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## The endomycorrhizal fungal species and their effects on the growth and nutrient characteristics of *Eucalyptus maidenii* seedlings in China

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**Abstract:** *Eucalyptus maidenii* plantations are typically fertilized to increase the yields of eucalyptus oil and timber. However, the application of chemical fertilizers is costly and can cause environmental pollution and soil erosion. Mycorrhiza fungi can facilitate the cultivation of optimal seedlings while promoting the growth of trees. To date, investigations into the inoculation of endomycorrhizal fungi to enhance the productivity of *E. maidenii* in Yunnan, China have not been undertaken.

The species/dominant species of endomycorrhizal fungi on *E. maidenii* in Yunnan were isolated and identified. The effect of endomycorrhizal fungi on *E. maidenii* was elucidated. The major species of endomycorrhizal fungi that affect *E. maidenii* were ascertained.

Bulk soil was collected from an *E. maidenii* plantation, and the species of endomycorrhizal fungi were investigated. The effect of the endomycorrhizal fungi on *E. maidenii* were analyzed via a single factor experimental design, where the number of spores of endomycorrhizal fungi in the seedling substrate were controlled. The major endomycorrhizal fungi species that affected *E. maidenii* were ascertained via stepwise regression analysis.

Five endomycorrhizal fungal species were isolated from the bulk soil, with the dominant being *Glomus aggregatum* and *Glomus mosseae*. With higher counts of endomycorrhizal fungal spores in the seedling substrate, the infection rate and intensity of seedlings were observed to increase, as well as the NPK concentrations of the bulk soil. With the increased nitrogen concentration in the bulk soil, the nitrogen concentration of the aerial components of the seedlings also increased. The NPK concentrations in the bulk soil and seedlings, the second order lateral roots, the root activity, and the seedling height were significantly promoted by the presence of endomycorrhizal fungi (the third experimental treatment).

The NPK concentrations of the bulk soil and seedlings, as well as the growth indices of seedlings increased through endomycorrhizal fungi inoculation. The major species of the endomycorrhizal fungi on *E. maidenii* were *G. aggregatum*, *Glomus multicaule*, and *Claroideoglomus etunicatum*.

**Keywords:** *Eucalyptus maidenii*, endomycorrhizal fungi, major species

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## Introduction

*Eucalyptus maidenii* has been extensively planted on a global scale due to its dual use for eucalyptus oil and timber. Owing to its broad ecological range, *E. maidenii* was initially introduced in Yunnan Province, China since 1951 (Wang, 2010; Zhou, 2011). At present, *E. maidenii* plantations are typically supplemented with chemical fertilizers to enhance the yields of eucalyptus oil and timber (Xiang et al., 2017; Omar et al., 2018). However, the application of chemical fertilizer is costly and can cause environmental contamination and soil erosion, which is not conducive to the sustainable management of *E. maidenii* (Ren et al., 2013). It was found that the mycorrhiza could facilitate the cultivation of optimum seedlings, while promoting the growth of trees (Hua, 1995).

Mycorrhizal fungi are important for trees, such as *Eucalyptus* and *Populus*, and they can improve the growth and stress resistance of trees (Lapeyrie & Chilvers, 1985; Lodge & Wentworth, 1990; Tan et al., 2000). *Eucalyptus* is a typical mycorrhizal nutritional plant, it has ectomycorrhiza, endomycorrhiza and dual mycorrhiza (Chen et al., 1998; Tan et al., 2000), and research has shown that inoculation with mycorrhizal fungi can positively affect *Eucalyptus* tree species; thus, it has evolved as a key technique toward matching tree species with mycorrhizal fungi in certain regions (Gong et al., 1997; Zhong et al., 2001). Currently, investigations into the effects of mycorrhizal fungi inoculation on *Eucalyptus globulus*, *Eucalyptus urophylla*, *Eucalyptus grandis* × *E.urophylla*, and other species of *Eucalyptus* have been conducted (Wang et al., 2003; Huang et al., 2008). However, investigations into the inoculation of endomycorrhizal fungi on *E. maidenii* in Yunnan, China have not been conducted as yet.

For this study, bulk soil was collected from an *E. maidenii* plantation at the Jindian tree farm in Kunming City, Yunnan Province. Endomycorrhizal fungi in the bulk soil were isolated, identified, and counted, whereafter the overall and dominant species were investigated. The effects of endomycorrhizal fungi on the growth indices and nutrient characteristics of the *E. maidenii* seedlings were analyzed via a single factor experimental design, where the populations of endomycorrhizal fungi in the seedling substrate were controlled.

The primary endomycorrhizal fungal species that facilitated growth promotion and nutrient absorption for *E. maidenii* seedlings were ascertained via stepwise regression analysis. The main objectives of this study were to: (1) isolate and identify the overall and dominant species of endomycorrhizal fungi on *E. maidenii* in Yunnan Province, (2) elucidate the effect of endomycorrhizal fungi on *E. maidenii* seedlings via a single factor experimental design, and (3)

determine the primary endomycorrhizal fungi species on the *E. maidenii* seedlings via stepwise regression analysis. This study provides a theoretical basis and practical guidance for the optimal cultivation of *E. maidenii* seedlings. The issues of soil degradation and the decline of forest productivity in *E. maidenii* plantations might be resolved through the use of mycorrhizal seedlings as afforestation materials.

## Methods

### Jindian tree farm profiles

Jindian tree farm is located in the northeast of Kunming City, Yunnan Province, and in north latitude 27 degrees 7 minutes and east longitude 102 degrees 43 minutes. The altitude of Jindian tree farm is 1,900–2,338 meters, the slope is 10–30 degrees, and the soil type is mountainous forest red soil. Jindian tree farm is located in the subtropical climate zone of China. *Pinus yunnanensis* natural forest, *E. maidenii* plantation, *Pinus armandii* plantation, and *Acacia mearnsii* plantation are distributed in Jindian tree farm, it is an important ecological protection barrier for Kunming City. The land of *E. maidenii* plantation was ever colonized by *P. yunnanensis* natural forest, then the native forest was cut down to grow *E. maidenii*. *E. maidenii* plantation is managed as an ecological forest, thus it has not being rotational felled, the trees of *E. maidenii* plantation are 23 yr old. Currently, investigations into the species of mycorrhizal fungi and the effects of mycorrhizal fungi inoculation on the trees in Jindian tree farm have not been conducted as yet.

### Bulk soil collection

The bulk soil collection zone was located at the *E. maidenii* plantation of Jindian tree farm. In April 2018, thirty *E. maidenii* samples were selected in the plantation for the extraction of bulk soil. In the canopy projection zone of each tree, about 1m from the trunk of the tree, approximately 500g bulk soil and fibrous roots were collected from each direction (i.e., east, south, west, and north), well mixed, and then placed into bags. Subsequently the bulk soil of each tree was taken back to the nursery, well mixed, and used for isolating endomycorrhizal fungi and cultivating seedlings.

### Isolation and identification of endomycorrhizal fungi

The endomycorrhizal fungi spores were isolated by the wet sieve method (Trappe & Schenck, 1982;

Zhu et al., 2001a). Using glycerine as the suspending agent and glass slides as carriers, the spores of endomycorrhizal fungi were observed under a microscope (Nikon YS100) ( $\times 400$ ). The endomycorrhizal fungi spores were identified according to their morphological characteristics (i.e., size, color, spore wall layers, thickness, spore inclusions) (Schenck & Perez, 1988; An et al., 1990; Redecker et al., 2013).

### Counting of endomycorrhizal fungi spores

Five grams of bulk soil was weighed, after which the resident endomycorrhizal fungi spores were isolated by the wet sieve method (Trappe & Schenck, 1982; Zhu et al., 2001a). Subsequently, the spores were counted under an anatomical lens (Nikon SMZ1500) ( $\times 10$ ), and the experiment was repeated three times.

$$\text{Spore density (pcs/g)} = \frac{\text{the number of spores}}{\text{the weight of bulk soil}}$$

### Cultivation of *Eucalyptus maidenii* seedlings

*E. maidenii* seedlings were cultivated in the permanent nursery of Southwest Forestry University in Kunming City, China. The seedlings were cultivated outdoors in full sun, the max, min, and mean temperatures were 30.3 °C, 2 °C, and 15.7 °C respectively during the experiment. The *E. maidenii* seeds were obtained from the Forest Seed and Seedling Station of Yunnan Province. The container seedlings of *E. maidenii* were cultivated via a single factor experiment, which was designed to include three experimental treatments. For the three experimental treatments, the weight of soil substrate per container was consistent at 1.5 kg. For the first experimental treatment, the seedling substrate was comprised of bulk soil that was sterilized at 121 °C for two hours, where the percentage of endomycorrhizal fungi was 0. For the second experimental treatment, the seedling substrate was comprised of bulk soil, half of which was sterilized at 121 °C for two hours, and half was not, where the percentage of endomycorrhizal fungi was 50%. For the third experimental treatment, the seedling substrate was comprised of bulk soil that was not sterilized, where the percentage of endomycorrhizal fungi was 100%. This experiment was designed by a random arrangement in the field, in triplicate, with thirty seedlings for each repetition. The seedling cultivation began in May 2018 and was concluded in March 2019. The containers of three experimental treatments were not fertilized at all

over the experimental period, and were watered with equal quantity duration of the experiment.

### Infection rate of endomycorrhizal fungi

At the completion of the experiment in March 2019, three seedlings were randomly selected to study the infection rate of endomycorrhizal fungi in each experimental treatment. The intact roots of the seedlings were dug out, after which the seedling substrate on the roots were gently washed off. The roots were then cut into several segments ( $\sim 1$  cm long), and placed into a FAA (Formalin–acetic acid–alcohol) fixative solution for 24 h. Afterwards, the roots were immersed in a 10% KOH solution and then a water bath at 90 °C for 1h. Subsequently, the roots were introduced into a 10% H<sub>2</sub>O<sub>2</sub> solution at room temperature for 20 min. The roots were then placed into a 1% HCl solution for 5 min, and then a 0.05% aniline blue solution, and water bath at 90 °C for 35 min for staining.

The roots were then soaked in lactic acid glycerol to remove any excess aniline blue, and used in the preparation of a glass slide (Berch & Kendrick, 1982; Schenck & Perez, 1988; Wu et al., 2014). The roots were observed under a microscope (Nikon YS100) ( $\times 400$ ), where the infection rate and intensity were calculated (Nicolson & Schenck, 1979; Li & Feng, 2001). According to the proportion of the number of root segments being infected to the number of root segments being observed, the infection intensities were distinguished by five grades (i.e., 0–5% for grade I, 6–25% for grade II, 26–50% for grade III, 51–75% for grade IV, and 76–100% for grade V).

$$\text{Infection rate (\%)} = \left( \frac{\text{the length of root segments being infected}}{\text{the length of root segments being observed}} \right) \times 100\%$$

### Determination of the growth and nutrient indices of *Eucalyptus maidenii* seedlings

At the completion of the experiment in March 2019, the seedling heights, stem diameters at ground level, dry matter weight (i.e., roots, stems, and leaves), and the number of primary and secondary lateral roots of the seedlings being cultivated under the three experimental treatments were measured. The experiments were conducted in triplicate, with thirty seedlings per repetition for the seedling heights, stem diameters at ground level, and the number of primary and secondary lateral roots, with twenty-seven seedlings per repetition for the dry matter weight (i.e., roots, stems, and leaves).

Three seedlings were randomly selected for each experimental treatment, and the roots of each seedling were weighed to 0.5 g for determining the root activity via the TTC (triphenyl tetrazolium chloride) method (Li, 2000). Briefly, this involved the reduction amount of TTC to reflect the activity of dehydrogenase in roots, and then explain the root activity. Subsequently, the roots, stems, and leaves of the seedlings were grinded, respectively, meanwhile the seedling substrate of each experimental treatment were grinded, respectively. The experiment was designed to be conducted in triplicate, after which the concentrations of total nitrogen (Kjeldahl method), total phosphorus (molybdenum antimony colorimetric method), total potassium (flame photometer), available phosphorus (sodium bicarbonate extraction – molybdenum antimony colorimetric method), available potassium (ammonium acetate extraction – flame photometer), and alkalitic nitrogen (alkali N-proliferation method) in the seedling samples and nursery substrates were determined, respectively (Li, 2000).

### Screening of major endomycorrhizal fungi species for their effect on the growth and nutrient indices of *Eucalyptus maidenii* seedlings

Correlation analyses were performed on the growth, biomass, root activity, and nutrient concentration of 11-month-old *E. maidenii* seedlings, with the species of endomycorrhizal fungi being identified in this study, respectively. According to the results of the correlation analyses, stepwise regression analyses were performed to establish regression equations, respectively. Based on the results of the correlation and stepwise regression analyses, the major species of endomycorrhizal fungi involved with the growth and nutrient absorption of the *E. maidenii* seedlings were ascertained. The data analyses were implemented by SPSS17.0 and Excel2007.

## Results

### Species of endomycorrhizal fungi in the *Eucalyptus maidenii* plantation

Five species of arbuscular endomycorrhizal fungi were isolated from the bulk soil of *E. maidenii*, which were derived from three genera (i.e., *Glomus*, *Claroideoglomus*, and *Septoglomus*) (Table 1). The morphological characteristics of the spores of these five species of endomycorrhizal fungi are shown in Fig. 1. Among the five species of endomycorrhizal fungi, the spore densities of *G. mosseae* and *G. aggregatum* were higher

Table 1. Species and spore density of endomycorrhizal fungi in the bulk soil of *Eucalyptus maidenii*

Genus	Species	Spore density of endomycorrhizal fungi (pcs/g)
Glomus	<i>Glomus mosseae</i>	32
	<i>Glomus multicaule</i>	10
	<i>Glomus aggregatum</i>	24
Claroideoglomus	<i>Claroideoglomus etunicatum</i>	13
Septoglomus	<i>Septoglomus constrictum</i>	6

than those of the others (Table 1). Furthermore, the ratio of the spore density of *G. mosseae* to that of the other five species of endomycorrhizal fungi was 37.65%, whereas the ratio of the spore density of *G. aggregatum* to that of the other five species of endomycorrhizal fungi was 28.24%, giving a sum total of 65.89%. Therefore, it was considered that *G. mosseae* and *G. aggregatum* were the dominant species of endomycorrhizal fungi in the bulk soil of *E. maidenii*.

### Endomycorrhizal infection of the *Eucalyptus maidenii* seedlings

In March 2019, the results of the endomycorrhizal infection rate and intensity of the seedlings of *E. maidenii* (Table 2) revealed that the endomycorrhiza fungi were formed on the roots of all seedlings of *E. maidenii* in the second and third experimental treatments. Furthermore, with the increased number of endomycorrhizal fungi spores in the seedling substrate, both the infection rate and intensity of the seedlings increased.

Table 2. Endomycorrhizal infection rate and intensity of the *Eucalyptus maidenii* seedlings by cultivating with different soil treatments

Experimental treatment	Endomycorrhizal infection rate (%)	Endomycorrhizal infection intensity
1	0	0
2	63.2	IV
3	93.5	V

### Effects of different soil treatments on the growth indices and root activities of the *Eucalyptus maidenii* seedlings

The results of the growth indices and root activities of the *E. maidenii* seedlings by culturing with different soil treatments (Table 3) revealed that the seedling height, stem diameter, dry weights of roots, stems, and leaves, root-shoot ratio, number of primary lateral roots, number of secondary lateral roots, and root activity all increased with the higher number of endomycorrhizal fungi spores in



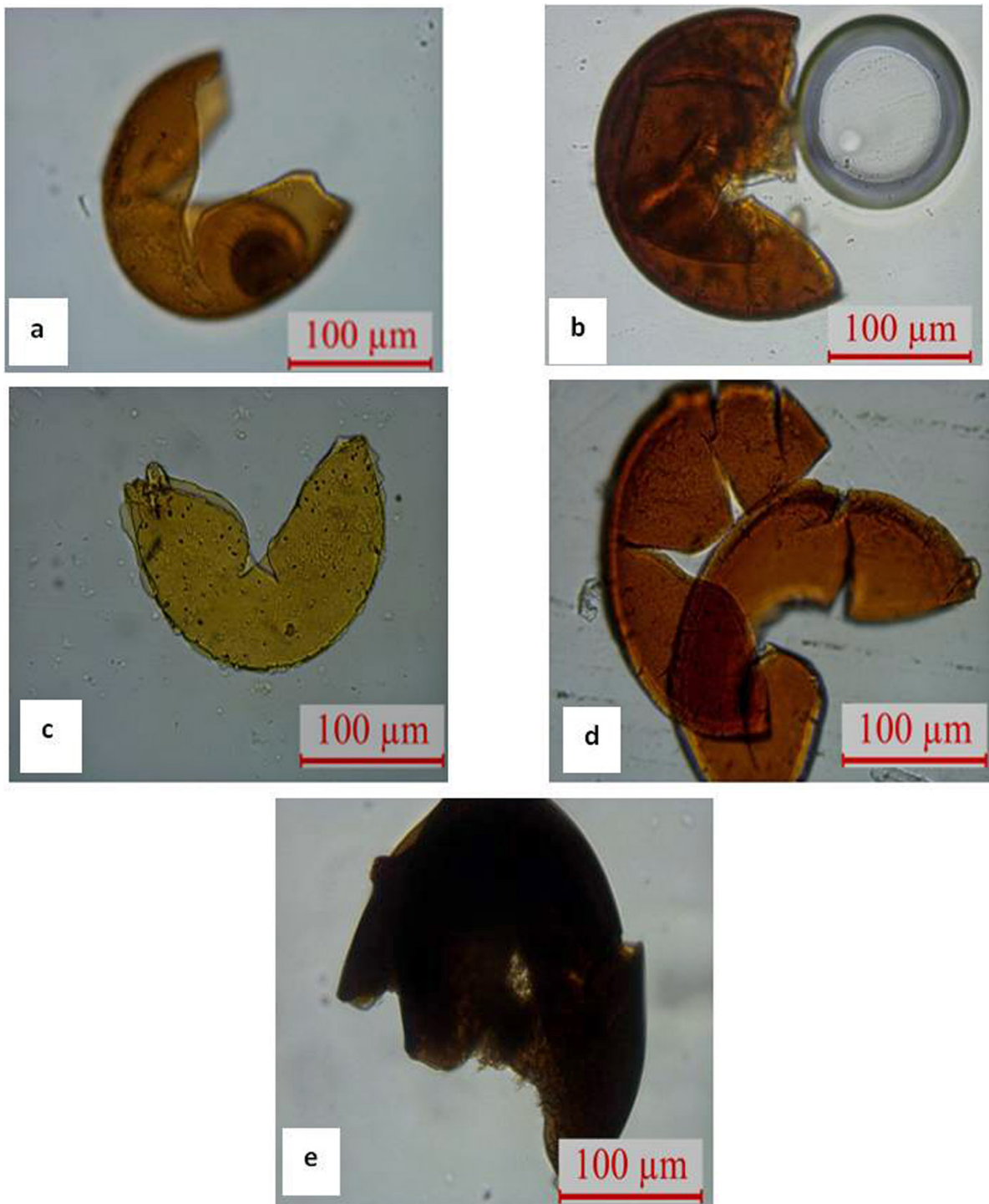


Fig. 1. Morphological characteristics of the endomycorrhizal fungi spores in bulk soil of *Eucalyptus maidenii* ( $\times 400$ ) (a. *Glomus mosseae*; b. *Glomus multicaule*; c. *Glomus aggregatum*; d. *Claroideoglomus etunicatum*; e. *Septoglomus constrictum*)

Table 3 Effects of different soil treatments on the growth indices and the root activities of *Eucalyptus maidenii* seedlings

Experimental treatment	Seedling height (cm)	Stem diameter (mm)	Root dry weight (g)	Stem dry weight (g)	Leaf dry weight (g)	Root-shoot ratio	Number of first order lateral roots (stick)	Number of second order lateral roots (stick)	Root activity ( $\mu\text{g/g/h}$ )
1	32.92 $\pm$ 8.70b	2.42 $\pm$ 0.75a	0.26 $\pm$ 0.08b	0.73 $\pm$ 0.25a	0.63 $\pm$ 0.01a	0.20 $\pm$ 0.31b	38.22 $\pm$ 8.18a	398.11 $\pm$ 85.59b	450.89 $\pm$ 81.16b
2	37.31 $\pm$ 3.28ab	2.43 $\pm$ 0.33a	0.36 $\pm$ 0.11b	0.81 $\pm$ 0.24a	0.69 $\pm$ 0.27a	0.24 $\pm$ 0.04ab	40.78 $\pm$ 8.35a	404.44 $\pm$ 121.08b	566.44 $\pm$ 72.60b
3	43.39 $\pm$ 3.62a	3.06 $\pm$ 0.75a	0.51 $\pm$ 0.16a	1.01 $\pm$ 0.28a	0.88 $\pm$ 0.35a	0.27 $\pm$ 0.05a	47.00 $\pm$ 9.37a	543.67 $\pm$ 12.47a	758.67 $\pm$ 313.42a

Note: Lowercase letters indicate significant difference at 0.05 level.

the seedling substrate. For the seedling height, there were significant differences between the three experimental treatments ( $P=0.031$ ). The seedling height of the third experimental treatment was significantly greater than that of the first experimental treatment, whereas the seedling height of the second experimental treatment was the transition group. The results of the variance analysis and multiple comparisons of the root-shoot ratio ( $P=0.038$ ) were similar to that of the seedling height.

For the dry weight of roots, there were significant differences between the three experimental treatments ( $P=0.017$ ), where that of the third experimental treatment was significantly greater than the first and second experimental treatments, while there was no significant difference between the first and second experimental treatments. The results of the variance analysis and multiple comparisons of the number of secondary lateral roots ( $P=0.024$ ) and root activities ( $P=0.015$ ) were similar to that of the dry weight of roots.

For the stem diameter, the dry weight of stems, the dry weight of leaves, and the number of primary lateral roots, there were no significant differences between the three experimental treatments (all were  $P>0.05$ ). Above all, the seedling roots, particularly the number of secondary lateral roots and the root activities were significantly promoted by the third experimental treatment. It was revealed that the major indices of the absorption capacity of roots were enhanced when a certain number of endomycorrhizal fungi spores were added to the seedling substrate for the cultivation of the *E. maidenii* seedlings.

### Effects of different soil treatments on the nutrient concentration in the bulk soil of the *Eucalyptus maidenii* seedlings

The results of the nutrient concentration in the bulk soil of the *E. maidenii* seedlings obtained through culturing with different soil treatments (Table 4) indicated that the concentrations of total nitrogen, total phosphorus, available nitrogen, available phosphorus, and available potassium increased with the higher number of endomycorrhizal fungi spores in the seedling substrate. Initially, the concentration of total potassium increased and then decreased,

whereas for the total nitrogen, the total phosphorus, the total potassium, the available nitrogen and the available potassium, there were significant differences between three experimental treatments (all were  $P<0.05$ ), while there was no significant difference between three experimental treatments for the available phosphorus ( $P=0.154$ ).

The results of the variance analysis and multiple comparisons of the total nitrogen, total phosphorus, and available potassium of the third experimental treatment were significantly greater than that of the first experimental treatment (all were  $P<0.05$ ). The total nitrogen, total phosphorus, and available potassium of the second experimental treatment was the transition group. The results of the variance analysis and multiple comparisons of the available nitrogen of the second and third experimental treatments were significantly larger than that of the first experimental treatment (all were  $P<0.05$ ), and there was no significant difference for the available nitrogen between the second and third experimental treatments ( $P=0.079$ ).

The results of the variance analysis and multiple comparisons of the total potassium of the second experimental treatment were significantly larger than that of the first experimental treatment ( $P=0.042$ ), and the total potassium of the third experimental treatment was the transition group. In summary, the concentrations of nitrogen, phosphorus, and potassium in the bulk soil of the *E. maidenii* seedlings increased significantly for the third experimental treatment. It was shown that the concentrations of the major nutrients in the bulk soil for the growth of seedlings were improved when a certain number of spores of endomycorrhizal fungi was added to the seedling substrate.

### Effects of different soil treatments on the nutrient concentration of *Eucalyptus maidenii* seedlings

The results of investigations into the nutrient concentration of different organs (i.e., roots, stems, and leaves) of *E. maidenii* seedlings by culturing with different soil treatments (Table 5) revealed that the concentration of nitrogen in roots, stems, and leaves; phosphorus in stems and leaves; and potassium in

Table 4. Effects of the different soil treatments on the nutrient concentration of the bulk soil of the *Eucalyptus maidenii* seedlings

Experimental treatment	Total nitrogen (g/kg)	Total phosphorus (g/kg)	Total potassium (g/kg)	Available nitrogen (mg/kg)	Available phosphorus (mg/kg)	Available potassium (mg/kg)
1	1.22±0.17b	0.10±0.02b	2.65±0.19b	46.93±3.49b	4.36±1.99a	65.98±4.80b
2	1.33±0.12ab	0.12±0.04ab	3.16±0.29a	50.31±1.37a	4.56±0.95a	68.13±3.87ab
3	1.45±0.14a	0.16±0.05a	2.88±0.41ab	51.89±2.16a	5.34±1.01a	78.45±19.46a

Note: Lowercase letters indicate significant difference at 0.05 level.

Table 5. Effects of the different soil treatments on the nutrient concentration of the *Eucalyptus maidenii* seedlings

Experimental treatment	Nitrogen (g/kg)			Phosphorus (g/kg)			Potassium (g/kg)		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
1	1.39±0.41b	1.31±0.22b	1.44±0.36b	0.17±0.03b	0.11±0.22b	0.42±0.16b	2.30±0.33a	1.27±0.39a	2.43±0.54a
2	1.54±0.28b	1.47±0.25b	1.59±0.31b	0.30±0.06a	0.18±0.07ab	0.45±0.08b	2.47±0.42a	1.29±0.33a	2.36±0.46a
3	2.17±0.21a	2.02±0.39a	1.76±0.28a	0.26±0.06a	0.20±0.09a	0.61±0.26a	2.48±0.34a	1.26±0.20a	2.19±0.30a

Note: Lowercase letters indicate significant difference at 0.05 level.

roots were increased with the higher number of endomycorrhizal fungi spores in the seedling substrate. Interestingly, the concentrations of phosphorus in roots and potassium in stems initially increased and then decreased. For the concentration of potassium in roots, stems, and leaves, there were no significant differences between the three experimental treatments (all were  $P > 0.05$ ).

The concentration of nitrogen in roots, stems, and leaves, were significantly different between the three experimental treatments (all were  $P < 0.05$ ). The results of multiple comparisons for the indices of the third experimental treatment were significantly greater than that of the first and second experimental treatments (all were  $P < 0.05$ ), and there was no significant difference for these indices between the first and second experimental treatments ( $P = 0.283$ ).

The concentration of phosphorus in roots, stems, and leaves, were significantly different between the three experimental treatments (all were  $P < 0.05$ ). Furthermore, the results of the multiple comparisons for the indices of the third experimental treatment were significantly greater than that of the first experimental treatment. From the above, the concentrations of nitrogen, phosphorus, and potassium in the roots, stems, and leaves of the seedlings increased significantly via the third experimental treatment. This signified that the concentrations of the major nutrients in the seedlings were enhanced when a certain number of endomycorrhizal fungi spores was added to the seedling substrate.

### Correlation analysis of the nutrient concentration in the bulk soil and seedlings of *Eucalyptus maidenii*

Correlation analysis of the nutrient concentration in the bulk soil and seedlings of *E. maidenii* was performed (Table 6). The concentration of nitrogen in the aerial components of seedlings (i.e., stems and leaves) had a significantly positive correlation with the concentration of nitrogen (i.e., total nitrogen and available nitrogen) in the bulk soil. The concentration of nitrogen in the stems and roots of seedlings exhibited a significantly positive correlation with the concentrations of total phosphorus and available phosphorus in the bulk soil, respectively. The concentration of phosphorus in the roots of seedlings had a significantly positive correlation with the concentrations of available nitrogen and total potassium in the bulk soil, respectively. To summarize, the concentrations of nutrients in the *E. maidenii* seedlings were significantly positively correlated with those in the bulk soil.

### Correlation analysis of the growth indices and nutrient concentration of the *Eucalyptus maidenii* seedlings

Correlation analysis of the growth indices with the concentrations of nutrients of the *E. maidenii* seedlings was performed (Table 7). The seedling height

Table 6. Correlation of the nutrient concentration in the bulk soil and seedlings of *Eucalyptus maidenii*

Nutrients in seedlings	Nutrients in rhizosphere soil	Total nitrogen of bulk soil	Total phosphorus of bulk soil	Total potassium of bulk soil	Available nitrogen of bulk soil	Available phosphorus of bulk soil	Available potassium of bulk soil
		Root	Nitrogen	0.271	0.220	0.059	0.344
	Phosphorus	0.210	0.071	0.460*	0.427*	-0.041	-0.253
	Potassium	0.320	-0.064	0.088	0.026	-0.100	-0.296
Stem	Nitrogen	0.396*	0.424*	0.097	0.563**	0.261	-0.292
	Phosphorus	0.052	0.331	0.319	0.242	0.038	-0.266
	Potassium	0.059	0.136	0.070	-0.231	0.096	-0.072
Leaf	Nitrogen	0.316	0.065	0.222	0.424*	-0.118	-0.127
	Phosphorus	0.044	0.226	0.060	0.104	0.192	-0.270
	Potassium	-0.112	-0.143	0.018	0.072	-0.236	0.027

Note: “\*\*\*” indicates significant correlation at 0.01 level; “\*\*” indicates significant correlation at 0.05 level.

Table 7. Correlation of the growth and the nutrient concentration of the *Eucalyptus maidenii* seedlings

Growth and roots physiological indicators of seedlings		Seedling height	Stem diameter	Number of first order lateral roots	Number of second order lateral roots	Root activity	Root dry weight	Stem dry weight	Leaf dry weight
Root	Nitrogen	0.573**	0.504**	0.216	0.521**	0.530**	0.539**	0.227	0.301
	Phosphorus	0.310	0.231	0.220	-0.117	0.454*	0.295	0.138	0.202
	Potassium	-0.182	-0.048	-0.012	-0.015	0.143	0.215	0.090	0.066
Stem	Nitrogen	0.518**	0.109	0.158	0.362	0.163	0.417*	0.261	0.194
	Phosphorus	0.330	0.362	0.428*	0.287	0.534**	0.475*	0.337	0.361
	Potassium	-0.226	-0.149	-0.019	-0.313	0.117	-0.005	-0.066	0.000
Leaf	Nitrogen	0.246	0.201	0.073	0.181	0.071	0.171	0.186	0.152
	Phosphorus	0.235	-0.242	0.320	0.531**	0.664**	0.262	-0.053	-0.017
	Potassium	-0.227	-0.242	-0.044	-0.210	-0.186	-0.236	-0.087	-0.154

Note: “\*\*\*” indicates significant correlation at 0.01 level; “\*\*” indicates significant correlation at 0.05 level.

was significantly positively correlated to the concentration of nitrogen in roots and stems, respectively. The stem diameter had significantly positive correlation with the concentration of nitrogen in roots. The root activity was significantly positively correlated to the concentration of nitrogen in roots, and the concentration of phosphorus in stems, leaves, and roots, respectively.

The number of lateral roots was significantly positively correlated with the concentration of phosphorus

in the aerial components of seedlings (i.e., stems and leaves) and the concentration of nitrogen in roots. Overall, the growth indices of the aerial components of seedlings had a significantly positive correlation with the concentration of nitrogen in the seedlings. Meanwhile the capacity of nutrient absorption (i.e., root activity and the number of lateral roots) was significantly positively correlated with the concentration of phosphorus of seedlings and the concentration of nitrogen in roots, respectively.

Table 8. Stepwise regression analysis between the five species of endomycorrhizal fungi and their growth indices for the *Eucalyptus maidenii* seedlings

Indexes	Regression equation	Correlation coefficient	Fitting coefficient	Significance
Seedling height	$y=30.841+0.299X_1+1.792X_4$	0.78	0.608	0.060
Stem diameter	-	-	-	-
Dry weight of root	$y=0.293+0.010X_3$	0.676	0.457	0.046*
Dry weight of stem	-	-	-	-
Dry weight of leaf	-	-	-	-
Total biomass (dry weight)	$y=1.571+0.182X_4$	0.524	0.275	0.147
Root shoot ratio	$y=0.192+0.008X_2+0.002X_4$	0.866	0.75	0.016*
Number of first order lateral roots	$y=37.943+1.299X_2$	0.891	0.793	0.001**
Number of second order lateral roots	$y=382.690+21.155X_2$	0.854	0.73	0.003**
Root activity	$y=419.571+10.238X_1+7.820X_3$	0.879	0.722	0.012*
Nitrogen concentration of root	$y=1.165+0.135X_2+0.182X_4-0.027X_1$	0.97	0.941	0.002**
Phosphorus concentration of root	$y=0.149+0.031X_4+0.003X_1$	0.902	0.814	0.006**
Potassium concentration of root	$y=2.318+0.032X_2$	0.584	0.341	0.099
Nitrogen concentration of stem	$y=1.295+0.029X_1$	0.722	0.521	0.029*
Phosphorus concentration of stem	$y=0.106+0.004X_1+0.003X_3$	0.916	0.838	0.004**
Potassium concentration of stem	-	-	-	-
Nitrogen concentration of leaf	$y=1.374+0.102X_4$	0.656	0.430	0.055
Phosphorus concentration of leaf	$y=0.422+0.023X_2$	0.722	0.521	0.028*
Potassium concentration of leaf	-	-	-	-
Total nitrogen concentration of bulk soil	$y=1.207+0.012X_2+0.011X_3$	0.952	0.905	0.001**
Total phosphorus concentration of bulk soil	$y=0.094+0.015X_4$	0.585	0.342	0.098
Total potassium concentration of bulk soil	-	-	-	-
Available nitrogen concentration of bulk soil	$y=47.581+0.202X_1$	0.630	0.397	0.069
Available phosphorus concentration of bulk soil	$y=4.310+0.142X_2$	0.538	0.290	0.135
Available potassium concentration of bulk soil	-	-	-	-

Note:  $X_1$  – *Glomus mosseae*,  $X_2$  – *Glomus multiclaue*,  $X_3$  – *Glomus aggregatum*,  $X_4$  – *Claroideoglossum etunicatum*,  $X_5$  – *Septoglossum constrictum*, “\*\*\*” indicates significant correlation at 0.01 level; “\*\*” indicates significant correlation at 0.05 level.



## Screening of the major species of endomycorrhizal fungi for the *Eucalyptus maidenii* seedlings

Regression equations were developed between the five species of endomycorrhizal fungi and each index (i.e., dry weight of roots, root-shoot ratio, number of lateral roots, root activity, concentrations of nitrogen and phosphorus in roots and stems, phosphorus in leaves, and total nitrogen in bulk soil). Furthermore, the five species of endomycorrhizal fungi were significantly linearly correlated with each index (Table 8). By exploiting stepwise regression, we identified the main species of endomycorrhizal fungi that affected the nutrient concentration and growth of *E. maidenii* seedlings, which were *G. multicaule*, *G. aggregatum*, and *C. etunicatum* (Table 8).

## Discussion

### Endomycorrhizal fungi species in the bulk soil of *Eucalyptus maidenii* plantation

In this study, five species of endomycorrhizal fungi were isolated from the bulk soil of an *E. maidenii* plantation in Kunming City, Yunnan Province, China, which were derived from three genera (i.e., *Glomus*, *Claroideoglomus*, and *Septoglomus*). The five species of endomycorrhizal fungi included *G. mosseae*, *G. multicaule*, *G. aggregatum*, *C. etunicatum*, and *S. constrictum*, with the dominant species of endomycorrhizal fungi being *G. mosseae* and *G. aggregatum*. The results of this study were different from another investigation of endomycorrhizal fungi in an *E. globulus* plantation in Kunming City of Yunnan Province, which had been done by the same research team, and published in a separate paper. Six species of endomycorrhizal fungi were isolated from the bulk soil in an *E. globulus* plantation in Kunming City of Yunnan Province, which were derived from three genera (i.e., *Glomus*, *Claroideoglomus* and *Septoglomus*). The six species were *G. mosseae*, *G. multicaule*, *G. aggregatum*, *C. etunicatum*, *S. constrictum*, and *Glomus hoi*, with the dominant species being *G. aggregatum*. Many scholars have intensively studied the endomycorrhizal fungi of *Eucalyptus*, and it was found that the endomycorrhizal fungi resources of *Eucalyptus* were very abundant. Further, that the endomycorrhizal fungi species of *Eucalyptus* were variable for different regions and tree species (Tan et al., 2000; Zhu et al., 2001a; Zhu et al., 2001b; Zhong et al., 2001). In the Yunnan region, endomycorrhizal fungi species in the bulk soil of *E. maidenii* plantation and that of *E. globulus* plantation were different, thus, it is a key content toward matching tree species with endomycorrhizal fungi in a certain region.

In previous investigations of endomycorrhizal fungi for *Eucalyptus*, *G. multicaule* and *G. hoi* were not isolated, while *G. multicaule* and *G. hoi* were isolated from the bulk soil of *Betula alnoides* in the Yunnan region (Jing, 2015), and from the soil in the dry-hot valleys of Yunnan (Wu et al., 2014). It was revealed that the distribution of the two species of endomycorrhizal fungi was closely associated with the geographical environment, particularly the soil environment of Yunnan.

### Effects of the endomycorrhizal fungi on the growth and nutrient characteristics of *Eucalyptus maidenii* seedlings

In this study, it was shown that the growth of roots (i.e., the number of secondary lateral roots and root activities) and aerial components of seedlings (i.e., seedling heights) of *E. maidenii* were significantly promoted by the third experimental treatment, where certain quantities of endomycorrhizal fungi spores were added to nursery substrates. These results were consistent with the promotional effects on the growth of *E. grandis* × *E. urophylla*, *E. urophylla*, and *Macadamia ternifolia* seedlings, all of which were inoculated by endomycorrhizal fungi (Zhong et al., 2001; Liu et al., 2005).

For 11 month old *E. maidenii* seedlings with an increased number of endomycorrhizal fungi spores in nursery substrates, the endomycorrhizal infection rate and intensity of the *E. maidenii* seedlings increased. The nitrogen, phosphorus, and potassium concentrations in the bulk soil also increased, as did the nitrogen concentration in the aerial components of seedlings, and phosphorus in the seedling roots. Furthermore, the concentrations of nutrients (i.e., nitrogen, phosphorus, and potassium) in the bulk soil and in the seedlings (i.e., roots, stems, and leaves) were significantly improved by the third experimental treatment.

The growth indices of the aerial components of the seedlings were significantly positively correlated with the nitrogen concentration of the seedlings. The indices of the nutrient absorption capacity of the seedlings (i.e., number of lateral roots and root activities) had a significantly positive correlation with the phosphorus concentration of the seedlings. Therefore, the promotional effect of endomycorrhiza for the growth of the *E. maidenii* seedlings was explained from the perspective of nutrient physiology. To summarize, the study of endomycorrhizal fungi for *E. maidenii* was done from the appearance to the mechanism, and the guidance of species and quantity of endomycorrhizal fungi spores for the cultivation of *E. maidenii* seedlings was provided.

Future studies should develop single spore cultures for each of the five species isolated in the present study using the *Trifolium repens* method (Zhu et al., 2001a). Once single spore cultures are available, additional studies would allow further exploration of the mechanisms by which these endomycorrhizal fungi promote growth in *E. maidenii* seedlings. Further in depth research may provide a theoretical basis and practical guidance for optimal seedling cultivation in sustainable *E. maidenii* plantations.

## Conclusions

In an *E. maidenii* plantation in Kunming, five species of endomycorrhizal fungi were identified and isolated from the bulk soil, where the dominant species of endomycorrhizal fungi were *G. mosseae* and *G. aggregatum*. We observed that when a certain quantity of endomycorrhizal fungi spores were added to the soil substrate, additional nutrients (i.e., nitrogen, phosphorus, and potassium) of the bulk soil were provided. Further, the nutrient (i.e., nitrogen, phosphorus, and potassium) absorption capacity of the seedling roots were enhanced, and the concentrations of nitrogen, phosphorus, and potassium in the seedlings were increased; thus, the seedling growth was promoted. The main endomycorrhizal fungi species that affected the nutrient concentration and growth of the *E. maidenii* seedlings were *G. multicaule*, *G. aggregatum*, and *C. etunicatum*.

## Availability of data and material

All data generated or analyzed during this study are included in this published article.

## Competing in interests

The authors declare that they have no competing interests.

## Author's contributions

WXL, KXN, ZYW, CY, and CZL conceived and designed the study. WXL and KXN implemented the seedling cultivation, as well as the determination of nutrient concentration and growth traits of the seedlings. ZYW, CY, and CZL performed bulk soil sample collection, data analysis, and prepared the tables and figures. WXL drafted the manuscript. All authors reviewed and edited the manuscript.

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