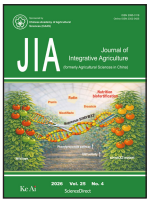
Journal of Integrative Agriculture 2026, 25(4): 1451–1462

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

Cultivar mixtures of maize enhance grain yield and nitrogen use efficiency by promoting canopy photosynthetically active radiation and root growth

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Highlights

- Cultivar mixtures enhanced late-initiated nodal root growth due to greater light interception at the whole canopy layer and leaf area at the lower canopy layer
- Cultivar mixtures can promote nitrogen utilization efficiency by improving the canopy structure and root functional traits.

Abstract

Cultivar mixtures increase crop diversification and grain yield stability. Achieving high grain yield and nitrogen use efficiency (NUE) with environmentally friendly practices is a major challenge, but it is currently unclear whether maize cultivar mixtures can improve NUE. A two-year field experiment was conducted using two maize cultivars with different roots angles and leaf angles planted in monoculture or in mixtures under four nitrogen levels N0 (0 kg N ha^{-1}), N140 (140 kg N ha^{-1}), N280 (280 kg N ha^{-1}) and N340 (340 kg N ha^{-1}). Cultivar mixtures significantly increased light interception of the middle canopy, dry matter accumulation and total root length under N0, N140, and N280 conditions. Light interception of the middle canopy was positively related to dry matter accumulation and thus increased grain yield. In addition, light interception of the whole canopy was positively related to total lateral root length, while the greater total lateral root length of outer nodal roots significantly improved nitrogen accumulation and NUE. Thus, cultivar mixtures promoted an optimal canopy structure and good root growth, thereby improving grain yield and NUE. These findings deepen our understanding of the facilitating effect of canopy structure and root traits of cultivar mixtures on the combined promotion of grain yield and NUE.

Keywords: cultivar mixtures, grain yield, nitrogen use efficiency, light interception, lateral roots of nodal roots

1. Introduction

Maize is a globally cultivated crop, and there is increasing pressure on agriculture to increase maize yields. Obtaining high yields of maize requires a large amount of nitrogen, so the supply of nitrogen is essential for its growth and development (Malhi and Lemke 2006). Farmers often use excessive N to achieve high yields (Wang *et al.* 2021), but this is often counterproductive and can lead to over-fertilization waste and low economic benefits. Over-fertilization exacerbates nitrogen leaching and volatilization, in addition to causing a range of environmental problems such as soil acidification and eutrophication of water bodies (Bai *et al.* 2020; Yao *et al.* 2021). Therefore, it is critical to protect the environment and ensure food security by improving yield and nitrogen use efficiency (NUE) while reducing nitrogen input.

Enhancing biodiversity in cropping systems (i.e., through cultivar mixtures and intercropping) can utilize intraspecific or interspecific diversity to improve yields and agricultural sustainability (Tamburini *et al.* 2020). Intercropping is the

Received 9 May 2024; Received in revised form 26 September 2024; Accepted 11 October 2024; Available online 4 November 2024
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doi: 10.1016/j.jia.2024.11.002

cultivation of two or more crops simultaneously on the same field (Martin *et al.* 2011). Previous studies have shown that intercropping can improve nutrient and water use efficiency, maintain soil fertility and reduce fertilizer demand, which can give full play to the production potential of crops and achieve high and stable crop yields (Beedy *et al.* 2010; Wang W *et al.* 2022; Li C J *et al.* 2023; Yin *et al.* 2025). Intercropping of maize and soybean increases the fraction of intercepted photosynthetically active radiation (PAR) of maize, enhances the leaf duration of photosynthesis and changes the spatial distribution of roots. It also promotes the vertical niche separation of roots in the soil to improve the absorptive capacity of roots (Gao *et al.* 2022). Therefore, the advantages of intercropping in improving maize photosynthesis and promoting root growth are beneficial for phosphorus and nitrogen absorption and grain yield increments (Lv *et al.* 2014; Zhou *et al.* 2021). In intercropped maize and alfalfa systems, root spatial distribution is optimized, and interspecific competition is reduced by increasing root dry weight, the number of lateral roots and root length density in the surface layer. Therefore, compared to monoculture, intercropping makes up for the root avoidance phenomenon, so nitrogen uptake by maize roots is increased in intercropping (Shao *et al.* 2021).

Cultivar mixtures, or the planting multiple cultivars of one crop in same field, can enrich the intra-specific and genetic diversity of crops in the field, and it may be particularly suitable for mechanized intensive production since it involves planting only one crop. It can not only reduce biotic stressors such as disease, weeds and insects, but also mitigate damage by abiotic stressors (Grettenberger and Tooker 2015; Reiss and Drinkwater 2018). Cultivar mixtures also gave full play to spatiotemporal niche complementary and interspecific promotion by staggering the period of maximal demand of the two different plants for light (Reiss and Drinkwater 2018). Light transmittance, ventilation of the plant population and intercepted PAR are improved in cultivar mixtures of maize plants with different phenotypes (such as leaf senescence, leaf angle), and thus light utilization in the population is promoted. Meanwhile, the time of anthesis and pollen shedding is also prolonged in mixture systems, and the maize fertilization rate in cultivar mixtures is higher than in monoculture under continuous rainfall and high temperature in the silking stage (Li *et al.* 2020). Cultivar mixtures can effectively reduce the ear bald tip and lack of grain caused by poor pollination, increase yield and stabilize production (Li *et al.* 2020). Cultivar mixtures with more functional trait diversity can promote plant growth and increase yield (Reiss and Drinkwater 2018). There is great variation of maize root morphology in response to nitrogen deficiency, and this variation often determines the NUE. Maize root growth in different maize cultivars, especially in the crown roots, and nitrogen uptake are coordinated with shoot growth and nitrogen demand (Peng *et al.* 2010). With a low nitrogen supply, total lateral root length mainly contributes to the high nitrogen accumulation and utilization in maize (Wang H *et al.* 2022). Greater root growth is guaranteed by the carbohydrate supply from leaves. With a sufficient nitrogen supply, efficient nitrogen absorption is related to shoot growth potential, rather than root size (Peng *et al.* 2010). Whether the leaf and root traits are coordinated with high nitrogen acquisition in cultivar mixtures remains unknown.

To date, studies have mostly focused on the resource complementarity of intercropping, so leaf and root trait coordination in intercropping or cultivar mixtures needs further study. In this study, we examined the effects of maize cultivar mixtures with different root angles and leaf angles on the growth of leaves and roots, nitrogen accumulation and NUE. We hypothesized that: (1) cultivar mixtures of maize can enhance the interception of PAR and leaf area; (2) the interception of PAR and leaf area duration has a positive correlation with root length and nitrogen accumulation; and (3) post-anthesis matter accumulation can be promoted in cultivar mixtures, which will promote grain yield and NUE. The results of this study will provide insights and theoretical support for improving crop yield and NUE by using cultivar mixtures of maize.

2. Materials and methods

2.1. Experimental design and crop management

Field experiments were conducted in 2021 and 2022 at the Experimental Station of Henan Agricultural University (33°19′48′′N, 114°01′01′′E) in Xiping County, Henan Province, China. The mean annual temperature and rainfall at this site are 15.81°C and 910.20 mm. The soil is a lime concretion black soil, with organic matter content of 13.65 g kg^{-1}, total N content of 1.25 g kg^{-1}, alkaline hydrolytic nitrogen (N) content of 25.57 mg kg^{-1}, Olsen phosphorus (P) content of 18.13 mg kg^{-1}, and NH_4OAc extractable potassium (K) of 124.91 mg kg^{-1} in the 0–20 cm soil layer.

Based on previous experimental results, two maize cultivars, Zhengdan 958 (ZD958, small root angle and high nitrogen efficiency under low nitrogen conditions) and Denghai 3622 (DH3622, large root angle and low nitrogen efficiency under low nitrogen conditions), were selected as the materials. The plant phenotype data (leaf angle, root angle, etc.) are shown in the supplementary material (Appendices A and B). Field experimental parameters, including planting patterns and N application, were arranged as a two-factor randomized block design with three replications (Appendix C). The planting patterns were composed of a cultivar mixture of ZD958 (CM958) and DH3622 (CM3622), and monocultures of ZD958 (M958) and DH3622 (M3622). The N applications consisted of four levels (0, 140, 280, and 340 kg N ha^{-1} denoted as N0, N140, N280 and N340, respectively). Plants in monoculture were planted in 8-row plots, and the cultivar mixture was planted in 12-row plots (with the two cultivars alternately planted in two rows), with 0.60 m interrow spacing and 0.25 m in-row spacing to give a plant population of 67,500 plants ha^{-1}. Calcium superphosphate, potassium chloride and urea were incorporated into a 10 cm depth at sowing, at rates of 90 kg P ha^{-1} and 90 kg K ha^{-1}. Half of the N was applied at sowing as basal fertilizer (banding the fertilizer in a 5 cm deep strip), with topdressing of the other half at the sixth leaf stage (V6) of maize. In the field, four neighboring plants in each treatment were separated by PVC plates to sample the roots. Plants were planted after wheat

harvests on June 15, 2021 and June 10, 2022, and harvested on September 28, 2021 and October 3, 2022.

2.2. Leaf area index (LAI), leaf area duration and fractions of intercepted PAR

According to the definition of LAI, four plants were selected from each plot to measure the maximum leaf length (L) and leaf width (W) at the silking stage (R1), blister stage (R2), milk stage (R3), and mature stage (R6). Leaves were classified as upper, middle and lower canopy layers, bounded by the middle canopy layer of the three leaves adjacent to the ear. Leaf area per plant was calculated by multiplying leaf area values by a weighting factor of 0.75 (Mckee 1964; Dwyer and Stewart 1986).

In the field experiment, 36 measurements for each treatment were taken by a linear quantum sensor for PAR (SunScan; Delta-T, UK) parallel to the rows from 11:00 to 14:00 on a sunny day at the R1 stage. The fraction of intercepted PAR was calculated from the PAR measurements at three sites: 20 cm above the canopy (PARa), middle canopy layer at the ear height position (PARe) and the whole canopy at 10 cm above the soil surface (PARs) (Li *et al.* 2021).

LAI (eq. (1)), intercepted PAR at the ear height position (fPARe, %) (eq. (2)) and the fraction of intercepted PAR at 10 cm above the soil surface (fPARs, %) (eq. (3)) were calculated as:

$$\text{LAI}=\text{Leaf area}/\text{Ground area} \quad (1)$$

$$\text{fPARe}=(\text{PARa}-\text{PARe})/\text{PARa} \quad (2)$$

$$\text{fPARs}=(\text{PARa}-\text{PARs})/\text{PARa} \quad (3)$$

2.3. Root length

The roots of four plants in each treatment were sampled at the R1 stage. The roots were classified as primary, seminal and nodal roots. The crown roots separated from each node were defined as C1, C2, C3, etc., from the inner to outer positions according to the root initiation order. The roots were scanned using a scanner (V750, Epson Japan) at a rate of 600 dots per inch (dpi) and analyzed for root length by WinRHIZO software (2016 WinRhizo Pro, Régent Instruments, Canada).

2.4. Post-anthesis dry matter accumulation, post-anthesis nitrogen accumulation (NA) and NUE

Four plants from each plot were randomly selected in a row with uniform growth and divided into stem, leaf, grain and other parts (including tassel, cob and husk) at the R1, R2 and R6 stages. Dry matter was measured after oven drying at 105°C for 30 min and then at 70°C to constant weight. Samples were powdered for N concentration determination by a flow injection auto-analyzer (AA3, Seal Germany) after digestion of the plant samples with H_2SO_4-H_2O_2.

Dry matter translocation (DMT, g/plant) (eq. (4)), post-anthesis dry matter accumulation (DMA, g/plant) (eq. (5)) and the contribution of dry matter translocation to grain yield (CDMT, %) (eq. (6)) were calculated as:

$$\text{DMT}=\text{VM}_{R2}-\text{VM}_{R6} \quad (4)$$

$$\text{DMA}=\text{DM}_{R6}-\text{DM}_{R1} \quad (5)$$

$$\text{CDMT}\ (\%)=\text{DMT}/\text{GY}\times 100 \quad (6)$$

where VM_{R1} is the dry weight of vegetative organs at R1, VM_{R6} is the dry matter weight of vegetative organs at maturity, DM_{R1} is dry matter weight at R1, DM_{R6} is dry matter weight at maturity and GY is grain yield at maturity (Chen et al. 2017).

Post-anthesis nitrogen accumulation (NA, mg/plant) (eq. (7)) and the contribution of nitrogen remobilization to grain yield (CNRA, %) (eq. (8)) were calculated as:

$$\text{NA}=\text{N}_{mat}-\text{N}_{R1} \quad (7)$$

$$\text{CNRA}\ (\%)=\text{NRA}/\text{GN}_{mat}\times 100 \quad (8)$$

where VN_{R2} is the nitrogen content of vegetative organs at R2, VN_{mat} is the nitrogen content of vegetative organs at maturity, N_{R1} is plant nitrogen content at R1, N_{mat} is plant nitrogen content at maturity and GN_{mat} is grain nitrogen content at maturity.

Nitrogen use efficiency (NUE, %) (eq. (9)) was calculated as:

$$\text{NUE}=(\text{N}-\text{N0})/\text{Na} \quad (9)$$

where N is the plant total nitrogen content in different nitrogen application treatments (140, 280, and 340 kg N ha^{-1}), N0 is plant total nitrogen content in the N0 treatment (0 kg N ha^{-1}), and Na is nitrogen application rate (140, 280, and 340 kg N ha^{-1}).

2.5. Yield and yield components

Grain yield was harvested in a sampling area of 2 rows×5 m length in each plot.

2.6. Data analyses

Data were analyzed statistically by SPSS 20.0 (SPSS, Inc., USA). Nitrogen levels, planting pattern, and their interactions were analyzed using two-way-ANOVA at the 5% level. Statistical analysis of cultivar mixture and monoculture with planting pattern and cultivar were assessed using two-way-ANOVA at the 5% level. Statistical analysis of planting patterns at the same nitrogen level was analyzed using one-way-ANOVA at the 5% level. All figures were plotted using Origin 2023 (Origin Lab, Inc., USA). The structural equation model (SEM) was analyzed using R language, version 4.0.2.

3. Results

3.1. Grain yield

Cultivar mixtures and nitrogen application significantly increased grain yield (Fig. 1). On average over the two years and four N levels, grain yield was significantly increased by 6.86% in the cultivar mixtures (Appendix D). The difference between cultivar mixtures and monoculture was inconsistent across nitrogen applications. Grain yields significantly increased in the cultivar mixtures by 12.72, 9.07 and 4.83% under N0, N140 and N280 treatments in 2021 and 2022, while there was no significant difference under the N340 treatment between monoculture and cultivar mixtures in 2021 (Fig. 1).

3.2. LAI, light interception and root length

Cultivar mixtures and nitrogen application had positive effects on the fraction of intercepted PAR (fPAR) (Fig. 2). Compared to those in monoculture, fPAR at the middle canopy layer and whole canopy layer in cultivar mixtures increased by 15.95 and 3.93% on average in the two years. In cultivar mixtures,

fPAR at the middle canopy layer increased by 22.84, 17.22, 13.40, and 11.34% under the N0, N140 N280, and N340 treatments, respectively, compared to monoculture. In addition, fPAR at the whole canopy layer increased by 6.94, 4.17, 2.68, and 2.24% under the N0, N140, N280, and N340 treatments, respectively, compared to monoculture.

Meanwhile, LAI was higher in cultivar mixtures than monoculture under the same nitrogen level (Fig. 3). Compared with those in monoculture, LAI at the middle and lower canopy layers in cultivar mixtures increased by 5.99 and 2.93% on average in the two years. The leaf angle at each canopy layer of ZD958 was lower than DH3622, in both

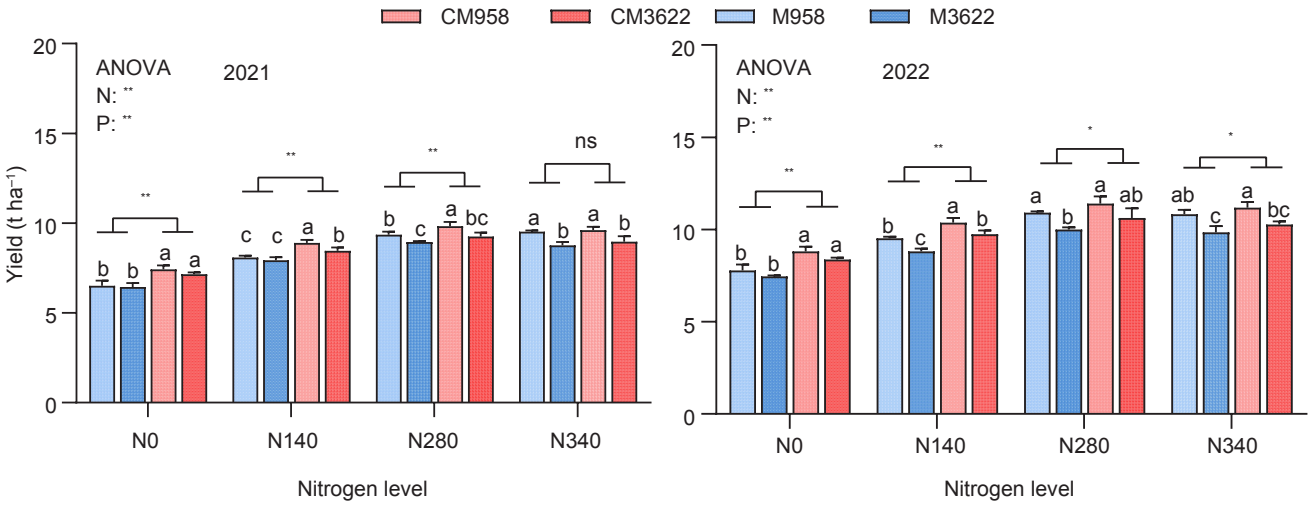


Fig. 1 Grain yields of two maize cultivars under different nitrogen levels (N) and planting patterns (P) in 2021 and 2022. M, monoculture; CM, cultivar mixtures. N0, N140, N280, and N340 indicate 0, 140, 280, and 340 kg N ha^{-1}, respectively. Based on one-way ANOVA, different lower-case letters indicate significant differences between the two planting patterns and two maize cultivars under the same N level. Bars mean SE (n=4). Based on two-way ANOVA considering the factors of planting pattern and cultivar, **, * and ns indicate differences between cultivar mixtures and monoculture under same N level. Based on two-way ANOVA considering the factors of N application and planting pattern, **, * and ns indicate their effects, **, P<0.01; *, P<0.05; ns, P>0.05.

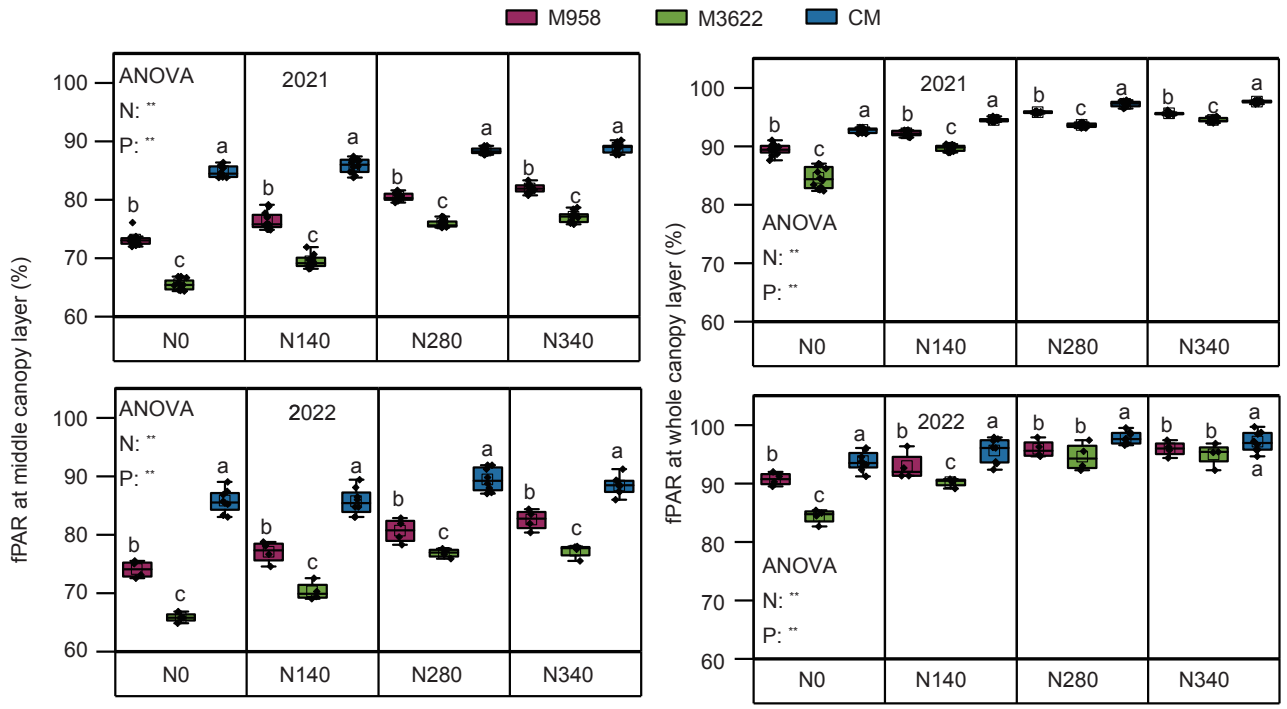


Fig. 2 Fractions of intercepted photosynthetically active radiation at the middle canopy layer and whole canopy layer in 2021 and 2022 under different nitrogen levels (N) and planting patterns (P). M, monoculture; CM, cultivar mixtures. N0, N140, N280, and N340 indicate 0, 140, 280, and 340 kg N ha^{-1}, respectively. Bars mean SE (n=4). Based on one-way ANOVA, different lower-case letters indicate significant differences between the different planting patterns under the same N level. Based on two-way ANOVA considering the factors of N application and planting pattern, **, * and ns indicate their effects, **, P<0.01; *, P<0.05; ns, P>0.05.

cultivar mixtures and monoculture (Appendix E).

There was a positive effect on root length in cultivar mixtures (Fig. 4), with 6.86% greater total root length, 5.70% greater axial root length and 6.37% greater lateral root length on average in cultivar mixtures compared to those in monoculture. Total root lengths increased significantly by 8.95, 6.88 and 4.84% under the N0, N140 and N280 treatments in cultivar mixtures, but there was no significant difference under the N340 treatment. The root angles of ZD958 were lower than DH3622 in both cultivar mixtures and monoculture (Appendix E). Clustering analysis showed three groups among the root nodes (Appendix E). We identified those groups as inner roots (seminal roots, 1st and 2nd nodal roots), middle roots (3rd, 4th, 5th, and 6th nodal roots) and outer roots (7th and 8th nodal roots) according to the order of root emergence (Appendix F). The lateral roots of outer roots and middle roots increased by 4.38 and 8.29% on average in cultivar mixtures compared to those in monoculture (Fig. 4). The axial roots of outer roots and middle roots increased by 5.01 and 6.17% on average in cultivar mixtures compared to those in monoculture (Fig. 4).

3.3. Dry matter weight, post-anthesis dry matter accumulation and dry matter translocation

Cultivar mixtures had a more positive effect on post-

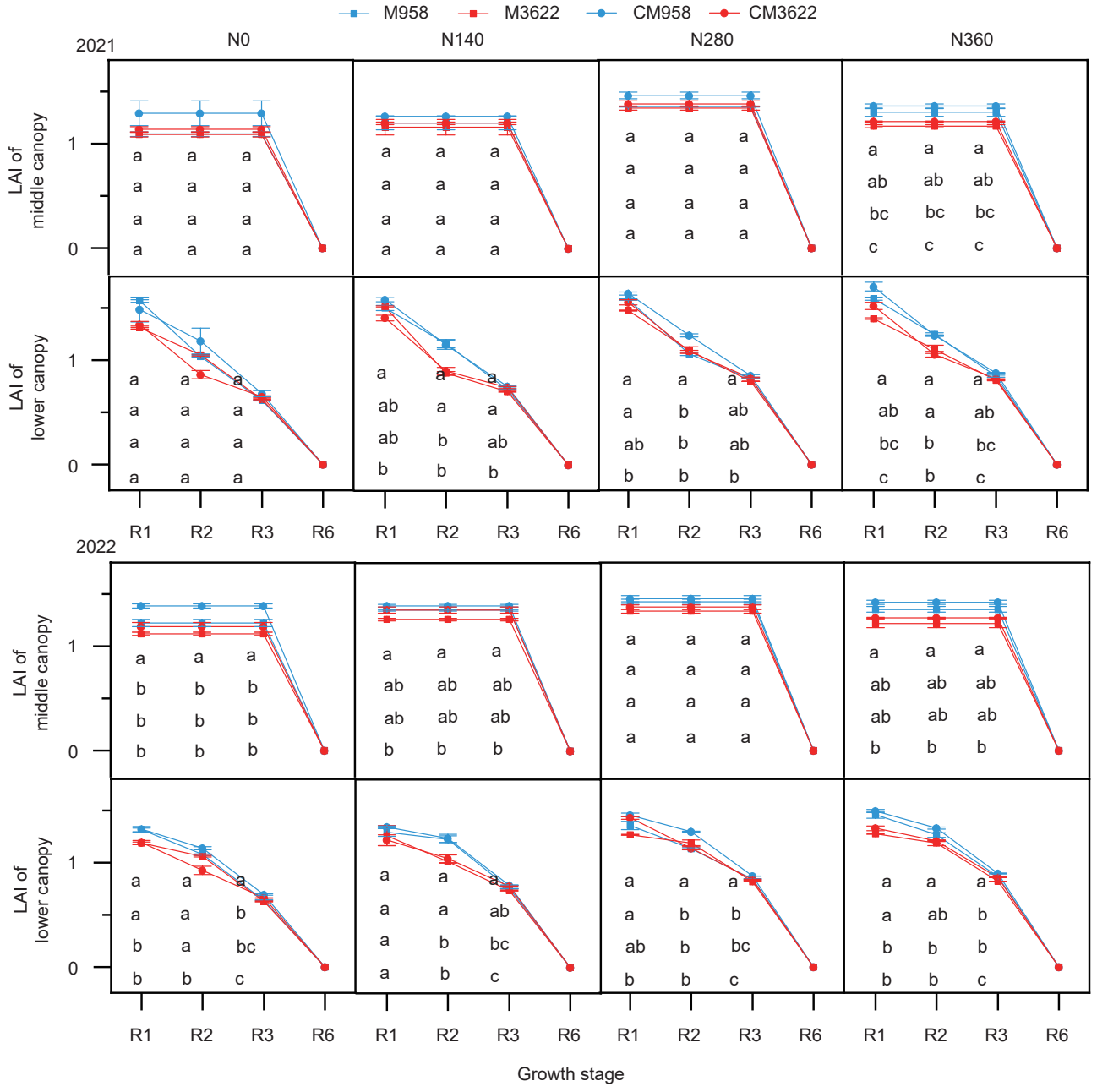


Fig. 3 Leaf area index (LAI) of the middle and lower canopy layers of two maize cultivars under different nitrogen levels (N) and planting patterns (P) in 2021 and 2022. M, monoculture; CM, cultivar mixtures. N0, N140, N280, and N340 indicate 0, 140, 280, and 340 kg N ha^{-1}, respectively. R1, R2, R3, and R6 indicate silking, blister, milk, and mature stages, respectively. Bars mean SE (n=4). Based on one-way ANOVA, different lower-case letters indicate significant differences between the different planting patterns under the same N level.

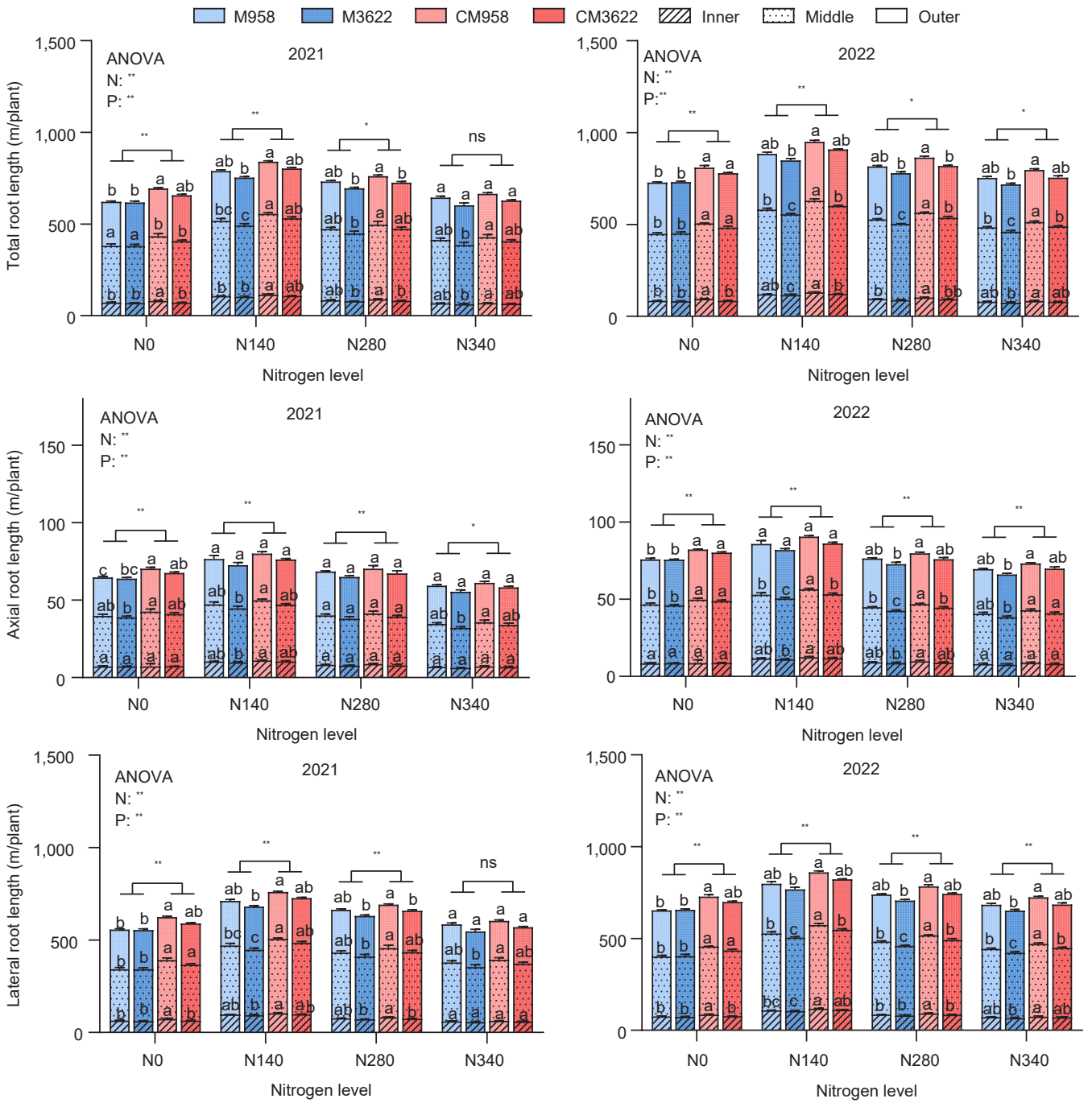


Fig. 4 Total , axial and lateral root lengths of two maize cultivars under different nitrogen levels (N) and planting patterns (P) in 2021 and 2022. M, monoculture; CM, cultivar mixtures. N0, N140, N280, and N340 indicate 0, 140, 280, and 340 kg N ha^{-1}, respectively. Bars mean SE (n=4). Based on two-way ANOVA, different lower-case letters indicate significant differences between different planting patterns under the same N level. Based on two-way ANOVA considering the factors of planting pattern and cultivar, **, * and ns indicate differences between cultivar mixtures and monoculture under the same N level. Based on three-way ANOVA considering the factors of N application, planting pattern and cultivar, **, * and ns indicate their effects, **, P<0.01; *, P<0.05; ns, P>0.05.

anthesis dry matter accumulation (DMA) than dry matter translocation (DMT) (Fig. 5), but they had no pronounced effect on the contribution of dry matter translocation (Appendix G). In cultivar mixtures, dry matter weight significantly increased from 273.09 to 287.60 g/plant, DMA significantly increased from 126.25 to 133.39 g/plant, while DMT increased from 18.53 to 20.47 g/plant, on average under the four N levels in the two years. The positive effect of cultivar mixtures on DMA was reduced with N application, as it decreased from 10.40% under the N0 treatment to 3.24% under the N340 treatment. N application caused clear increases in DMA and DMT, especially in DMA, which increased from 101.85 g/plant under the N0 treatment to 145.60 g/plant under the N340 treatment over the two years (Fig. 5).

3.4. Nitrogen content, NA and NUE

Cultivar mixtures had a greater positive effect on NA than nitrogen remobilization (NRA) (Fig. 6), but they had no pronounced effect on the contribution of nitrogen remobilization (Appendix F). In cultivar mixtures, the nitrogen content significantly increased from 2,806.75 to 2,995.50 mg/plant, NA significantly increased from 1,043.64 to 1,136.96 mg/plant, and NRA increased from 796.90 to 849.39 mg/plant over the two years. Under the N0 treatment, nitrogen remobilization mainly came from the stems and leaves. The proportion of leaf nitrogen remobilization gradually increased with N application, but the positive effect of cultivar mixtures on NA and NRA were reduced with greater N application. NA decreased from 17.32% under the N0 treatment to 5.67% under the N340 treatment, and NRA decreased from 12.72 to 3.31%. However, NA and NRA reached their highest values under the N240 treatment, with values of 1,272.98 and 1,005.06 mg/plant, respectively (Fig. 6). Therefore, there were obvious

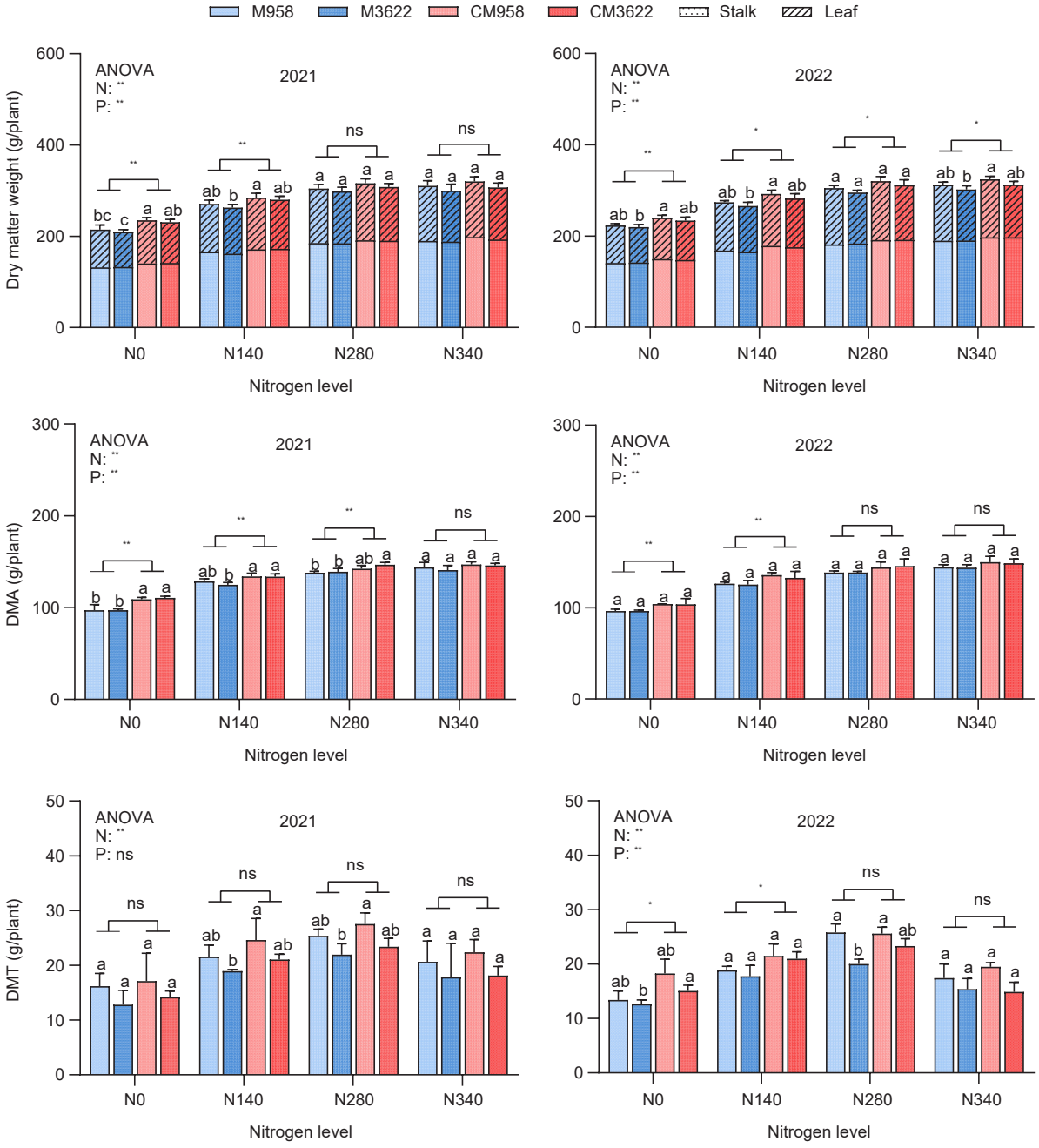


Fig. 5 Dry matter weight at maturity stage (R6), post-anthesis dry matter accumulation (DMA) and dry matter translocation (DMT) of two maize cultivars under different nitrogen levels (N) and planting patterns (P) in 2021 and 2022. M, monoculture; CM, cultivar mixtures. N0, N140, N280, and N340 indicate 0, 140, 280, and 340 kg N ha^{-1}, respectively. Bars mean SE (n=4). Based on one-way ANOVA, different lower-case letters indicate significant differences between the two planting patterns and two maize cultivars under the same N level. Based on two-way ANOVA considering the factors of planting pattern and cultivar, **, * and ns indicate differences between cultivar mixtures and monoculture under the same N level. Based on three-way ANOVA considering the factors of N application, planting pattern and cultivar, **, * and ns indicate their effects, **, P<0.01; *, P<0.05; ns, P>0.05.

increases in NA and NRA with the N application rate.

Cultivar mixtures had positive effects on NUE. Compared to monoculture, NUE values increased by 16.48, 8.50 and 28.09% in cultivar mixtures (Fig. 7). NUE decreased obviously with higher N application rates. Compared to monoculture, NUE increased by 26.60, 11.51 and 9.18% under the N140, N280 and N340 treatments in cultivar mixtures, respectively (Fig. 7).

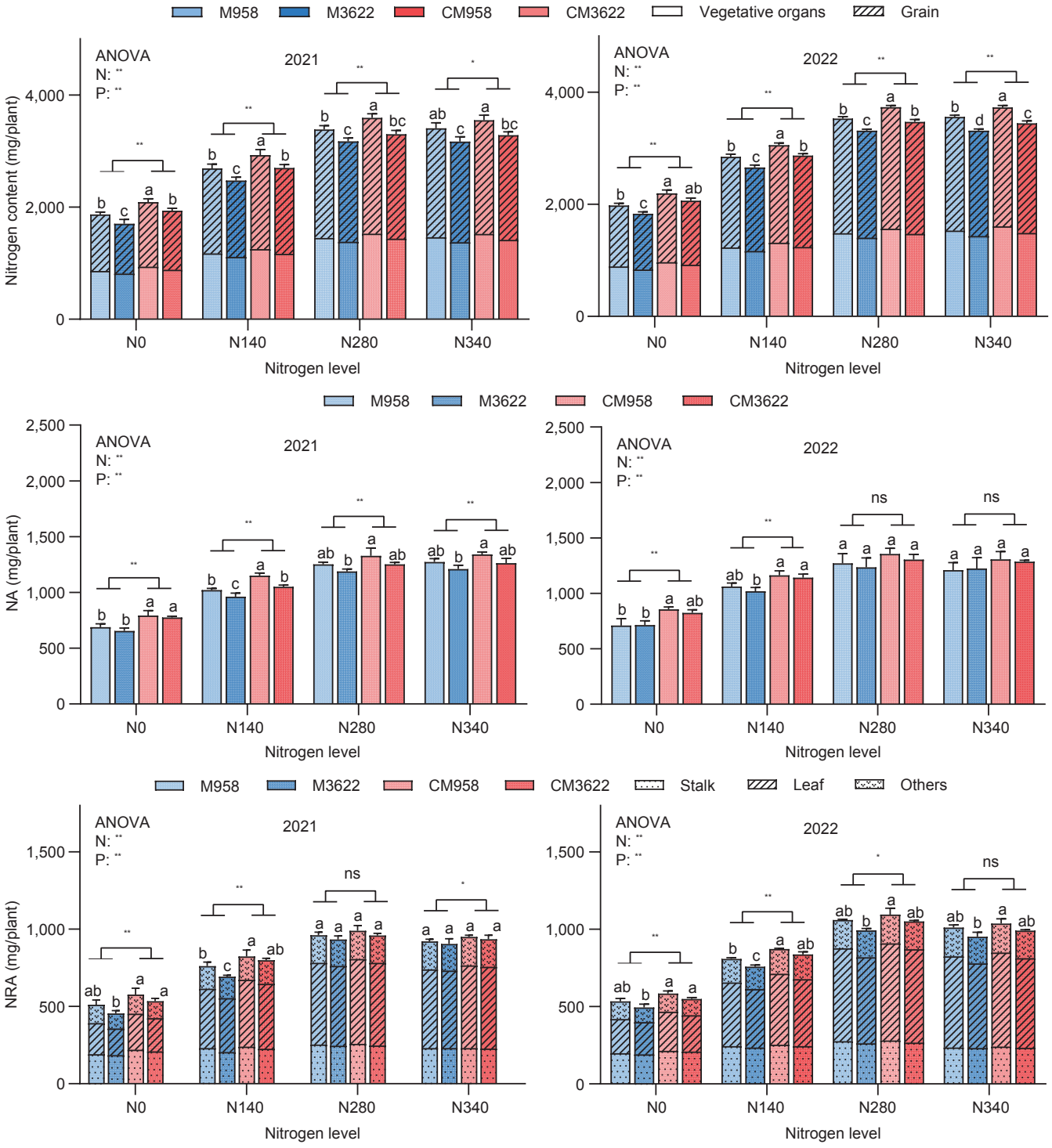


Fig. 6 Nitrogen content at maturity stage (R6), post-anthesis nitrogen accumulation (NA) and nitrogen remobilization (NRA) of two maize cultivars under different nitrogen levels (N) and planting pattern (P) in 2021 and 2022. M, monoculture; CM, cultivar mixtures. N0, N140, N280, and N340 indicate 0, 140, 280, and 340 kg N ha^{-1}, respectively. Bars mean SE (n=4). Based on one-way ANOVA, different lower-case letters indicate significant differences between the two planting patterns and two maize cultivars under the same N level. Based on two-way ANOVA considering the factors of planting pattern and cultivar, **, * and ns indicate differences between cultivar mixtures and monoculture under the same N level. Based on three-way ANOVA considering the factors of N application, planting pattern and cultivar, **, * and ns indicate their effects, **, P<0.01; *, P<0.05; ns, P>0.05.

3.5. Relationships between plant patterns, NUE and grain yield

Cultivar mixtures increased dry matter weight, possibly because cultivar mixtures promote plant growth through the interception of PAR at the middle canopy layer and leaf area at the silking stage, enhancing the capacity of supply sources (Fig. 8; Appendix H). The grain yield was mainly determined by dry matter accumulation, and the effect of dry matter translocation was greater than that of dry matter accumulation after anthesis (Fig. 8). Cultivar mixtures had a significant positive effect on PARS, which further positively affected the lateral roots length of crown roots (Fig. 8). The outer lateral roots of nodal roots were significantly related to nitrogen accumulation and contributed more to NUE (Fig. 8).

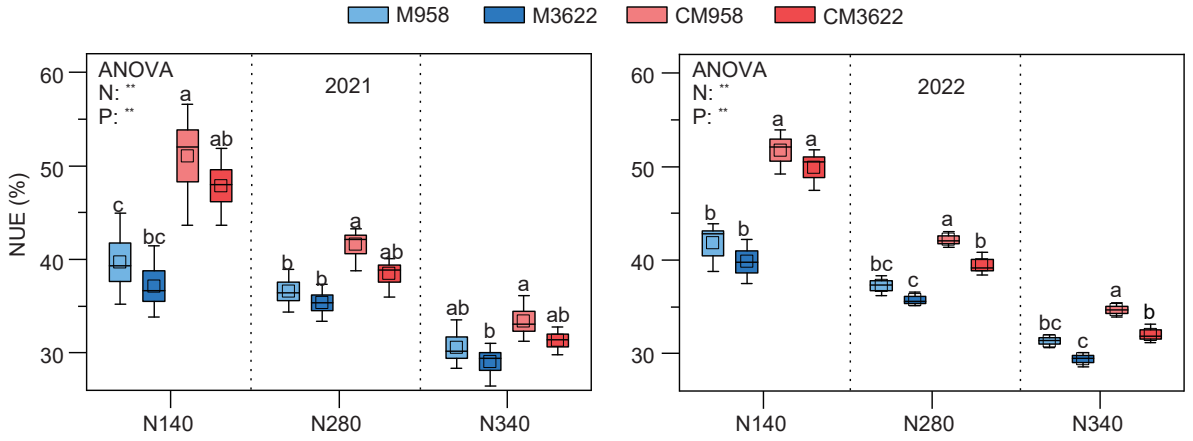


Fig. 7 Nitrogen use efficiency (NUE) of two maize cultivars under different nitrogen levels (N) and planting patterns (P) in 2021 and 2022. M, monoculture; CM, cultivar mixtures. N140, N280, and N340 indicate 140, 280, and 340 kg N ha^{-1}, respectively. Bars mean SD SE (n=4). Based on one-way ANOVA, different lower-case letters indicate significant differences between the two planting patterns and two maize cultivars under the same N level. Based on one-way ANOVA, different lower-case letters indicate significant differences between the two planting patterns and two maize cultivars under the same N level. Based on three-way ANOVA considering the factors of N application, planting pattern and cultivar, **, * and ns indicate their effects, **, P<0.01; *, P<0.05; ns, P>0.05.

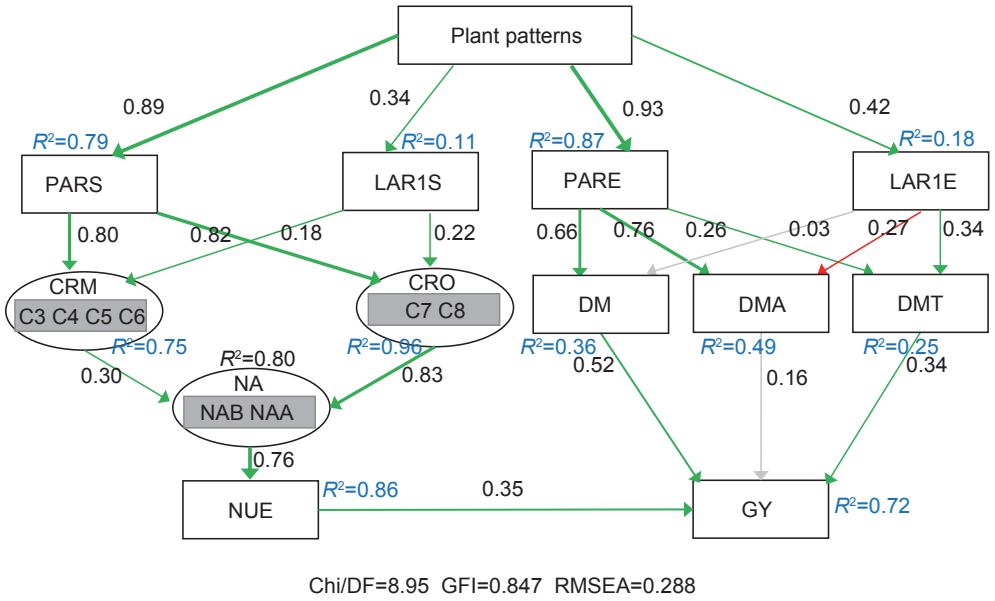


Fig. 8 Structural equation model of plant patterns as related to nitrogen use efficiency (NUE) and yield. Green and red lines represent positive and negative impacts, respectively; gray line path indicates an insignificant coefficient; width of each arrow indicates the strength of the normalized path coefficients; numbers on the lines represent the standardized total effects. GY, grain yield; DM, dry matter weight at the sixth leaf stage (R6); NA, nitrogen accumulation; CRO, lateral root length of outer nodal roots; CRM, lateral root length of middle nodal roots; C3–C8, the 3rd, 4th, 5th, 6th, 7th, and 8th nodal roots; PARE, light interception at the middle canopy layer; PARS, light interception at the whole canopy layer; DMA, post-anthesis dry matter accumulation; NAA, post-anthesis nitrogen accumulation; DMT, dry matter translocation; NAB, nitrogen accumulation at anthesis. LAR1S, leaf area index at the lower canopy layer; LAR1E, leaf area index at the middle canopy layer; Chi/DF, Chi-square/df; GFI, Goodness-of-Fit Index; RMSEA, root mean square error of approximation.

4. Discussion

4.1. Cultivar mixtures promote PAR

The intercropping system can present either a positive or negative effect depending on the promotion and competition between different crop types (Loreau and Hector 2001; Cardinale *et al.* 2007; Barry *et al.* 2019). Crop biomass is linearly correlated with PAR in the field. Light distribution is determined by plant architecture (such as leaf angle and leaf area) and further influenced by plant physiology (such as leaf senescence) (Huang *et al.* 2017). Light transmittance, ventilation of the plant population and intercepted PAR are all improved in interplanting systems with different plant phenotypes for features such as plant height, leaf angle in corn, and thus light utilization of the population is promoted (Liu *et al.* 2008). On the one hand, intercropped maize and soybean were shown to inhibit the growth of soybean and reduce PAR due to the shading effect of maize on soybean (Pelech *et al.* 2023). The same result was also obtained with intercropping maize and wheat (Lv *et al.* 2014). On the other hand, appropriate intercropping can fully utilize species diversity and give play to the spatiotemporal niche complementarity effect and interspecific promotional effect of differential crop growth compared with monoculture (Li *et al.* 2021; Zhou *et al.* 2021). Intercropped plants can capture much more PAR radiation to improve the light utilization rate and promote the growth of crops (Wang *et al.* 2017). This study showed that cultivar mixtures had significantly more light interception in the whole canopy layer, especially in the middle canopy layer (Fig. 4), indicating a higher PAR for the maize canopy. In the present study, the two cultivars displayed different spatial distributions of leaf area (Appendix F), implying a canopy structure complementarity that is related to high PAR in the leaves at middle positions in the canopy. When intercropped with soybean, the light distribution is more uniform and the penetration of light is deeper for intercropped maize, which can enhance the photosynthetic capacity compared with maize monoculture (Migliavacca *et al.* 2017; Zhu *et al.* 2020). In cultivar mixture systems, there are also complementary effects for reducing the risk of lower yield caused by biotic and abiotic stresses (Zhu *et al.* 2000; Li H P *et al.* 2023). In this study, we found that there were complementary effects on growth, yield and nitrogen utilization due to mixtures of cultivars with different nitrogen use efficiencies. Therefore, the key to increasing crop yield and stress resistance is choosing cultivars with complementary traits in the cultivar mixtures.

4.2. Cultivar mixtures promote root growth

Carbohydrates are usually translocated from the nearest leaf to the roots through the phloem. When the photosynthetic capacity is enhanced, leaves at the bottom of the canopy can provide more carbohydrates to root growth. Dry matter accumulation promotes the distribution of assimilates to roots (Hou *et al.* 2023). In the present study, cultivar mixtures enhanced light interception at the whole canopy layer and the leaf area at the lower canopy layer, which in turn increased root length (Fig. 8), implying greater root growth under cultivar mixtures. Meanwhile, plants regulate their own root morphological characters (such as root length, root diameter, etc.) according to the adjacent plants, and attain the optimal root system distribution (Craine *et al.* 2006). Total root length was greater for cultivar mixtures in this study (Fig. 3-B), which was consistent with previous research on improving root length in two maize cultivar mixtures (Jia *et al.* 2024). Maize roots grow along with faba bean roots when maize and faba bean are intercropped, but the maize roots avoid growing in shared soil and show enhanced growth away from their neighbors when grown with maize (Zhang *et al.* 2020). In maize cultivar mixtures, the mechanism for increasing roots was probably root competitive growth, which promotes root elongation and vertical distribution in deep soil for acquiring nutrients. Meanwhile, this study also found that the root length of cultivar mixtures was restricted with excessive nitrogen application, but it was promoted in low nitrogen applications (Fig. 3-B), indicating that excessive nitrogen application can suppress root facilitation.

Improved root growth can increase nitrogen acquisition, thus increasing NUE (Lynch *et al.* 2023). In the present study, cultivar mixtures showed increased axial root length and lateral roots (Fig. 5). On the one hand, the elongation of axial roots is conducive to root foraging depth for nitrogen in deep soil, with nitrate leached to deep soil at the late growth stage of maize (Wang H *et al.* 2022; Wu *et al.* 2022). On the other hand, lateral roots make a major contribution to nutrient absorption (Chen *et al.* 2022). Interestingly, the lateral roots of outer nodal roots presented a stronger positive relation with nitrogen accumulation (Fig. 8). The outer nodal roots are initiated late, implying unoccupied soil and high uptake capacity in new roots (Volder *et al.* 2005). Thus, cultivar mixtures demonstrated better root growth and higher NUE in this study, especially in suboptimal nitrogen applications, indicating an efficient practice to reduce nitrogen input.

4.3. Cultivar mixtures promote dry matter accumulation and translocation

Grain yield is formed not only from direct transfer to grains but also from the remobilization and transfer of assimilates pre-stored in vegetative organs (Laza *et al.* 2003; Yang and Zhang 2006). Most assimilates in grains (74.80–92.80%) are from the supplies of post-anthesis leaf photosynthesis (Yang and Zhang 2006). The level of 84.51% in this study was consistent with the findings from previous studies. Light interception at the middle canopy was the key to increasing or maintaining maize yield in the present study (Fig. 8). Cultivar mixtures significantly improved total dry matter accumulation and post-anthesis dry matter translocation (Fig. 5-B). During the reproductive growth stage, grain development competes with vegetative organs for photosynthetic products, accelerating the senescence of vegetative organs. Vegetative organs undergo photosynthetic product transfer so they play a critical role in grain yield during the reproductive stage of crops (Dreccer *et al*. 2000). Cultivar mixtures enhance disease resistance by intraspecific complementation to achieve a stable yield (Li *et al*. 2002; Duan and Zhao 2017). Cultivar mixtures can effectively reduce the ear bald tip and lack of grain caused by poor pollination, while also

increasing yield and stabilizing production (Tao *et al.* 2016; Li H P *et al.* 2023). This study showed that cultivar mixtures could promote the dry matter translocation amount by 10.4% compared to monoculture, but the contribution of dry matter translocation showed no significant difference (14.5% on average) between cultivar mixtures and monoculture (Appendix G). The dry matter translocation during post-anthesis was low and contributed less (8.4–14.6%) to grain yield in maize, which limited the contribution of dry matter translocation during post-anthesis to grain yield (Feng *et al.* 2017; Gao *et al.* 2022). When nitrogen application was within a reasonable range (within the range of 0–280 kg ha^{-1}), cultivar mixtures increased dry matter translocation. However, cultivar mixtures lost their positive effect on dry matter translocation as the nitrogen application rate exceeded the optimal range. Although dry matter translocation was positively correlated with grain yield in this study, the contribution of dry matter accumulation to grain yield was greater (Fig. 8), suggesting that cultivar mixtures increased grain yield by promoting dry matter accumulation. In this study, the promotional effect of cultivar mixtures on grain yield was much stronger in suboptimal nitrogen applications, confirming cultivar mixtures as a useful practice in low-input systems.

5. Conclusion

Cultivar mixtures exhibited improved light interception and dry matter accumulation, thus increasing grain yield. Higher light interception at the whole canopy layer and leaf area at the lower canopy layer promoted axial and lateral root length in cultivar mixtures. Furthermore, the enhanced root growth of late-initiated nodal roots increased nitrogen accumulation and nitrogen use efficiency. As a result, cultivar mixtures can promote crop growth, increase yield, and improve nitrogen utilization efficiency when considering the optimal canopy structure and root functional traits.

Acknowledgements

We are grateful for grants from the China Agriculture Research System (CARS-02-20), the Henan Province Agro-ecosystem Field Observation and Research Station, China (30602535), the Natural Science Foundation of Henan Province, China (202300410216 and 222300420180), and the Key Laboratory of Agricultural Monitoring and Early Warming Technology, China (2023KLAMEWT03).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2024.11.002

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