil depth and then increased with increasing soil depth. In 2015, the soil water content at 0–100 cm depth decreased under N250, but not at 0–20 cm depth under the NM-N0 treatment. In 2016, the soil water content in the 0–40 cm soil profile increased under FM-N250 compared to NM, and the overall soil water content at 0–100 cm depth was reduced by N application.

3.3 Root architecture

The crown root angle (Tab. 2, Fig. 3A) decreased by 21% from 53° to 42° from the horizontal in 2015 and by 22% from 56° to 43° from the horizontal in 2016 from NM to FM treatments. Similarly, the crown root angle was shallower in N250 treatments than in N0 treatments. The crown root angle decreased by 34% from 57° to 38° from the horizontal in 2015 and by 28% from 57° to 42° from the horizontal in 2016. The brace root angle also decreased by 17% from 64° to 52° from the horizontal in 2015 and by 11% from 60° to 54° from the horizontal in 2016 (Fig. 3F). Crown roots tended to be shallower than brace roots overall, with means of 47° in 2015 and 50° in 2016 from the horizontal in crown roots, and 58° in 2015 and 57° in 2016 from the horizontal in brace roots.

The distance to branching of crown roots decreased considerably under FM compared to NM (Tab. 2, Fig. 3B) by 21% (5.8 cm to 4.6 cm) in 2015 and by 16% (4.7 cm to 4.0 cm) in 2016. The crown root distance to branching decreased by 22% (5.8 cm to 4.6 cm) in 2015 and by 44% (5.6 cm to 3.1 cm) in 2016 from N0 to N250 treatments. In contrast, FM treatment showed longer distances to branching in brace roots compared to NM treatment (Tab. 2, Fig. 3G). The brace roots delayed branching from 9.3 cm to 10.3 cm in 2015 and from 5.7 cm to 7.0 cm in 2016 from NM to FM treatments. Brace roots branched at 7.1 cm and 4.9 cm under N0 and at 12.5 cm and 7.8 cm under N250 in 2015 and 2016, respectively.

The interaction of M × N produced significant effects on the lateral root length of crown roots (Tab. 2, Fig. 3C). Compared to NM-N0, lateral root length of crown roots under NM-N250 and FM-N250 was 105% and 67% higher in 2015, and 55% and 27% higher in 2016 (Tab. 2, Fig. 3C). Both FM and N250 treatments showed longer lateral root length in brace roots compared to NM and N0 treatments (Tab. 2, Fig. 3H); the lateral root length in 2015 and 2016 increased from 32 cm to 55 cm, and 34 cm to 48 cm from NM to FM treatments, and increased from 35 cm to 51 cm, and 23 cm to 60 cm from N0 to N250 treatment, respectively. M did not affect the lateral root densities in crown roots, but N application resulted in more compact and denser lateral roots in crown roots (Tab. 2, Fig. 3D). The lateral root density in crown roots increased by 57% (from 2.0 branch cm^{-1} to 3.0 branch cm^{-1}) and by 62% (from 2.4 branch cm^{-1} to 3.9 branch cm^{-1}) under N0 compared to N250, in 2015 and 2016, respectively. M × N × Y interaction significantly affected the lateral root density of brace roots (Tab. 2, Fig. 3I). Compared to NM-N0, the lateral root density of brace roots was increased by 22% and 25% under NM-N250 and FM-N250 in 2015 and by 306%, 103%, and 214% under NM-N250, FM-N0, and FM-N250 in 2016.

The average root diameters of crown and brace roots (Fig. 3 E, J) increased slightly from NM to FM treatments. The largest difference in average root diameters was observed between N0 to N250 treatments, increased by 74% (2015) and 65% (2016) for crown roots, and by 47% (2015) and 74% (2016) for brace roots.

Nodal root number in an entire root crown (Tab. 2, Fig. 4) increased by 12% from 36 to 40 (2015), and 21% from 37 to 44 (2016) from NM to FM treatments. Similarly, we counted a higher nodal root number from N0 to N250 treatments. The nodal root number increased by 40% from 42 to 45 (2015), and 38% from 34 to 47 (2016).

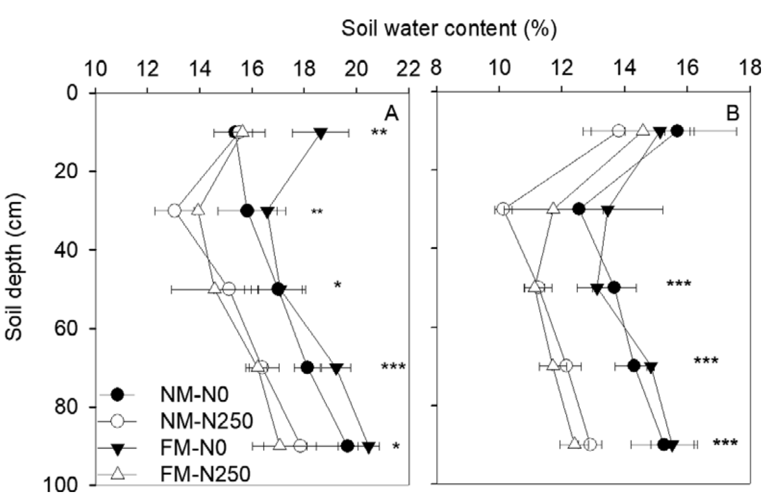

Figure 2: Gravimetric soil water content in the 0–100 cm soil profile at the silking stage (R1) in 2015 (A) and 2016 (B) under the indicated treatments (NM, non-mulching; FM, plastic film mulching; N0, without N supply; N250, 250 kg N ha^{-1} supply). Data are means ± SE of three replicates. Significant at $^{*}P \leq 5\%$; significant at $^{**}P \leq 1\%$; significant at $^{***}P \leq 0.1\%$.

To investigate the root distribution in the soil, we collected root cores from 0 to 70 cm soil profile at the R1 stage in 2016 (Fig. 5). Across all treatments, the root system was predominantly distributed in the 0–30 cm soil layer, which accounted for 76% of the total root length density in the 0–70 cm soil layer. Root length and density declined at deeper soil levels. In addition, we observed that the impact of mulching practices on root distribution in the soil profile was dependent on N supply. Under the N0 treatment, root length and density did not significantly differ between FM and NM. Increased N supply, however, led to higher root length and density throughout the root system under FM compared to NM. Therefore, the highest root length density among the four treatments was found in the FM-N250 treatment.

Table 2: ANOVA table of crown root (CR) and brace root (BR) architectural and anatomical measurements. The F values and significance of all factors and factor interactions are shown (NS, not significant; $^{*}P \leq 5\%$, $^{**}P \leq 1\%$, and $^{***}P \leq 0.1\%$). Architectural and anatomical traits, and their abbreviations (Abr.) and descriptions are also listed.

Factor	Abr.	Description	Mulching practice (M)	N level (N)	Year (Y)	M × N	M × Y	N × Y	M × N × Y
Architecture trait									
Root Number	Nodal R #	Number of nodal roots in root crown	21.64***	100.47***	3.13NS	0.35NS	1.89NS	0.04NS	3.13NS
Root diameter	CR Diam.	Diameter of crown root (mm)	36.94***	113.03***	0.30NS	0.86NS	0.34NS	0.43NS	0.74NS
Distance to branching	CR DTB	Distance from cut to first lateral emergence on crown root (mm)	15.72***	54.99***	11.18**	0.55NS	0.86NS	5.58*	2.20NS
Lateral root density	CR LRD	Number of lateral roots within a centimeter of crown root	1.82NS	88.31***	22.56***	2.16NS	1.00NS	1.99NS	1.02NS
Lateral root length	CR LRL	Average length of three lateral roots of crown root (mm)	10.43**	117.46***	46.42***	5.74*	0.31NS	1.00NS	0.02NS
Angle	CR Ang	Angle from horizontal of crown root (°)	76.27***	171.88***	2.97NS	1.13NS	0.24NS	1.48NS	3.94NS
Root diameter	BR Diam.	Diameter of brace root (mm)	8.15*	48.07***	0.95NS	0.28NS	0.19NS	0.91NS	1.40NS
Distance to branching	BR DTB	Distance from cut to first lateral emergence on brace root (mm)	6.27*	77.38***	53.61***	1.37NS	0.06NS	6.92*	0.23NS
Lateral root density	BR LRD	Number of lateral roots within a centimeter of brace root	3.79NS	188.91***	74.62***	1.52NS	5.73*	6.14*	38.88***
Lateral root length	BR LRL	Average length of three lateral roots of brace root (mm)	22.92***	46.50***	0.26NS	0.38NS	1.23NS	7.22*	1.02NS
Angle	BR Ang	Angle from horizontal of brace root (°)	4.15NS	25.39***	0.36NS	0.09NS	1.05NS	1.85NS	3.40NS
Anatomy traits									
Percent aerenchyma	%A	Percentage of the cortical area occupied by aerenchyma (%)	40.91***	59.98***	2.07NS	11.37***	0.01NS	0.09NS	13.73**
Cross-sectional area	RXSA	Total cross-sectional area of a nodal root (mm^2)	190.74***	0.63NS	6.14*	3.12NS	0.60NS	2.62NS	0.83NS
Cell size	CS	Average area of individual cortical cells (μm^2)	16.04***	51.22***	199.59***	6.50*	0.46NS	28.57**	5.45*
Stele area	SA	Total area of the stele (mm^2)	412.92***	4.24*	61.95***	9.78**	0.66NS	0.19NS	0.00NS
Late metaxylem vessel diameter	V Diam.	Average diameter of all late metaxylem vessels (mm)	438.97***	20.77***	1.82NS	0.62NS	1.56NS	0.11NS	0.34NS
Late metaxylem vessel number	XV#	Total number of late metaxylem vessels	0.25NS	0.08NS	0.47NS	2.45NS	3.95NS	0.00NS	0.01NS

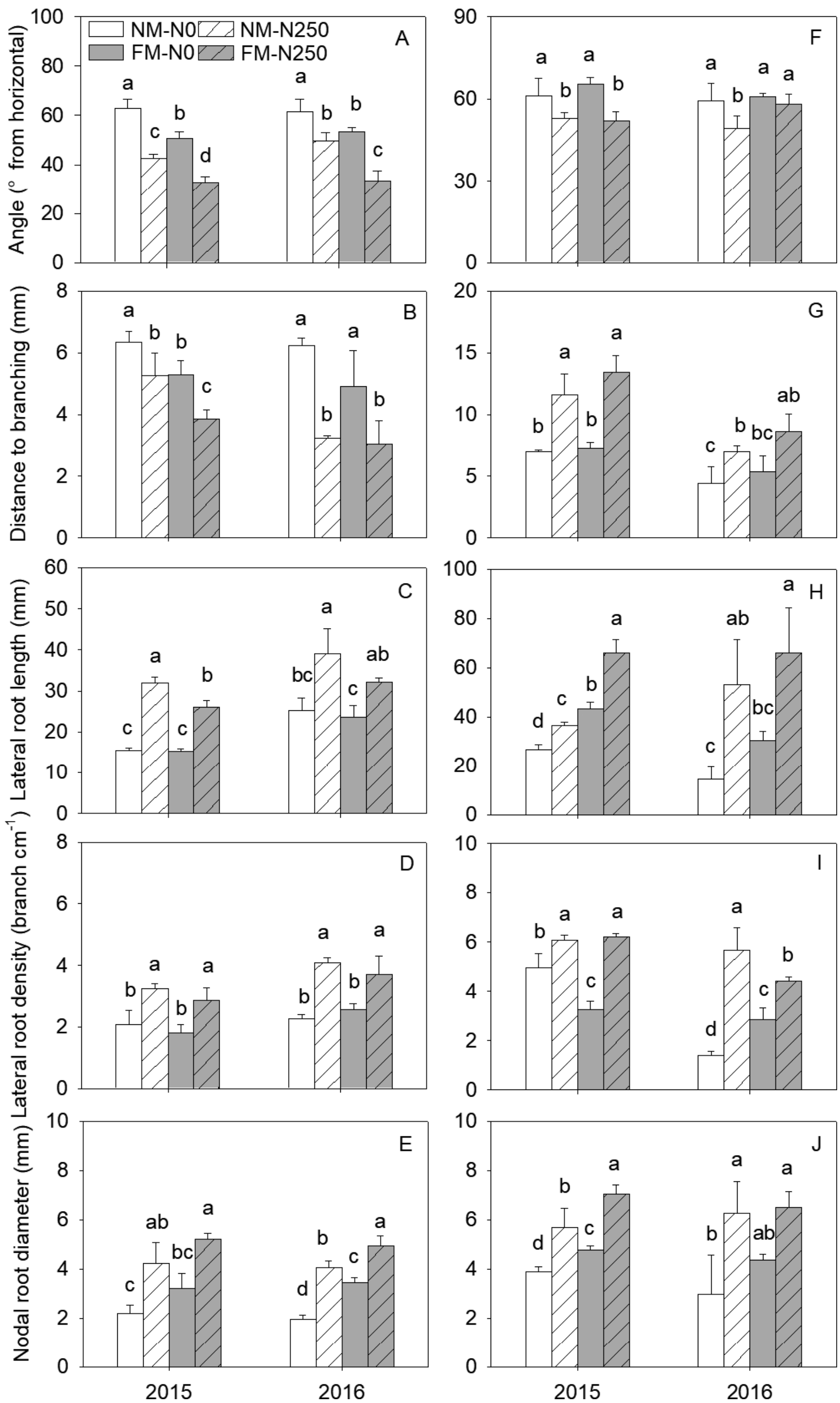

Figure 3: Root architecture traits at the silking stage (R1) in 2015 and 2016 under the indicated treatments (NM, non-mulching; FM, plastic film mulching; N0, without N supply; N250, 250 kg N ha^{-1} supply), A–E, crown root, F–J, brace root. Data are means ± SE of three replicates. Different letters represent significant differences ($P \le 5\%$) among the four treatments within the same year.

3.4 Root anatomical traits

The proportion of cortical aerenchyma was significantly affected by M × N × Y interaction (Tab. 2, Fig. 6A). Compared to NM-N0, the proportion of cortical aerenchyma was increased by 217% under FM-N0, but decreased by 49% and 21% under NM-N250 and FM-N250 in 2015, and increased by 116% under FM-N0, but decreased by 76% and 18% under NM-N250 and FM-N250 in 2016.

FM significantly increased the root cross section area by 91% (2015) and 131% (2016) compared with NM (Tab. 2, Fig. 6B). However, no significant differences were found between N0 and N250 treatments. The mean cortical cell size was significantly affected by the interactions of M × N, N × Y, and M × N × Y (Tab. 2, Fig. 6C). Compared to NM-N0, the mean cortical cell size was decreased by 34%, 16%, and 31% under NM-N250, FM-N0, and FM-N250 in 2015, and by 7%, 17%, and 22% under NM-N250, FM-N0, and FM-N250 in 2016. The stele area was significantly affected by the interaction of M × N (Tab. 2, Fig. 6D). Compared to NM-N0, the stele area was 4%, 74%, and 59% larger under NM-N250, FM-N0, and FM-N250 in 2015, and 3%, 110%, and 84% larger under NM-N250, FM-N0, and FM-N250 in 2016. The late metaxylem vessel diameter increased by 31% (2015) and 36% (2016) from NM to FM treatments (Tab. 2, Fig. 7A). Late metaxylem vessel diameter increased by 6% in 2015 and 7% in 2016, under N250 compared to N0 (Fig. 7A). The number of late metaxylem vessels was not impacted by mulching practice or N application (Tab. 2, Fig. 7B).

4 Discussion

4.1 Responses of maize root architectural traits to plastic film mulching and N application

Root systems modulate their angle, rate of growth, and root type to improve the efficiency of water and nutrient uptake (*Lynch*, 1995). In the present study, the root angle from the horizontal, distance to branching, density and length of lateral roots, as well as the diameter and number of nodal roots, were evaluated. The angles of crown and brace roots were shallower under the FM treatment than the NM treatment (Fig. 3), possibly due to improved soil water content, physical properties, and enhanced nutrient cycling under mulching conditions (*Fang* et al., 2011). High N application

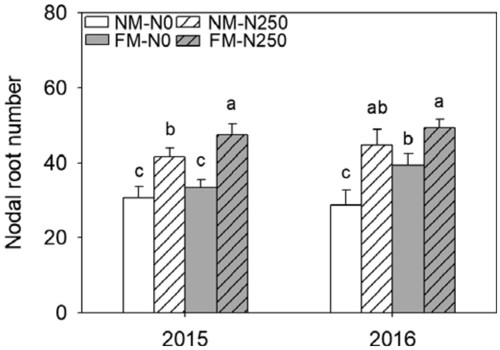

Figure 4: Total number of nodal roots. Number of nodal roots at the silking stage (R1) in 2015 and 2016 under the indicated treatments (NM, non-mulching; FM, plastic film mulching; N0, without N supply; N250, 250 kg N ha^{-1} supply). Data are means ± SE of three replicates. Different letters represent significant differences (P ≤ 5%) among the four treatments within the same year.

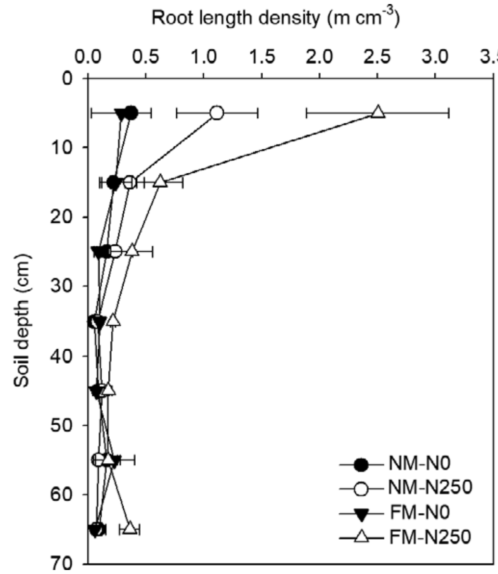

Figure 5: Root length density in the 0–70 cm soil profile at the silking stage (R1) in 2016 under the indicated treatments (NM, non-mulching; FM, plastic film mulching; N0, without N supply; N250, 250 kg N ha^{-1} supply). Data are means ± SE of three replicates.

also led to shallower angles of crown and brace roots (Fig. 3). Our observations confirm a recent study that reported up to 18° shallower nodal roots in maize under high-N treatment compared to low-N treatment (*Trachsel* et al., 2013). The root length density in the 0–70 cm soil layer was the highest under the FM-N250 treatment, compared to NM-N0, NM-N250, and FM-N0 treatments (Fig. 5). This suggests that mulching combined with N fertilization is beneficial for the overall extension and distribution of roots in the soil, possibly due to improved soil water content and nutrient cycling (*Sharratt* and *Gesch*, 2004). Improved root distribution has practical implications, especially in the deep soil layers, since N easily converts to nitrate and leaches into deep soil layers if applied early in the season as nitrate or ammonium (*Wiesler* and *Horst*, 1993; *Raun* and *Johnson*, 1999; *Cassman* et al., 2002; *Chen* et al., 2011). Plants with deeper roots are believed to be plastic in order to respond to the environment if N uptake from the deep soil layers is improved (*Zhan* et al., 2015), which ultimately promotes plant growth and decreases nitrate leaching.

Distance to branching (*i.e.*, distance from the base of the root to the first lateral branch) is a new trait which seems to be related to the allocation of metabolic resources in root and shoot (*York* et al., 2013, 2015). In the present study, the distance to branching of crown roots decreased significantly under the FM and N250 treatments (Fig. 3B), potentially as the result of increased soil water content, nutrient availability, and topsoil physiology, and thus promoting the emergence of

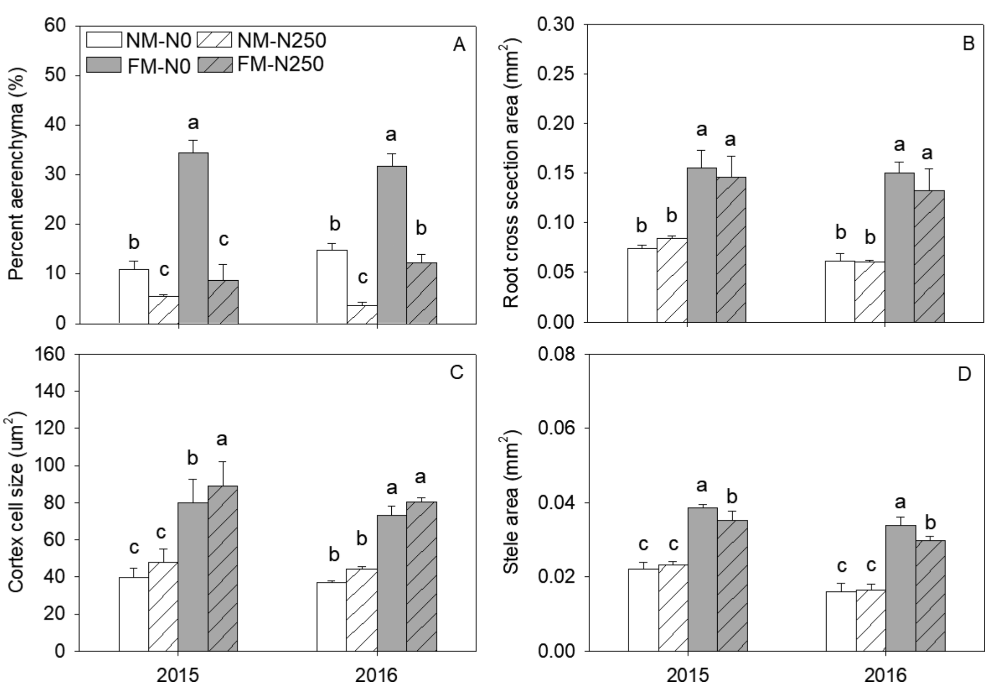

Figure 6: Root anatomical traits at the silking stage (R1) in 2015 and 2016 under the indicated treatments (NM, non-mulching; FM, plastic film mulching; N0, without N supply; N250, 250 kg N ha^{-1} supply). Data are means ± SE of three replicates. Different letters represent significant differences (P ≤ 5%) among the four treatments within the same year.

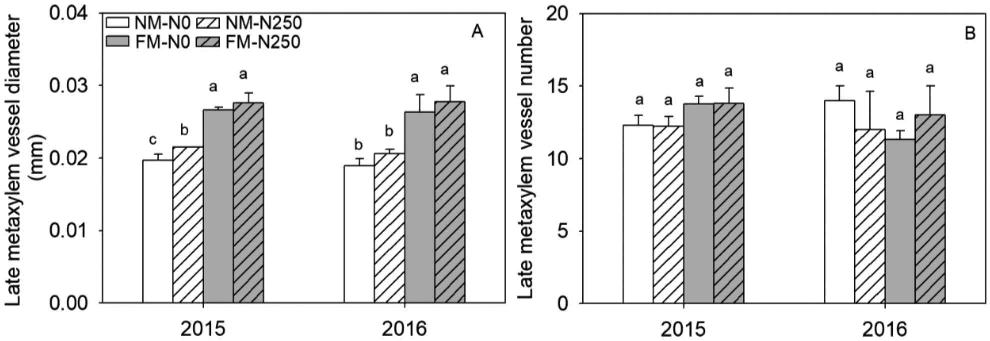

Figure 7: Late metaxylem vessel diameter (A) and late metaxylem vessel number (B) at the silking stage (R1) in 2015 and 2016 under the indicated treatments (NM, non-mulching; FM, plastic film mulching; N0, without N supply; N250, 250 kg N ha^{-1} supply). Data are means ± SE of three replicates. Different letters represent significant differences ($P \leq 5\%$) among the four treatments within the same year.

lateral roots. Emergence of lateral roots benefits nutrient and water uptake in the topsoil layer, especially during early growth stages when fertilizer was recently applied (*Lynch*, 2013).

In our study, lateral root length and density increased significantly under the N250 treatment (Fig. 3C, D). This is consistent with previous reports of modulation of lateral root number and length by external N application (*Zhang* and *Forde*, 1998; *Hodge*, 2004). From a functional perspective, our observations suggest that the formation of lateral roots increases the sink strength of the root system and promotes the development of longer roots, thereby improving soil resource acquisition (*Yoshida* and *Hasegawa*, 1982; *Postma* et al., 2014).

Root diameter is correlated with the root's ability to penetrate hard soil (*Materechera* et al., 1992; *Clark* et al., 2008). Furthermore, larger root diameters are correlated with greater sink strength and growth potential (*Thaler* and *Pagès*, 1996). The crown and brace root diameters were increased under the FM and N250 treatments, which is associated with a higher root system growth rate (Fig. 3E, J). This association is the result of improved root distribution in the 0–70 cm soil profile under the FM and N250 treatments (Fig. 5). Therefore, we speculate that plastic film mulching and N supply positively affect soil exploration, as well as water and nutrient acquisition.

The number of nodal roots is an important indicator to estimate the overall degree of soil exploration and the carbon budget of the plant (*Lynch*, 2013; *York* et al., 2015). In the present work, the number of nodal roots increased significantly under FM and N250 treatments (Fig. 4), which potentially improved root distribution in the 0–70 cm soil profile under the FM and N250 treatments (Fig. 5).

4.2 Responses of maize root anatomical traits to plastic film mulching and N application

The plasticity of anatomical traits facilitates efficient use of available water and N (*Lynch*, 2013). Root cortical aerenchyma (RCA), *i.e.*, enlarged gas spaces in the root cortex that form through either cell death or cell separation (*Evans*, 2004), is formed constitutively and in response to a variety of stimuli including suboptimal availability of N, P, S, and water as well as under the presence of hypoxia, high temperature, and mechanical impedance (*Drew* et al., 2000; *Evans*, 2004; *Bouranis* et al., 2006; *Lynch* and *Brown*, 2008). In the present study, under both FM and NM treatment, compared to N250 treatment, a significantly higher RCA percentage was observed in the N0 treatment (Fig. 6A), which is consistent with previous reports (*Drew* et al., 1989; *Saengwilai* et al., 2014). It has been reported that RCA formation is an adaptation to nutrient deficiency by reducing the metabolic cost of soil exploration (*Fan* et al., 2003; *Lynch* and *Brown*, 2008). By converting living root cortical cells to air space, RCA formation significantly reduces the respiratory and nutrient requirements of root tissue, permitting greater root growth and therefore can improve soil resource capture for a given metabolic investment.

It should be noted that, contrary to previous studies which reported that water shortage induced the formation of RCA (*Jaramillo* et al., 2013), we measured a higher percentage of RCA under FM treatment than under NM treatment. This is likely because plastic film mulching isolates the soil from the air, which impedes soil–air gas exchange. Oxygen in the soil is quickly consumed by plants, insects, worms, and other organisms without replenishment from the air. Under plastic mulching conditions, the soil is therefore no longer able to continuously supply the root with oxygen similar to a waterlogging event (*Drew*, 1990; *Visser* and *Voesenek*, 2005). Previous studies in maize demonstrated that hypoxia conditions induced the formation of RCA (*Drew* et al., 1981; *He* et al., 1996). In addition, numerous studies have reported an increase in soil temperature under plastic-film mulching conditions (*Liu* et al., 2014a; *Wang* et al., 2015).

Noteworthy are the RCA studies by *Evans* (2004), who reported that the development of RCA can be induced by high temperature. Therefore, the increased soil temperature under mulching conditions may be another factor that led to the increased formation of RCA. Aerenchyma has been associated with deeper rooting (*Zhu* et al., 2010; *Lynch* et al., 2014). Thus, the increased extension and distribution of roots throughout the soil under FM treatment can be partly explained in terms of the increased formation of aerenchyma under FM conditions. The factors that influence RCA formation under mulching conditions deserve special attention because they may have more potential for yield gains relative to other traits.

Larger cortical cells are hypothesized to decrease cortical burden as they have a higher vacuole-to-cytoplasm volume ratio and a reduced respiratory and nutrient burden (*Lynch*, 2013). Recent studies in maize revealed that roots with large cortical cells exhibited a 59% reduction in respiration compared to those with small cortical cells and have up to 145% greater yield in the field (*Chimungu* et al., 2014). Large stele area under the FM treatment may be related with increased water and nutrient flow rates which further promote water and nutrient uptake. Bulk flow of water or axial conductance is closely related to the diameter of xylem vessels (*Niklas*, 1985). Greater xylem vessel areas indicate more efficient resource transport from the root to support larger shoots (*Lewis* and *Boose*, 1995). Previous studies in rice demonstrated improved resource transport based on measurements of increased late metaxylem vessel diameter under well-watered conditions and reported an enhanced axial conductance (*Mostajeran* and *Rahimi-Eichi*, 2008). In our opinion, the availability of extra water under mulching conditions fostered the production for larger xylem vessels. Therefore, the larger late metaxylem vessel diameter might be beneficial for water and nutrient transportation, as well as nutrient acquisition.

5 Conclusion

Root architectural and anatomical traits are markedly affected by plastic film mulching and N fertilization. Some of the observed effects of plastic film mulching are similar to the effects of N fertilization, yet being the result of presumably hypoxic soil conditions and high soil temperature. Hence, future challenges remain to measure, describe, and understand the multivariate effects of treatments on root architecture and aerenchyma variation (*Bucksch* et al., 2017; *Puttonen* et al., 2018) in greater detail.

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