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Effect of inoculation with arbuscular mycorrhizal fungi on growth of *Catalpa bungei*

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Abstract

Background: *Catalpa bungei* is a well-known, valuable, ornamental, high-quality timber tree traditionally cultivated in China because of its excellent quality, decay resistance, wide applicability, and attractive form. The aim of this study was to evaluate the effects of inoculation with arbuscular mycorrhizal fungus (AMF) on the transplant survival rate and growth of *C. bungei* seedlings under greenhouse conditions.

Methods: Two treatments were applied in a completely randomised experimental design: inoculation with *Rhizophagus intraradices*, and, as a control, soil without inoculum.

Results: Four months after inoculation, AMF had colonised 76.05% of plant roots and significantly improved plant growth. With the establishment of the symbiotic relationship, AMF inoculation significantly improved the seedling transplant survival rate by 20%, promoted major growth traits (plant height, basal diameter, leaf area, and specific leaf area), accelerated biomass accumulation (roots, stems, and leaves), and changed the biomass allocation patterns. In addition, compared with non-inoculated treatments, inoculation with AMF increased photosynthetic parameters and chlorophyll contents, elevated major root morphological parameters, changed the proportion of particle sizes of soil micro-aggregates, and promoted the accumulation of nutrients in roots and leaves.

Conclusions: The effects on transplant survival, growth and development were more pronounced in *C. bungei* seedlings inoculated with *R. intraradices*. Mycorrhizal seedlings of *C. bungei* can therefore be widely applied in plant transplantation and production practices.

Keywords: *Rhizophagus intraradices*, seedling production, transplant survival, growth performance, soil micro-aggregates.

Introduction

Climate change is projected to alter precipitation amounts and patterns and soil moisture availability, all of which have direct impacts on plant growth and nutrient acquisition and, potentially, ecosystem functions such as nutrient cycling and losses (Bowles et al. 2018). Soil microorganisms are widely recognised as playing an important role in the biogeochemical cycling of soil inorganic and organic nutrients and maintenance of soil quality (Jeffries et al. 2003). Some telluric beneficial microorganisms, especially bacteria and fungi, can overcome adverse environmental effects and improve plant performance (Lenoir et al. 2016). Among them, arbuscular mycorrhizal fungi (AMF), which are naturally occurring soil fungi, form a mutualistic symbiosis with the roots of over 80% of terrestrial plant species (Smith & Read 2008; H.H. Zhang et al. 2018; Ye et al. 2019) and are widely used in agriculture, horticulture, and landscape restoration (Vani et al. 2018).

Reciprocal nutrient exchange is the foundation of a stable symbiosis between AMF and most land plants (Kiers et al. 2011; Roth & Paszkowski 2017). On the one hand, AMF are obligate mutualists that benefit from host plants through the receipt of organic carbon

in the form of glucose and lipids (Keymer & Gutjahr 2018; Adeyemi et al. 2020; Tchiechoua et al. 2020). On the other hand, the hyphae of AMF are an important channel for soil nutrients and water to enter plants (Karagiannidis et al. 2002; H.H. Zhang et al. 2018) and can extend several centimeters into the soil (Vani et al. 2018). AMF hyphae can therefore contribute to plantlet growth (Wicaksono et al. 2018; Toprak, 2020; Chenchouni et al. 2020), root morphogenesis (Giri et al. 2003; Gamalero et al. 2004; Shao et al. 2018), uptake of mineral nutrients, such as phosphorus (P), nitrogen (N), and micronutrients (Gamalero et al. 2004; Koch et al. 2006; Machineski et al. 2018; Pel et al. 2018), improvement of plant photosynthetic capacity and PSII function (Chen et al. 2017; Mathur et al. 2018), increased disease resistance (Douds et al. 2016; Jacott et al. 2017), stabilisation of soil aggregates (Wright & Upadhyaya 1998; Miller & Jastrow 2000; Rillig et al. 2002; Zhang et al. 2016), and enhancement of environmental stress tolerance (Zhang et al. 2014; Bitterlich et al. 2018; Turrini et al. 2018). Inoculation with AMF may therefore increase the survival and establishment of seedlings following transplantation (Machineski et al. 2018) via the mycorrhizal pathway and through indirect effects of morphological and physiological changes to roots (Cavagnaro 2008; Chen et al. 2014). In recent years, researches on the effects of AMF on plant growth and physiological characteristics have been widely carried out and successfully contributed to the cultivation of fruit trees (Wu & Xia 2006; Machineski et al. 2018), vegetables (Estrada-Luna & Davies 2003; Douds et al. 2016), and other trees (Zhang et al. 2016; Wicaksono et al. 2018; Toprak, 2020).

Catalpa bungei, native to China, is an important tree species widely planted in warm temperate and subtropical regions of the country (Qiu et al. 2015). *Catalpa bungei* has the advantages of wide distribution, fast growth, and strong adaptability (Wu et al. 2018; E.L. Zhang et al. 2018). In addition, *C. bungei* is a valuable, well-known ornamental, high-quality timber tree traditionally cultivated in China because of its excellent quality, decay resistance, wide applicability, and attractive form (Shi et al. 2017; Zheng et al. 2017). Nevertheless, afforestation with *C. bungei* is limited because of its self-sterility, poor seed production, and low germination and seedling emergence rates (Meng et al. 2020; Xiao et al. 2019). The development of new methods and techniques is therefore needed to improve the survival rate and growth performance of *C. bungei* seedlings used for afforestation in northern China. In recent studies, drought stress (Luo & Luo 2017; Shi et al. 2017; Zheng et al. 2017; Huang et al. 2019), light conditions (Wu et al. 2018), and fertiliser application (Wang et al. 2012a; Qiu et al. 2015) have been shown to impact the growth of *C. bungei* seedlings. Limited information is available, however, on how AMF affect *C. bungei* seedling growth and performance. Wang et al. (2012b) reported that inoculation with the endomycorrhizal fungus *Glomus mosseae* had a significant effect on the root morphology of *C. bungei* under drought stress, whereas the ectomycorrhizal fungus *Pisolithus tinctorius* had no

significant influence. Consequently, the aim of the present study was to evaluate the effects of inoculation with AMF on *C. bungei* seedling survival, growth, root morphology, photosynthesis, and nutrient contents and soil micro-aggregates under greenhouse conditions. The ultimate goal of this research is to improve the survival ability of *C. bungei* seedlings, reduce seedling production costs, shorten the growth cycle, and increase the application of mycorrhizal seedlings in production practice.

Methods

Experiment design
Pot experiments using tissue culture seedlings of *C. bungei* were conducted in a greenhouse. To minimise the genetic differences and their influence on the experiments, the *C. bungei* genotype Hybrid No. 2 was used, which was provided by the Henan Academy of Agricultural Sciences, China. *Rhizophagus intraradices* was obtained from the Beijing Academy of Agriculture and Forestry Sciences, Beijing, China. The *R. intraradices* inoculum consisted of a mixture of rhizospheric soil from potted *Zea mays* cultures containing spores, hyphae, and mycorrhizal root fragments. Cylindrical plastic resin flowerpots (20 cm depth and 19 cm diameter) were sterilised with 0.5% sodium hypochlorite for 48 h. For use as a substrate, soil was collected from an area of cultivation in Yangling, Shaanxi Province. The soil, with a low nutrient content, was typical of northwestern China. In addition, river sand was washed 8–9 times with water and dried under natural light. After filtering through a 2-mm sieve, the soil and river sand were sterilised at 0.1 MPa and 121 °C for 2 h and then used in a proportion of 1:1 (v:v) as the substrate for cultivation. Total N and available P and potassium (K) in the substrate were 830, 25.43, and 183.88 mg/kg, respectively. Two treatments were applied: inoculation with *R. intraradices*, and, as a control, soil without inoculum. To establish AMF symbiosis, each plastic pot was filled with 4.0 kg substrate and 5 g AMF inoculum, with an equivalent amount of inactivated inoculum used for the control. During transplantation, inoculum was spread evenly on the roots of *C. bungei* seedlings to ensure complete contact. We transplanted 100 AMF-treated seedlings and 100 non-treated ones. Pots were arranged in a completely randomised experimental design. All plants were well watered, and Hoagland's nutrient solution (Hoagland & Arnon 1950) was applied every 2 weeks throughout the plant growth period to ensure nutrient supply. The experiment was conducted in the greenhouse in Yangling, Shaanxi Province, China (34°15′59″N, 108°03′39″E), from March to July 2019. After 2 months, the transplant survival rate of the seedlings was measured. Ambient conditions during the treatment period were as follows: day temperature, 20–30 °C; night temperature, 10–20 °C; and relative humidity, 40%–85%.

Measurement of mycorrhizal colonisation
Plant roots were collected, rinsed with running water, placed in a clean centrifuge tube, and softened in 10% KOH at 90 °C for 30 min. After roots became transparent,

segments were stained with trypan blue (0.05%) at 90 °C for 30 min and then bleached three times with a 1:1 (v/v) mixture of lactic acid and glycerin. Fungal colonisation rate (Col) was estimated according to Trouvelot et al. (1986). For the Col measurements, at least 100 stained root segments were examined under a digital computerised microscope (model TL4, Olympus Bx43, Japan) at 400× magnification. The percentage of mycorrhizal structures in each 1-cm root fragment was assessed as 0%, 10%, 20%...100%. Col (%) was calculated as $\sum(0\% \times N_0 + 10\% \times N_{10} + 20\% \times N_{20} + \cdots 100\% \times N_{100}) / (N_0 + N_{10} + N_{20} + \cdots N_{100})$, where N represents the number of root segments (Mcgonigle et al. 1990).

Plant growth variables

Prior to harvest, plant heights were measured with a ruler, and basal diameters were determined with a vernier caliper. Fresh leaves collected from treated plants were immersed in water for 24 h and then removed. After quickly blotting with absorbent paper to remove surface water, leaves were weighed on an electronic balance to determine their saturated fresh weights. Leaf area was measured by the transparent grid method (Gao, 2006). Leaves were then dried in a 75 °C oven for 48 h and weighed. Specific leaf area (SLA), which is the ratio of the area of a single leaf and its dry weight, was determined. We also calculated specific leaf weight (SLW), the dry weight of a leaf per unit area— that is, the ratio of the dry weight of a single leaf to its area. Leaf relative water content (LRWC) was calculated by the following equation: LRWC (%) = $100 \times$ (FW – DW) / (SW – DW). In this equation, FW, DW, and SW are leaf fresh weight, dry weight, and saturated fresh weight, respectively.

Leaves, stems, and roots were harvested separately and dried for 48 h in an oven at 80 °C to a constant weight. Total biomass and the following biomass distribution ratios were determined: root mass ratio (RMR; the ratio of root biomass to total biomass), stem mass ratio (SMR; the ratio of stem biomass to total biomass), leaf mass ratio (LMR; the ratio of leaf biomass to total biomass), root/leaf ratio (RLR; the ratio of root biomass to leaf biomass), and root/shoot ratio (RSR; the ratio of underground biomass to aboveground biomass).

Photosynthetic parameters and chlorophyll contents

Before harvest, gas exchange parameters, such as net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr), were measured using a LI-6400 portable open-flow gas-exchange system (LI-COR, USA) with an attached LED light source. The measurements were performed from 9:00 a.m. to 11:00 a.m. on the nine youngest leaves of each plant according to the method described by Cao et al. (2011).

Chlorophyll (Chl a and Chl b) and carotenoid contents were determined by the direct extraction method (Gao, 2006). Specifically, fresh leaves (0.2 g) were cut into small pieces (approximately 0.2 cm) and placed into 50-mL centrifuge tubes to which was added 0.5 mL of pure acetone and 10 mL of 80% (v/v) acetone. The tubes were incubated in darkness overnight at room temperature with shaking three to four times. Then, the leaf tissue had turned completely white, which indicated that the chlorophyll had been completely extracted. After filtration, the volume was fixed to 25 mL with 80% acetone, and the absorbance of the extracted mixtures was measured with spectrophotometer.

Root morphogenesis

Root morphological variables, including root length, root surface area, root volume, root tip number, branch number, and fine root length, were measured for five roots using a root scanner system (WinRHIZO 2013e, China).

Mechanical composition of soil micro-aggregates

First, we measured the glomalin content of the rhizosphere soil of the inoculated group. Secondly, mechanical compositions of soil micro-aggregates between inoculated and control groups were determined with a Marvin laser particle-size meter (MS2000, UK). The small stones and coarse roots were removed from seedling rhizosphere soil. The soil was transported to the laboratory and gently broken down along the natural structural surface of the soil into small clumps having a diameter of approximately 1 cm. After natural air drying, the soil was then used for determination of the mechanical composition of soil micro-aggregates. Two to three spoonfuls of each soil sample were placed in a disposable plastic cup preloaded with 150 ml distilled water. After 3 days, the scum on the top was discarded, and the residue was used for analysis.

Nutrient contents

All oven-dried plant samples were ground into a fine powder and passed through a 100-mesh screen. A representative sample (0.15 g) was digested with H_2SO_4–H_2O_2 in a digestion oven and subsequently diluted in deionised water to a final volume of 10 mL. After filtration of the digested solution, the N concentration was determined using an automatic Kjeldahl apparatus (KjeltecTM 8400, Foss, Sweden), and P concentration was quantified by molybdenum–antimony anti-colorimetry (Unico UV-2600A, Unico, US). Finally, the K concentration was measured by atomic absorption spectrometry (AA-7003A, Beijing, China).

Data statistics and analysis

SPSS 21.0 was used for ANOVA and LSD multiple comparisons ($P < 0.05$). Mapping was carried out in SigmaPlot 12.0.

Results

Root colonisation, transplant survival rate, and growth traits

Non AMF-treated seedlings exhibited no root colonisation by AMF (Fig. 1b), whereas clear colonisation by AMF was observed in AMF-inoculated seedlings. Mycorrhizal hyphae ultimately formed distinct morphological structures: intraradical hyphae (Fig. 1a), arbuscules
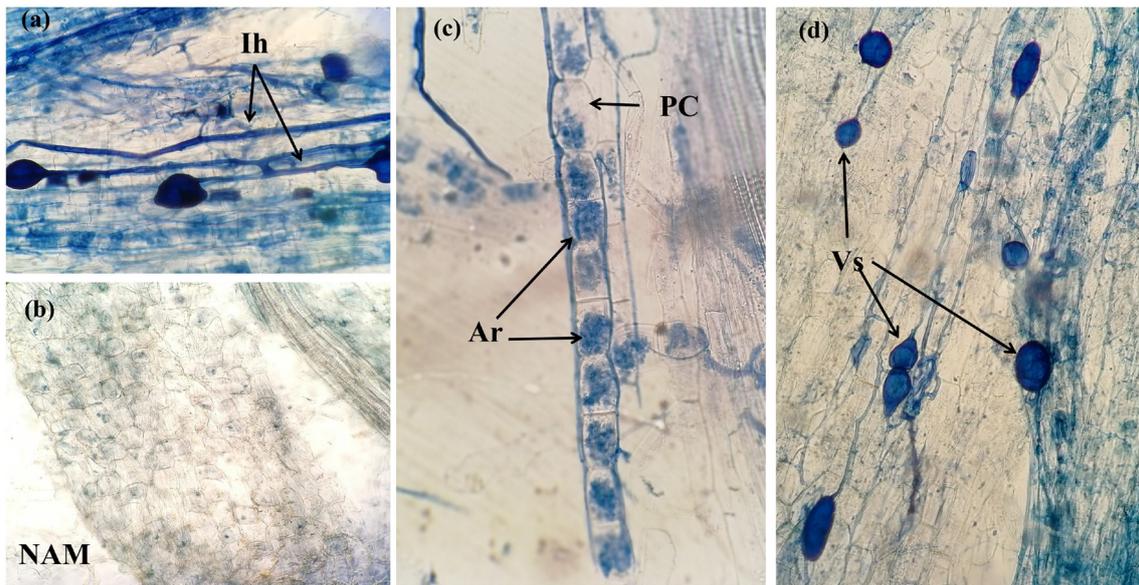
FIGURE 1: Photomicrographs of root colonisation of *Catalpa bungei* illustrating structural components of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus intraradices*. *Ih* intraradical hyphae; *NAM* non-AMF-inoculated; *PC* plant cell; *Ar* arbuscule; *Vs* vesicles; (**a**, **b**, **c** and **d**, G: ×400).

(Fig. 1c), and vesicles (Fig. 1d). The colonisation rate was 76.05% after inoculation (Fig. 2b). The survival rate of non-inoculated *C. bungei* seedlings was 53%. The survival rate after inoculation was 73%, which was 20% higher than the control (Fig. 2c). Moreover, AMF significantly affected the morphology of *C. bungei* seedlings (Fig. 2a). Four months after inoculation, major growth traits of *C. bungei* seedlings, such as plant height, basal diameter, leaf area, and SLA, were significantly higher in mycorrhizal seedlings than non-inoculated plants, in contrast, SLW was notably reduced (Fig. 3a–e). In particular, the plant height and basal diameter of inoculated plants were 4.0 and 2.25 times those of control plants, and the leaf area of a single plant and SLA were 16.4 and 1.23 times larger, respectively ($P < 0.05$). SLW, however, was significantly decreased, by 20.04%,
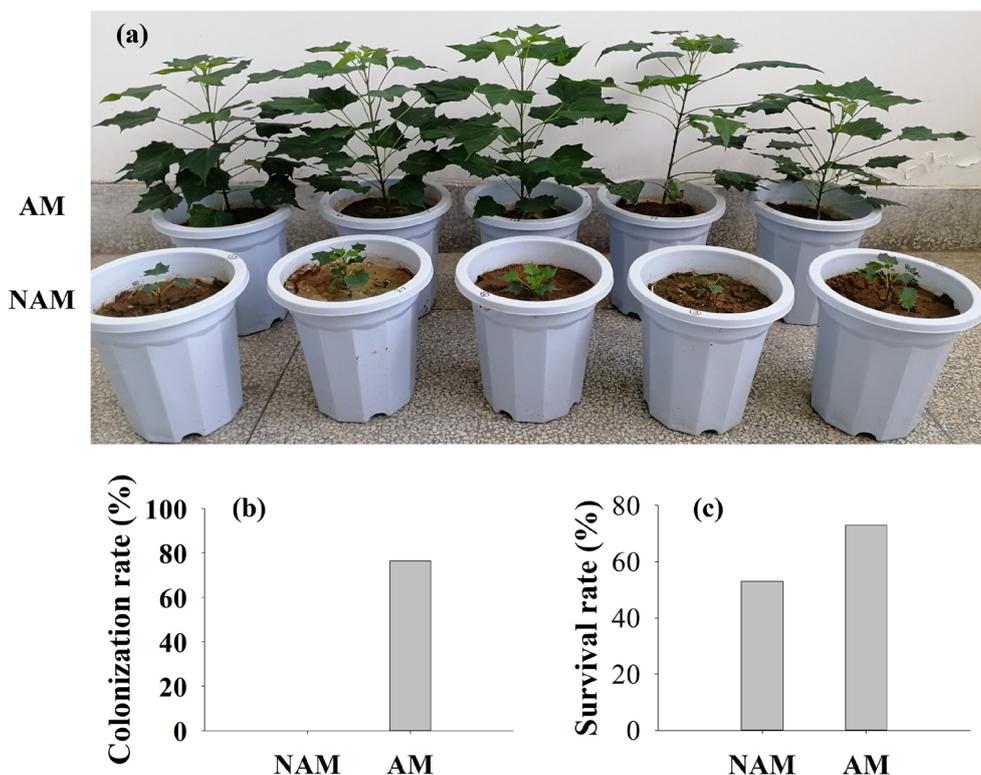

FIGURE 2: Effect of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus intraradices* on the: (**a**) morphology, (**b**) colonisation rate; and (**c**) survival rate of *Catalpa bungei* seedlings. *NAM* non-AMF-inoculated; *AM* AMF-inoculated.

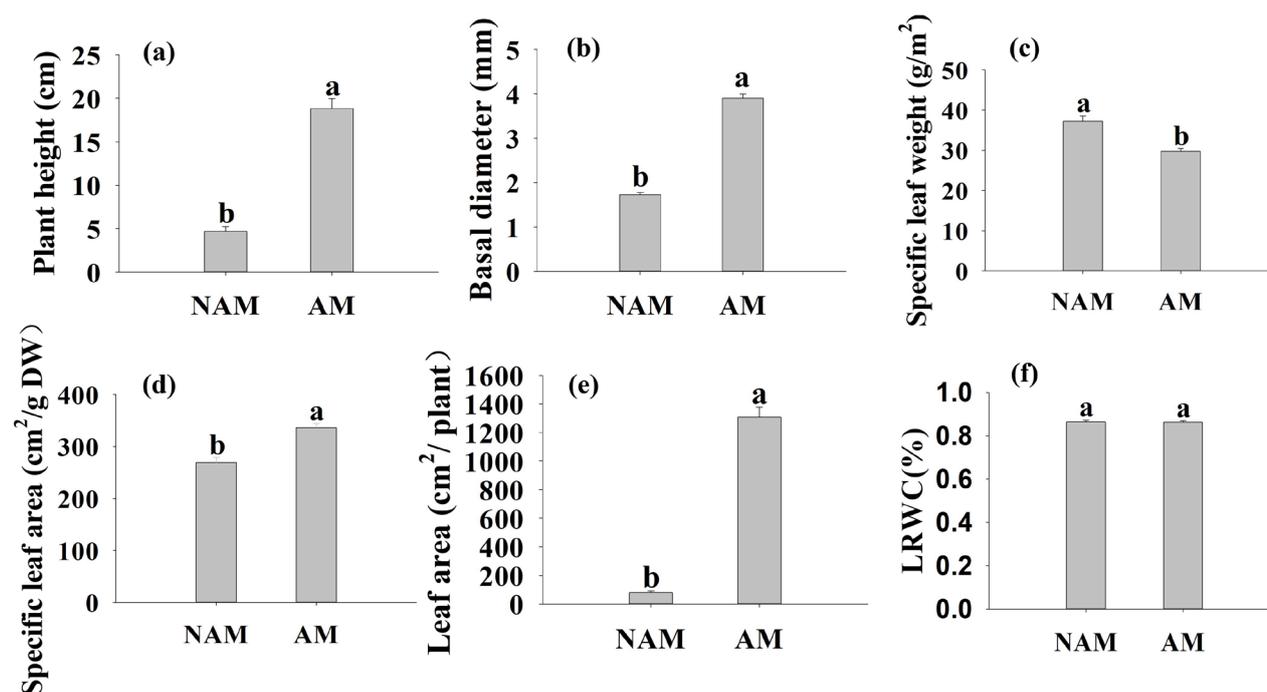FIGURE 3: Effect of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus intraradices* on: (**a**) plant height; (**b**) basal diameter; (**c**) specific leaf weight (SLW); (**d**) specific leaf area (SLA); (**e**) leaf area; and (**f**) leaf relative water content (LRWC) of *Catalpa bungei* seedlings. Different lowercase letters above bars indicate significant differences ($P < 0.05$) between AMF-inoculated (AM) and non-AMF-inoculated (NAM) treatments. Values are means ± SE. Plant heights and basal diameters are based on 15 biological replicates, while SLW, SLA, leaf area, and LRWC used 3 biological replicates.

compared with the control group, while no significant difference in LRWC was observed ($P > 0.05$) (Fig. 3f).

Biomass accumulation and allocation patterns
Compared with the control, inoculation with AMF significantly promoted root, stem, leaf, and total biomass accumulations of *C. bungei* seedlings (Table 1). The total biomass of AMF-inoculated seedlings was 6.82 g per plant, which was 4.6 times higher than that of the

control. Furthermore, the leaf, stem, and root biomass of a single plant inoculated with AMF was 3.76 g, 1.6 g, and 1.46 g, respectively, which corresponded to 7.0, 3.3, and 3.2 times higher than the control ($P < 0.05$). Four months after AMF inoculation, the biomass allocation pattern of *C. bungei* seedlings was significantly different from that of the control (Table 1). To a large extent, the fraction of biomass in leaves was usually higher in inoculated plants than in non-treated ones, while the

TABLE 1: Effect of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus intraradices* on biomass accumulation and allocation patterns of *Catalpa bungei* seedlings.

Index		Treatment	
		NAM	AM
Biomass (g)	Root biomass	0.46±0.07b	1.46±0.14a
	Shoot biomass	0.48±0.16b	1.6±0.14a
	Leaf biomass	0.54±0.15b	3.76±0.20a
	Total biomass	1.49±0.32b	6.82±0.32a
Biomass allocation	RMR	0.35±0.05a	0.21±0.02b
	SMR	0.29±0.07a	0.23±0.02b
	LMR	0.37±0.06b	0.55±0.02a
	RLR	1.12±0.29a	0.39±0.04b
	RSR	0.56±0.11a	0.27±0.03b

Different lowercase letters in each row indicate significant differences ($P < 0.05$) between AMF-inoculated (AM) and non-AMF-inoculated (NAM) treatments. *RMR* root mass ratio; *SMR* shoot mass ratio; *LMR* leaf mass ratio; *RSR* root/shoot ratio. Values are means ± SE ($n = 5$).

TABLE 2: Effect of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus intraradices* on photosynthetic parameters and chlorophyll contents of *Catalpa bungei* seedlings.

Index		Treatment	
		NAM	**AM**
Photosynthesis	Pn [μmol/(m²·s)]	5.75±1.12b	8.88±0.74a
	Gs [mol/(m²·s)]	0.08±0.02a	0.10±0.01a
	Ci (μmol/mol)	279.29±6.75a	239.59±4.46b
	Tr [mmol/(m²·s]	2.13±0.37a	2.53±0.26a
Chloroplast pigment content	Chlorophyll a content (mg/g)	1.60±0.09b	1.92±0.07a
	Chlorophyll b content (mg/g)	0.48±0.02b	0.58±0.02a
	Carotenoid content (mg/g)	0.32±0.02b	0.41±0.02a
	Chlorophyll a/b	3.30±0.04a	3.33±0.010a

Different lowercase letters in each row indicate significant differences ($P < 0.05$) between AMF-inoculated (AM) and non-AMF-inoculated (NAM) treatments. *Pn* net photosynthetic rate; *Gs* stomatal conductance; *Ci* intercellular CO_2 concentration; *Tr* transpiration rate. Values are means ± SE ($n = 5$).

fraction in stems and roots was usually higher under non-inoculated conditions. More specifically, LMR increased by 48.65% compared with the control group, whereas SMR and RMR decreased by 20.69% and 40%, respectively ($P < 0.05$). Most of the biomass of the seedlings was distributed in leaves, with the distribution to stems and roots having decreased. In addition, RLR and RSR decreased significantly after AMF inoculation, by 65.18% and 51.79%, respectively, compared with the control ($P < 0.05$).

Photosynthesis and chlorophyll contents
Inoculation with AMF had a significant effect on the gas exchange parameters and photosynthetic pigment contents of *C. bungei* seedlings (Table 2). AMF inoculation significantly increased the Pn of seedlings, by 54.43%, compared with the control group ($P < 0.05$). Gs and Tr also increased, by 25% and 18.78%, respectively, but the differences were not significant ($P < 0.05$). Ci was significantly reduced, by 14.21% ($P < 0.05$). Compared with the control group, inoculation with AMF significantly
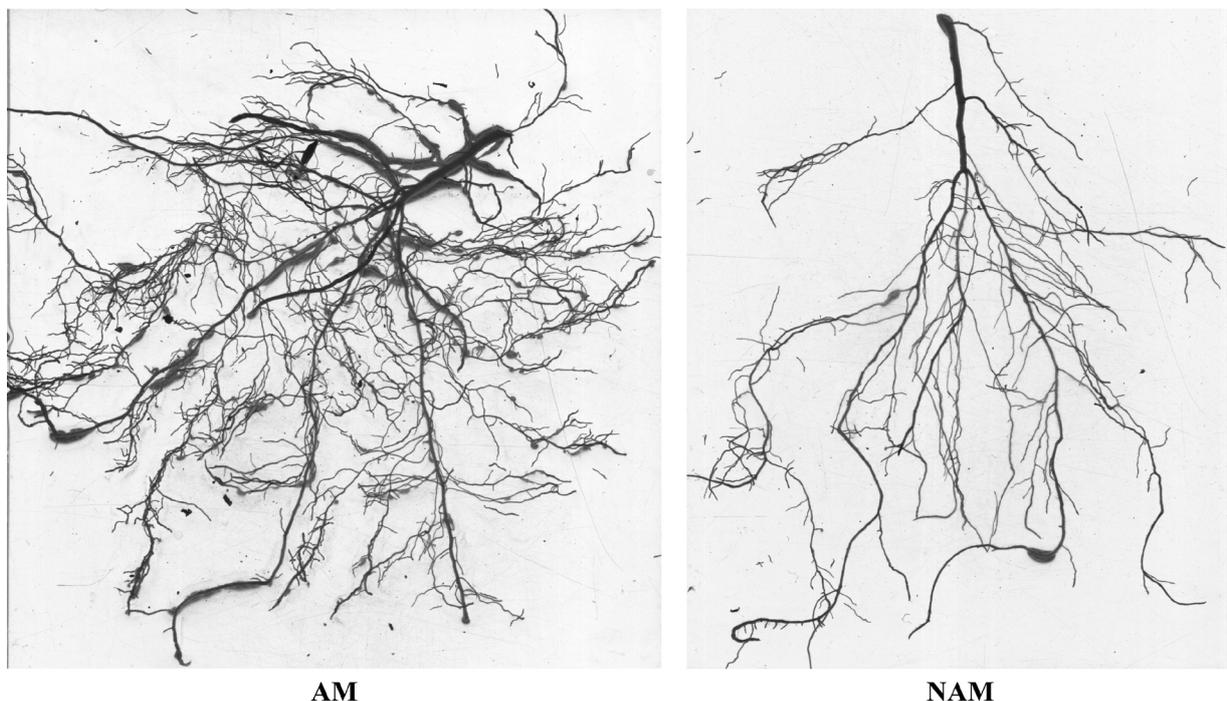

AM   **NAM**

FIGURE 4: Effect of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus intraradices* on the root morphology of *Catalpa bungei* seedlings. *NAM* non-AMF-inoculated; *AM* AMF-inoculated.

TABLE 3: Effect of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus intraradices* on root morphological parameters of *Catalpa bungei* seedlings.

Index	Treatment	
	NAM	AM
Total root length (cm)	751.88±162.11b	2 929.39±355.38a
Max root length (cm)	445.67±57.37b	817.37±44.08a
Total root surface area (cm²)	135.02±17.81b	594.98±57.40a
Root projected area (cm²)	47.39±10.02b	189.39±18.27a
Total root volume (cm³)	2.16±0.30b	9.79±0.85a
Mean root diameter (mm)	0.63±0.02a	0.66±0.02a
Root tips	1 135±244.31b	5 891±933.53a
Branching number	1 563±254.42b	11 000±1912.43a
LF (cm)	366.06±41.15b	1 844.72±262.61a
SAF (cm²)	36.53±4.18b	171.64±24.79a

Different lowercase letters in each row indicate significant differences ($P < 0.05$) between AMF-inoculated (AM) and non-AMF-inoculated (NAM) treatments. *LF* length of fine roots (0< d ≤ 0.5 mm); *SAF* surface area of fine roots (0< d ≤ 0.5 mm). Values are means ± SE (n = 5)

increased seedling chlorophyll a, chlorophyll b, and carotenoid contents, which were 20.00%, 20.83%, and 21.95% respectively higher than in the control group ($P < 0.05$). No significant difference in the ratio of chlorophyll a to chlorophyll b was detected ($P > 0.05$).
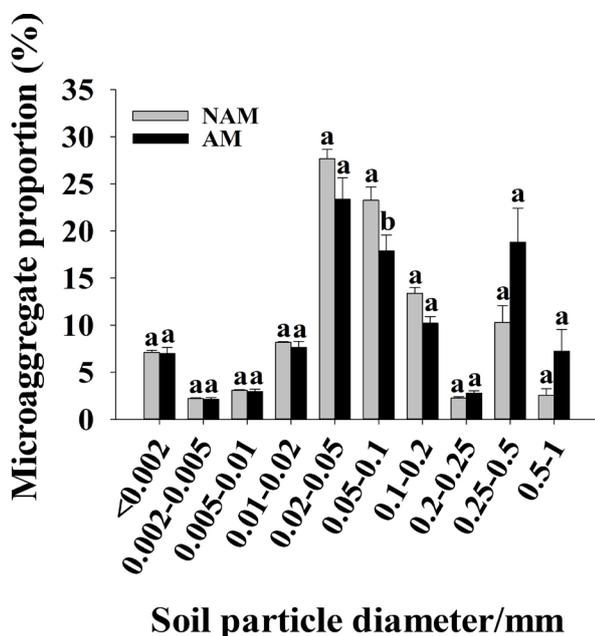

FIGURE 5: Effect of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus intraradices* on the mechanical composition of soil micro-aggregates. Different lowercase letters above bars indicate significant differences ($P < 0.05$) between AMF-inoculated (AM) and non-AMF-inoculated (NAM) treatments. Values are means ± SE (n = 3).

Root morphogenesis

Treatment with AMF significantly improved root development (Fig. 4). Four months after the inoculation treatment, major morphological parameters of the *C. bungei* root system, such as root length, root surface area, projected root area, root volume, root tip number, branch number, and fine root length and surface area had increased significantly under inoculated conditions ($P < 0.05$) (Table 3); the exception was mean root diameter, which was not significantly increased ($P > 0.05$). In particular, root tip and branch numbers were significantly higher than those of the control, 5.19 and 7.04 times, respectively, which indicates that AMF can significantly promote tiller root formation in *C. bungei* seedlings. Moreover, the length and surface area of fine roots of inoculated plants were 5.04 and 4.70 times those of the control group, respectively, thus indicating that AMF can enhance the production of fine roots in seedlings.

Soil micro-aggregates

The concentration of easily extracted glomalin was 325.63 μg/mL, while that of total glomalin was 334.64 μg/mL. Particle sizes of soil micro-aggregates in the non-inoculated group were mainly distributed between 0.02 and 0.2 mm, while those in the inoculated group were mainly concentrated in the range of 0.02 to 0.1 mm and also between 0.25 and 0.5 mm (Fig. 5). The proportion of soil micro-aggregates with a particle size of 0.02 to 0.1 mm was lower in the inoculation treatment than in the control; conversely, the proportion in the range of 0.25 to 0.5 mm was higher (Fig. 5).

Nutrient contents

Compared with levels in non-inoculated plants, the N content of roots and leaves of mycorrhizal plants increased by 86.90% and 31.07%, respectively, while

FIGURE 6: Effect of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus intraradices* on contents of: (**a**) nitrogen (N); (**b**) phosphorus (P); and (**c**) potassium (K) in *Catalpa bungei* seedlings. Different lowercase letters above bars indicate significant differences ($P < 0.05$) between AMF-inoculated (AM) and non-AMF-inoculated (NAM) treatments. Values are means ± SE (n = 3).
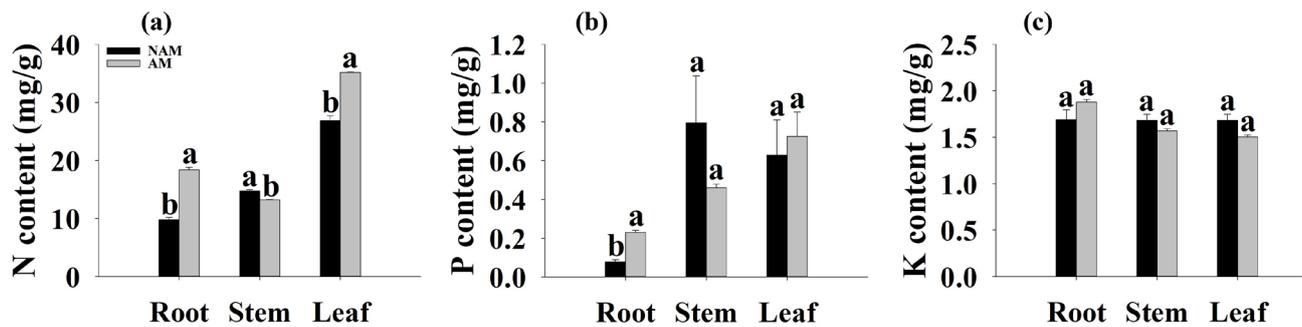
that of stems decreased by 10.22% ($P < 0.05$) (Fig. 6a). Compared with the control, the P content of leaves and roots increased by 1.15 and 2.97 times, respectively. The P content of stems decreased by 42.25%, but the difference was not significant ($P > 0.05$) (Fig. 6b). After AMF inoculation, the K content of roots increased and that of stems and leaves decreased, but none of these differences were significant ($P > 0.05$) (Fig. 6c).

Discussion

Most plants live in close collaboration with a variety of soil organisms. Among them, AMF play a remarkable role in plant growth, development, and productivity, as they can help their host plants better acclimatise to biotic and abiotic conditions (Lenoir et al. 2016; Machineski et al. 2018; Vani et al. 2018).

The role of AMF in promoting plant growth has been confirmed in several plant species, such as *Cyclobalanopsis glauca* (Zhang et al. 2014), *Camellia sinensis* (Shao et al. 2018), *Cedrus libani* (Toprak 2020), and *Prunus africana* (Tchiechoua et al. 2020). In the present study, AMF was able to form a good mutualistic symbiosis with *C. bungei* seedlings and inoculation significantly improved the survival rate of seedlings, in agreement with previous investigations (Lata et al. 2003; Yadav et al. 2013). This enhancement may be due to the fact that AMF can promote the absorption of water and nutrients by seedlings, thus helping them better adapt to the environment. In our study, major growth traits of *C. bungei* seedlings, such as plant height, basal diameter, leaf area, and SLA, were significantly higher in mycorrhizal seedlings than non-inoculated plants, while SLW was notably reduced; these observations are consistent with previous research. The decrease of SLW may be because inoculation promoted cell expansion and reduced the number of cells per unit area; the cell wall then became thinner, and the number of cell layers also decreased, indicating that inoculation can reduce leaf density and thickness. SLA is a major leaf functional trait that mirrors plant nutrient retention capacity, reflecting the ability of plants to obtain environmental resources (Huang et al. 2010). Inoculation increased the SLA of seedlings, mainly by increasing the leaf area of single plants. This increase in SLA indicates that the ability of seedlings to capture light energy was enhanced. Furthermore, inoculation with AMF had no significant effect on the LRWC of *C. bungei*. This result is not in line with the findings of Wu and Xia (2006), who reported that *Citrus tangerine* seedlings inoculated with *Glomus versiforme* had a higher LRWC than corresponding non-AMF seedlings. These conflicting results may be due to various responses of AMF to different tree species.

In the current study, inoculation with AMF significantly promoted root, stem, leaf, and total biomass accumulations of *C. bungei* seedlings relative to the control. In regard to the positive effect of AMF on plant biomass accumulation, the present results are in agreement with the findings of Giri et al. (2003), Solís-Domínguez et al. (2011), Chen et al. (2014), and Chenchouni et al. (2020). The biomass ratio of plant organs can be used to analyse patterns of biomass distribution, with LMR, SMR, and RMR reflecting the proportion of biomass distributed in leaves, stems, and roots, respectively (Yang et al. 2010). The biomass allocation pattern is an important determinant of plant resource acquisition, competition, and reproductive capacity as well as a major indicator of plant competitiveness (Fan et al. 2017). During the growth process, trees constantly adjust the proportion of biomass allocated to each organ to maximise access to limited resources (Wang et al. 2018). In the present work, the fraction of biomass accumulated in leaves was overall usually higher in inoculated plants than in non-inoculated ones, whereas the fraction in stems and roots was generally higher under non-inoculated conditions. These results indicate that the biomass distribution pattern of *C. bungei* seedlings changed significantly after AMF inoculation. Most of the biomass of seedlings was distributed in leaves, but the distribution to stems and roots decreased; this indicates that the balance of carbon fixation and nutrient uptake of seedlings changed. In addition, RLR and RSR decreased significantly after AMF inoculation compared with the control. This result is consistent with the observations of Estrada-Luna and Davies (2003), who indicated that *Capsicum annuum* inoculated with AMF had a lower RSR compared with non-AMF plantlets. A study conducted by Gavito et al. (2000) additionally revealed that inoculation with *Glomus caledonium* significantly reduced the RSR of *Pisum sativum* L.

Inoculation with AMF increased Pn, Gs, and Tr and significantly decreased Ci. An increase in the Gs of the epidermis of seedling leaves would enhance the entry of CO_2 from the external environment into mesophyll cells, thereby providing sufficient raw materials for leaf photosynthesis. In addition, the observed reduction in Ci indicates that inoculation promoted the assimilation efficiency of CO_2, further increased photosynthetic efficiency, and indirectly allowed plants to obtain more carbohydrates. This observation is in accord with previous results reported by Wu and Xia (2006) in a study of tangerine (*Citrus tangerine*) colonised by *Glomus versiforme*. In addition, research on the effect of AMF on *Citrullus lanatus* L. uncovered higher photosynthesis-related parameters than those found under normal conditions (Ye et al. 2019). The above results indicate that AMF colonisation can improve gas exchange capacity by keeping stomata open, reducing stomatal resistance, and increasing transpiration fluxes. The symbiotic effect between AMF and plants may be related to endogenous hormone levels as well as water and nutrient absorption and transport in mycorrhizal plants (Chen et al. 2014). Chlorophylls are important photosynthetic pigments that participate in energy production via photosynthesis during plant growth and development (Alam et al. 2019). Estrada-Luna and Davies (2003) reported that *Capsicum annuum* inoculated with AMF had a greater leaf chlorophyll content compared with non-AMF plantlets. Lone et al. (2015) also discovered that the chlorophyll content of two mycorrhizal potato cultivars (*Solanum tuberosum*) was higher than that of non-inoculated plants. The findings of our study are in keeping with the results of previous studies, namely, AMF inoculation can promote an increase in chlorophyll contents.

Root morphology is often influenced by plant hormones, soil pH, and soil microorganisms (Shao et al. 2018). Previous studies have shown that AMF can modify the morphology and structure of plant roots (Yang et al. 2015). Bi et al. (2018) reported that single inoculation with *R. intraradices* significantly increased the root projected area, number of root tips, and root surface area of *Amygdalus pedunculata* compared with a non-inoculated control treatment. Analogously, inoculation of *Robinia pseudoacacia* L. (black locust) with *Rhizophagus irregularis* or *Glomus versiforme* elevated root morphological parameters. In that study, both AMF increased root lengths, average root diameters, root volumes, root tips, and root branching (Zhang et al. 2016). A study conducted by Giri et al. (2003), however, found that *Acacia auriculiformis* inoculated with two AMF, *Glomus fasciculatum* and *G. macrocarpum*, both alone and in combination, had a greater number of lateral roots. Mycorrhizal fungi decrease root-tip meristem activity, which leads to an increase in adventitious roots. These results indicate that AMF can strongly regulate root morphology, with the magnitude of the effect dependent on AMF species, soil water status, and host species. Results from the present study indicate that the major morphological parameters of the *C. bungei* root system, except for mean root diameter, were increased considerably under inoculated conditions 4 months

after treatment. Although AMF can influence root plasticity in different ways, the most common effects are increased root branching and improved ability to absorb and transport nutrients (Gamalero et al. 2004). In the present study, root tip and root branch numbers were significantly higher than those of the control, which indicates that AMF can significantly promote root tiller formation in *C. bungei* seedlings. Moreover, the length and surface area of fine roots of the inoculated plants were markedly higher than those of the control, thus indicating that AMF can enhance the production of fine roots in seedlings.

Soil structure is the core of soil and ecosystem functions because it controls water transport and gas and nutrient exchange (Wright & Upadhyaya 1998). Soil is also a large-scale carbon repository, with its carbon storage capacity dependent on soil structure to a large extent (Rillig et al. 2002). The soil aggregate is the basic unit of soil structure, and soil particle size distribution and stability are affected by the distribution of soil pores as well as water storage and movement (Zhang et al. 2016). Among fungi, AMF appear to be the most important mediators of soil aggregation (Rillig et al. 2002). Hyphae of AMF and fibrous roots can be regarded as a "sticky-string bag" contributing to the entanglement of soil particles to form macro-aggregates, a basic building block of soil structure (Miller & Jastrow 2000; Adeyemi et al. 2020). In the present study, the particle sizes of soil micro-aggregates in the non-inoculated treatment were mainly distributed between 0.02 to 0.2 mm, while those in the inoculation group were mainly concentrated between 0.02 and 0.1 mm and between 0.25 and 0.5 mm. The proportion of soil micro-aggregates with a particle size between 0.02 and 0.1 mm was lower under inoculated than non-inoculated conditions, whereas the opposite was true for the proportion between 0.25 and 0.5mm. These results indicate that AMF can hold soil particles together, a finding similar to the observations of Zhang et al. (2016) for black locust colonised by *R. irregularis* or *G. versiforme*. This outcome may be due to the formation of a skeleton structure by AMF through the physical entanglement of its extraradical mycelium. This action is also conducive to the formation of micro-aggregates and the transformation of smaller micro-aggregates into macro-aggregate structures.

Earlier studies have shown that AMF can promote plant absorption of nutrients from the soil via external hyphae (Estrada-Luna & Davies 2003). Zhang et al. (2014) discovered that *Cyclobalanopsis glauca* inoculated with *Glomus mosseae* and *Glomus intraradices* enhanced P and K contents of seedling shoots under both well-watered and drought-stress conditions. Machineski et al. (2018) reported that inoculation with *Dentiscutata heterogama* and *Acaulospora morrowiae* increased levels of all estimated nutrients in *Diospyros kaki* L. roots. Shao et al. (2018) conducted similar research on *Camellia sinensis*, and the results suggested that leaf N, P, K, and other trace elements were significantly higher in AMF-inoculated plants than in non-inoculated ones. A recent study conducted by Toprak (2020) also found that mycorrhizal-inoculated Taurus cedar (*Cedrus libani*)

seedlings had higher nutrient concentrations than non-inoculated seedlings. In the present work, root N, P, and K contents of *C. bungei* seedlings increased after AMF inoculation compared with the control. N, P, and K contents decreased in stems, while leaf N and P contents increased and leaf K decreased. These results indicate that AMF can promote nutrient accumulation in roots and leaves. The higher nutrient contents in response to AMF treatment may be due to the larger absorption area in mycorrhizal plants available to external hyphae (Zhang et al. 2014).

With the assistance of AMF, the growth and performance of *C. bungei* seedlings was greatly promoted. This finding suggests that AMF can be applied to *C. bungei* plantations in northern China. The ubiquitous symbiosis between AMF and plants, however, is characterised by low host specificity, although plant responses may differ under variable environmental conditions (Bitterlich et al. 2018). The effect of AMF on *C. bungei* growth and development therefore needs to be verified by field experiments. In addition, a plant species can coexist with different AMF, depending on specific conditions (Machineski et al. 2018). Because different AMF species may have different effects on plants, several AMF strains need to be tested.

Conclusions

In short, inoculation with *R. intraradices* had pronounced effects on the growth and development of *C. bungei* seedlings. Upon the establishment of a symbiotic relationship, AMF were able to (1) promote major growth traits (plant height, basal diameter, leaf area, and specific leaf area); (2) accelerate biomass accumulation (roots, stems, and leaves) and change biomass allocation patterns; (3) increase photosynthesis and chlorophyll contents; (4) change major morphological parameters (root length, root surface area, root area, root volume, root tip number, and branch number); (5) change the proportion of particle sizes of soil micro-aggregates; and (6) promote nutrient accumulation in roots and leaves.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

CW and GY obtained the funds to support the study. CW, PM and WC planned the experiments. PM and WC conducted the experiments. HF, SZ, JW, and WM analysed the data. WC and PM wrote the manuscript. CW, GY, WC and PM revised the manuscript. All authors read and approved the final manuscript.

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Data availability

The datasets analysed in the current study are available from the corresponding author on reasonable request.

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