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ORIGINAL PAPER

Arbuscular mycorrhiza enhances maize grain yield and nitrogen uptake during the grain filling stage with contrasting nitrogen status in two types of soils

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Abstract

Arbuscular mycorrhizal fungi (AMF) generally improve crop nutrient acquisition and grain yield, especially under nutrient deficiency. It is uncertain, however, whether and how AMF colonization affects maize nitrogen (N) uptake during grain filling stage and grain yield under varying soil N status. To investigate this role under the conditions of poor and rich agricultural soils, two pot experiments were conducted with AMF inoculated and non-inoculated maize growing at low (180 kg N hm^{-2}) and high N (270 kg N hm^{-2}) input and two different nutrient areas. Compared to the non-inoculation treatment, AMF inoculation increased maize grain yield, plant biomass, and N accumulation during the filling stage under different soil N conditions. Other responses included increasing root length, root surface area, activities of grain nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase (GOGAT) and their gene expressions. All enzyme activities and *GOGAT* gene expression were significantly correlated with grain yield. Grain yield and N accumulation were significantly higher at the nutrient rich site than poor site. Inoculation with AMF significantly increased grain yield with either lower or higher N input at both sites, whereas increased efficiency was greater with lower N input than higher N input. These results showed that AMF inoculation can increase maize yield and N uptake during the filling stage through regulating root traits and grain N metabolic enzyme activities and their gene expressions independent of soil N status. This enhances our knowledge of the role of AMF in the context of conventional agricultural management.

Keywords Nitrogen fertilizer · Arbuscular mycorrhizal fungi · Nitrogen uptake · Grain enzyme activity · Gene expression

Introduction

Maize (*Zea mays* L.) is one of the more important cereal crops cultivated globally with an area of > 197 million hectares producing 1134.8 million tons in 2017 (FAO 2019). High grain yield per plant is mainly related to maize kernel weight (Ruiz et al. 2022), which is highly dependent on the assimilation of nitrogen (N) during the grain-filling stage, a stage that is important in determining maize kernel weight and grain yield (Gambín et al. 2006a; Hisse et al. 2019). Indeed, limitations of the supply of N during the grain-filling stage influence final kernel weight and grain yield (Paponov et al. 2005; Gambín et al. 2006a; Olmedo and Vyn 2021). Understanding the mechanisms of maize grain yield and N uptake during the grain filling stage could improve plant N uptake and maximize maize productivity and consequently minimize N pollution risk for fulfilling sustainable development of agroecosystems.

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Arbuscular mycorrhizal fungi (AMF) are important rhizosphere microorganisms forming symbioses with the roots of over 80% of terrestrial plant species (Hodge et al. 2001; He et al. 2003; Kiers et al. 2011). Many studies have shown that inoculation with AMF can effectively increase corn grains by increasing N absorption (Zhang et al. 2022), with up to 74% of shoot N being derived from the fungal partner in maize (Tanaka and Yano 2005). Zhang et al. (2022) found that AMF can effectively increase maize grain yield, mainly by increasing maize N uptake (Leigh et al. 2008; Saia et al. 2015), although Zhang et al. (2018) demonstrated that AMF increased the allocation of shoot biomass to panicles and grains through increased N redistribution to panicles particularly under low fertilizer levels. Despite unequivocal evidence indicating that AMF can acquire and transfer substantial amounts of N to their host plants (Leigh et al. 2008; Fellbaum et al. 2012; Yang et al. 2018), reports are contradictory regarding the net effects of AMF symbioses on plant growth in N-deficient soil. Some studies show that AMF increase N uptake and biomass of their host plants (Tu et al. 2006; Zhang et al. 2022), whereas others show that AMF symbioses have no benefit for ameliorating N limitation (Reynolds et al. 2005). Varinderpal-Singh et al. (2020) found that mean grain yield obtained via seed coating with mycorrhizae (5.59 t ha^{-1}) was equivalent to non-mycorrhizal seed coating (5.51 t ha^{-1}), suggesting ineffectiveness of mycorrhizae in improving the grain yield. These conflicting results may be originated from inconsistencies in N fertilizer rates, soil structure (Rillig and Mummey 2006), soil nutritional status (Zhang et al. 2022), and pH (Bago et al. 1996). Hence, soil properties may have strong influence on AMF's function in plant growth and grain yield (Karagiannidis and Hadjisavva-Zinoviadi 1998; Lehmann and Rillig 2015).

Among abiotic factors, the nutritional status of the soil plays a key role in mitigating AMF effects (Yang et al. 2011; Chen et al. 2023). Some studies found that AMF improved plant nutrient uptake in nutrient-limited soils (Tajini et al. 2011; Begum et al. 2019). Varinderpal-Singh et al. (2020) reported that the mycorrhizal inoculation increased grain yield by 17.1% in no-N plots, whereas grain yield was independent of mycorrhizal inoculation in N-fertilized plots. Although AMF play an important role in plant N uptake, it is often believed that AMF is less important in the conventional agricultural systems where high fertilizer inputs often suppress AMF infection (Gilliam 2006; Johnson et al. 2015; Treseder et al. 2018; Han et al. 2020). It is further generally thought that under high nutrient conditions AMF can shift from a net benefit to a cost for the host (Werner and Kiers 2015; Zhang et al. 2019). Zhang et al. (2022), however, reported that even under conditions of high N availability the presence of AMF could increase maize plant biomass and their N accumulation (Begum et al. 2019). Excessive levels of N fertilizer are commonly applied in modern agriculture to meet the requirement of crop yield for the increasing human population, even though such application has resulted in negative effects on soil quality, such as soil acidification (Guo et al. 2010; Zhang et al. 2015). Thus, it is urgent not only to increase food production but also to maintain soil biodiversity and functionality by implementing sustainable management practices. AMF is considered as an effective measure to increase crop yield and their N uptake, and improve the quality of soil (Zhang et al. 2019; Shi et al. 2023). It remains largely unclear, however, how AMF affect maize grain yield during the grain filling period under high N input. Furthermore, few studies have examined the potential roles through which AMF affect activities of enzymes associated with grain N metabolism or pathways of genes at transcriptional level that modify important functions such as grain N uptake.

To investigate the effects of AMF on maize grain yield under contrasting soil N conditions, we selected two agroecosystems sites of contrasting soil fertility: Zhengzhou, a nutrient rich site, and Shangqiu, a nutrient poor site, and established both low and high N fertilizer rate at each site. The purpose of this study was to determine (i) whether AMF could increase maize grain yield and N uptake during the grain filling stage in soils of contrasting soil N status, and (ii) whether the role of AMF in improving grain yield and their N uptake is regulated by activities of enzymes that regulate plant N and associate gene expression at the transcription levels.

Materials and methods

Site description and experimental materials

To quantify the effects of AMF inoculation on maize grain yield and N uptake during grain filling stage, two pot experiments were established under ambient conditions of field temperature and rainfall at two open fields at the campus of Henan Agricultural University in Zhengzhou City (113°48′E, 34°47′N, mean elevation 80 m) and at the Farmland Ecosystem Research Station, Institute of Farmland Irrigation, Chinese Academy of Agricultural Sciences in Shangqiu city (115°48′E, 34°35′N, mean elevation 73 m), Henan Province, China (Figure S1). The soil type in Zhengzhou was lime concretion black soil, and was fluventic Ustochrept in Shangqiu. Soils were collected to a 20-cm depth in June 2020 in the local farmlands with wheat and maize annual rotation in both sites. All soil samples were air dried and sieved (2 mm). Basic chemical properties in Zhengzhou and Shangqiu were as follows: total N 2.76 and 0.45 g kg^{-1}, total phosphorus 4.46 and 0.8 g kg^{-1}, available phosphorus 0.02 and 0.005 g kg^{-1}, available potassium 0.33 and 0.038 g kg^{-1}, organic matter

8.17 and 8.3 g kg^{-1}, pH (water) 6.81 and 7.16, respectively. During maize growth periods from June 23 to October 10 2020 (Fig. 1), daily mean temperatures in Zhengzhou and Shangqiu were 25.5 and 24 °C, 29.8 and 29.5 °C for daily mean maximum temperature, 21.2 and 19.5 °C for daily mean minimum temperature, respectively; mean annual rainfall was 327.4 and 489.3 mm, respectively.

The AMF strain *Funneliformis mosseae* (Nicol. & Gerd.) Schüßler & Walker (1511C0001BGCAM0063) was obtained from Bank of Glomeromycota in China (BGC), Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, China. Then, the AM fungus was propagated with both the identified fungal spores and maize (*Zea mays* L.) in sterilized sand for 3 months until sporulation in the greenhouse of Henan Agricultural University. Sand substrates with chopped roots, external hyphae, and spores were stored in plastic bags at room temperature. The spore number in the inoculum was 30 spores per gram (g), based on wet sieving and centrifugation (Gerdemann and Nicolson 1963; Giovannetti et al. 1991) and microscopic examination (Nikon SMZ800). A widely-grown maize variety Zhengdan 958 was used as host plant in this propagation.

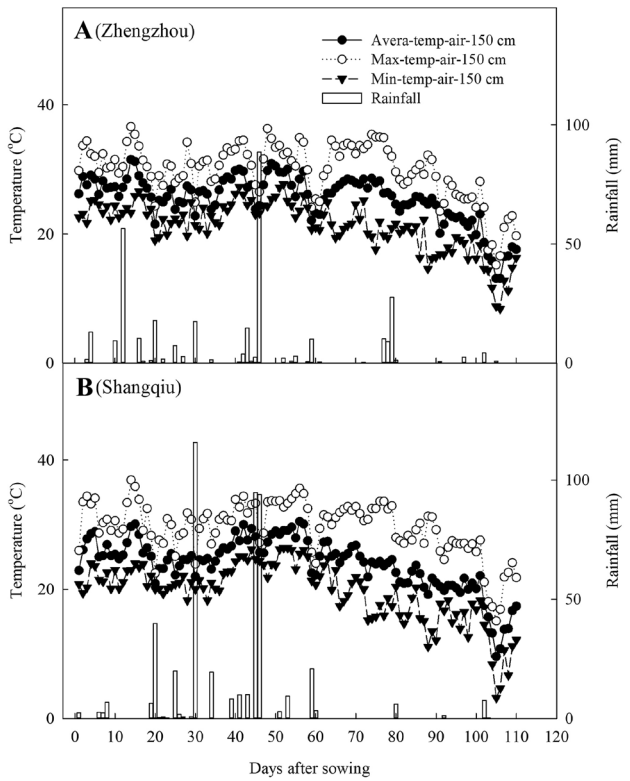

Fig. 1 Average (Avera-temp-air-150 cm), maximum (max-temp-air-150 cm) and minimum (min-temp-air-150 cm) daily temperature measured 150 cm above the soil surface and daily rainfall (Rainfall) in Zhengzhou (**A**) and Shangqiu (**B**) during maize growing season (from June 23 to October 10) in 2020

Experimental design

A split randomized design was used in the both experiments consisting two AMF inoculations treatments, two N fertilizer levels, five sampling times (10, 20, 30, 40 and 50 d after the silking stage), and four replicates (pots) per treatment (totaling 80 pots). The two AMF treatments were without (M-) or with AMF inoculation (M+), with the M- treatment having 100 g of sterilized sand per pot and the M+treatment having 100 g AMF inoculum. The two N fertilizer treatments were 180 kg N hm^{-2} (N1) and 270 kg N hm^{-2} (N2) as urea. An ^{15}N tracer technique was used for the experiment in Zhengzhou to evaluate the impacts of AMF on maize grain N uptake. Each pot was 33 cm in diameter and 22 cm in height and filled with 10 kg of soil. The 10 kg of soil was mixed with 100 g of the AMF inoculum or sterilized sand before maize sowing.

Maize seeds of variety Zhengdan 958 were sown on 23 June 2020 in both Zhengzhou and Shangqiu. Seeds of uniform size were surface-sterilized in a 10% (v/v) solution of H_2O_2 for 10 min and then thoroughly washed with deionized water. Three seeds were sown in each pot, with only one plant was maintained after the five-leaf stage. Soil moisture was maintained at 60–70% of field capacity with periodical watering with deionized water during the maize growth periods. Before maize seed sowing, all phosphate (at 90 kg P_2O_5 hm^{-2}) and potassium (at 120 kg K_2O hm^{-2}) fertilizers mixed with all soils were applied along with half of N fertilizer rate. The remaining half of the N fertilizer rate for the experiment in Shangqiu was dissolved in H_2O and applied to the soil 30 d after seed sowing. In contrast, in Zhengzhou, the remaining half of the N fertilizer rate including the labelled fertilizer $K^{15}NO_3$ (13.9% ^{15}N) was dissolved in H_2O and applied to the soil 30 d after seed sowing. Stable isotope ^{15}N abundance was utilized as a tracer to determine ^{15}N allocation within maize organs during the growth periods. The labelled fertilizer $K^{15}NO_3$ at 0.09 g per plant was applied with plastic syringes on both sides of the plant root after diluting in 30 ml of water (de Oliveira Silva et al. 2017). A total of 48 potted plants were applied with the ^{15}N-labelling technique, and the isotopic concentrations of grains were sampled at 10, 30 and 50 d after the silking stage, while the isotopic concentrations of roots, stems and leaves were sampled at 50 d after the silking stage.

Plant and soil sample collection and determination

At 10, 20, 30, 40 and 50 d after the silking stage, leaf length, width and area and relative content of chlorophyll (Soil–Plant Analysis Development Section, SPAD) of the ear leaf were measured from 10 to 12 am, and leaf area index (LAI) was calculated based on the coefficient 0.75, and the SPAD was analyzed by SPAD-502 (Minolta Camera Co.,

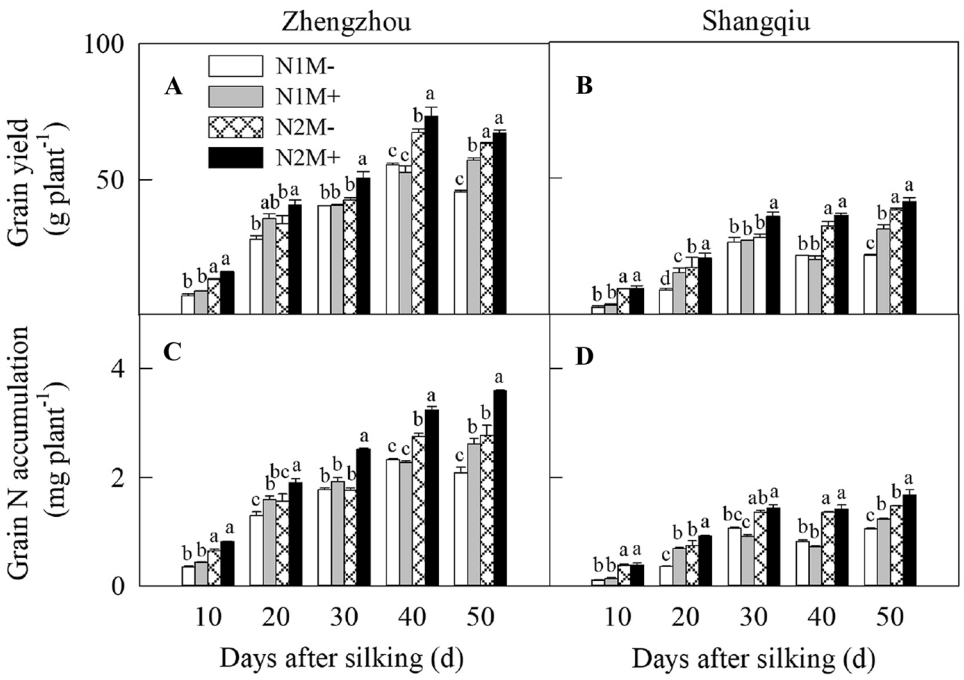

Fig. 2 Effects of nitrogen (N) fertilizer and arbuscular mycorrhizal fungal inoculation (M) on grain yield per plant and grain N accumulation during the grain filling stage in maize variety Zhengdan 958 in two experiments in Zhengzhou (**A** and **C**) and Shangqiu (**B** and **D**). N1 and N2 represent the N fertilization rates of 180 and 270 kg N hm^{-2}. M- represent without arbuscular mycorrhizal fungal inoculation in the pots; and M+ represent with the arbuscular mycorrhizal fungal inoculation in the pots. Values are means ± SE, n = 4. Different letters above the column indicate significant difference in $P < 0.05$ level among treatments

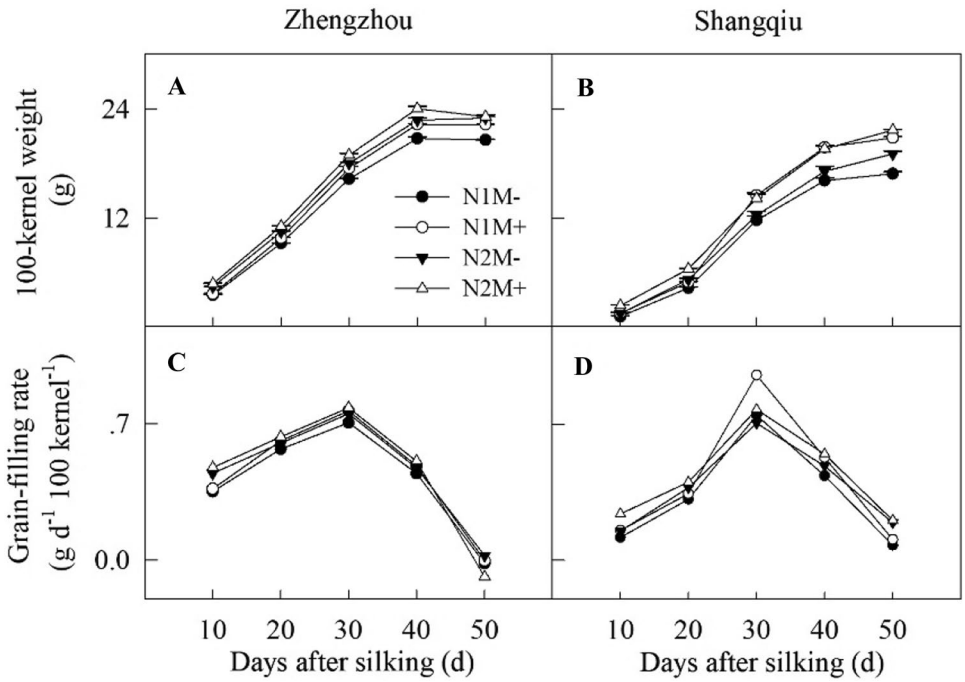

Fig. 3 Effects of nitrogen (N) fertilizer and arbuscular mycorrhizal fungal inoculation (M) on maize 100-kemel weight and grain filling rates during grain filling stages in maize variety Zhengdan 958 in two experiments in Zhengzhou (**A** and **C**) and Shangqiu (**B** and **D**). N1 and N2 represent the N fertilizer rates of 180 and 270 kg N $hm.^{-2}$. M- represent without arbuscular mycorrhizal fungal inoculation in the pots; and M+ represent with the arbuscular mycorrhizal fungal inoculation in the pots. Values are means ± SE, n = 4

Osaka, Japan). Maize plants in each sampling time were also sampled and divided into five parts: grains, roots, stems, leaves, and the other part organs (maize tassel, silk, bract and cob). Plant material was dried in an oven at 105 °C for 30 min and oven-dried at 75 °C to a constant weight to (1) determine their biomass, (2) randomly weigh the 100-kernel weight, and (3) calculate the grain filling rates (Gambín et al. 2006b). Grain filling rate was calculated as the difference of total grain yield per plant between two sampling times (10 d interval). The dried tissues of maize plant were digested and analyzed for their total N content by the flow analyzer (AA3, SEAL-Analytical, Germany). At 10, 30, and 50 d after the silking stage, 10 g fresh grain was taken from the middle part of the ear, pre-cooled in liquid N for 30 s, and stored in a refrigerator at – 80 °C for subsequent measurement of grain enzyme activities and gene expressions related to N metabolism. After cleaning the maize roots with water, root traits (root length, root surface area, root

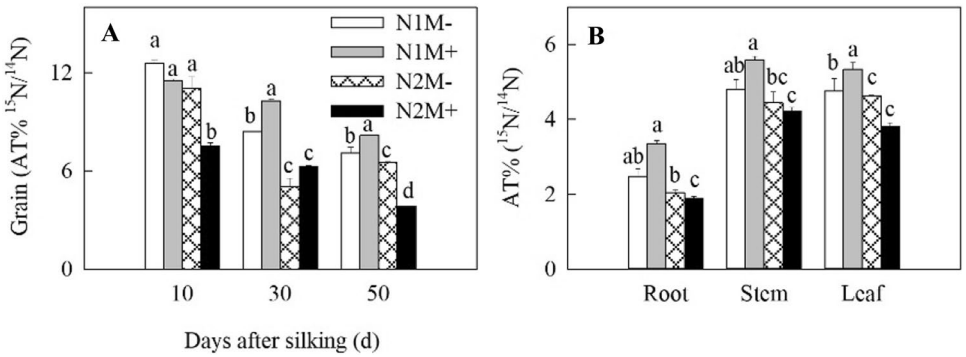

Fig. 4 Effects of nitrogen (N) fertilizer and arbuscular mycorrhizal fungal inoculation (M) on maize grain ^{15}N isotope content (**A**) during grain filling stage, and ^{15}N isotope content in roots, stems and leaves (**B**) at 50 days after silking in maize variety Zhengdan 958 in Zhengzhou. N1 and N2 represent the N fertilizer rates of 180 and 270 kg N hm^{-2}. M- represent without arbuscular mycorrhizal fungal inoculation in the pots; and M + represent with the arbuscular mycorrhizal fungal inoculation in the pots. Values are means ± SE, n = 4. Different letters above the column indicate significant difference in $P < 0.05$ level among treatments

volume and root diameter) at 10 d after the silking stage were scanned with EPSON EXPRESSION 10000XL root scanner (Seiko Epson Corp., Japan) and root images were analyzed with WinRHIZO software (Pro 2013e, Regent Instruments Inc., Canada). The infection rate of mycorrhiza colonization (Figure S2) at 10 d after the silking stage was analyzed using decolorized-acid fuchsin staining method (Koske and Gemma 1989). Briefly, root samples were heated in 2.5% KOH_3 at 90 °C for 30 min and rinsed roots in water, bleached roots in alkaline H_2O_2 for 30 min and rinsed in water. Roots were soaked in 5% HCL for 5 min and stained in acid fuchsin for 30 min at 90 °C, after which they were stored in acidic glycerol. Stained roots were spread on a Petri dish with gridlines to determine AMF colonization by a dissecting microscope at × 40 magnification. Soil spore numbers at 10 and 50 d after the silking stage were measured on the basis of wet sieving and centrifugation method on a sucrose gradient (Gerdemann and Nicolson 1963) and microscopic examination (Nikon SMZ800). Approximately 10 g each soil sample was stirred vigorously with 500 ml tap water in a beaker. The soil suspension was passed through two sieves of 500 µm and 50 µm mesh under a water jet with the suspension of 50 µm sieve stored in distilled water. The spore suspension was centrifuged for 5 min at 9000 rev/min, and a viscosity gradient was created by adding 15 ml of sucrose solution at 60% to each centrifuge tube. The mixture was stirred rapidly and centrifuged for 4 min at 3000 rev/min. The spore-containing supernatant was filtered and rinsed through a 50 µm sieve with distilled water to remove sucrose. Spores were recovered with distilled water in a petri dish, and were quantified using a binocular magnifying glass by estimating the number of spores for each soil sample.

Soil samples in the pots were taken at 10, 30, and 50 d after the silking stage and mixed evenly. Soil inorganic N (NH_4^+ and NO_3^-) was measured by the following method: 10 g of moist soil was extracted with 50 ml 2 mol L^{-1} KCl solution and shaken for 1 h at a speed of 200 r in an oscillator, and the filtered solution was used to determine soil inorganic N content with a flow analyzer (AA3, SEAL-Analytical, Germany).

Sampling and measurement of ^{15}N plant traits for the experiment in Zhengzhou

Maize grain ^{15}N concentrations were sampled and measured at 10, 30, and 50 d after the silking stage, whereas the ^{15}N concentration of maize roots, stems and leaves was sampled and measured at 50 d after the silking stage: All samples were dried at 75 ℃ to a constant weight and ground to pass through a 0.15 mm sieve for laboratory analyses. Using gas chromatography-combustion-isotope ratio mass spectrometry (Delta V Advantage stable isotope mass spectrometer), the combustion products were dehydrated and flowed into a chromatographic column for separation. After magnetic separation and continuous automatic injection, $^{15}N/^{14}N$ isotope ratios were measured and read. The ^{15}N concentrations of maize samples were measured at the Yangling Xinhua Ecological Technology Co., Ltd, China.

Determination of N metabolism-related enzyme activities

The activities of N metabolizing enzymes, including nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) and glutamate synthase (GOGAT), were measured in the fresh grains of maize and determined using the kits NR-2-W, NIR-2-G, GS-2-Y and GOGAT-2-Y, respectively (Rizwan et al. 2019), purchased from Comin Biotechnology Co. Ltd, China (http://www.cominbio.com). Approximately 0.1 g fresh grains were added to 1 mL of extract and homogenized in an ice bath. These were centrifuged at 8000 g for 10 min at 4 °C

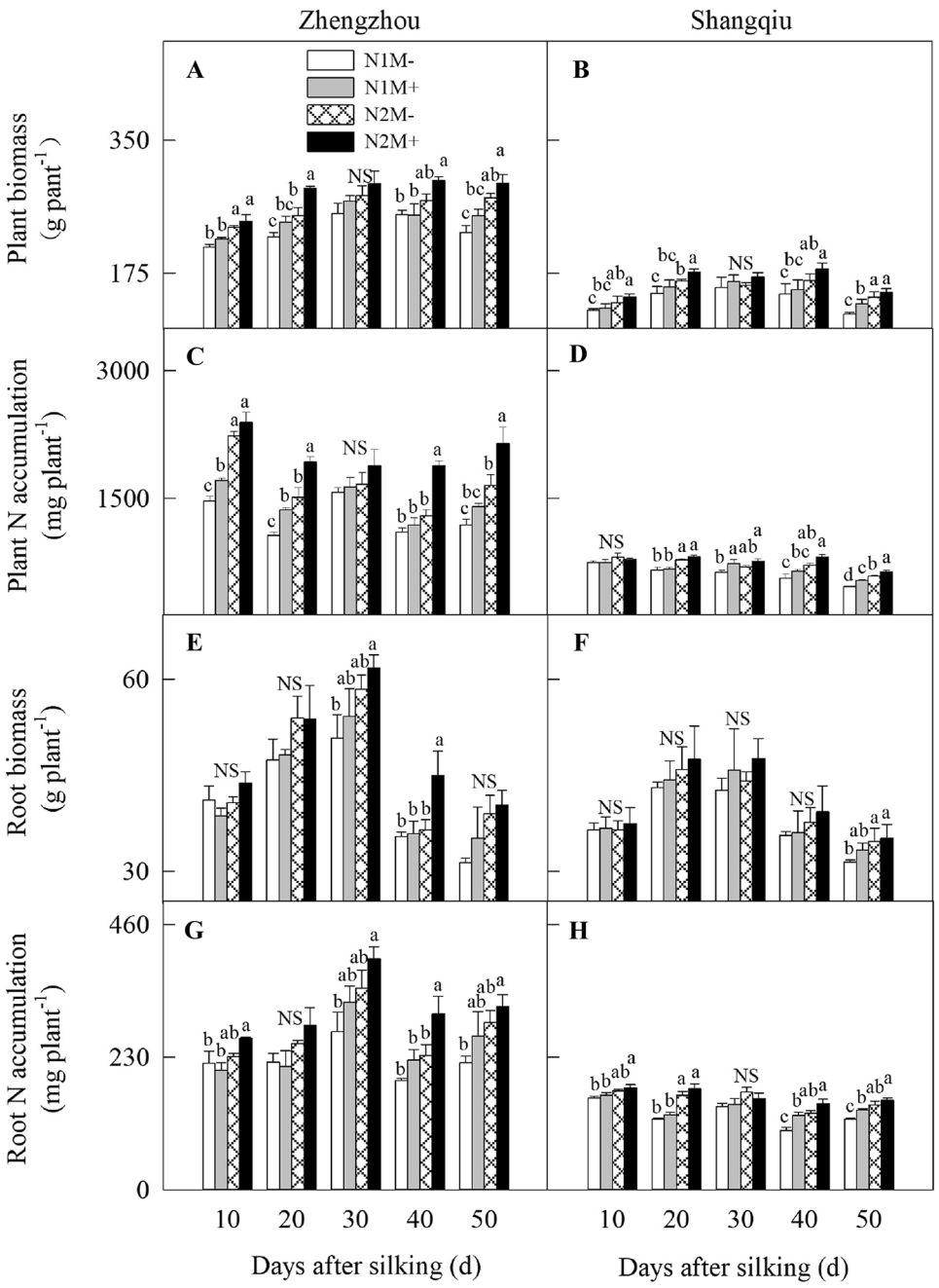

Fig. 5 Effects of nitrogen (N) fertilizer and arbuscular mycorrhizal fungal inoculation (M) on plant biomass, plant N accumulation, root biomass, root N accumulation in maize variety Zhengdan 958 in two experiments in Zhengzhou (**A**, **C**, **E**, **G**) and Shangqiu (**B**, **D**, **F**, **H**). N1 and N2 represent the N fertilizer rates of 180 and 270 kg N hm^{-2}. M- represent without arbuscular mycorrhizal fungal inoculation in the pots; and M + represent with the arbuscular mycorrhizal fungal inoculation in the pots. Values are means ± SE, n = 4. Different letters above the column indicate significant difference in $P < 0.05$ level among treatments

to obtain crude enzymes and placed on ice for testing. Activities of NR, NIR, and GS were measured by visible spectrophotometer, whereas GOGAT enzyme activity was measured by UV spectrophotometer. Activity of NR was defined as the amount of 1 μmol NADH catalyzed per gram tissue protein per hour. Activity of NIR was defined as the amount of reduction of 1 μmol NO_2^- per gram of tissue per hour. Activity of GS was defined as the production of 1 μmol γ-glutamyl hydroxamic acid per hour per mL of reaction system per gram of grain. Finally, activity of GOGAT was defined as the consumption of 1 μmol NADH per gram tissue per hour.

Total RNA extraction, cDNA synthesis, and quantitative real-time polymerase chain reaction (qRT-PCR) analysis for gene sequences of N metabolism-related enzymes

Total RNA from grains of maize was extracted using RNA kit (Tap SYBR©Premix Ex TaqTM II, Biotech, Beijing) with manufacturer specifications. To remove genomic DNA contamination, 10 μg of total RNA were digested with RNase-free DNaseI (Promega, Madison, WI). RNA concentration was measured with a NanoDrop ND-2000 UV

Table 1 Effects of nitrogen fertilizer (N) and arbuscular mycorrhizal fungal inoculation (M) on AMF root colonization, soil spore numbers, soil NO_3^--N, NH_4^+-N and inorganic N (INN) concentration in maize variety Zhengdan 958 in two experiments in Zhengzhou (ZZ) and Shangqiu (SQ)

	AMF root colonization (%)		Soil spore numbers (50 soil g^{-1})		NO_3^- -N (mg kg^{-1})		NH_4^+-N (mg kg^{-1})		INN (mg kg^{-1})	
	ZZ	SQ	ZZ	SQ	ZZ	SQ	ZZ	SQ	ZZ	SQ
N1M–	12.8 ± 4.3b	12.7 ± 0.2c	10.3 ± 0.3c	4.5 ± 0.6c	13.2 ± 0.1bc	11.0 ± 0.2bc	3.35 ± 0.03b	3.3 ± 0.03b	16.6 ± 0.2bc	14.3 ± 0.2bc
N1M+	81.9 ± 2.5a	79.6 ± 0.3a	41.9 ± 1.1a	48.1 ± 0.6a	12.7 ± 0.3c	10.7 ± 0.2c	3.20 ± 0.03c	3.2 ± 0.03c	16.0 ± 0.3c	14.0 ± 0.2c
N2M–	13.8 ± 1.8b	16.3 ± 0.2b	9.5 ± 0.4c	4.6 ± 0.3c	14.1 ± 0.3a	11.8 ± 0.3a	3.50 ± 0.03a	3.4 ± 0.02a	17.6 ± 0.3a	15.2 ± 0.3a
N2M+	82.3 ± 2.7a	80.1 ± 0.2a	38.4 ± 0.9b	46.1 ± 0.4a	13.6 ± 0.3ab	11.5 ± 0.2ab	3.33 ± 0.04b	3.3 ± 0.02ab	16.9 ± 0.3ab	14.9 ± 0.2ab
N	0.05	83.8***	8.1*	3.7	12.4**	12.6**	17.6***	14.7**	16.6**	16.1**
M	542.5***	84,067.1***	1649.7***	7520.5***	4.7	1.2	23.7***	9.1*	7.8*	2.1
N × M	0.02	44.3***	3.4	4.7	0.03	0.01	0.02	0.02	0.03	0.01

N, M, and N × M represent the N fertilizer effect, mycorrhizae effect, and their interaction effect, respectively. N1 and N2 represent the N fertilizer rates of 180 and 270 kg N hm^{-2}. M- represent without arbuscular mycorrhizal fungal inoculation in the pots; and M + represent with the arbuscular mycorrhizal fungal inoculation in the pots. Values are means ± SE, n = 4. *, **, and *** indicate the significant difference at the 0.05, 0.01 and 0.001 levels, respectively. Different letters after the data in the same column indicate significant difference in $P < 0.05$ level among treatments

spectrophotometer (Thermo Scientific, USA) after DNaseI treatment. All cDNA samples were diluted tenfold with sterile water to obtain qRT-PCR. Sequences of the genes encoding N metabolic enzymes were obtained through GenBank (www.maizegdb.org/ and www.nebi.nlm.nih.gov/). Primers of individual genes were applied (Table S1). The qRT-PCR was accomplished by denaturing the DNA at 95 °C for 30 s, followed by 40 cycles of 95 °C (5 s) and 60 °C (40 s). The relative expression of transcription levels for target genes in each treatment was were calculated using Equation $2^{-\Delta\Delta CT}$ (Livak and Schmittgen 2001; Guo et al. 2014) with the maize *ACTIN* (GenBank Accession No. XM_008656735.3).

Statistical analysis

Effects of sampling times, N fertilizer rates, and AMF inoculation treatments on maize grain yield, grain N accumulation, plant biomass, plant N accumulation were tested using repeated measures analysis of variance (ANOVA) in a general linear model, with sampling time, N fertilizer rate and AMF treatments used as the main effects. General linear model analysis of variance (GLM-ANOVA) was used to examine the effects of N fertilizer rates and AMF treatments on grain yield, grain N accumulation, plant biomass and plant N accumulation, N metabolizing enzymes activities and their gene expression, and least significant difference (LSD) multiple range tests were used to separate treatment means. For the four N and AMF combination treatments, one-way ANOVA was used to examine and compare their difference on grain N accumulation, plant biomass, plant N accumulation, ^{15}N concentration of plant samples, N metabolizing enzymes activities and their gene expression, respectively. And means were compared using Tukey's honestly significant difference test, and plotting with Sigmaplot 12.5. The difference of root colonization and soil spore number between Zhengzhou and Shangqiu were compared by paired t-test. Linear regressions of LAI, SPAD, soil inorganic N, NR, NiR, GS, GOGAT enzyme activities, and NR, NiR, GS, GOGAT gene expressions on grain yield per plant were analyzed. All statistical analyses were performed using the software program SPSS, ver. 10.0 (SPSS Inc., Chicago, IL, USA).

Results

Maize grain yield, grain N accumulation, 100-kernel weight, grain filling rates and grain ^{15}N content

In both experiments in Zhengzhou and Shangqiu, maize grain yield and N uptake varied significantly with sampling time, N fertilizer, and AMF inoculation (Fig. 2, Tables S2 and S3), with yield per plant increasing during the grain filling stage (Fig. 2). Compared with Shangqiu, yield and uptake in Zhengzhou increased by 89% and 107%, and by 33% and 13% for 100-kernel weight and grain filling rates, respectively (Figs. 2, 3 and S3). In the N1 treatment, M + (AMF inoculation) increased grain yield and grain N accumulation relative to M- by 10% and 13%, respectively, in Zhengzhou, and by 19% and 10%, respectively, in Shangqiu. For the N2 treatment, these increases were14% and 25% in Zhengzhou, and by 17% and 13% in Shangqiu (Fig. 2). The AMF inoculation also increased 100-kernel weight and grain filling rate with N1 and N2 input in both Zhengzhou

Table 2 Effect of nitrogen fertilizer (N) and arbuscular mycorrhizal fungal inoculation (M) on maize root traits in maize variety Zhengdan 958 in two experiments in Zhengzhou (ZZ) and Shangqiu (SQ)

	Root length (cm plant^{-1})		Root surface area (cm^2 plant^{-1})		Root volume (cm^3 plant^{-1})		Root diameter (mm plant^{-1})		Root tips (plant^{-1})	
	ZZ	SQ	ZZ	SQ	ZZ	SQ	ZZ	SQ	ZZ	SQ
N1M-	43,368 ± 147c	31,815 ± 22d	7947 ± 17d	6048 ± 89c	103.7 ± 0.7c	86.8 ± 0.2d	0.60 ± 0.10b	0.60 ± 0.01bc	137,934 ± 265d	90,877 ± 267c
N1M +	44,286 ± 174b	35,438 ± 58a	8629 ± 29c	6763 ± 391a	102.9 ± 1.0c	95.3 ± 0.3b	0.61 ± 0.10b	0.59 ± 0.01c	142,448 ± 605c	114,052 ± 1664a
N2M-	43,627 ± 206c	32,423 ± 106c	9386 ± 118b	5994 ± 165d	116.6 ± 1.1b	92.1 ± 0.1c	0.61 ± 0.10b	0.61 ± 0.01b	168,224 ± 345b	95,808 ± 349b
N2M +	47,561 ± 168a	33,666 ± 63b	10,146 ± 201a	6295 ± 135b	123.5 ± 0.4a	100.8 ± 0.3a	0.65 ± 0.01a	0.64 ± 0.01a	170,889 ± 399a	96,674 ± 280b
N	101.3***	70.4***	157.1***	940.5***	404.9***	569.3***	47.1***	48.4***	4820.3***	50.9***
M	190.9***	1230.6***	37.4***	3562.9***	13.8**	1445.1***	37.7***	6.4*	72.1***	190.1***
N × M	73.8***	294.3***	0.1	592.8***	21.3**	0.5	15.8**	19.6**	4.8*	163.6***

N, M, and N × M represent the N fertilizer effect, mycorrhizae effect, and their interaction effect, respectively. N1 and N2 represent the N fertilizer rates of 180 and 270 kg N hm^{-2}. M- represent without arbuscular mycorrhizal fungal inoculation in the pots; and M + represent with the arbuscular mycorrhizal fungal inoculation in the pots. Values are means ± SE, n = 4. The *, **, and *** indicate the significant difference at the 0.05, 0.01 and 0.001 levels, respectively. Different letters after the data in the same column indicate significant difference in $P < 0.05$ level among treatments

and Shangqiu (Fig. 3), and increased the maize grain ^{15}N, leaf ^{15}N, stem ^{15}N and root ^{15}N concentration with N1 input in Zhengzhou (Fig. 4, Tables S2 and S4).

Plant biomass and their N accumulation, root biomass and their N accumulation, root traits and soil inorganic N

Compared with Shangqiu, plant biomass and plant N accumulation, root biomass, and root N accumulation in Zhengzhou increased by 33%, 67%, 12%, and 78%, respectively (Figs. 5 and S3, Table S5), whereas maize root colonization and soil spore numbers did not vary significantly between sites (Table 1). Compared with M-, M + increased plant biomass, plant N accumulation, root biomass, and root N accumulation with both N treatments and at both sites (Fig. 5), and showed a similar pattern for root length, root surface area, root tips, root colonization, and soil spore numbers (Tables 1 and 2, Figure S2), while reducing soil inorganic N concentration (Table 1).

Lear area index, ear leaf SPAD, grain N metabolism-related enzymes activities and their gene expression

Compared with Shangqiu, LAI, SPAD, grain NR, GOGAT enzymes activities and *GOGAT* gene expression in Zhengzhou increased by 133%, 10%, 129%, 191% and 101%, respectively, while decreased by 10% and 17% for *NR* and *NiR* gene expressions (Figs. 6, 7 and S4, Tables 3 and 4). The AMF inoculation significantly increased maize LAI, SPAD with N1 and N2 input in both Zhengzhou and Shangqiu (Figure S4), and increased the enzymes activities of NR, NIR, GS and GOGAT by 423%, 23%, 25% and 435% in Zhengzhou, and 85%, 26%, 25% and 37% in Shangqiu with N1 input, and by 341%, 49%, 32% and 133% in Zhengzhou, and 109%, 16%, 36% and 48% in Shangqiu with N2 input, respectively (Fig. 6 and Table 3). The AMF inoculation also increased the relative gene expressions of *NR*, *NIR*, *GS* and *GOGAT* by 66%, 66%, 30% and 7% in Zhengzhou, and 69%, 61%, 22% and 30% in Shangqiu with N1 input, and by 45%, 35%, 12% and 40% in Zhengzhou, and 30%, 10%, 45% and 12% in Shangqiu with N2 input, respectively (Fig. 7 and Table 4). Maize grain yield was significantly and positively correlated with maize ear leaf SPAD, LAI, the grain enzyme activities of NR, NIR, GS and GOGAT, and the relative expression *of GOGAT* gene (Fig. 8).

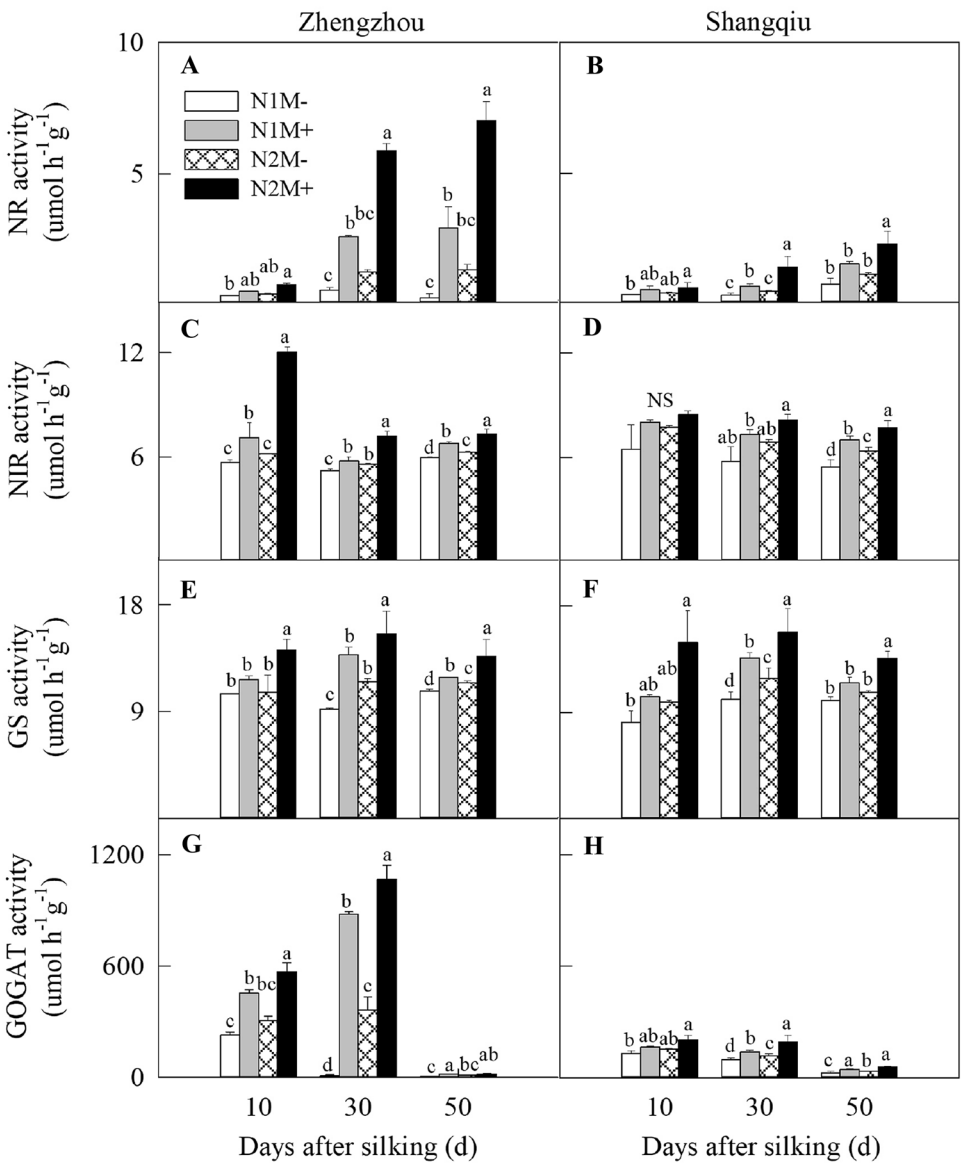

Fig. 6 Effects of nitrogen (N) fertilizer and arbuscular mycorrhizal fungal inoculation (M) on maize grain nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) and glutamate synthase (GOGAT) enzyme activities during grain filling stage in maize variety Zhengdan 958 in two experiments in Zhengzhou (**A**, **C**, **E**, **G**) and Shangqiu (**B**, **D**, **F**, **H**). N1 and N2 represent the N fertilizer rates of 180 and 270 kg N hm^{-2}. M- represent without arbuscular mycorrhizal fungal inoculation in the pots; and M + represent with the arbuscular mycorrhizal fungal inoculation in the pots. Values are means ± SE, n = 4. Different letters above the column indicate significant difference in $P < 0.05$ level among treatments

Discussion

The two pot experiments in Zhengzhou and Shangqiu showed that AMF inoculation could increase maize grain yield and their N uptake during grain filling stages through modifying maize root traits and improving enzyme activities associated with grain N metabolism and their gene expression. This even occurred under high inputs of N independent of nutritional status of the soil. Studies have shown that increase in grain yield and N uptake in maize was associated with high AMF root colonization (Garcia-Gonzalez et al. 2018; Zhang et al. 2022). Using different optical sensing tools, Varinderpal-Singh et al. (2021) found that the increase in AMF root infection rate can significantly improve N absorption and utilization efficiency of maize root and increase maize yield. AMF extraradical mycelial networks increase host plant root absorption area and soil contact points, thereby enhancing the absorption and utilization of inorganic N (Hawkins et al. 2000; Püschel et al. 2017). AMF inoculation can also promote plant N uptake by improving root traits (Hodge and Storer 2015). Previous researchers found that single or dual inoculation of AMF markedly increased root length, root projected area, root surface area, and root volume (Liu et al. 2022; Shen et al. 2022), which stimulated N uptake and plant biomass. The results of our study also showed that the presence of AMF under different N status in the two soils significantly improved root traits, increased root colonization, and maize N uptake.

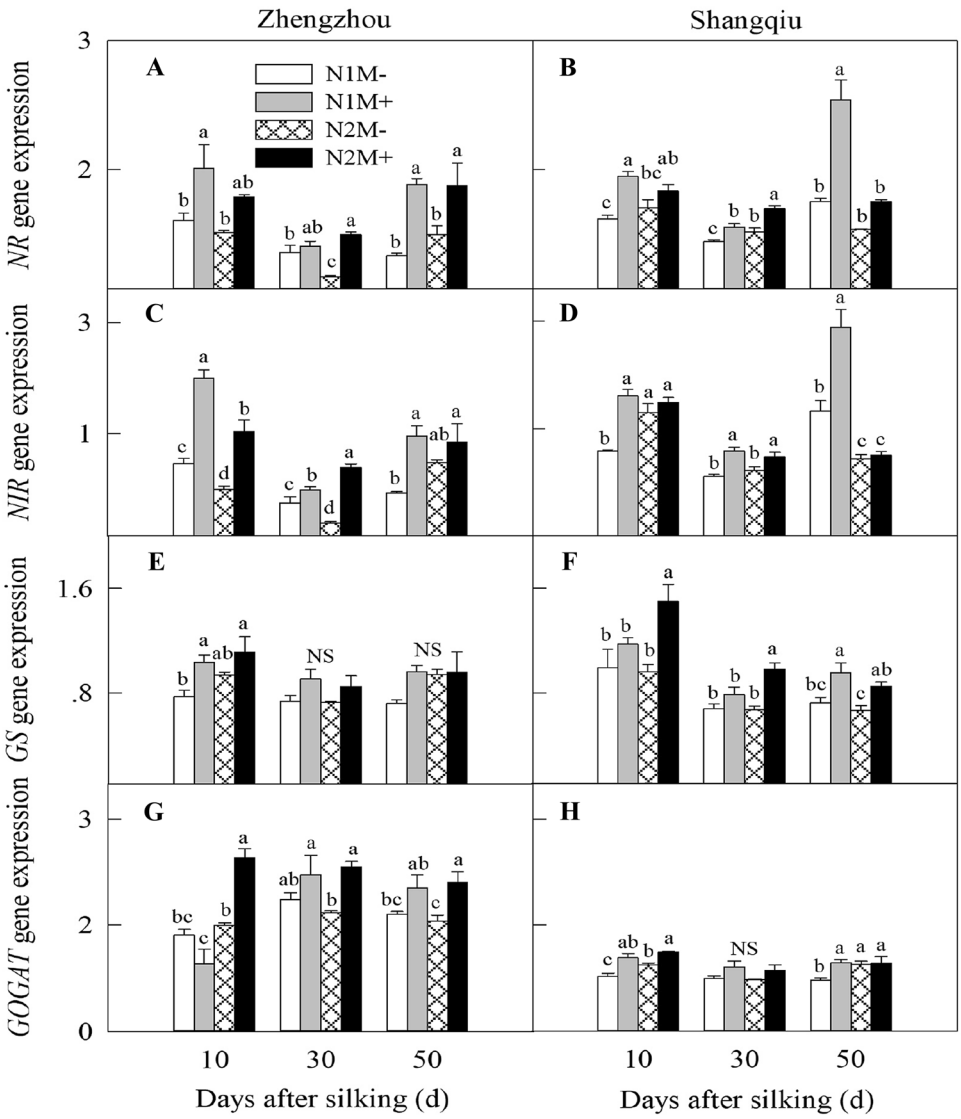

Fig. 7 Effects of nitrogen (N) fertilizer and arbuscular mycorrhizal fungal inoculation (M) on the relative expression of grain *NR, NiR, GS, and GOGAT* gene of transcript levels during grain filling stage in maize variety Zhengdan 958 in two experiments in Zhengzhou (**A**, **C**, **E**, **G**) and Shangqiu (**B**, **D**, **F**, **H**). N1 and N2 represent the N fertilizer rates of 180 and 270 kg N hm^{-2}. M- represent without arbuscular mycorrhizal fungal inoculation in the pots; and M + represent with the arbuscular mycorrhizal fungal inoculation in the pots. Values are means ± SE, n = 4. Different letters above the column indicate significant difference in $P < 0.05$ level among treatments

Table 3 Effects of nitrogen (N) fertilizer and arbuscular mycorrhizal fungal inoculation (M) on maize grain N nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) and glutamate synthase (GOGAT) enzyme activities in maize variety Zhengdan 958 in two experiments in Zhengzhou (ZZ) and Shangqiu (SQ)

Treatments	NR (umol h^{-1} g^{-1})		NiR (umol h^{-1} g^{-1})		GS (umol h^{-1} g^{-1})		GOGAT (umol h^{-1} g^{-1})	
	ZZ	SQ	ZZ	SQ	ZZ	SQ	ZZ	SQ
N1M−	0.42 ± 0.05c	0.51 ± 0.03d	5.61 ± 0.11c	5.89 ± 0.42c	9.98 ± 0.11c	9.44 ± 0.31c	82.22 ± 4.56d	83.75 ± 5.78c
N1M+	2.20 ± 0.36b	0.94 ± 0.03b	6.92 ± 0.22b	7.44 ± 0.10ab	12.47 ± 0.31b	11.84 ± 0.18b	440.02 ± 20.37b	114.71 ± 3.40b
N2M−	1.05 ± 0.06c	0.71 ± 0.01c	6.04 ± 0.03c	7.00 ± 0.07b	11.38 ± 0.08bc	10.84 ± 0.20bc	238.01 ± 15.05c	101.52 ± 2.24bc
N2M+	4.65 ± 0.27a	1.47 ± 0.08a	9.00 ± 0.16a	8.09 ± 0.16a	15.00 ± 0.90a	14.77 ± 0.84a	554.66 ± 31.01a	150.64 ± 10.91a
N	45.50***	70.36***	69.72***	14.27**	16.67**	21.45***	45.04***	17.06**
M	138.23***	192.75***	201.32***	32.43***	40.29***	45.84***	280.15***	37.94***
N × M	15.73**	15.02**	30.08***	1.01	1.73	2.71	1.04	1.95

N, M, and N × M represent the N fertilizer effect, mycorrhizae effect, and their interaction effect, respectively. N1 and N2 represent the N fertilizer rates of 180 and 270 kg N hm^{-2}. M- represent without arbuscular mycorrhizal fungal inoculation in the pots; and M + represent with the arbuscular mycorrhizal fungal inoculation in the pots. Values are means ± SE, n = 4. The *, **, and *** indicate the significant difference at the 0.05, 0.01 and 0.001 levels, respectively. Different letters after the data in the same column indicate significant difference in $P < 0.05$ level among treatments

Table 4 Effects of nitrogen (N) fertilizer and arbuscular mycorrhizal fungal inoculation (M) on the relative expression of grain *NR*, *NiR*, *GS* and *GOGAT* gene of transcript levels in maize variety Zhengdan 958 in two experiments in Zhengzhou (ZZ) and Shangqiu (SQ)

Treatment	*NR*		*NiR*		*GS*		*GOGAT*	
	ZZ	SQ	ZZ	SQ	ZZ	SQ	ZZ	SQ
N1M−	0.71 ± 0.08a	0.9 ± 0.02c	0.78 ± 0.09a	1.09 ± 0.04b	0.75 ± 0.02b	0.80 ± 0.06c	1.62 ± 0.06b	0.75 ± 0.02c
N1M+	1.18 ± 0.10a	1.52 ± 0.10a	1.29 ± 0.07a	1.75 ± 0.08a	0.97 ± 0.03a	0.97 ± 0.03b	1.73 ± 0.16b	0.97 ± 0.03a
N2M−	0.86 ± 0.22a	0.87 ± 0.03c	0.93 ± 0.24a	1.08 ± 0.04b	0.87 ± 0.02a	0.77 ± 0.01c	1.58 ± 0.03b	0.87 ± 0.01b
N2M+	1.24 ± 0.25a	1.14 ± 0.04b	1.25 ± 0.19a	1.19 ± 0.01b	0.97 ± 0.06a	1.11 ± 0.05a	2.21 ± 0.10a	0.97 ± 0.04a
N	0.32	13.76**	0.10	34.76***	2.84	1.80	4.82*	6.23*
M	5.71*	64.09***	6.66*	63.44***	18.73**	38.85***	13.64**	41.09***
N × M	0.06	10.31**	0.36	32.96***	2.41	3.99	7.13*	5.29*

N, M, and N × M represent the N fertilizer effect, mycorrhizae effect, and their interaction effect, respectively. N1 and N2 represent the N fertilizer rates of 180 and 270 kg N hm^{-2}. M- represent without arbuscular mycorrhizal fungal inoculation in the pots; and M + represent with the arbuscular mycorrhizal fungal inoculation in the pots. Values are means ± SE, n = 4. The *, **, and *** indicate the significant difference at the 0.05, 0.01 and 0.001 levels, respectively. Different letters after the data in the same column indicate significant difference in $P < 0.05$ level among treatments

Leaf area index is an important biophysical factor that explains differences in canopy phenology, assimilation, and biomass production, and is a key variable used to evaluate the exchanges of mass and energy (Schmitt et al. 2010; Restrepo-Coupe et al. 2013; Yang et al. 2022). Studies have found that AMF can significantly improve plant leaf area coefficient, SPAD, and the chlorophyll content of leaves with the increasing LAI (Bidinger et al. 1977). Increased chlorophyll content can affect the phosphorylation process of adenosine triphosphate (ATP) enzyme and delay leaf senescence, thereby improving plant photosynthetic performance, promoting the production of photosynthetic products and carbon filling into seeds, and increasing grain filling rate and grain weight (Masclaux-Daubresse et al. 2010; Kant et al. 2011). Some studies have shown that inoculation with AMF significantly increased leaf area index and yield of sweet pepper (*Capsicum annuum* L.) under greenhouse conditions (Ombodi et al. 2019), and leaf area index and green pod yield of garden pea (*Pisum sativum* L.) under different irrigation conditions and phosphorus fertilizer levels (Yadav et al. 2015). In this study, AMF inoculation increased maize leaf area index and ear leaf SPAD under different N status in the two soils, indicating that AMF could improve leaf physiological characters and increase plant biomass production.

AMF is coupled with the transfer and metabolism of N in the symbiosis (Fellbaum et al. 2012; Yang et al. 2018), and significantly increases the activities of NR, NIR, GS, and GOGAT in crop leaves and grains, improving grain yield and N content (Liu et al. 2022; Zhang et al. 2022). On one hand, the increase of NR and NIR activities in grains promoted the conversion of NO_3^--N to NH_4^+-N, decreased NO_3^--N in grains and increased NH_4^+-N content (Masclaux-Daubresse et al. 2010; Wang 2021). Work has shown that the amino acids formed by NH_4^+-N are mainly assimilated through the GS/GOGAT cycle (Oliveira et al. 2001; Goel and Singh 2015), and the increase of GS and GOGAT enzyme activities promotes the synthesis of glutamate in grains and improves the accumulation of proteins in grains (Wang 2021). On the other hand, the increase in the activities of NR and GS in grain can also promote the decomposition of proteins in plant leaves into amino acids, which in turn promotes the transfer of amino acids into grains (Canas et al. 2010; Kant et al. 2011), and synthesis of proteins in grain by NR, NIR, GS and GOGAT. Increased protein content in grains, however, can significantly increase grain N content, N accumulation and yield (Masclaux-Daubresse et al. 2010). The present study found that AMF significantly increased the activities of NR, NIR, GS, and GOGAT in maize grains during grain filling stages, and the activities of these four enzymes and *GOGAT* gene expression were positively correlated with grain yield (Fig. 8), indicating that the mechanism by which AMF increases grain N uptake and yield is mainly through increasing grain N metabolism enzyme activities and their gene expression via transcription.

Previous studies have found that the contributions of AM fungi to host plants production was more efficient under the conditions of nutrient-poor soils (Shi et al. 2023). In our study, however, AMF inoculation increased maize grain yield in both poor (Shangqiu) and rich (Zhengzhou) nutrient soil, with increased efficiency even higher in poor soil (Fig. 2), which expands the application of AMF in current modern agriculture production. The interaction between AMF and N fertilizer rate could affect the growth and development of crops, especially in improving nutrient uptake and increasing yield depending on the soil nutritional status (Yang et al. 2018; Varinderpal-Singh et al. 2020). Previous studies have reported that N fertilizer had significant effects on the genes/enzymes related to N metabolism in wheat (He et al. 2022) and on the expression of many critical genes

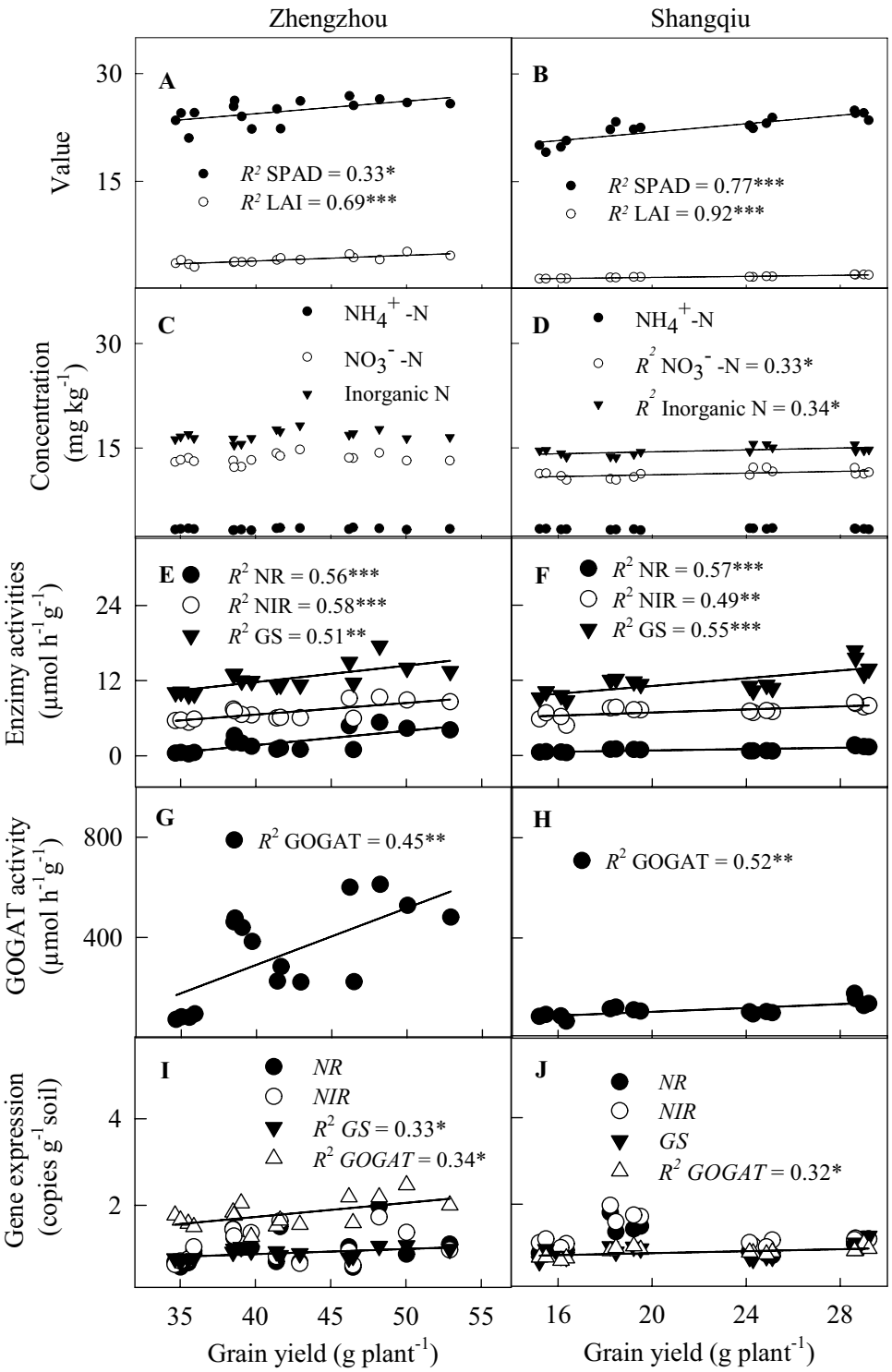

Fig. 8 Linear regressions of grain yield per plant with maize leaf area index (LAI) and SPAD (**A** and **B**), soil NH_4^+-N, NO_3^--N and inorganic N (INN) (**C** and **D**), grain NR, NiR, GS and GOGAT enzyme activities (**E**, **F**, **G** and **H**), and the relative expressions of *NR, NiR, GS, GOGAT* gene of transcript levels on grain yield per plant (**I** and **J**) in Zhengzhou and Shangqiu, respectively. The significance levels are labelled as: $*P<0.05$; $**P<0.01$; $***P<0.001$, respectively

in N metabolism for the developing endosperm of maize grain (Ning et al. 2023). AMF has a more prominent effect on crop growth and their N uptake under low-N conditions (Mäder et al. 2011; Zhu et al. 2018). We found that higher N input increased maize grain yield, grain N metabolism enzyme activities, and their gene expressions (Tables 3 and 4). The presence of AMF increased maize grain yield and N accumulation even with high N input independent of soil nutrient status, suggesting that the role of AMF in maize grain N uptake during filling stages in N-rich agroecosystems is underestimated. Some studies suggest that high-N will inhibit the symbiotic relationship between AMF and crops, resulting in a decrease in root colonization, N uptake and yield reduction (Bakhshandeh et al. 2017; Gezahagn

et al. 2019), whereas others have shown that under high-N conditions, AMF also can form a mycorrhizal network structure with the root of the host plant, and significantly improve the absorption of nutrients such as N and phosphorus by plants (Liu et al. 2021). Paungfoo-Lonhienne et al. (2015) found that high-N can reduce the antagonism between nutrients, promote the absorption of nutrients in the soil by AMF, and improve the crop utilization efficiency of nutrients such as N. Our results demonstrate that plant roots and their symbiotic mycorrhizal fungi may interact with a wide range of other grain N metabolism enzymes to modify grain N transformation and redistribution during the grain filling stage. All of these findings by pot experiments suggest that AMF play a critical role in mediating maize grain yield and grain N metabolism during grain filling periods under the contrasting soil status. These results should be followed up with field experiments to examine true effect sizes. Future field trials should be conducted to estimate the effects of AMF on maize grain yield under the conditions of higher N fertilizer input for current modern agriculture, and to reveal their role for managing crop production in the future.

Conclusion

Our results showed that AMF inoculation effectively promoted maize grain yield and N accumulation during the grain filling stage by modifying maize root traits and regulating grain N enzyme activities of NR, NIR, GS and GOGAT, and the relative expression *of GOGAT* gene in soils of contrasting nutritional status. The effect of AMF on grain yield was associated with the alterations in the grain N metabolism enzyme and the *GOGAT* gene expression, indicating that AMF could regulate maize grain N transformation and redistribution during the grain filling period. Maize grain yield and N accumulation were significantly higher in Zhengzhou than those in Shangqiu even with same N fertilizer input, AMF inoculation increased maize grain yield with either lower (180 kg N hm^{-2}) or higher N input (270 kg N hm^{-2}) independent of soil nutritional status. Our findings suggest that AMF may play a more significant role in mediating maize kernel weight and N uptake in both N-poor and N-rich agroecosystems than previously appreciated. Future investigations should examine the effects of AMF on maize grain yield under field conditions to reveal their role for managing crop production under current agricultural production scenarios with higher N fertilizer input.

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Author contributions Conceptualization, XZ; data collection and analysis, MT and CF; writing—original draft, MT, CF, and XZ; meteorological data FZ; writing—review & editing, FSG, BG, YC, and XZ; experimental design, project management and manuscript preparation, XZ, HZ, TL, QY. All authors have read and agreed to the published version of the manuscript.

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Data availability All of the data supporting the findings of this study are included in this article.

Declarations

Conflict of interest The authors declare no conflict of interest.

Experiments involving human and/or animals participants Not applicable.

Informed consent Not applicable.

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