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GROWTH RESPONSES OF *SORGHUM BICOLOR* (L.) MOENCH TO ARBUSCULAR MYCORRHIZAL FUNGI UNDER SIMULATED NITROGEN DEPOSITION

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Abstract

Anthropogenic nitrogen (N) deposition leads to a dramatically increase in biologically available N in many ecosystems, which can change the symbiotic relationship between AMF and host plants. However, how and to what extent exogenous N-induced AMF could affect plants remains poorly understood. In this work, mycorrhizal growth responses of *Sorghum bicolor* to AMF under simulated N deposition were conducted in a glasshouse experiment. Results demonstrated that AMF elevated the growth performance and nutrient uptake (N, P) of *S. bicolor* at almost all treatments, although mycorrhizal colonization decreased with N addition. In addition, mycorrhizal response (MR) showed identical trend of first fall and then increase, and the lowest value was at the N1 treatment. The present study provided the first pot-based evidence that AMF can alleviate the mischief induced by high N addition, implying that AMF has a considerable significance in the farmland ecosystem under anthropogenic N deposition.

Introduction

With the massive burning of fossil fuels and excessive use of nitrogen (N) fertilizers, the N content at the atmosphere has been increased sharply since the middle of the 20th century (Kontunen-Soppela *et al*. 2000). Relevant studies have shown that the N deposition has reached more than 30 kg N hm^{-1} a^{-1} in the North China Plain in 2010 (Zheng *et al*. 2014). The increase in soil N content caused by N deposition has also brought a series of severe ecological and environmental problems. The impact of N on land plants has also attracted more attention to the scientists (Sun *et al*. 2015, Yi *et al*. 2016).

N is an essential element for plant growth and participates in important physiological and biochemical reactions in the entire life of plants. Hao *et al.* (2014) reported that the introduction of external N can promote plant growth in N-deficient areas, but inhibit plant growth in N-rich areas, and directly affect the diversity of ecosystems to a certain degree.

Arbuscular mycorrhizal fungi (AMF) are widely distributed in nature and can form symbiotic mycorrhizas with more than 80% of terrestrial vascular plants (Smith and Read 2008). AMF can transfer the absorbed water and mineral nutrients to the host plant, and plants conversely transport carbohydrates synthesized by photosynthesis to AMF as carbon sources. This symbiotic relationship not only enhances the absorption of nutrients by plants but also improves their ability

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to resist adversity to a certain extent (van der Heijden *et al.* 2015). Compared with the N absorption by plant roots, the mycorrhiza formed by AMF reshape the structure of the root system and expand the absorption range of roots, which increase the absorption capacity of host plant roots for nutrients such as N and phosphorus (P) (Hodge and Fitter. 2010). Simultaneously, Xue *et al.* (2004) found that soil N content can significantly affect the ability of AMF to infect plant roots. The increase of soil N content can increase the mycorrhizal infection rate in N-deficient areas and reduce the mycorrhizal infection rate in N-rich areas.

S. bicolor is a common coarse grain crop in northern China. Considering its close symbiosis relationship with AM fungi, it is often used as research material for AM fungi expansion and inoculation experiments (Yamato *et al*. 2008). Hence, the present study was conducted to evaluate the effect of AMF under simulated N deposition to the growth responses of *S. bicolor* in greenhouse conditions.

Materials and Methods

The AMF (*Funneliformis coronaturm*) (Schüßler and Walker 2010) inoculum in the form of spores and infected roots of Sorghum (approximately 150 spores per gram inoculum) were obtained from the department of school of life science, Lanzhou University, Lanzhou, Gansu, China. Seeds of *S. bicolor* collected from the Shaanxi Prefecture Institute of Agronomy, was disinfected with 0.5% NaClO solution for 100 s, rinsed with running water, and soaked in distilled water for 10 min, after that the germination in a 25°C constant temperature light incubator was accelerated (Bahadur *et al.* 2019). Germinative seeds were sown in a seedling pot (16 × 14cm).

Soil collected from an arid agriculture demonstration area with an N deposition background value of 27.6 kg N/hm/a, was sieved through a 2 mm mesh as a pot substrate. Each inoculated pot (+AMF) received 10 g of inoculum, and non-inoculated pot (–AMF) were applied with an equal amount of sterilized inoculum. To minimize differences in microbial communities and nutrient between + AMF and –AMF treatments, 10 ml of AMF -free soil filtrate was added to each non-inoculated pot.

The plant seeding was thinned to five plants per pot. Three levels of N fertilization (0, 30 and 60g kg N hm^{-1} a^{-1}; hereafter referred to as N0, N1 and N2, respectively) were selected for this experiment. The N addition gradient was generated with different amounts of NH_4NO_3 fertilizer. The 150 ml of quantitative N addition solution was poured once every 15 days per pot, and N addition was applied 10 times in total. The pots were watered every 3 days with 100 ml water each time throughout the experimental period. Hoagland solution was applied per pot to fulfill the increasing nutrient demand of the plants once a week.

The experiment was arranged in a factorial randomized complete design with two factors. The first factor was AMF inoculation treatment (–AMF and +AMF), and the second factor was N fertilization treatment (N0, N1, N2). Thence, 6 treatments and 5 replicates of each treatment, were generated yielding a total of 30 pots. The experiment was carried out in the greenhouse of Institute of Land Engineering and Technology, Shaanxi Srovincial Land Engineering Construction Group Co., Ltd., Shaanxi P.R., China from May 15, 2020 to July 30, 2020. The temperature was maintained between 18 and 25°C and photoperiod hrs 14/10 (day/night) with 25% relative humidity and the average of 120 μmol/m^2/s photosynthetic photon flux (PPF).

At the end of the experiment, plant shoots and roots were carefully separated. The fresh roots were cut into 1 cm segments in length and stained and mycorrhizal colonization was determined by examining root segments under the microscope (200x) (Bahadur *et al*. 2019). Subsequently, the shoots and remaining roots were kept in the oven for 48 hrs at 80 °C (Fariduddin *et al.* 2014), and then used for the determination of dry weight and shoot N and P content.

The mycorrhizal growth response (MGR), mycorrhizal N-uptake response (MNR) and P-uptake response (MPR) of plants were used to evaluate the effect of AMF to plant biomass and tissue contents of N or P under different N treatments, and they were calculated according to the formulas of Veiga *et al*. (2011) and Jiang *et al.* (2018).

Statistical analyses were carried out using IBM SPSS Statistics 19.0 (StatSoft Inc., Tulsa, USA). All measured data were tested for normality before data analysis. The effects of N and AMF treatments on root length colonization, plant biomass and N/P content were analyzed using linear mixed-effects models. "t-test" was used to analysis significant differences in biomass, N and P content, MGR, MPR and MNP of *S. bicolor* between +AMF and -AMF treatments.

Results and Discussion

Mycorrhizal colonization of –AMF treatments was zero in all cases, but all +AMF pots showed high levels of colonization (Fig.1). N addition significantly affect the root length colonization (RLC) (Fig.1a; $F = 4.2$, $P = 0.023$) and vesicles colonization (VC) (Fig.1c; $F = 6.4$, $P = 0.004$) except for arbuscular colonization (AC) (Fig.1b; $F = 2.4$, $P = 0.102$). The RLC has a downward trend with the N application; the highest RLC at N0 (66.78%), and the lowest at N1 (47.56%). Similarly, the highest VC was discovered at N0 (38.05%), and observed lowest at N1 (22.81%). The decrease of mycorrhizal colonization with N addition due to N enrichment of systems reduces host plant carbon allocation to AMF (Johnson 2010).

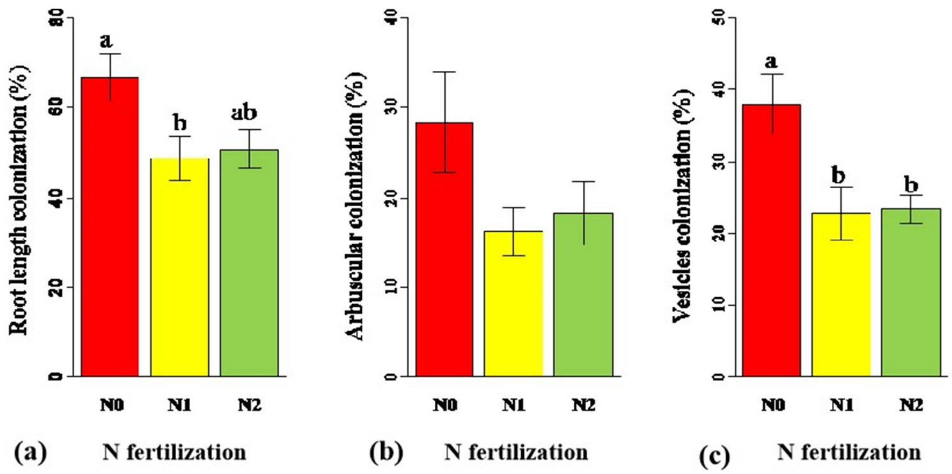

Fig.1. Effects of nitrogen (N) addition and AMF inoculation on mycorrhizal root colonization. (a). Root length colonization (RLC), (b). Arbuscular colonization (AC) and (c). Veslcles colonization (VC). Bars represent means ± se (n = 5). Different letters represent significant difference ($P < 0.05$) between bars.

Both AMF inoculum and N addition had a significant effect on plant total biomass, shoot and root biomass (all $P < 0.05$). Inoculation with AMF significantly increased *S. bicolor* total biomass, shoot and root biomass at a same N addition level except for root biomass at N1 treatment. Interestingly, shoot, root and total biomass of *S. bicolor* increased by about 3 times at the N0 and N2 treatment after inoculating AMF, while they increased by less than double at the NI treatment (Fig. 2). It is understood that N and P are important elements to participate metabolism and synthesis of tissues of organs. Increased biologically available N, P uptake through AMF extramycelium might have contributed to increase synthesis of prerequisite of chlorophyll and enhanced photosynthesis, and eventually lead to an increase in biomass (Begum *et al*. 2019).

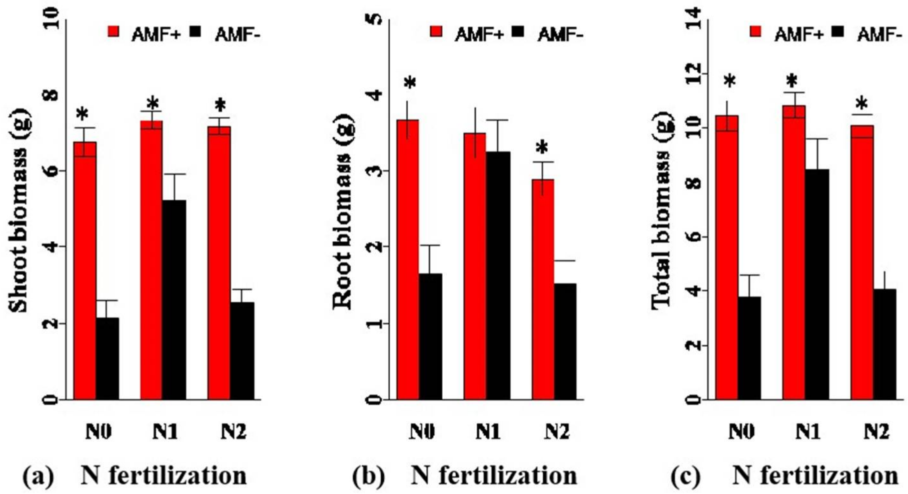

Fig. 2. Effects of nitrogen (N) addition and AMF inoculation on biomass of *S. bicolor*. (a). Shoot biomass, (b). Root biomass and (c). total biomass. Bars represent means ± se (n = 5). * indicate significant difference between +AMF and –AMF treatment ($P < 0.05$).

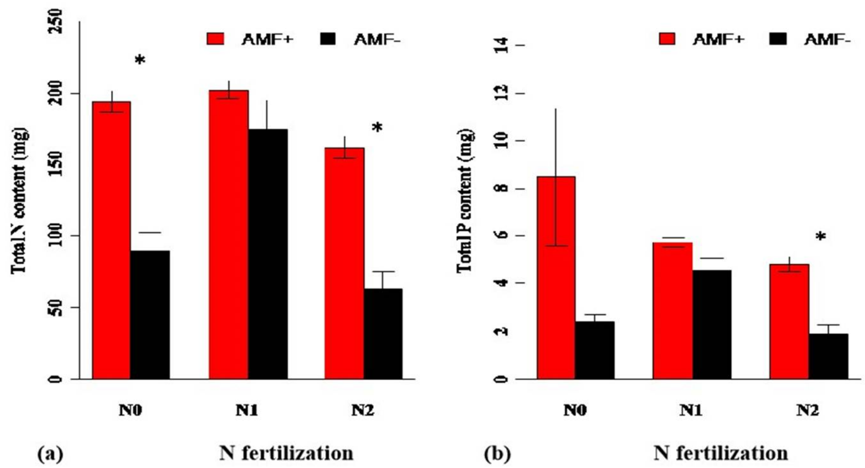

Fig. 3. Effects of nitrogen (N) addition and AMF inoculation on total N and P content of *S. bicolor.* (a) total nitrogen (N) content and (b) total phosphorus (P) content. Bars represent means ± se (n = 5). * indicate significant difference between +AMF and –AMF treatment ($P < 0.05$).

Inoculation with AMF lead to a significant total N content increase in all cases except N1 treatment. Under N0 treatment, inoculating AMF increased the total N content more than twice, rise from 86.77 to 191.65 mg per pot, and the total N content more than doubled (Fig.3a). Analogously, total N content were significantly lower on -AMF than on +AMF treatment under N2 treatment (Fig.3a). According to the results of linear mixed-effects models, inoculating AMF significantly affect issue total P content (Fig.3b), while N addition showed no statistically significance (Fig.3b). Compared to N0 and N1 treatment, significant difference was discovered according to the *t-test* (Fig.3b). Inoculation with AMF help to establish a mutually beneficial symbiotic relationship between AMF and host plant, by which AMF provide inorganic nutrients to host plant (van der Heijden *et al.* 1998). This is why total N and P content of *S. bicolor* in +AMF treatment was significantly higher than –AMF treatment.

Figure 4 showed that AMF have a positive effect on MGR, MNR and MPR of *S. bicolor* and MR except MNR at N1 treatment. MR all showed a trend of first decreasing and then increasing, and the lowest value was at N1 treatment. Jiang *et al.* (2018) showed that MR declined with N addition. However, in the present study MR strangely increased instead of continuing to decline with the N gradient continued to increase. There are two possible reasons for the analysis: on one hand, AMF need numerous N for their survival, presumably by which alleviates the stress of high soil N levels on the host plant (Castellanos-Morales *et al.* 2010). On the other hand, AMF can improve the absorption of P to better maintain the N to phosphorus ratio of the plant, thereby helping the host plant to resist the stress of high N.

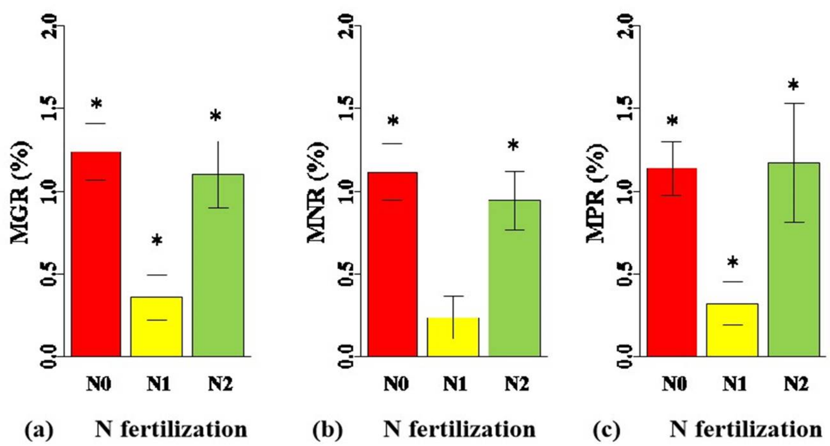

Fig.4. Mycorrhizal growth response (MGR, a), mycorrhizal N uptake response (MNR, b) and mycorrhizal P uptake response (MPR, c) of *S. bicolor* in glasshouse. * indicates a significant difference between the mycorrhizal response and zero according to *t-test* ($P < 0.05$).

It may be interred that AMF improved the growth performance and nutrient uptake (N, P) of *S. bicolor* at almost all treatment. Furthermore, it also demonstrated that N addition induced a great shift on MGR, MNR and MPR, which unanimously reduced first and escalated later. In addition, the present study also provided the first pot-based evidence that AMF can alleviate the mischief induced by high N addition, implying that AMF has an considerable significance in the farmland ecosystem under gradually increased anthropogenic N deposition. Considering the ecological significance of AM symbiosis in nature, future studies are needed to address how and to what extent N-induced AMF could affect crop growth in the field test.

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