Nitrate Transporter Gene Expression and Kinetics of Nitrate Uptake by *Populus × canadensis* ‘Neva’ in Relation to Arbuscular Mycorrhizal Fungi and Nitrogen Availability

Fei Wu1,2,3†, Fengru Fang2†, Na Wu4, Li Li2 and Ming Tang1*

1 State Key Laboratory of Conservation and Utilization of Subtropical Agro-bioresources, Guangdong Laboratory of Lingnan Modern Agriculture, Guangdong Key Laboratory for Innovative Development and Utilization of Forest Plant Germplasm, College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou, China, 2 College of Forestry, Northwest A&F University, Yangling, China, 3 Key Laboratory of State Forestry and Grassland Administration on Forest Ecosystem Protection and Restoration of Poyang Lake Watershed, College of Forestry, Jiangxi Agricultural University, Nanchang, China, 4 School of Life Science, Shanxi Datong University, Datong, China

Plants and other organisms in the ecosystem compete for the limited nitrogen (N) in the soil. Formation of a symbiotic relationship with arbuscular mycorrhizal fungi (AMF) may influence plant competitiveness for N. However, the effects of AMF on plant nitrate (NO$_3^-$) uptake capacity remain unknown. In this study, a pot experiment was conducted to investigate the effects of N application and *Rhizophagus irregularis* inoculation on the root absorbing area, uptake kinetics of NO$_3^-$, and the expression of NO$_3^-$ transporter (*NRT*) genes in *Populus × canadensis* ‘Neva’. The results showed that *R. irregularis* colonized more than 70% of the roots of the poplar and increased root active absorbing area/total absorbing area. The uptake kinetics of NO$_3^-$ by poplar fitted the Michaelis–Menten equation. Mycorrhizal plants had a higher maximum uptake rate ($V_{\text{max}}$) value than non-mycorrhizal plants, indicating that *R. irregularis* enhanced the NO$_3^-$ uptake capacity of poplar. The expression of *NRTs* in roots, namely, *NRT1;2, NRT2;4B, NRT2;4C, NRT3;1A, NRT3;1B, and NRT3;1C*, was decreased by *R. irregularis* under conditions of 0 and 1 mM NH$_4$NO$_3$. This study demonstrated that the improved NO$_3^-$ uptake capacity by *R. irregularis* was not achieved by up-regulating the expression of *NRTs* in roots. The mycorrhizal pathway might repress root direct pathway in the NO$_3^-$ uptake by mycorrhizal plants.

**Keywords:** arbuscular mycorrhiza, poplar, nitrate transporter gene, nitrate uptake kinetics, nitrogen fertilization

**INTRODUCTION**

Nitrogen (N) is an essential component of nucleic acids, proteins, and many other critical biomolecules of living organisms (Castro-Rodriguez et al., 2017). Limited N in terrestrial ecosystems generally causes strong competition between different players, such as plants and/or soil microorganisms (Geisseler et al., 2010). Microorganisms are considered to be more competitive than plants in regard to N uptake due to their unique properties, such as faster growth rates and
larger surface-area-to-volume ratios (Kuzyakov and Xu, 2013). In order to acquire enough N for growth, plants have evolved several N uptake strategies, including changing their root physiological characteristics and gene expression levels in N uptake systems, and establishing symbiosis with microorganisms (Kraiser et al., 2011).

Several different forms of N can be available to plant roots, such as ammonium (NH$_4^+$), nitrate (NO$_3^-$), and amino acids (Fan et al., 2017). Nitrate is usually the primary form of inorganic N absorbed by plants in aerobic soils (Xu et al., 2012). The NO$_3^-$ uptake rate is mainly determined by NO$_3^-$ uptake kinetics, which can be modeled by the Michaelis–Menten equation, which denotes the absorption rate of NO$_3^-$ as a function of external NO$_3^-$ concentration (McMurtrie and Nåsholm, 2017). Two parameters of Michaelis–Menten kinetics, the maximum uptake rate ($V_{max}$) and the Michaelis constant ($K_m$), can describe N absorption characteristics (York et al., 2016). The $V_{max}$ value is the maximum influx rate of NO$_3^-$, whereas the $K_m$ value is the external NO$_3^-$ concentration when the absorption rate is half of $V_{max}$ and presents the affinity of transporters (York et al., 2016).

Uptake of NO$_3^-$ from soil is mediated by low- (LATS) and high-affinity transport systems (HATS) (Fan et al., 2017). The molecular basis of LATS and HATS mainly includes two families of NO$_3^-$ transporters (NRT): NRT1/PTR (renamed NPF by Léran et al., 2014) family and NRT2 family (Castro-Rodriguez et al., 2017). The NPF family members are responsible for the LATS, except for NPF6;3/NRT1;1, which has a dual low and high affinity for NO$_3^-$ (von Wittgenstein et al., 2014). Among the 53 known NPF family members in Arabidopsis, only AtNPF6;2/AtNRT1;1 and AtNPF4;6/AtNRT1;2 are involved in root NO$_3^-$ influx (Kant, 2018). The NRT2 family has seven members and is responsible for the HATS (von Wittgenstein et al., 2014). The coding genes are induced by a low concentration of NO$_3^-$ and repressed by a high concentration of NO$_3^-$ (Kant, 2018). The pH value was 7.7 (1:5, soil:water). Of these, AtNRT2;1, in interaction with a NO$_3^-$ assimilation-related protein (NAR2/NRT3), accounts for approximately 75% of the high-affinity NO$_3^-$ absorption (Filleur et al., 2001).

Arbuscular mycorrhizal fungi (AMF), belonging to the monophyletic phylum Glomeromycota, are the most widespread root symbionts and can colonize at least 80% of terrestrial plants (Smith and Read, 2008). AMF acquire up to 20% of their host photosynthates and provide water and mineral nutrients in return (Bago et al., 2000; Kiers et al., 2011). Many $^{15}$N-labeling experiments confirmed the ability of AMF to transport N from soil to plants (Govindarajulu et al., 2005; Jin et al., 2005). Plants can acquire large amounts of N through the mycorrhizal pathway (Smith and Smith, 2011). The effects of AMF on the expression of NRT genes have been observed in several plant species, for example, in grapevine (Balestrini et al., 2017), Solanum lycopersicum (Ruzicka et al., 2012), and Lotus japonicus (Guether et al., 2009). The arbuscular mycorrhizal (AM)-induced alterations in NRTs expression suggest that AMF may affect plant uptake capacity for NO$_3^-$ (Smith and Read, 2008). AMF acquire up to 20% of their root symbionts and can colonize at least 80% of terrestrial plants (Smith and Read, 2008). AMF acquire up to 20% of their root symbionts and can colonize at least 80% of terrestrial plants (Smith and Read, 2008).

Poplars have great ecological and economic values and can form symbiotic relationships with AMF (Wu et al., 2017). With the publication of the genome of Populus trichocarpa (Tuskan et al., 2006), poplar has become a model species for woody plant research (Jansson and Douglas, 2007). In the genome database of P. trichocarpa, 68 NPF/NRT1 and 11 NRT2/NRT3 have been putatively identified (Willmann et al., 2014). The genes NRT1;1, NRT1;2, NRT2;4B, and NRT2;4C of poplar were the closest homologs to AtNRT1;1, AtNRT1;2, AtNRT2;4, and AtNRT2;1 of Arabidopsis, respectively; and NRT3;1A, NRT3;1B, and NRT3;1C of poplar were the closest homologs to AtNRT3;1 of Arabidopsis (Li et al., 2012; Luo et al., 2013). These genes may play important roles in NO$_3^-$ uptake by poplar because their expression levels are markedly affected by changes in available N (Li et al., 2012; Gan et al., 2015). The objectives of the current research were to (i) evaluate the effects of AMF on NO$_3^-$ uptake kinetics of poplar and (ii) investigate the influence of AMF on NRTs expression under different N levels. The results may provide new insights into the parts AMF play in plant N uptake capacity.

**MATERIALS AND METHODS**

**Plant Material and Fungal Inoculum**

Cuttings (6 cm long) of Populus × canadensis ‘Neva’ were obtained from a plant nursery in Yangling, Shaanxi Province, China. The cuttings were surface sterilized with 75% ethanol and rinsed with sterile water. After surface sterilization, each cutting was planted in a plastic pot (10 × 8 cm) containing 450 g of growth substrate.

Rhizophagus irregularis (B109) was commercially provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, China. The fungal inoculum contained colonized root fragments, hyphae, spores (approximately 50 spores g$^{-1}$), and cultured sands.

**Growth Substrates**

The growth substrate consisted of topsoil (0–20 cm) collected from a poplar field in Yangling, Shaanxi Province, China. Before being put into the pots, the growth substrate was sieved (2 mm) and autoclaved (0.11 MPa, 121°C, 2 h). The soil contained soil organic matter, 18.97 mg kg$^{-1}$; available N, 38.62 mg kg$^{-1}$; available phosphorus, 12.31 mg kg$^{-1}$; and available potassium, 140.26 mg kg$^{-1}$. The pH value was 7.7 (1:5, soil:water).

**Experimental Design and Growth Conditions**

The experiment was conducted in a greenhouse using a randomized complete block design with two AM status (inoculated with R. irregularis or not inoculated with R. irregularis) and five N levels (0, 1, 5, 10, or 15 mM NH$_4$NO$_3$), with 30 replicates per treatment. For AM fungal inoculation, each pot received 20 g of inoculum, whereas the non-mycorrhizal treatment received 20 g of autoclaved inoculum and the filtrate (<20 μm) of the inoculum to provide a typical microbial population free of AM propagules (Wu et al., 2017). The cuttings...
were grown at a temperature of 25–30°C with 12 h of light per day. Ninety days after planting, N application started. NH₄NO₃ solution was applied to the corresponding treatments every other day. The N treatment lasted for 28 days. The pots were kept at field capacity during the experiment.

**AM Colonization**

The fresh roots (<2-mm diameter) were cut into 1- to 2-cm fragments and stained with trypan blue according to the method from Phillips and Hayman (1970). The proportion of root length colonized by AMF was estimated under a light microscope (×200 magnification; Olympus, Tokyo, Japan) using the gridline intersection method (McGonigle et al., 1990). The arbuscular, vesicular, hyphal, and total colonization values were expressed as a percentage of the length of root fragments containing arbuscules, vesicles, hyphae, and all AMF structures, respectively.

**Root Absorbing Area**

The root absorbing area was determined by a methylene blue colorimetric method (Liu et al., 2014). The roots were sequentially immersed for 90 s in three beakers containing a known volume of 0.2 mM methylene blue solution. Then, the concentration and volume of the remaining methylene blue solution in each beaker were determined by a spectrophotometer (UV mini 1240; Shimadzu, Kyoto, Japan) at 660 nm. Methylene blue was used as the standard. The equations are as follows:

Total absorbing area (m²) = [(C - C₁) × V₁] + [(C - C₂) × V₂] × L

Active absorbing area (m²) = [(C - C₃) × V₃] × L

where C (mg mL⁻¹) was the initial concentration of the methylene blue solution; C₁, C₂, and C₃ (mg mL⁻¹) were the concentrations of the methylene blue solution after three root immersions; V₁, V₂, and V₃ (mL) were the volumes of the methylene blue solution after three root immersions.

**Determination of NO₃⁻ Uptake Kinetics**

Plant NO₃⁻ uptake was measured using the solution depletion method (Gobert and Plussard, 2002) with some modifications. The roots of the intact plants were carefully washed and transferred to a 0.2 mM CaSO₄ solution for pretreatment for 48 h. After pretreatment, the plants were transferred to a Ca(NO₃)₂ solution (containing 0, 0.01, 0.05, 0.1, 0.2, 0.5, or 1 mM NO₃⁻) for 6 h. The NO₃⁻ concentration in the Ca(NO₃)₂ solution was determined by an AA₃ continuous flow analytical system (AA₃ autoanalyzer; Bran + Luebbe GmbH, Norderstedt, Germany) (Hu et al., 2016). The uptake rates were calculated as follows:

Uptake rates = (C₁ - C₄)/(t × m)

where C₁ is the initial concentration of NO₃⁻ (µmol mL⁻¹), C₄ is the final concentration of NO₃⁻ (µmol mL⁻¹), t is the absorption time (h), and m is the root dry weight (g). The kinetic parameters, V₅₀ and Kₚ, were calculated using a non-linear curve-fitting model (Michaelis–Menten kinetics) using the Enzyme Kinetics Module of SigmaPlot 13.0 (SPSS Inc., Chicago, IL, United States).

**Quantitative Real-Time PCR Analysis**

At harvest, the root and leaf samples were immediately snap-frozen in liquid N and stored at −80°C. RNA was extracted using the cetyl trimethyl ammonium bromide method (Shao et al., 2014). A DNase treatment was performed to remove residual DNA by using RNase-free DNase I (Invitrogen, Carlsbad, CA, United States). The quantity and quality of RNA were analyzed by measuring the A260/A280 ratio with a spectrophotometer (Nanodrop 2000c; Thermo Scientific, Pittsburgh, PA, United States). The first-strand cDNA was synthesized with 2 µg of total RNA, using a RevertAid first-strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, United States). The gene-specific primers used are listed in Table 1. Quantitative real-time polymerase chain reaction (PCR) was performed on a Bio-Rad CFX96 real-time PCR instrument (Bio-Rad, Hercules, CA, United States). The reactions were performed in 20-µL volumes containing 10 µL of SYBR Premix Ex Taq II (TaKaRa, Dalian, China), 0.8 µL of gene-specific primers, 1.0 µL of cDNA template and 8.2 µL of sterile distilled water. The PCR amplification conditions were 94°C for 10 s and then 40 cycles at 58°C for 20 s and 72°C for 30 s. The ubiquitin gene (UBQ) was selected as an internal standard (Couturier et al., 2007). Four biological replicates were utilized for each assay. The relative RNA levels of each gene were determined using the 2−ΔΔCₚ method (Livak and Schmittgen, 2001).

**Statistical Analyses**

Experimental data were statistically analyzed using the statistical software package SPSS 13.0 (SPSS Inc., Chicago, IL, United States). The data were tested for normality and homogeneity of variances. A two-way analysis of variance (ANOVA) was used to analyze the effects of N treatment and AM treatment and their interactions on the measured parameters, at a significance level of P = 0.05. The means were compared by Tukey’s multiple comparison test (P = 0.05). Figures were drawn using SigmaPlot 10.0 (Systat Software, San Jose, CA, United States).

**RESULTS**

**AM Colonization**

AM colonization structures were observed in more than 70% of the poplar roots after inoculation with*R. irregularis*, whereas no AM structure was observed in NM plants (Figure 1). The hyphal colonization was prominent in mycorrhizal poplar roots (Figure 1C). With increasing N level, the arbuscular, hyphal, and total colonization rates increased substantially. The highest arbuscular and hyphal colonization rates (58.85 and 90.42%, respectively) were observed in plants treated with 15 mM NH₄NO₃.

**Root Absorbing Area**

According to the two-way ANOVA, the total absorbing area was notably affected by N treatment and the interaction of N treatment and AMF treatment (Table 2). The active absorbing area was affected by N treatment, AMF treatment, and their interaction. The ratio of active absorbing area to total absorbing area was affected by AMF treatment. Nitrogen fertilization increased the total absorbing area and active absorbing area in both NM and AM plants (Table 3). The active absorbing area/total absorbing area of AM plants was...
higher than that of NM plants under each N level (3.47, 2.51, 2.73, 3.65, and 2.21% under 0, 1, 5, 10, and 15 mM NH₄NO₃, respectively).

**NO₃⁻ Uptake Kinetics**

The NO₃⁻ uptake rates of both AM and NM plants increased with increasing external NO₃⁻ concentrations (Figure 2). The NO₃⁻ uptake rates were fitted using a non-linear regression of the Michaelis–Menten equation \( V = \frac{V_{\text{max}}}{K_m + [\text{NO}_3^-]} \). According to the two-way ANOVA results, \( V_{\text{max}} \) values were affected by N treatment and AMF treatment, whereas \( K_m \) values were affected by N treatment (Table 2). The N × AMF interaction had no effect on the \( V_{\text{max}} \) and \( K_m \) values. Nitrogen decreased \( V_{\text{max}} \) values but increased \( K_m \) values (Table 4). \( V_{\text{max}} \) values were higher in AM plants than in NM plants under each N level (46.83, 37.24, 67.07, 86.11, and 64.02% under 0, 1, 5, 10, and 15 mM NH₄NO₃, respectively). \( K_m \) values were not significantly different between AM and NM plants.

**NRTs Expression in Leaves and Roots**

Nitrogen treatment significantly affected the expression of all the tested N transporter genes (Table 2). AMF treatment significantly affected the expression of NRT1;1, NRT1;2, NRT3;1A, NRT3;1B, and NRT3;1C in leaves and NRT1;1, NRT1;2, NRT2;4B, NRT2;4C, NRT3;1A, NRT3;1B, and NRT 3;1C in roots. The interaction of N treatment and AMF treatment significantly affected the expression of NRT1;1, NRT2;4B, NRT3;1A, NRT3;1B, and NRT3;1C in leaves and NRT1;1, NRT1;2, NRT2;4B, NRT2;4C, NRT3;1A, NRT3;1B, and NRT3;1C in roots. In roots, the expression of NRT2;4B, NRT2;4C, NRT3;1A, NRT3;1B, and NRT3;1C in NM plants decreased with increasing N application (Figure 3). In leaves, the expression of NRT1;1 and NRT2;4B increased with increasing N application (Figure 4). AMF reduced the expression of NRT1;2, NRT2;4B, NRT2;4C, NRT3;1A, NRT3;1B, and NRT3;1C in roots (Figure 3) and up-regulated the expression of NRT2;4B in leaves under 0 and 1 mM NH₄NO₃ (Figure 4). Under 10 and 15 mM NH₄NO₃, AMF up-regulated the expression of NRT1;1 in roots and reduced the expression of NRT1;1, NRT1;2, and NRT2;4B in leaves. Correlation analysis showed that the expression levels of NRT3;1A, NRT3;1B, and NRT3;1C were positively correlated with the expression levels of NRT1;2, NRT2;4B, and NRT2;4C (Table 5).

**DISCUSSION**

The formation of symbiosis is the starting point of AMF to improve plant nutrient uptake and stress resistance (Zhang et al., 2017). In this study, the structures of arbuscules, vesicles, and internal hyphae were clearly observed inside the roots of...
TABLE 2 | Effects of nitrogen treatment (N), AMF treatment (AMF), and N × AMF on the physiological parameters and expression levels of nitrate transporter genes of poplar.

| Parameters                                      | N       | AMF      | N × AMF |
|-------------------------------------------------|---------|----------|---------|
| Total absorbing area                            | 2496.90 | 1.37     | 3.93    |
| Active absorbing area                           | 568.02  | 597.54   | 357.72  |
| Active absorbing area/total absorbing area      | 2.03    | 39.96    | 0.36    |
| $V_{\text{max}}$                               | 23.75   | 44.74    | 0.37    |
| $K_{\text{m}}$                                  | 8.63    | 1.56     | 0.57    |
| NRT1:1 (leaf)                                  | 62.10   | 31.12    | 6.84    |
| NRT2:4B (leaf)                                  | 5.39    | 20.94    | 2.23    |
| NRT2:4C (leaf)                                  | 20.67   | 3.71     | 23.63   |
| NRT3:1A (leaf)                                  | 5.40    | 53.94    | 15.88   |
| NRT3:1B (leaf)                                  | 103.03  | 164.53   | 80.57   |
| NRT3:1C (leaf)                                  | 258.62  | 373.58   | 187.58  |
| NRT1:1 (root)                                   | 31.05   | 23.77    | 13.52   |
| NRT1:2 (root)                                   | 45.76   | 54.13    | 26.78   |
| NRT2:4B (root)                                  | 123.35  | 125.58   | 55.95   |
| NRT2:4C (root)                                  | 8.16    | 18.81    | 1.45    |
| NRT3:1A (root)                                  | 14.78   | 38.09    | 3.97    |
| NRT3:1B (root)                                  | 223.50  | 115.53   | 67.93   |
| NRT3:1C (root)                                  | 145.86  | 17.31    | 20.89   |

*P < 0.05; **P < 0.01; ***non-significant.

TABLE 3 | Effects of N fertilization and AMF inoculation on root total absorbing area, active absorbing area, and active absorbing area/total absorbing area of poplar.

| NH$_4$NO$_3$ (mM) | AMF | Total absorbing area (m$^2$) | Active absorbing area (m$^2$) | Active absorbing area/total absorbing area (%) |
|-------------------|-----|----------------------------|------------------------------|-----------------------------------------------|
| 0                 | NM  | 12.54 ± 0.15$^c$           | 6.26 ± 0.14$^d$              | 49.91 ± 0.76$^{bc}$                           |
|                   | AM  | 12.86 ± 0.37$^{bc}$        | 6.64 ± 0.09$^a$              | 51.64 ± 0.91$^{a}$                           |
| 1                 | NM  | 19.13 ± 0.48$^b$           | 9.52 ± 0.19$^c$              | 49.76 ± 0.46$^{bc}$                           |
|                   | AM  | 19.76 ± 0.25$^b$           | 10.08 ± 0.09$^b$             | 51.01 ± 1.00$^{ab}$                           |
| 5                 | NM  | 25.50 ± 0.16$^a$           | 12.63 ± 0.19$^a$             | 49.54 ± 0.43$^{bc}$                           |
|                   | AM  | 25.76 ± 0.27$^{ab}$        | 13.11 ± 0.17$^a$             | 50.89 ± 0.40$^{ab}$                           |
| 10                | NM  | 25.78 ± 0.21$^a$           | 12.65 ± 0.20$^a$             | 49.09 ± 0.38$^c$                             |
|                   | AM  | 25.78 ± 0.16$^a$           | 13.12 ± 0.09$^a$             | 50.88 ± 0.69$^{ab}$                           |
| 15                | NM  | 26.23 ± 0.18$^a$           | 12.93 ± 0.06$^a$             | 49.30 ± 0.17$^c$                             |
|                   | AM  | 25.63 ± 0.40$^{ab}$        | 12.92 ± 0.09$^{ab}$          | 50.39 ± 0.56$^{abc}$                          |

Values are presented as means ± SD (n = 3); within columns, values followed by the same lowercase letter do not differ significantly at P < 0.05 by Tukey’s test. NM, non-mycorrhizal; AM, arbuscular mycorrhizal.

More than 70% of the roots of the P. × canadensis ‘Neva’ cuttings were colonized by R. irregularis, which accords with previous findings that P. × canadensis ‘Neva’ was capable of establishing AM symbiosis (Liu et al., 2014). Nitrogen application influences AMF colonization rate, but inconsistent results have been reported (Calabrese et al., 2017; Wheeler et al., 2017). Using the same intersection method as this study, Wheeler et al. (2017) found a negative effect of chronic N application on the AMF colonization rate of Acer rubrum, whereas Calabrese et al. (2017) observed the opposite result in P. trichocarpa.

The higher photosynthetic capacity in N-applied plants may provide more photosynthates for AMF and may promote AMF colonization (Wu et al., 2017). The establishment of AM in roots can trigger the changes in root activities and gene expression patterns of root systems, for example, in grapevine (Balestrini et al., 2017; Torres et al., 2018), Robinia pseudoacacia (Huang et al., 2019), L. japonicus (Guether et al., 2009), and Lycopersicon esculentum (Hildebrandt et al., 2002). The results of this study showed that the active absorbing area/total absorbing area ratios of AM plants were higher than those in NM plants, which was in keeping with observations by Liu et al. (2014) and Huang et al. (2019). An increase in root activities suggests a stronger element uptake capacity (Yang et al., 2008). Uptake of NO$_3^-$ by plants relies on NO$_3^-$ transport systems (Garnett et al., 2010). In this study, the absorption of NO$_3^-$ by P. × canadensis ‘Neva’ followed...
Michaelis–Menten kinetics, as with other plant species, for example, *Solanum tuberosum* (Sharifi and Zebarth, 2006), *Fagus sylvatica* (Kreuzwieser et al., 2000), *Populus tremuloides* Michx., and *Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm. (Min et al., 2000). Nitrogen application decreased the $V_{max}$ and increased the $K_m$ value, which was consistent with previous studies showing that HATS was under feedback repression by N levels, and was down-regulated by N supply and up-regulated by N limitation (Bassirirad, 2000; Rothstein et al., 2000). Inoculation with *R. irregularis* increased the $V_{max}$ value of poplar in absorption of NO$_3^-$, irrespective of N treatment. So far, the effects of AMF on the kinetic analysis of NO$_3^-$ uptake have never been reported. In ectomycorrhizal research, inoculation with *Rhizopogon roseolus* increased the $V_{max}$ of *Pinus pinaster* for NO$_3^-$ absorption, indicating that ectomycorrhiza conferred plants with higher ability to utilize NO$_3^-$ (Gobert and Plassard, 2002). Kuzyakov and Xu (2013) found a higher $V_{max}$ and smaller $K_m$ for NO$_3^-$ in microorganisms than in plants, indicating that microorganisms have an advantage in the absorption of NO$_3^-$, especially at low concentrations. In general, microorganisms absorb the majority of N immediately after N is mineralized from soil organic matter (Kuzyakov and Xu, 2013). The higher NO$_3^-$ uptake rates in AM plants than in NM plants were observed regardless of the external NO$_3^-$ concentration, suggesting that AMF increased the competitive ability of the host to use fluctuating concentrations of NO$_3^-$ in the soil solution (Gobert and Plassard, 2002; Kuzyakov and Xu, 2013).

 Regulation of NO$_3^-$ transporters is an essential factor for elucidating NO$_3^-$ uptake and assimilation. Nitrate transport systems have been well identified in *Arabidopsis* (Kant, 2018). AtNPF6;2/AtNRT1;1 was found to be a dual-affinity NO$_3^-$ transporter, participating in low- and high-affinity NO$_3^-$ uptake and function as a NO$_3^-$ sensor (Liu et al., 1999; Ho et al., 2009). AtNPF4;6/AtNRT1;2 was found to be a low-affinity NO$_3^-$ transporter, and AtNPF4;6/AtNRT1;2 has been observed being constitutively expressed in root NO$_3^-$ uptake (Huang et al., 1999). AtNRT2;1 is the main NO$_3^-$ inducible member of the HATS, whereas NRT2;2 plays a minor role in NO$_3^-$ absorption (Li et al., 2007). AtNRT2;5 has a similar role to AtNRT2;4, which plays a main role in NO$_3^-$ uptake under N-starvation condition (Kiba et al., 2012; Lezhneva et al., 2014). Moreover, the interaction of NRT2 members with NAR2;1 (NRT3;1) is necessary to function in the NO$_3^-$ transport capacity of NRT2 (Okamoto et al., 2006; Tsay et al., 2007). The results of this study showed that the expression levels of NRT2;4B, NRT2;4C, NRT3;1A, NRT3;1B, and NRT3;1C in the roots of poplar were negatively correlated with external NO$_3^-$ concentrations, supporting the suggestion that NRT2 family members are high-affinity transporters (Li et al., 2012). The positive correlations between the expression levels of NRT3;1A, NRT3;1B, or NRT3;1C and the expression levels of NRT1;2, NRT2;4B, or NRT2;4C

**TABLE 4** | The kinetic parameters of NO$_3^-$ absorption in the roots of poplar.

| N treatment | AMF treatment | $V_{max}$ (µmol g$^{-1}$ h$^{-1}$) | $K_m$ (mM) | $R$ |
|---|---|---|---|---|
| 0 | NM | 4.42 ± 0.38$^{abc}$ | 0.13 ± 0.05$^{abc}$ | 0.92 |
| | AM | 6.49 ± 1.09$^a$ | 0.10 ± 0.02$^c$ | 0.91 |
| 1 | NM | 3.84 ± 0.52$^{cd}$ | 0.15 ± 0.03$^{bc}$ | 0.92 |
| | AM | 5.27 ± 0.57$^{ab}$ | 0.11 ± 0.03$^c$ | 0.90 |
| 5 | NM | 2.49 ± 0.72$^{ds}$ | 0.16 ± 0.02$^{abc}$ | 0.79 |
| | AM | 4.16 ± 0.67$^{bcd}$ | 0.18 ± 0.07$^{abc}$ | 0.93 |
| 10 | NM | 1.80 ± 0.83$^a$ | 0.21 ± 0.06$^{abc}$ | 0.97 |
| | AM | 3.35 ± 0.21$^{cd}$ | 0.19 ± 0.03$^{abc}$ | 0.98 |
| 15 | NM | 1.89 ± 0.56$^a$ | 0.24 ± 0.02$^d$ | 0.86 |
| | AM | 3.10 ± 0.53$^{cd}$ | 0.21 ± 0.03$^{abc}$ | 0.90 |

Values are presented as means ± SD (n = 3); within columns, values followed by the same lowercase letter do not differ significantly at P < 0.05 by Tukey’s test. NM, non-mycorrhizal; AM, arbuscular mycorrhizal.
indicated that they might combine to function in $\text{NO}_3^-$ absorption (Tsay et al., 2007). In leaves, the expression of $\text{NRT}1;1$ and $\text{NRT}2;4B$ were up-regulated by N application, which may be due to a higher amount of N transferred to leaves with increasing N application. Under N-limiting condition, the transcript levels of $\text{NRT}3$s in roots increased, and the expression of $\text{NRT}3$s in leaves decreased, which may be a possible mechanism for poplar adaptation to an N-deficient environment (Luo et al., 2013).

Previous studies reported that AMF can influence $\text{NO}_3^-$ uptake of plants by regulating the transcript levels of $\text{NRT}3$s (Chen et al., 2018; Hildebrandt et al., 2002). With transcriptomics techniques, Balestrini et al. (2017) demonstrated that $\text{Funneliformis mosseae}$ up-regulated the expression of two putative $\text{NO}_3^-$ transporters in the roots of grapevine. In $\text{L. japonicus}$, four $\text{NRT}3$s showed fourfold to 18-fold up-regulation by $\text{Gigaspora margarita}$ colonization (Guether et al., 2009). Tian et al. (2017) found both up- and down-regulated expression of $\text{NRT}3$s in the roots of $\text{Triticum aestivum}$ by $\text{R. irregularis}$ colonization. The differential responses of $\text{NRT}3$s to AM colonization may be related to the different functions of $\text{NRT}3$s. Some $\text{NRT}3$s induced by AMF were expressed in arbuscule-containing cortical cells and may be involved in symbiosis development or N transfer through the periarbuscular membrane (Gaude et al., 2012). Hildebrandt et al. (2002) found that $\text{LeNRT2;3}$ was expressed in inner cortical cells of $\text{L. esculentum}$, in which arbuscules and vesicles were mainly distributed. The expression of $\text{LeNRT2;3}$ was higher in AMF-colonized roots than in non-colonized controls, which probably mediated the positive effects of AMF on $\text{NO}_3^-$ uptake from soil and the distribution of $\text{NO}_3^-$ to the host (Hildebrandt et al., 2002). In this research, the absorption capacity of poplar for $\text{NO}_3^-$ was improved by $\text{R. irregularis}$, but no AM-specific $\text{NRT}$ was detected. The expression levels of $\text{NRT1;2}$, $\text{NRT2;4B}$, $\text{NRT2;4C}$, $\text{NRT3;1A}$, $\text{NRT3;1B}$, and $\text{NRT3;1C}$ were lower in $\text{R. irregularis}$-colonized poplar roots compared with non-colonized roots at 0 and 1 mM $\text{NH}_4\text{NO}_3$. The mycorrhizal plants have two N absorption pathways, the root direct pathway and the mycorrhizal pathway (Smith and Smith, 2011). Plants that form AM may use the mycorrhizal pathway to absorb $\text{NO}_3^-$ and down-regulate the transcript levels of $\text{NRT}3$s involved in the absorption pathway.
FIGURE 4 | (A–G) NRT1;1, NRT1;2, NRT2;4B, NRT2;4C, NRT3;1A, NRT3;1B, and NRT3;1C expression levels in leaves of mycorrhizal and non-mycorrhizal poplar in response to increasing nitrogen concentrations. Values are presented as means ± SD (n = 4). Means followed by the same letter do not differ significantly at P < 0.05 by Tukey’s test. NM, non-mycorrhizal; AM, arbuscular mycorrhizal.

root direct pathway (Tian et al., 2017). Ruzicka et al. (2012) demonstrated that, under low inorganic N concentrations in the soil, the N uptake of mycorrhizal tomatoes shifted toward mycorrhizal-mediated N uptake over the root direct pathway. NRT1;2, NRT2;4B, NRT2;4C, NRT3;1A, NRT3;1B, and NRT3;1C may be involved in the root direct pathway and repressed in the presence of the mycorrhizal pathway (Ruzicka et al., 2012).

The diverse N sources and N conversion reactions lead to complex N pools in the field, of which organic N is the largest N pool (Kuypers et al., 2018). Most organisms prefer bioavailable N forms, such as NH₄⁺ and NO₃⁻, for which production is mainly controlled by microbial reactions (Kuypers et al., 2018). Nitrate is the main inorganic N form in aerobic soils (Xu et al., 2012). Compared with microorganisms, plants have shown a lower V_max and higher K_m for NO₃⁻, which indicated a disadvantage in competing for NO₃⁻ (Kuzyakov and Xu, 2013). Previous studies demonstrated that AMF could change the microbial community that degrades organic matter and promote plant uptake of inorganic N produced during the degradation of organic matter (Hodge and Fitter, 2010; Nuccio et al., 2013). This study showed that AMF improved the ratio of root active absorbing area/total absorbing area and increased the V_max value of the Michaelis–Menten equation for NO₃⁻ uptake of poplar, resulting in a promotion in NO₃⁻ uptake capacity. The key genes in NO₃⁻ uptake of poplar were repressed by AMF under 0 and 1 mM NH₄NO₃, indicating that the mycorrhizal pathway may have an impact on the root direct pathway for NO₃⁻ uptake. These results implied that the formation of AM symbiosis may be an efficient strategy for plants to compete with microorganisms for the finite available N in the soil. However, the NO₃⁻ uptake kinetics of R. irregularis remains unclear (Kuzyakov and Xu, 2013). Further studies are needed to investigate the NO₃⁻ uptake.

| TABLE 5 | Correlation coefficients between the expression levels of the nitrate transporter genes in roots of poplar. |
|-------------|-----------|-----------|-----------|-----------|
| Index       | NRT1;1    | NRT1;2    | NRT2;4B   | NRT2;4C   |
| NRT3;1A     | −0.088    | 0.666*    | 0.664*    | 0.798*    |
| NRT3;1B     | −0.262    | 0.777*    | 0.943*    | 0.709*    |
| NRT3;1C     | −0.173    | 0.744*    | 0.869*    | 0.655*    |

Pearson correlation coefficients r were determined across all nitrogen treatments and inoculation treatments (n = 40). *P < 0.017 (Bonferroni correction).
characteristics of AMF, in order to elucidate the mechanism of AMF in plant N uptake.

**DATA AVAILABILITY STATEMENT**

All datasets generated for this study are included in the manuscript.

**AUTHOR CONTRIBUTIONS**

FW, FF, and MT designed the experiments. FW, FF, NW, and LL performed the experiments. FW and FF analyzed the data. FW and MT wrote the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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