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Legume Green Manuring Improves Soil Fertility and Plant Growth of *Eucalyptus* Plantation in South Subtropical China

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ABSTRACT

Legume green manure is extensively planted to improve soil fertility in crop field. However, the application of legume in *Eucalyptus* plantation is still limited and depends on site specific and species. Therefore, the objective of this study was to determine the effects of green manure inter-plantation on soil fertility and plant growth of *Eucalyptus* plantation in a short term. A field experiment of one year was established to investigate the green manure growth, forest soil nutrients and *Eucalyptus* plant growth inter-planted with two legume species (*Tephrosia candida*, TC and *Sesbania cannabina*, SC) at south subtropical China. Legumes were inter-planted in linear among the tree space of *Eucalyptus* stand. Result showed that the green manure inter-plantation increased soil organic matter by 9.66% of TC and 18.44% of SC. Soil available nitrogen, phosphorus and potassium were improved significantly by the legume treatments as well. The increment of height and diameter at breast height of *Eucalyptus* during the experiment was significant in legume treatments. Thus, the timber volume increment was improved significantly by 46.81% of TC and 35.47% of SC compared with the control treatment. Therefore, the inter-plantation of legume green manure under the *Eucalyptus* plantation is effective to improve soil fertility and tree growth. Such a measure is potential and referenced for the sustainable forest management.

1. Introduction

Originated from Australia, *Eucalyptus* is the most widespread wood species by human beings. Due to its fast-growing, versatile use and high economic profit, *Eucalyptus* plantations have been cultivated extensively and cover roughly 20 million ha over the world. Nowadays, China is the country with the largest *Eucalyptus* plantation

area of 4.6 million ha in 2017 and the timber volume was more than 30 million m³^[1]. Compared with 1.5 million ha in 2008, the area of *Eucalyptus* plantations in China has been increased greatly. Certainly, *Eucalyptus* makes a significant contribution to the timber market and local social economics.

Generally, the cultivation rotations of *Eucalyptus* are seven years or less with a high stem wood production

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of more than 15 Mg ha⁻¹ yr⁻¹ [2]. Accordingly, *Eucalyptus* plantations require large quantities of nutrients from soil for growth. As estimated, the aboveground biomass in high-productivity stands in Brazil contained 483 kg N ha⁻¹ [3]. However, there is usually approximate 80 kg N ha⁻¹ as fertilizer used in an entire rotation in plantations [4,5]. Furthermore, other soil nutrients have been paid few attentions and less applied. Therefore, soil nutrient budgets are negative in balance in many *Eucalyptus* plantations after several rotations.

Effects of afforestation with *Eucalyptus* on soil properties were reported in different results. Many researchers found a positive effect of *Eucalyptus* on degraded soils, while little effects were observed in plantations [6-8]. The discrepancy is dependent on land use history, soil texture, management practices, plantation age, number of rotation and climate [9]. In China, *Eucalyptus* is mainly planted in southern region accompanied with suitable natural conditions of water, heat and sunlight. Successive short-rotation plantations of *Eucalyptus* exerted a great effect on soil in a long term. Liao [10] reviewed that *Eucalyptus* soil degraded significantly with rotations in China due to the fragile pure mono-plantation system, heavy water and soil erosion, and unreasonable management. Hence, it is important to improve soil quality for a sustainable *Eucalyptus* production.

As well known, legume green manure can provide N to soil through biological N fixation, enhance the soil N supply to subsequent crops, and partially substitute for chemical N fertilizers [11]. Moreover, green manure can improve soil physiochemical properties, stimulate soil enzyme activity and enrich soil microbe [12-14]. Hence, inter-plantation of legume green manure became one of effective measures to improve soil quality of *Eucalyptus* plantation [15,16]. However, the effects of legume green manure on soil quality are greatly different from species, time, region, climate, soil and so on. Therefore, the objective of this study is to demonstrate the effects of two legume species on soil fertility of *Eucalyptus* plantation in southern tropical region of China in a short-term.

2. Materials and Methods

2.1 Study Site

The study was conducted in Leika branch (107°55'711"E, 22°25'277"N) of National Dongmen Forest Farm of Guangxi Autonomous Region, China. This site belongs to the south subtropical monsoon region with a rainfall of 1,121 mm, mean temperature of 21.7 °C and annual sunshine time of 1,550 hours per year. Usually, high temperature with heavy rainfall occurs in summer.

The landform is hilly with mostly lower than 150 m. Soil is derived from Quaternary sandstone parent material and classified as Ultisol. The area of selected study site is 3.3 ha of pure *Eucalyptus* plantation. The tree species is *Eucalyptus urophylla* × *E. grandis* DH 32-29. The plantation afforestation was made at February of 2017 with a density of 4 m × 2 m. Before the experimentation, three times of fertilization were conducted at April of 2017, September of 2017 and April of 2018, respectively. The application rate of each time was 622.5 kg ha⁻¹ in complex fertilizer (N-P-K=6-12-7). Additionally, a rate of 4,500 kg organic manure (5% of total nutrient) was applied. The soil basic chemical properties were as follows: organic matter, 18.06 g·kg⁻¹; available nitrogen, 104.6 mg·kg⁻¹; available phosphorus, 1.49 mg·kg⁻¹; and available potassium, 11.18 mg·kg⁻¹. The stand was carried out for one time of pesticide and herbicide every year. Soil samples were collected from the depth of 0-40 cm for soil basic physicochemical properties.

2.2 Experimental Design

A field experiment was setup at June of 2019 and finished at June of 2020. There were 3 treatments with 3 replicates as *Tephrosia candida* (TC), *Sesbania cannabina* (SC) and control (CK), respectively. The area of each plot was 0.4 ha. *Tephrosia candida* and *Sesbania cannabina* are legume plant. Before the seed sowing, organic fertilizer in a rate of 4,500 kg ha⁻¹ was used and paved among the row space of 2 m in width. After dealt with soil tillage, legume seed was sowed in a strip along with the tree line. The application rate of seed was 30 kg ha⁻¹ and the seed was treated with germination accelerating treatment for a better growth before sowing. In the control treatment, organic fertilizer was used as the same but without seed sowing. When the experiment finished after one year later, soil samples were collected and legume plants were investigated. In each plot, soil samples were collected from the depth of soil in 0-10, 10-20 and 20-40 cm, respectively, using a "S" shape sampling method. Soil samples were air-dried and prepared for analysis. At the same time, five quadrats in 1 m × 1 m were selected for legume investigation. The legume plant was collected completely from the aboveground and underground in each quadrat. The plant sample was taken back to laboratory to separate into root, stem and leaf. Under 60 °C, plant samples were oven dried to stable weight after 24 hrs. Plant biomass was calculated on the basis of dried weight. At the same time, the diameter at breast height (DBH) and height (H) of *Eucalyptus* trees were measured in each plot before and after the experiment.

2.3 Laboratory Analysis

Soil bulk density, organic matter, pH, nitrogen (N), phosphorus (P), potassium (K) were determined according to the operation manual [17]. N, P and K in legume plant were measured as well. Soil bulk density was determined by a core method. Soil pH was measured using a pH meter in a 1:2.5 (w/v) soil/water extract. Soil organic matter (SOM) was determined through the wet-combustion method with $K_2Cr_2O_7$ and concentrated H_2SO_4 at 220–230°C; the total N in the digest was measured using a semi-micro Kjeldahl method. After melted by NaOH, the total P was measured using Mo-Sb colorimetric method and total K was measured by flame spectrophotometer. Soil available phosphorus (P) and potassium (K) were determined through the $HCl-NH_4F$ extraction–colorimetry method and the NH_4OAC extraction–flame photometry method, respectively. Soil available nitrogen (NH_4^+-N and $NO_3^- -N$) was analyzed through KCl extraction–colorimetry.

2.4 Statistical Analysis

According to Guangxi Forestry Inventory & Planning Institute, the tree timber volume of *Eucalyptus* was calculated as: $V=c_0 \times DBH^{[c_1-c_2 \times (DBH+H)]} \times H^{[c_3+c_4(D-BH+H)]}$, where V is the volume of single tree, m^3 ; DBH is the diameter at breast height, m; H is the height of the tree, m; c_0, c_1, c_2, c_3 and c_4 are parameters, specific for the study site, $c_0=1.09154150 \times 10^{-4}$; $c_1=1.87892370$; $c_2=5.69185503 \times 10^{-3}$; $c_3=0.65259805$; $c_4=7.84753507 \times 10^{-3}$. In the text, data were presented as the average of replicates. ANOVA of Kruskal Wallis with the Dunn test was used for pairwise comparisons. A linear relationship between soil nutrient and tree growth was conducted. An alpha level of 0.05 for significance determination was used in all statistical analyses. SPSS software (version 20, IBM, Chicago, IL, USA) was used to perform the statistical analysis.

3. Results

3.1 Effects of Legume Green Manure on Soil Organic Matter

As shown in Figure 1, SOM content ranged from 15.13 to 27.40 $g\ kg^{-1}$, decreased with the increasing soil depth in all treatments ($P<0.05$). Compared with the control (CK), SC treatment showed an improvement of SOM by 13.2% ($P<0.05$) in the layer of 0-10 cm. TC showed a notable increase of SOM in the layer of 10-20 cm. While in the deeper layer (20-40 cm), legume treatments showed no significant effect on SOM content ($P>0.05$). The results suggested that there was a great difference between le-

gume species and the effect of legume green manure on soil organic matter was weak in a short-term.

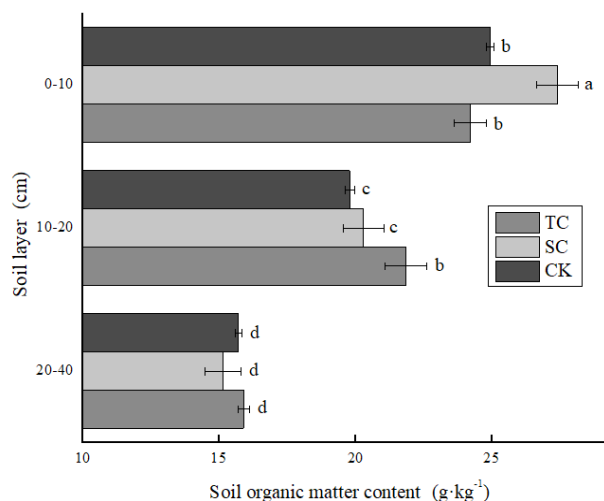


Figure 1. Effects of legume inter-plantation on soil organic matter of *Eucalyptus* plantation (Different letters indicate a significant difference at the level of 5%. The same below.)

After one year of the experimentation, SOM content increased in all treatments by 0.75- 3.57 $g\ kg^{-1}$ in the surface layer (Table 1). The treatment of SC had the largest increment by 18.44% compared to CK. The result indicated that SOM increased with time in *Eucalyptus* plantation and the legume green manure improved the increment under the intensive management. Of course, the legume green manure might play an important role in improving SOM and such a measure is critical to maintain SOM stable of the stand when in a long term production.

Table 1. Soil organic matter content in *Eucalyptus* plantation before and after legume inter-plantation

Treatment	SOM before test ($g\ kg^{-1}$)	SOM after test ($g\ kg^{-1}$)	Proportion of increment (%)	Compared to CK (%)
CK	18.06 b	18.81 a	4.16 a	-
TC	16.16 a	18.39 a	13.81 b	9.65 a
SC	15.87 a	19.46 b	22.60 c	18.44 b

3.2 Effects of Legume Green Manure on Soil Available Nitrogen

Indicated in Figure 2, soil available nitrogen (SAN) content of CK treatment ranged from 147.8 to 150.2 $mg\ kg^{-1}$, had no significant difference among various layers. However, the treatments of legume TC and SC showed a

lower content of SAN in the layer of 10-20 cm. Compared with CK treatment, TC and SC treatments had higher SAN contents in the layer of 0-10 cm, but lower in 10-20 cm, and no difference in 20-40 cm. Results suggested that legume green manure improved soil nitrogen supply significantly in the surface layer, but consumed the deeper soil nitrogen associated with the legume root system.

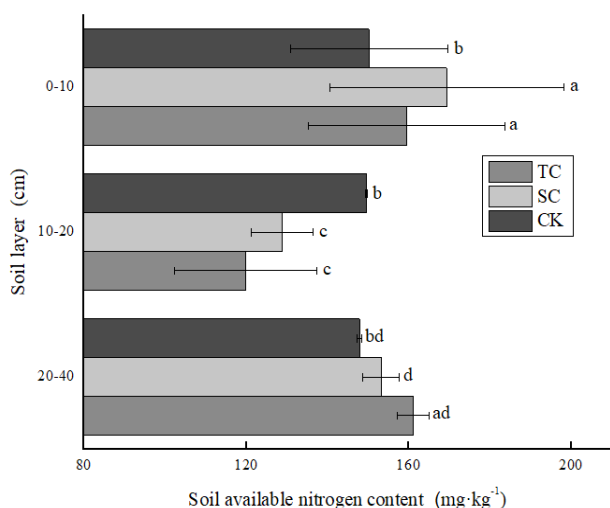


Figure 2. Effects of legume inter-plantation on soil available nitrogen of *Eucalyptus* plantation

Comparatively, SAN increased significantly in TC treatments after one year of the experiment (Table 2). TC treatment had a positive effect on SAN, but SC was negative on SAN. A short-term of the experiment and other factors should be responsible for such a result because the decomposition of biomass needs sufficient time or/and the legume might improve N uptake by the tree.

Table 2. Soil available nitrogen in *Eucalyptus* plantation before and after legume inter-plantation

Treatment	SAN before test (mg·kg ⁻¹)	SAN after test (mg·kg ⁻¹)	Proportion of increment (%)	Compared to CK (%)
CK	104.6 a	148.9 b	42.35 b	-
TC	106.1 b	154.9 c	46.10 c	3.75 b
SC	110.4 c	144.2 a	30.56 a	-11.79 a

3.3 Effects of Legume Green Manure on Soil Available Phosphorus

In the soil profile, soil available phosphorus (SAP) content of CK decreased significantly with the soil depth from 19.36 to 0.02 mg kg⁻¹ (Figure 3). The SC treatment had a same trend as CK from 23.55 to 0.35 mg kg⁻¹. However, TC was different and had a high content in the layer

of 10-20 cm as 19.33 mg kg⁻¹. Comparatively, in the layer of 0-10 cm, SC had the highest SAP content, while in the layer of 10-20 cm, TC had the highest content. In total, legume inter-plantation increased SAP content of *Eucalyptus* plantation.

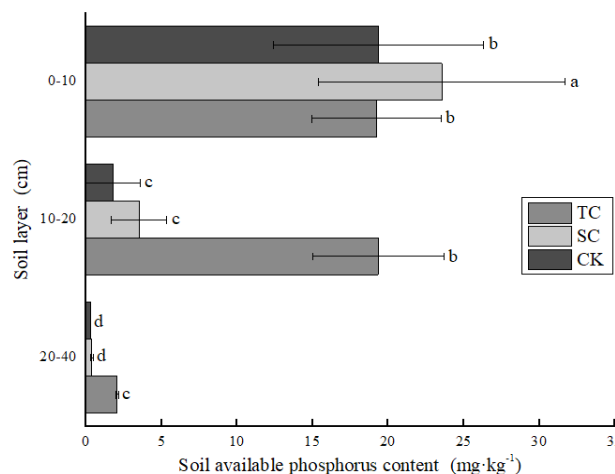


Figure 3. Effects of legume inter-plantation on soil available phosphorus of *Eucalyptus* plantation

3.4 Effects of Legume Green Manure on Soil Available Potassium

Figure 4 showed that soil available potassium (SAK) almost decreased with soil depth in all treatments. In the same layer, TC had the highest SAK content of 134.9 mg kg⁻¹, and SC had higher than CK. In the second layer, TC and SC had higher SAK than CK significantly as well. The result suggested that legume green manure could improve SAK due to a stimulation of K release from inert mineral to active form.

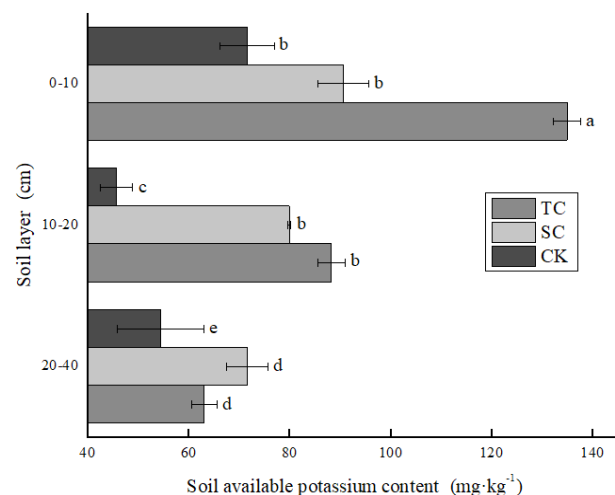


Figure 4. Effects of legume inter-plantation on soil available potassium of *Eucalyptus* plantation

3.5 Effects of Legume Inter-plantation on the Growth of *Eucalyptus*

As indicated by Table 3, the height, DBH and timber volume of *Eucalyptus* tree increased in all treatments after one-year experiment. The increases of tree height were 2.32, 2.61 and 2.76 m, respectively for CK, TC and SC. TC and SC had an increase rate of 12.50% and 18.97% compared with CK. Similarly, TC and SC had an increase in DBH as 17.61% and 10.30%. Accordingly, TC and SC increased the tree timber volume by 46.81% and 35.47%. Results showed that legume green manure could improve *Eucalyptus* growth and timber production effectively.

3.6 Correlation Analysis of *Eucalyptus* Growth and Soil Nutrients

As indicated in Table 3, the inter-plantation of legume green manure significantly increased *Eucalyptus* timber volume that could be explained by the improvement of soil nutrient availability. Table 4 showed that the tree height was co-related to soil available nitrogen and potassium ($P < 0.01$). There were many factors contributing to tree growth, but soil nutrient improvement by legume green manure could be one of the most important ones. Moreover, the difference of legume species in effect on *Eucalyptus* growth should be noted in practice for specific site. Lopez et al. [18] reported that soil nutrient management could be improved by specifically designed harvest

residue management and site preparation. Therefore, a suitable legume plantation regime is critical to improve *Eucalyptus* stand production.

4. Discussion

Forest management practices play an important role on *Eucalyptus* stand productivity and soil carbon sequestration [19,20]. Mendham et al. [21] have shown that the removal of harvest residues after two successive rotations reduced plantation productivity of *Eucalyptus globulus* Labill. in south eastern Australia, even on fertile soils. Li et al. [22] found that soil organic carbon stocks decreased over successive rotations in *Eucalyptus* stands of subtropical China possibly due to poor site preparation and short-rotation forestry strategies. In this study, soil organic matter increased after one year mainly due to organic matter fertilization and being a stage of growth (3 years old). When inter-planting legume plant of *Tephrosia candida* and *Sesbania cannabina*, soil organic matter improved significantly in *Eucalyptus* stands. Similar results were reported by Liang et al. [23]. However, Liu et al. [24] observed that legume green manure had no effect on soil organic matter. Probably, the effects of legume on soil organic matter are related to legume species and residue management. Our results showed a great difference between TC and SC on soil organic matter. Moreover, a large quantity of nutrient uptake by fast-growing *Eucalyptus* tree decreased soil nutrient. With two years of legume inter-plantation, soil

Table 3. Effects of legume inter-plantation on tree height, DBH and volume of *Eucalyptus* plantation

Treatment	Change in height		Change in DBH		Change in timber volume	
	(m)	Compared to CK (%)	(cm)	Compared to CK (%)	(m ³ ha ⁻¹)	Compared to CK (%)
CK	2.32 a	-	1.51 a	-	0.516 a	-
TC	2.61 b	12.50 a	1.77 c	17.61 b	0.757 b	46.81 b
SC	2.76 c	18.97 b	1.66 b	10.30 a	0.699 b	35.47 a

Table 4. Correlation analysis of *Eucalyptus* growth and soil nutrients

Soil nutrients	<i>Tephrosia candida</i> (TC)		<i>Sesbania cannabina</i> (SC)	
	Height	DBH	Height	DBH
Available nitrogen	0.856**	0.648	0.841**	0.420
Available phosphorus	0.610	0.650	0.521	0.154
Available potassium	0.925**	0.415	0.881**	0.409

organic matter increased significantly by 9.66-18.44% compared to the control ^[19].

The results in this study showed that legume plants increased soil available nitrogen significantly as well as soil organic matter. It was consistent with the studies of Zhu et al. ^[25] and Fungo et al. ^[26]. Three reasons of legumes increased soil available nitrogen could be summarized. Firstly, legumes returned to soil could stimulate a positive effect of soil organic nitrogen mineralization. Secondly, legume materials could improve soil enzyme activity. Thirdly, legume roots inoculated with rhizobia directly fix atmospheric nitrogen to active nitrogen for plant ^[26-28]. In this study, the decline of available nitrogen in the soil layer of 10-20 cm might be resulted from the consumption of legume growth and rhizobia formation. When harvesting the green manure, we observed that a large amount of rhizobia on the root of *Tephrosia candida* (TC), however, a few on *Sesbania cannabina* (SC), suggesting a great difference between this two species in nitrogen fixation.

Moreover, the inter-plantation of legumes increased soil available phosphorus and potassium. As reported by Du et al. ^[29], active nitrogen increased by legumes improved the uptake of soil phosphorus and thus stimulated soil phosphorus cycling. The legume inter-plantation prompted soil phosphorus enzyme activity and activated soil stable phosphorus ^[30]. Comparatively, *Tephrosia candida* (TC) had a significant effect on soil phosphorus activation in the surface layer. However, *Sesbania cannabina* (SC) had higher available phosphorus content in the layer of 10-20 cm. Such a result might be related to the difference of phosphorus required by various legume species. This study indicated that *Sesbania cannabina* (SC) requires phosphorus fertilization for a better growth in practice. Similarly, a method of phosphogypsum application was recommended for *Sesbania cannabina* in field ^[31]. The inter-plantation of legumes greatly increased soil available potassium in this study, much higher than other studies ^[32,33]. The application of chemical fertilizer and organic manure could be responsible for the higher soil available potassium content in *Eucalyptus* plantation. As well, the decomposition of green manure improved soil microbial activity to increasing soil nutrient availability ^[34-36].

5. Conclusions

The legume green manure inter-plantation increased soil organic matter, soil available nitrogen, available phosphorus and available potassium in *Eucalyptus* plantation. Moreover, the inter-plantation of legume improved *Eucalyptus* tree growth. The improved soil nutrient by legume green manure could be responsible for the growth stimulation of *Eucalyptus* tree. The legume inter-plantation

should be an effective measure in successive *Eucalyptus* plantation for a long term. The difference in effect of legume species on *Eucalyptus* stand merits a special attention in practice for a better forest management.

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