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Nutrient allocation and photochemical responses of *Populus × canadensis* 'Neva' to nitrogen fertilization and exogenous *Rhizophagus irregularis* inoculation

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Abstract

Arbuscular mycorrhizal fungi (AMF) can promote plant growth performance, but their effectiveness varies depending on soil nitrogen (N) availability. To clarify the effectiveness of exogenous AMF along an N-fertilization gradient (0, 2, 10, 20, and 30 mM), the impacts of exogenous *Rhizophagus irregularis* and N on the growth, photochemical activity, and nutritional status of *Populus × canadensis* 'Neva' in natural soil were evaluated in a pot experiment. The results showed that the 10 mM N level was the optimal fertilization regime with the highest promotion effect on plant growth and the maximum quantum yield of photosystem II (PSII) (F_v/F_m). Excess N (20 and 30 mM) fertilization reduced the actual quantum yield of PSII (ΦPSII) and the F_v/F_m of the plants. Regardless of the N availability, inoculated plants exhibited greater F_v/F_m values than did non-inoculated plants. The biomass of inoculated plants was significantly higher compared with the control under low N levels (0 and 2 mM). Under high N levels, inoculated plants showed significant increases in ΦPSII. Moreover, the nutrient imbalance of plants inoculated with exogenous *R. irregularis* was eased by increasing P, Fe, Mn and Cu uptake in roots and higher P, Ca, Mg, Fe, Mn and Zn concentrations in leaves. Moreover, the F_v/F_m and ΦPSII exhibited positive correlations with P, Ca, Mg and Zn concentrations in leaves. In conclusion, inoculation with exogenous *R. irregularis* can benefit plant fitness by improving the photochemical capacity and nutrient composition of poplar under different N levels.

Keywords Nitrogen fertilization · Arbuscular mycorrhiza · Poplar · Chlorophyll fluorescence · Nutrient allocation

Introduction

Nitrogen (N) is required in large quantities for plant growth and its shortage is a main factor limiting plant productivity (Cooke et al. 2005). Chemical N fertilizers are increasingly used to improve agro-forestry production in the world (Li and Korpelainen 2015). However, the manufacture and extensive application of N fertilizers not only are economically expensive but also cause serious environmental issues, such as the water eutrophication and NO_x emission (Rockström et al. 2009).

Nutrient cycling, and consequent reduction of chemical fertilizers, can be promoted by beneficial microorganisms, such as arbuscular mycorrhizal fungi (AMF) (Philippot et al. 2013). AMF are obligate symbiotic fungi and are able to establish symbiosis with the root of about 80% of land plants (Smith and Read 2008). AMF could enhance plant nutrient uptake, promote plant growth and protect plants from environmental stresses (Smith and Read 2008). Increased attention has been received to the utilization of non-native strains of AMF as bio-fertilizers owing to their beneficial influence on plant nutrition (Pellegrino et al. 2012). Successful examples of *Zea mays* (Hu et al. 2009), *Medicago sativa* (Pellegrino et al. 2012), *Gossypium hirsutum* (Cely et al. 2016), *Hedysarum coronarium* (Labidi et al. 2015) and

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other plants inoculated with non-native strains of AMF have been achieved both in laboratory and field trials.

However, the influence of AMF on plant growth and nutrition are dependent on environmental conditions and the genotypes of the partners (Smith and Smith 2011). AMF showed large variations in effects on plant growth after N application (Treseder 2004). Fonseca et al. (2001) investigated the influence of *Glomus etunicatum* and P and N supplies on the total biomass of *Sorghum bicolor* and found enhanced effects of AMF on plant biomass by supplying N. This result probably occurred because a high N supply eased the AMF-plant competition for N (Johnson 2010). Conversely, Johansen et al. (1994) demonstrated the opposite result: the effect of *G. intraradices* on the total biomass of *Cucumis sativus* was more pronounced under low N status. A possible reason might be because plants are more dependent on AMF for nutrients when exposed to nutrient-limiting environments (Smith and Read 2008). Consequently, there is an urgent need to study the impact of N availability on the efficiency of exogenous AMF.

AMF are reported to increase plant fitness by promoting nutrient absorption (Cely et al. 2016; Zhu et al. 2011). Alterations in the uptake, allocation and composition of nutrients were observed in maize and olive plantlets inoculated with AMF under different soil conditions (Bati et al. 2015; Cabral et al. 2016; Labidi et al. 2015). Mineral elements are crucial for plant cellular structure and metabolism (Mechri et al. 2011; Smethurst et al. 2005). Alterations in nutrient concentrations and allocation may lead to changes in photochemical activity (Cabral et al. 2016). Previous studies indicated that nutrient deficiency is the reason for impaired photosystem II (PSII) functionality (Mechri et al. 2011; Smethurst et al. 2005). Chlorophyll fluorescence techniques detect the function of PSII, including the capacity for electron transfer and associated photochemical activities (Kalaji et al. 2014). Analysis of chlorophyll fluorescence parameters have revealed evidence for abiotic stresses such as nutrient deficiency (Kalaji et al. 2014), drought (Li et al. 2015) and extreme temperature (Zhu et al. 2011). A beneficial role of AMF on plant stress tolerance has also been observed via chlorophyll fluorescence techniques, with improved photochemical activity in plants inoculated with AMF compared to controls (Sheng et al. 2008; Zhu et al. 2014). Nevertheless, the impact of N supply on the effects of AMF on photochemical activities is unclear.

Poplars have important economic implications for the paper industry as well as for bioenergy resources (Li et al. 2015). Large amounts of N are required for the production of poplar. Previous work has confirmed that the biomass and bioenergy production of poplar could be increased by exogenous *Rhizophagus irregularis* and *G. versiforme* in unsterilized soil (Liu et al. 2014; Wu et al. 2017b). Nevertheless, the impacts of inoculation with exogenous AMF on the nutritional and photochemical responses of poplar under different N levels is poorly understood. In this work, the impacts of exogenous *R. irregularis* on the growth, chlorophyll fluorescence and nutrient composition of *Populus × canadensis* 'Neva' was investigated under a large range of N supply—from deficient to excess. We hypothesized that N availability would influence the effectiveness of exogenous *R. irregularis* by nutritional and photochemical processes.

Materials and methods

Plant, growth substrate and AM inoculum

Hybrid poplar (*P. × canadensis* 'Neva') cuttings (15 cm long) were collected from a nursery of poplar seedlings in Yangling, Shaanxi Province, China. The cuttings were sterilized in a 75% ethanol for 15 s, and washed with deionized water before being inserted into the growth substrate.

The growth substrate consisted of surface soil (0–20 cm) gathered from a nursery of poplars. The soil type was cinnamon and contained 0.52 g kg^{-1} total N, 0.31 g kg^{-1} total P, 36.62 mg kg^{-1} available N, 11.71 mg kg^{-1} available P, 143.26 mg kg^{-1} available K and 18.19 g kg^{-1} organic matter. Additionally, the pH value was 7.6 (soil:water = 1.0:2.5 w/v).

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

Experimental design and plant growth conditions

Treatments were arranged in a randomized block design with two AMF treatments (inoculated with exogenous *R. irregularis* or non-inoculated control) and five N levels (0, 2, 10, 20, and 30 mM). Each treatment had 30 replicates. The experiment was conducted at a greenhouse with the relative humidity of 55–65% and the average temperature of 25–35 °C in early April 2013. One cutting was planted in each pot (4 L, 22.5 × 22.5 cm) containing approximately 4 kg growth substrate. Half of the pots received 20 g inoculum, while the other half received an equal amount of autoclaved (121 °C, 2 h) inoculum with the filtrate (10 mL) from the non-autoclaved inoculum. Ninety days after transplantation, N fertilization (0, 1, 5, 10, and 15 mM NH$_4$NO$_3$) was applied every other day. The plants were treated with N fertilization for 30 days. Thirty days after fertilization, chlorophyll fluorescence was measured and the plants were harvested.

Chlorophyll fluorescence and relative chlorophyll content

Before harvest, chlorophyll fluorescence was measured by a modulated chlorophyll fluorometer (Mini-Imaging-PAM, Walz, Germany) in accordance with the manual (Li et al. 2015). Plants were placed in the dark for 30 min previous to the fluorescence measurements. The sixth fully expanded leaf without visible injury was selected for fluorescence measurements. After 30 min of dark adaption, the minimal fluorescence (F_o) of dark-adapted state was determined. The maximal fluorescence (F_m) of dark-adapted state was measured after irradiating the leaves for 3 s at a saturating pulse of irradiation (2000 μmol m^{-2} s^{-1}). Then, the minimal fluorescence (F'_o), maximal fluorescence (F'_m) and steady-state value of fluorescence (F_s) were determined after actinic light illumination (300 μmol m^{-2} s^{-1}, 10 min). The maximal quantum efficiency of PSII was calculated by $F_v/F_m = (F_m - F_o)/F_m$, which shows the intrinsic efficiency of PSII. The actual quantum yield of PSII was determined as ΦPSII $= (F'_m - F_s)/F'_m$, which shows the proportion of absorbed light energy utilized in photochemistry. Quenching parameters were calculated by the following equations: NPQ $= (F_m - F'_m)/F'_m$ for non-photochemical quenching, which is closely related to heat dissipation; qP $= (F'_m - F_s)/(F'_m - F'_o)$ for photochemical quenching, which shows the degree of the PSII reaction centers that are open (Maxwell and Johnson 2000).

The leaves that were measured for chlorophyll fluorescence were also used for relative chlorophyll content measurements. A SPAD-502 chlorophyll meter (Konica–Minolta Holdings, Inc., Osaka, Japan) was used to determine the relative chlorophyll content.

Mycorrhizal colonization

Fresh roots (< 2 mm diameter) were collected from each treatment and rinsed lightly with running water, and then cut into segments of 1–2 cm, followed by trypan blue staining (60 segments per treatment) (Phillips and Hayman 1970). Then, the root samples were microscopically (200 × magnification; Olympus, Tokyo, Japan) investigated for AMF colonization by examining structures containing hyphae, vesicles and arbuscules. The total colonization and arbuscular colonization were measured using the grid line intersection method (McGonigle et al. 1990).

Plant biomass and nutrient analysis

At harvest, six plants were randomly selected for each treatment to analyse growth parameters. Root and shoot samples were dried at 80 °C for 48 h to measure the biomass (Liu et al. 2014).

After the biomass was measured, the leaves and roots were ground and homogenized. The concentration of N was measured by the Kjeldahl method on a Kjeltec™ 8400 analyser unit (FOSS-Tecator, Hoganas, Sweden) (Wu et al. 2017a). For other element determinations, the dry samples were digested with HNO$_3$-HClO$_4$. The concentration of phosphorus (P) was analysed by the vanadomolybdate method (Cabral et al. 2016), and the potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) concentrations were measured by atomic absorption spectroscopy (Z-5000; Hitachi, Tokyo, Japan) (Azcón et al. 2003).

Statistical analysis

The SPSS 17.0 (SPSS Inc., IL. USA) was used to perform data analysis. The normality and homogeneity of variances were tested. NPQ was logarithmically transformed to achieve homogeneity of variance. The significance of the effects of N treatment, AMF treatment and their interaction on the experimental parameters was determined by a two-way analysis of variance (ANOVA) at $p \leq 0.05$. Means were compared by Duncan's multiple range test. Correlations between chlorophyll fluorescence parameters and leaf nutrient concentrations were analysed by Pearson's correlation coefficients ($p < 0.005$, Bonferroni correction). Principal component analysis (PCA) was conducted using the prcomp() function of the R (version 3.2.3) statistical computing language (He et al. 2015).

Results

Mycorrhizal colonization

The two-way ANOVA results suggested that the total colonization rate was influenced by N treatment ($p \leq 0.01$) and AMF treatment ($p \leq 0.01$) (Supplemental Table S1). The arbuscular colonization rate was affected by N treatment ($p \leq 0.01$), AMF treatment ($p \leq 0.01$) and the interaction ($p \leq 0.01$) (Supplemental Table S1). The total colonization rate and arbuscular colonization rate in both the non-inoculated and inoculated poplars increased with increasing N fertilization (Fig. 1). Inoculation with exogenous *R. irregularis* increased the total colonization rate and arbuscular colonization rate under each N level ($p \leq 0.05$).
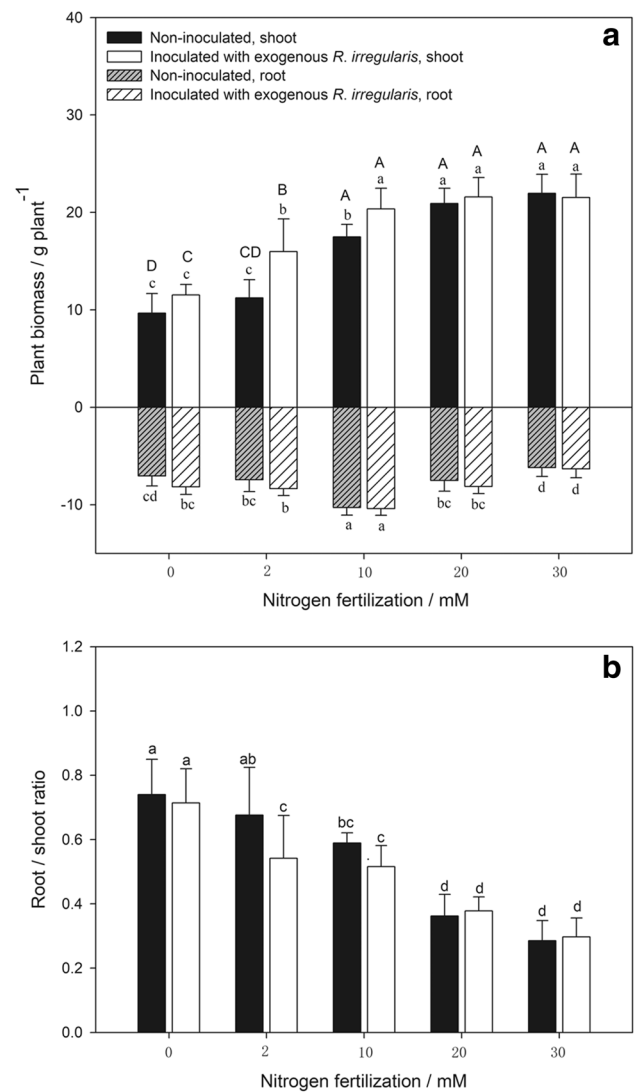
Plant biomass

The shoot and total biomass were influenced by N treatment ($p \leq 0.01$) and AMF treatment ($p \leq 0.05$), whereas the root biomass and root/shoot ratio were affected only by N treatment ($p \leq 0.01$) (Supplemental Table S1). The shoot biomass
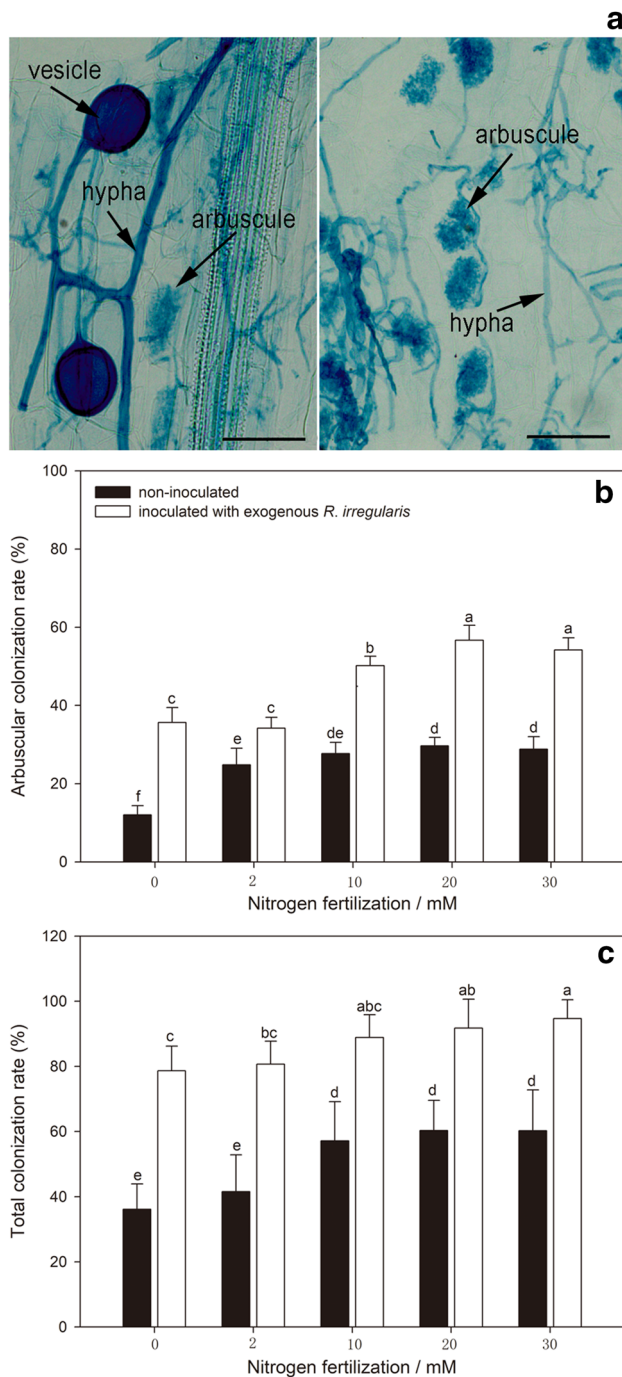
Fig. 1 Arbuscular mycorrhizal fungi structures in poplar roots (**a**) as well as the arbuscular colonization rate (**b**) and total colonization rate (**c**) in poplar with or without exogenous *R. irregularis* under five N levels. Values are presented as the means ± SD (*n* = 6). Values followed by the same letter show no significant difference at *p* ≤ 0.05 according to Duncan's test. Scale bar = 50 μm

Fig. 2 Effects of N and exogenous *R. irregularis* on the biomass (**a**) and root:shoot ratio (**b**) of poplar. Values are presented as the means ± SD (*n* = 6). Values followed by the same letter show no significant difference at *p* ≤ 0.05 according to Duncan's test. The lowercase letters are used for comparison of shoot biomass and root biomass, while the uppercase letters are used for comparison of total biomass

increased and tended to be stable with increasing levels of N fertilization (Fig. 2). At low N levels (0 and 2 mM), the total biomass was increased by inoculation (*p* ≤ 0.05). No obvious difference in root biomass or the root/shoot ratio

was found between the non-inoculated and inoculated plants under all N levels.

Chlorophyll fluorescence and chlorophyll content

The F_v/F_m, ΦPSII and NPQ were affected by N treatment and AMF treatment, whereas the qP was affected only by N treatment ($p \leq 0.01$, Supplemental Table S1). The F_v/F_m initially increased but then decreased by N fertilization, with peak values at 10 mM N (Fig. 3). The ΦPSII decreased with increasing N fertilization ($p \leq 0.05$). Inoculated plants had higher F_v/F_m value than non-inoculated
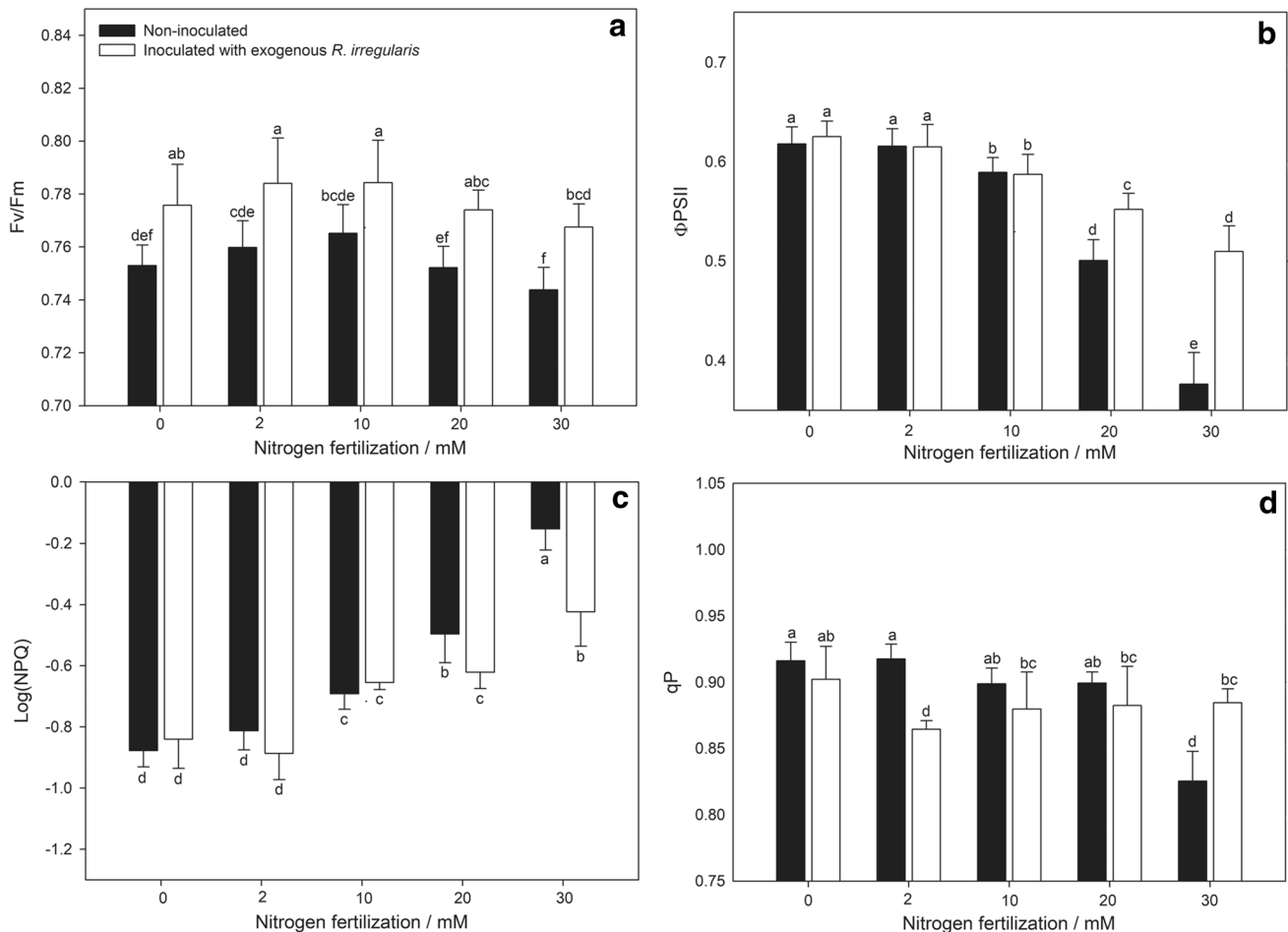
Fig. 3 Effects of N and exogenous *R. irregularis* on maximum quantum yield of PSII (F_v/F_m) (**a**), actual quantum yield of PSII (ΦPSII) (**b**), non-photochemical quenching (NPQ) (**c**), and photochemical quenching (qP) (**d**) of poplar. Values are presented as the means ± SD ($n = 6$). Values followed by the same letter show no significant difference at $p \leq 0.05$ according to Duncan's test

plants at all N levels ($p \leq 0.05$). No obvious difference in ΦPSII was observed between AMF treatments under 0, 2, or 10 mM N; however, the ΦPSII of the inoculated plants was higher than that of the non-inoculated ones at high N levels (20 and 30 mM) ($p \leq 0.05$).

The quenching analysis revealed an increase in NPQ with increasing N fertilization ($p \leq 0.05$, Fig. 3c, d). At high N levels (20 and 30 mM), the NPQ was improved by inoculation ($p \leq 0.05$, Fig. 3). The reduction in qP at higher N levels relative to the 0 mM level was larger in non-inoculated than inoculated plants (Fig. 3).

N fertilization had significant ($p \leq 0.01$) influence on the SPAD values (Supplemental Table S1). N fertilization enhanced the SPAD values of inoculated and non-inoculated plants (Supplemental Fig. S1). The differences in SPAD values were not significant between the AMF treatments under low N levels (0, 2 mM). Inoculated plants had higher SPAD values than non-inoculated ones at 30 mM N ($p \leq 0.05$).

Plant nutrients

Nitrogen treatment and AMF treatment had significant influences on the analysed nutrient concentrations in the leaves and roots (Supplemental Table S1) except for Mg, whose level was unaffected in the roots. In the roots, the concentration of N increased with increasing N fertilization, whereas P, K and Ca concentrations decreased (Table 1). Root N and P concentrations were improved by inoculation at 10 and 30 mM N, respectively (Table 1). At high N levels (20 and 30 mM), the concentration of K was decreased by inoculation ($p \leq 0.05$, Table 1). In non-inoculated plants, leaf N concentration increased at higher N levels relative to 0 mM N, whereas the corresponding concentrations of P, Ca and Mg decreased (Table 2). No obvious differences were noted in the concentrations of K, Ca, and Mg between the AMF treatments at 0, 2, or 10 mM N. At high N levels, the K concentration was decreased by inoculation, whereas the concentrations of Ca and Mg were increased (Table 2).

Table 1 Effects of N and exogenous *R. irregularis* on the concentrations of macronutrients and micronutrients in the roots of poplar

Nutrient	Non-inoculated					Inoculated with exogenous *R. irregularis*				
	0 mM	2 mM	10 mM	20 mM	30 mM	0 mM	2 mM	10 mM	20 mM	30 mM
Macronutrients (g kg⁻¹ DW)										
N	6.5±0.5e	6.7±0.7e	8.6±0.8d	13.4±1.0b	15.5±0.5a	6.8±0.3e	7.0±0.7e	9.5±0.8c	13.4±1.2b	16.3±0.6a
P	0.83±0.07ab	0.78±0.05bc	0.74±0.08cd	0.72±0.08cd	0.66±0.07d	0.89±0.03a	0.81±0.06bc	0.74±0.07cd	0.73±0.06cd	0.80±0.04bc
K	8.1±0.5a	7.3±0.9b	6.2±0.6c	6.2±0.6c	5.6±0.3cd	8.0±0.3ab	7.2±0.7b	5.9±0.8c	5.1±0.6de	4.8±0.4e
Ca	24.8±2.9a	19.9±2.0bc	20.1±1.9bc	18.7±1.9bc	18.0±1.0bc	20.3±2.4b	15.6±0.6d	18.6±2.3bc	17.6±0.8cd	19.9±2.5bc
Mg	2.9±0.4	2.7±0.4	2.7±0.3	2.7±0.3	2.7±0.2	2.7±0.1	2.5±0.2	2.7±0.3	2.7±0.3	3.1±0.3
Micronutrients (mg kg⁻¹ DW)										
Fe	1418.1±141.4c	1398.7±141.2c	1615.0±65.9b	1600.7±238.3b	1609.1±107.2b	1302.5±144.4c	1251.3±90.1c	1835.8±194.7a	1846.2±125.6a	1906.5±178.1a
Mn	161.5±33.3e	176.5±21.9e	259.3±21.9bc	262.2±20.4bc	203.5±21.8d	170.8±16.2e	186.9±15.0de	276.2±20.9ab	294.0±12.1a	248.1±20.0c
Cu	28.1±4.0de	23.1±1.6f	31.6±4.4cd	38.5±2.9b	36.9±2.3b	28.3±3.4de	25.7±4.0ef	34.5±3.8bc	46.0±2.9a	48.7±4.0a
Zn	38.3±2.1a	34.6±2.0bc	31.2±2.4c	30.0±2.5c	31.6±4.6c	39.6±6.3ab	30.3±3.3c	30.0±2.3c	30.0±3.4c	30.0±4.4c

Values are presented as the means ± SD ($n=6$). Values followed by the same letter show no significant difference at $p \leq 0.05$ according to Duncan's test

Table 2 Effects of N and exogenous *R. irregularis* on the concentrations of macronutrients and micronutrients in the leaves of poplar

Nutrient	Non-inoculated					Inoculated with exogenous *R. irregularis*				
	0 mM	2 mM	10 mM	20 mM	30 mM	0 mM	2 mM	10 mM	20 mM	30 mM
Macronutrients (g kg⁻¹ DW)										
N	8.4±0.6f	10.0±1.3e	12.3±0.7d	14.5±0.8c	15.6±0.9b	8.4±0.6f	9.6±0.6e	12.6±0.7d	15.3±1.1bc	17.0±0.8a
P	0.41±0.03b	0.45±0.04a	0.40±0.03b	0.33±0.04c	0.25±0.02d	0.40±0.03b	0.45±0.02a	0.42±0.02ab	0.39±0.03b	0.31±0.04c
K	3.8±0.3c	4.1±0.4bc	4.4±0.3abc	4.5±0.5ab	4.4±0.4abc	3.8±0.6c	4.4±0.3abc	4.9±0.4a	4.0±0.4c	4.0±0.4c
Ca	14.5±1.8ab	13.1±1.7bc	11.1±1.4c	8.7±1.2d	12.1±1.7cd	13.7±1.1bc	13.5±1.5bc	12.1±1.6cd	13.6±2.0bc	15.9±1.8a
Mg	4.8±0.3b	4.3±0.4cd	4.0±0.3d	3.3±0.4e	4.1±0.2d	4.6±0.3bc	4.6±0.3bc	4.4±0.5bcd	4.6±0.3bc	5.3±0.3a
K/Ca	0.27±0.04c	0.31±0.01c	0.40±0.07b	0.53±0.05a	0.50±0.17a	0.28±0.06c	0.33±0.02bc	0.41±0.05b	0.30±0.08c	0.25±0.02c
Micronutrients (mg kg⁻¹ DW)										
Fe	116.4±12.1a	109.1±13.0a	62.5±14.2cd	50.7±11.3d	69.3±13.3bc	81.8±14.1b	61.1±7.9cd	58.4±15.1cd	70.4±9.2bc	102.6±11.9a
Mn	122.5±5.4bcde	116.3±8.0cde	93.3±12.2f	109.0±11.1e	114.3±8.0cde	124.9±18.0abcd	126.4±11.0abc	112.1±9.9de	134.5±6.3ab	136.9±9.3a
Cu	3.2±0.3b	3.4±0.3ab	3.7±0.2a	3.8±0.3a	3.8±0.4a	2.0±0.5d	2.5±0.3c	3.1±0.3b	3.3±0.2b	3.3±0.1b
Zn	40.8±5.4de	41.6±3.3de	37.8±3.1e	24.5±1.9f	44.9±4.6cd	50.0±3.6b	49.3±2.1b	47.0±3.1bc	40.8±3.0de	59.9±3.2a

Values are presented as the means ± SD ($n=6$). Values followed by the same letter show no significant difference at $p \leq 0.05$ according to Duncan's test

The ratio of leaf K:Ca in non-inoculated plants increased in response to N fertilization, whereas the K:Ca ratio in inoculated plants remained consistent across N levels (Table 2). The K:Ca ratio was decreased by inoculation at 10, 20 and 30 mM N ($p \leq 0.05$).

With respect to micronutrients, N fertilization increased the Cu, Fe, and Mn concentrations in the roots (Table 1). In the leaves, the concentration of Fe decreased with increasing N fertilization, but the concentration of Cu increased (Table 2). Leaf Cu concentration was decreased by inoculation, but the concentration of Zn was increased (Table 2). Under low N levels, the Mn concentration in leaves and the Fe, Mn and Cu concentrations in the roots showed no significant difference between AMF treatments (Tables 1, 2). At high N levels, inoculated plants had higher Fe and Mn concentrations in both leaves and roots as well as a higher concentration of Cu in the roots ($p \leq 0.05$, Tables 1, 2).

PCA and correlation analysis

To investigate the response patterns of the non-inoculated and inoculated plants to N treatment, PCA was performed using physiological data related to plant growth (Fig. 4; Table 3). The PCA results indicated that principle component 1 (PC1) and PC2 explained 42.7 and 16.9% of the variance, respectively (Fig. 4). PC1 tended to uncouple the influence of N fertilization, and PC2 uncoupled the differences in exogenous *R. irregularis* inoculation. The main drivers for PC1 were increases in leaf N concentration, shoot biomass
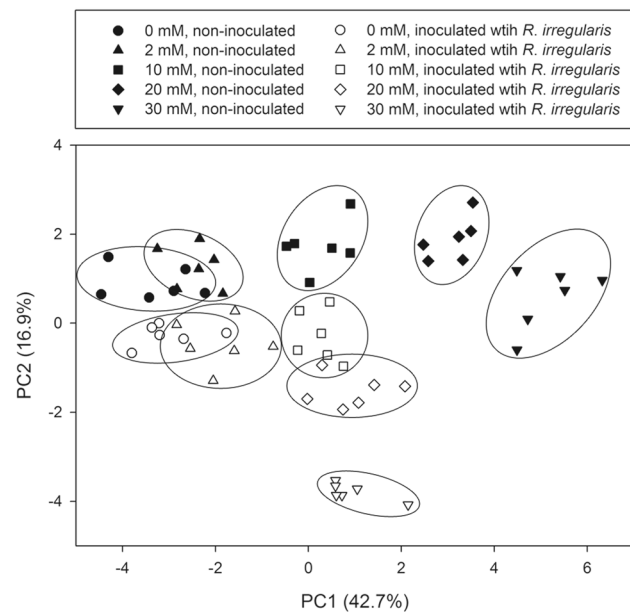
Fig. 4 Principal component analysis (PCA) plots of chlorophyll fluorescence parameters and nutrient elements in the leaves of poplar under N fertilization and exogenous *R. irregularis* inoculation

Table 3 Contributions of physiological parameters to PC1 and PC2 of poplar

Parameter	PC1	PC2
N	0.302	− 0.264
P	− 0.320	0.138
K	0.107	0.115
Ca	− 0.219	− 0.348
Mg	− 0.204	− 0.415
Fe	− 0.212	− 0.112
Mn	− 0.110	− 0.398
Cu	0.224	0.172
Zn	− 0.193	− 0.435
Shoot biomass	0.318	− 0.226
Root biomass	− 0.010	− 0.013
Total biomass	0.283	− 0.207
Root:shoot ratio	− 0.299	0.194
F_v/F_m	− 0.118	− 0.234
ΦPSII	− 0.333	0.039
NPQ	0.336	− 0.109
qP	− 0.231	0.125

F_v/F_m maximum quantum efficiency of photosystem II (PSII), ΦPSII actual quantum yield of PSII, *NPQ* non-photochemical quenching, *qP* photochemical quenching

and NPQ and decreases in leaf P concentration and ΦPSII (Table 3). PC2 was mainly determined by the Ca, Mg, Mn and Zn concentrations in the leaves (Table 3). The results of the PCA indicated that inoculated and non-inoculated plants exhibited distinct physiological responses to N fertilization.

The F_v/F_m showed positive correlation with P, Mg and Zn concentrations in leaves and showed negative correlation with leaf Cu concentration ($p \leq 0.005$, Table 4). In addition, ΦPSII was positively related to the leaf P, Ca, Mg and Zn concentrations and negatively correlated with SPAD values, leaf N and Cu concentrations ($p \leq 0.005$).

Table 4 Correlation coefficients between the chlorophyll fluorescence parameters and SPAD values and nutrient concentrations in the leaves of poplar

Index	F_v/F_m	PSII
SPAD	− 0.138	− 0.579*
N	− 0.143	− 0.727*
P	0.444*	0.831*
K	0.041	− 0.145
Ca	0.285	0.453*
Mg	0.388*	0.406*
Fe	− 0.184	0.316
Mn	0.217	0.166
Cu	− 0.456*	− 0.505*
Zn	0.531*	0.414*

*$p \leq 0.005$ (Bonferroni correction)

Discussion

In the present work, five levels of N were used to provide a wide range of N availabilities to examine the efficiency of exogenous *R. irregularis* along an N-fertilization gradient. We assessed the mycorrhizal colonization parameters, growth parameters, nutrient status and PSII efficiency of poplar subjected to N and AMF treatments to further clarify the mechanisms of the effect of exogenous AMF on plant growth. The results showed that exogenous *R. irregularis* differed in its effectiveness depending on the N availabilities. Under limiting N levels, exogenous *R. irregularis* had positive effects on plant biomass accumulation and photochemical activity. Under excessive N levels, exogenous *R. irregularis* enhanced the photochemical capacity of PSII and alleviated the nutrient imbalance due to excess N fertilization by increasing leaf P, Ca, Mg, Fe, Mn, and Zn concentrations and root P, Fe, Mn, and Cu concentrations.

In keeping with previous studies (Liu et al. 2014), inoculation with exogenous *R. irregularis* increased the AMF colonization rate. This increase suggested that inoculation with exogenous AMF was effective even with an abundant indigenous AMF community, as shown by the increased colonization rate and the differential effects compared to the non-inoculated ones (Sýkorová et al. 2012). The results of present study demonstrated that the AMF colonization rate increased in response to N fertilization, which agreed with similar findings reported previously (Treseder and Allen 2002). Such an effect may be due to the essential role of N in lignin composition (Bago et al. 2004); therefore, N fertilization may provide AMF with enough nutrients to grow.

With increasing N fertilization, the shoot and total biomass of both non-inoculated and inoculated poplars rose and tended to stabilize, which was consistent with findings reported for this species (van den Driessche 1999). These findings indicated that the optimal N supply was important for plant growth; however, supra-optimal N supply did not further increase productivity and even reduced plant fitness (Mauromicale et al. 2006). Alterations in N availability may impact the effectiveness of exogenous AMF on plant growth (Treseder 2004). In the present study, exogenous *R. irregularis* inoculation had a greater effect on biomass accumulation in poplar under limiting N levels, which supported previous findings in which plants relied more heavily on AMF to take up nutrients under nutrient-insufficient environments (Smith and Read 2008). Nitrogen fertilization might have weakened the reliance of poplars on exogenous *R. irregularis* by the supply of available N.

Plants absorb light for photosynthesis (Liu et al. 2014). The acquired light energy is either utilized to drive photosynthesis (photochemistry), release as light in the form of chlorophyll fluorescence (non-photochemistry), or lost through thermal dissipation (Sheng et al. 2008). Chlorophyll fluorescence can reveal the photosynthetic efficiency of leaves; therefore, chlorophyll fluorescence parameters are often used to evaluate plant photosynthesis and related mechanisms (Smethurst et al. 2005). In the present work, plants had the highest F_v/F_m value at 10 mM N, which is in line with previous reports showing that an optimum N supply is crucial for the function of the photosynthetic machinery and that high N inputs damaged the photosystem due to nutrient imbalance and NH_4^+ toxicity (Mauromicale et al. 2006; Zhou et al. 2006). The decreases in ΦPSII and qP observed in the present study further indicated harmful effects of excess N on plant health (Sheng et al. 2008). Several earlier studies have showed that AMF are able to enhance the photochemical capability of the host under adverse conditions, such as salt (Sheng et al. 2008), water (Li et al. 2015), and heat stresses (Zhu et al. 2011); however, the influence of AMF under different N levels has not been investigated. This study showed that the plants inoculated with exogenous *R. irregularis* maintained relatively higher F_v/F_m values than did non-inoculated plants regardless of N-fertilization level, suggesting that the inoculated plants had a higher potential photochemistry efficiency of PSII. Moreover, the NPQ represents the energy that is dissipated as heat (Osmond 1994). The lower NPQ and higher ΦPSII observed in the plants inoculated with exogenous *R. irregularis* under excessive N levels indicated that more energy was allocated to photochemical use and that less energy was dissipated as heat, which would increase the photosynthetic capability of PSII.

Plant nutrient composition is closely associated with cellular metabolism and can be altered by environmental changes (Mechri et al. 2011). Nitrogen fertilization led to considerable alterations in nutrient concentrations in both the leaves and roots. Consistence with the previous studies (Barunawati et al. 2013), the N and Cu concentrations in both the leaves and roots increased with increasing N fertilization. Specifically, in the present study, compared with non-fertilized controls, non-inoculated plants exposed to N fertilization presented lower concentrations of P, K, Ca, and Zn in the roots and lower concentrations of P, Ca, Mg, Fe, Mn, and Zn in the leaves. The decreases in element concentrations with increasing N fertilization might be explained by the dilution of nutrient concentrations as plant biomass increases (Lambers et al. 2008) or by the competitive effect between N and other ions (Pegoraro et al. 2013). A common characteristic of NH_4^+ toxicity under high N loads is the inhibition of plant cation content (Nakaji et al. 2001; Szczerba et al. 2008), which leads to a nutrient imbalance and physiological changes under excessive N levels. This

idea is supported by our results in that the non-inoculated plants showed a decrease in photochemical capability under excessive N levels.

Improving nutrition is generally considered a vital mycorrhizal benefit to the host (Corrêa et al. 2015). Altered nutrient compositions by AMF have been reported for Ca, Fe, Mn, Cu and Zn in *Olea europaea* (Bati et al. 2015) and for N, P, K, Ca, Fe and Mn in *Triticum aestivum* (Cabral et al. 2016). The differential influence of AMF on element absorption might be related to the combination of AMF and the host genotypes (Cabral et al. 2016; Munkvold et al. 2004). In the current study, almost all the macronutrients and micronutrients showed no improvement in the inoculated plants in comparison with the non-inoculated ones under limiting N levels. The lack of differences in nutrient concentrations in the present study might be attributed to a dilution effect due to the greater biomass in the plants inoculated with exogenous *R. irregularis* under low N levels (Marulanda et al. 2003). Under excessive N levels, significant increases in several element concentrations (N, P, Ca, Mg, Fe, Mn and Zn in the leaves and P, Fe, Mn, and Cu in the roots) were noted in the inoculated poplars compared with non-inoculated ones. Similar findings were noted in wheat exposed to heat stress by Cabral et al. (2016). The K:Ca ratio has been used as an indice of ion mobility in plants (Cabral et al. 2016) and the lower ratio of K:Ca in inoculated than non-inoculated plants under excessive N levels further indicated the altered plant nutrient composition by exogenous *R. irregularis*. Cabral et al. (2016) suggested that AMF-induced changes in plant nutrient composition under adverse conditions were related to the increased number of arbuscules, which agrees with our findings, as we observed a greater arbuscular colonization rate under excessive N levels. Arbuscules are involved in nutrient transfer between mycorrhizal symbionts, and an increase in arbuscular colonization rate may facilitate nutrient exchange between two symbiotic partners (Smith and Smith 2011; Treseder 2013).

Exogenous *R. irregularis* affected the allocation of different nutrient elements in different manners. A higher concentration of N was observed only in leaves under high N levels, which is in line with a report by Johnson (2010), who reported that AMF had no effect on plant N content when soil N limited the growth of both plants and fungi. The extra N in the leaves of plants inoculated with exogenous *R. irregularis* under excessive N levels might be stored in chlorophyll (Wu et al. 2017a; Zhang et al. 2018), as the inoculated plants exhibited greater SPAD values than did the non-inoculated ones. The magnitude of the decrease in the concentration of P with increasing N fertilization was lower in inoculated than non-inoculated poplars; this discrepancy might be owing to the function of exogenous *R. irregularis* in the P nutrition of the host (Johnson 2010). The decrease in the concentration of K observed in inoculated plants under

excessive N levels might be related to the competition for uptake sites between NH_4^+ and K^+ (Hoopen et al. 2010). The high Cu-binding capability of *R. irregularis* and the immobilization of Cu within root tissues containing mycorrhizal structures may contribute to the high Cu concentration in roots inoculated with exogenous *R. irregularis* (Carvalho et al. 2006). The increased allocation of Ca, Mg, Fe, Mn and Zn to the leaves due to exogenous *R. irregularis* inoculation might be explained by the 'stress severity hypothesis', which states that there is a reinforcement of the mutualistic relationship when one or two symbionts are exposed to environmental stress, as mutual exchanges alleviate environmental influences such as plant nutrient deficiency (Brooker et al. 2005).

Alterations to nutrient composition may lead to changes in photochemical activity (Cabral et al. 2016). Mechri et al. (2011) and Smethurst et al. (2005) observed that nutrient imbalances or deficiencies were major contributors to a decrease in PSII photochemistry. Similar to these findings, the results of present study indicated that chlorophyll fluorescence parameters were positively correlated with leaf P, Ca, Mg and Zn concentrations. Thus, the reductions in P, Ca, Mg, and Zn in the leaves of excessive N-fertilized poplars potentially contributed to the reduced efficiency of PSII under excessive N levels. Exogenous *R. irregularis* inoculation might enhance the photochemical activities of poplar by increasing the uptake of these elements. Similar findings were noted by Hajiboland et al. (2010), who observed that AMF relieved salt-induced reductions in P, Ca and K absorption and protected the photochemical processes of PSII. Hence, in addition to the previous works, the present study indicated a beneficial role of exogenous AMF in alleviating nutrient imbalance and the functional disruption of PSII caused by adverse conditions. We assumed that these nutritional and photochemical adjustments induced by exogenous *R. irregularis* may enhance plant adaptability to environmental change.

In summary, using a wide N availability gradient provided by applying five levels of mineral N inputs, we showed that the effect of N fertilizer on plants changed from beneficial to detrimental. Under limiting N levels, exogenous *R. irregularis* resulted in improved plant growth and F_v/F_m. Under excessive N levels, however, exogenous *R. irregularis* increased the photosynthetic capability of PSII and alleviated nutrient imbalance. These findings suggested that N availability influenced the efficacy of exogenous *R. irregularis* and that exogenous *R. irregularis* benefited plant fitness by improving plant photochemical and nutritional characteristics under different N availabilities.

to the collection and statistical analysis of the data. FW, HZ and MT wrote the manuscript. In addition, all authors have read the final manuscript and approved for publication.

Compliance with ethical standards

Conflict of interest The author(s) declare no conflict of interest.

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