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Soil Nutrient, Enzyme Activity, and Microbial Community Characteristics of *E. urophylla* × *E. grandis* Plantations in a Chronosequence

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Abstract: The effects of continuous *Eucalyptus* cropping on soil properties and microbial characteristics and the specific factors influencing tree species growth remain elusive. In this study, three *Eucalyptus* stands of three different ages were selected, and soil nutrients, microbial biomass, enzyme activity, microbial community composition, and diversity were quantified for each. The findings indicated a significant decline in soil pH, soil cation exchange, soil organic matter, and available phosphorus content with the plantation age. Simultaneously, there was an observed increase in soil alkaline hydrolyzed nitrogen content. In addition, urease and acid phosphatase activities did not show a significant difference with age. In spite of this, catalase activity exhibited a decline corresponding to the advancement in plantation age. The carbon and nitrogen content of the soil microbial biomass increased with the progression of *Eucalyptus* planting time. The high-throughput sequencing data demonstrated a reduction in microbial diversity in *Eucalyptus* soils as the planting age increased. Interestingly, the microbial community structure exhibited minimal alterations, and did not exhibit a predominantly oligotrophic state overall. In conclusion, the study results showed that short-term successive *Eucalyptus* cropping exerts a significant negative impact on the soil system.

Keywords: *Eucalyptus*; plantation age; soil nutrient; soil microbial biomass; soil enzyme; growth

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1. Introduction

Eucalyptus, an Australian native plant, encompasses approximately nine hundred species and subspecies [1]. *Eucalyptus urophylla* × *Eucalyptus grandis*, a distinctive asexual lineage, is extensively cultivated in the provinces of Hainan, Guangxi, and Guangdong in China [2], owing to its favorable attributes for pulp, timber, and industrial raw material production within forest ecosystems [3]. Nevertheless, the establishment of extensive *Eucalyptus* plantations has induced significant modifications in the composition and functioning of forest communities, resulting in a substantial reduction in the richness of native species [4]. In the southeastern region of Guangxi in particular, the continuous cultivation of *Eucalyptus* has presented potential ecological and environmental challenges, including reduced biodiversity, groundwater contamination, and soil nutrient imbalances [5]. Consequently, the decline in biodiversity, yield, and soil quality within fast-growing *Eucalyptus* monocultures has emerged as a critical concern, posing a significant threat to the short-term sustainability of forest management practices [6].

The soil serves as a fundamental component for sustainable woodland development, with its nutrient content exerting a profound influence on plant growth and development [7]. Previous research has shown that the implementation of poor management practices

during the planting phase, such as unsuitable crop rotation, planting density, monoculture systems, and planting age [8], can negatively impact soil properties. The authors of one study investigated the effect of the continuous cultivation of *E. urophylla* × *E. grandis* on soil physicochemical properties, enzyme activity, and microbial community diversity [9]. The physicochemical properties of soil, such as pH, moisture content, organic matter content, and nutrient availability, significantly influence extracellular enzymes [10] and soil microbial communities [11]. Notably, these soil extracellular enzymes are critical in driving the biochemical cycling of carbon, nitrogen, and phosphorus in ecological systems, and they are actively involved in the mineralization of soil organic matter and the release of nutrients within *Eucalyptus* forests [12]. Soil nutrients also determine the composition and function of microbial communities [13]. Simultaneously, as *E. urophylla* × *E. grandis* has been planted for many years, it has been observed to induce modifications in the size of soil nutrient pools and the diversity of soil microorganisms [14].

Soil microbial communities represent a pivotal component of forest ecosystems [15], playing a crucial role in the decomposition of plant material and contributing to the maintenance of ecosystem stability [16]. Therefore, there is a critical need to investigate alterations in soil microbial community dynamics resulting from short-term *Eucalyptus* cultivation, in order to enhance the sustainable stewardship of *Eucalyptus* plantations [17]. Although it is recognized that permanent *Eucalyptus* cultivation causes changes in soil properties and microbial characteristics, the specific factors influencing *Eucalyptus* growth remain elusive. Despite the existing literature containing studies on the effects of continuous *Eucalyptus* cultivation on the physicochemical properties of soil, enzyme activities, and microorganisms, there are significant discrepancies due to differences in study sites, cultivation practices, and age of planting [18,19]. These variations contribute to uncertainties in identifying the key factors responsible for soil degradation and the patterns of soil microbial changes over the course of *Eucalyptus* planting. As a result, the impact of short-term *Eucalyptus* plantation on soil nutrients and microorganisms, as well as the underlying mechanisms, remains inadequately elucidated. Thus far, the influence of *Eucalyptus* planting on these aspects has not been extensively investigated.

Accordingly, the objective of this research was to evaluate the effect of plantation age on soil quality. Specifically, *E. urophylla* × *E. grandis* plantations in a chronosequence were selected as samples to evaluate changes in soil physicochemical characteristics, enzyme activities, microbial biomass, and soil bacterial biodiversity and community structure. The primary objectives of this study included (1) evaluating the impact of *Eucalyptus* plantation age on soil physicochemical characteristics and enzyme activities; (2) observing the influence of the duration of *Eucalyptus* planting on the diversity and community structure of soil bacteria; and (3) conducting correlation analysis on the soil physicochemical factors, enzyme activities, and planting ages to elucidate the relationship among planting ages, soil physicochemical factors, and soil microbes, providing an evidence-based reference for the sustainable management of *Eucalyptus* plantation forests.

2. Materials and Methods

2.1. Study Area

The experimental site was located in the Guangxi state-owned Dongmen Forest Farm in Dongmen town, Fusui county, Chongzuo city, Guangxi, China (22°1′15″–22°58′05″ N, 107°3′35″–108°6′46″ E) (Figure 1). The region falls within the tropical humid monsoon zone, characterized by an average annual temperature ranging from 16.5 to 25.1 °C, average annual rainfall of approximately 1080–1200 mm, annual sunshine hours of 1634~1719 h, and a frost-free period lasting over 346 days per year. The soil of the stand was quartz sandstone, with sand shale development of reddish soil. In the year 2020, the *Eucalyptus* plantation forests within this forest farm were projected to cover an area of 4625 hm^2, constituting a total volume of 441,693 m^3. Among the *Eucalyptus* trees present,

42.91% were aged between 1–3 years, 35.01% fell within the 4–5 years age range, and 22.08% exceeded 5 years of age. The favorable ground conditions and natural environment described above established a pivotal foundation for this study.

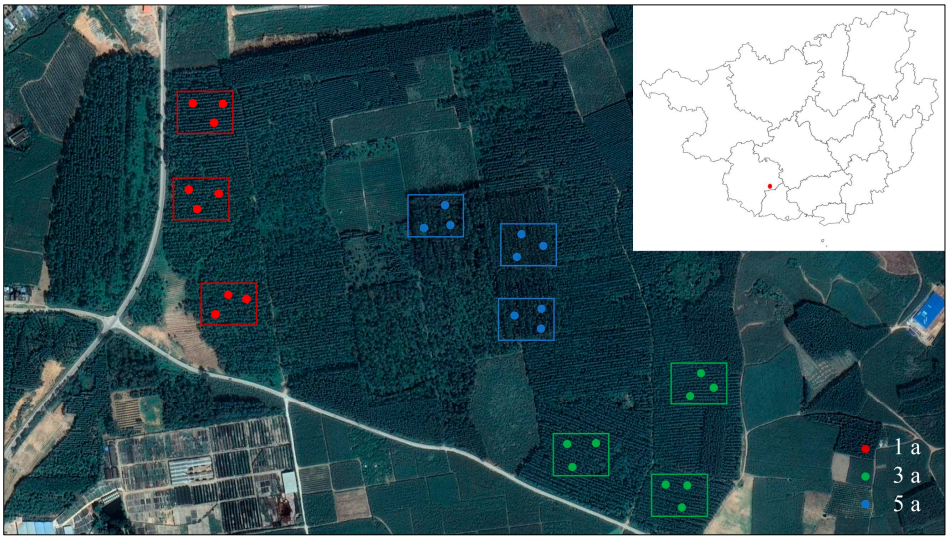


Figure 1. Design and location of the sample in Dongmen town of Chongzuo city, Guangxi, China. 1a, 3a, and 5a represent the age of *Eucalyptus* plantations at 1, 3, and 5 years, respectively. The red dot on the map in the upper right corner represents the location of the Forest Farm in Guangxi.

2.2. Design of Experiments and Collection of Soil Samples

The experiments carried out in this study were based on the "space-for-time substitution" method [20]. The experimental sites included *E. urophylla* × *E. grandis* plantations aged 1, 3, and 5 years, denoted as 1a, 3a, and 5a, respectively. The *Eucalyptus* plants in this experiment were planted for the first time using externally grown *Eucalyptus* seedlings transplanted into the forest. These sites were subject to identical management practices and exhibited comparable stand conditions. During site preparation, plant residues were mixed with surface soil via conventional ploughing. Prior to afforestation, each *Eucalyptus* plant underwent treatment with 0.5 kg of compound fertilizer (N:P:K = 15:6:9, consisting of urea for nitrogen, monoammonium phosphate for phosphorus, and potassium chloride for potash). Subsequent to afforestation, each plant was provided with 0.5 kg of compound fertilizer annually (N:P:K = 15:6:9) in the month of April. Weed control activities were conducted twice per year in the months of June and October for *Eucalyptus* forests aged 1–3 years, and once per year for *Eucalyptus* forests aged 4–5 years.

Due to the harvest time of 6 years in this region, the selected age was set to 5 years. Three sites of *Eucalyptus* forests with three plantation ages (1a, 3a, and 5a) were selected. Within each site, three 20 m × 30 m plots were randomly designated in each stand, from which soil samples were subsequently collected. To ensure the representativeness of the sampling process, replicated plots with the same stand ages were spatially segregated by a minimum distance of 100 m, while the separation between plots with differing stand age categories generally exceeded 1 km. Table 1 presents the fundamental information regarding the plantations of varying stand ages.

Table 1. Basic information of the sample plots.

Stand Age/a	Stand Density/N·hm^{-2}	Average Height/m	Average Diameter at Breast Height/cm
1	1250	3.74	3.49
2	1250	17.08	11.58
3	1250	21.69	14.43

In each designated plot, the soil was cleared of debris and litter, and three soil cores were taken from depths of 0–20 cm and 20–40 cm. Cores of the same layer were then combined to create two composite soil samples. The aforementioned sampling protocol was iterated thrice within *Eucalyptus* plantations of every stand age. A total of eighteen composite soil samples were collected from *Eucalyptus* plantations of three varying ages situated along a consistent gradient and elevation. The composite soil samples were immediately preserved in ice packs and transferred to the laboratory for further processing. In order to eliminate roots, litter, stones, and other extraneous materials, all soil samples were subjected to thorough filtration through utilization of a 2 mm mesh. The resulting composite sample was then placed in two groups for subsequent analyses. One group was preserved at 4 °C for the evaluation of soil characteristics and enzyme activities. Another group comprised nine composite samples obtained by blending the 0–20 cm and 20–40 cm soil layers, which were subsequently cryopreserved at −80 °C for the extraction of soil microbial DNA.

2.3. Soil Characteristics Analysis

Soils of *Eucalyptus* plantations of three different planting ages were tested for bulk density, pH, soil organic matter (SOM), soil cation exchange capacity (CEC), alkaline hydrolytic N (AN), available PO_4^{3-} (AP), Available K^+ (AK), and soil urease activity (UA). Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were also recorded. The soil bulk density was determined using the cutting ring method. The pH of the soils was measured with a pH-measuring instrument (FiveEasy Plus pH meter FP20, Mettler Toledo Co., Ltd., Shanghai, China) in a soil–water suspension (1:2.5, *w*/*v*). SOM was determined using the high-temperature exothermic–potassium dichromate oxidation–volumetric method [21]. The soil AN was determined using the alkaline diffusion method [22]. The hydrochloric acid-ammonium fluoride leaching-molybdenum-antimony colorimetric method was used to measure AP [23]. AK was assessed using ammonium acetate leaching flame spectrophotometry [24]. The soil CEC was measured using ammonium acetate exchange.

Basic soil extracellular enzyme activities associated with soil nutrient cycling, such as soil urease activity (UA), sucrase activity (SA), acid phosphatase activity (APA), and catalase activity (CA), were determined in this study. Soil urease activity was assessed utilizing the sodium salicylate-sodium dichloroisocyanurate colorimetric method to quantify the ammonium nitrogen yield per gram of soil hydrolyzed urea over a specific duration [25]. Sucrase activity was determined via the colorimetric method by employing dinitrosalicylic acid to estimate the glucose content per gram of soil following a 24 h incubation period [26]. Acid phosphatase activity was evaluated using the colorimetric method with disodium p-nitrophenylphosphate to ascertain the p-nitrophenol quantity per gram of soil per unit time [27]. Catalase activity was analyzed using ultraviolet spectrophotometry to determine the quantity of hydrogen peroxide decomposed per gram of soil per unit time [28].

The chloroform fumigation extraction method was used to measure the soil microbial biomass [29]. Specifically, 50 g of fresh soil was treated with ethanol-free chloroform in a vacuum desiccator at 25 °C for 24 h under dark conditions. An equal amount of fresh unfumigated soil sample was also weighed and used as a control sample. The fumigated soil samples were aseptically transferred into polyethylene plastic bottles, to which 200 mL of 0.5 mol/L K_2SO_4 solution (soil to solution ratio 1:4) was added. Subsequently, the leachate was subjected to agitation using a horizontal oscillator at 200 r/min for 30 min followed by filtration through filter paper. The extraction and filtration procedures for the unfumigated control samples were conducted in an identical manner. The oxidation method with $K_2Cr_2O_7$ was used to determine the soil MBC in the filtrates. The MBN was analyzed using colorimetric UV spectrophotometry at 280 nm.

2.4. Extraction of DNA and 16S rRNA Illumina Sequencing

The DNA was extracted from the soil samples stored at −80 degrees Celsius with an E.Z.N.A. Gel Extraction Kit ((Omega Bio-tek, Norcross, GA, USA) Co., Ltd., Beijing, China) according to the manufacturer's instructions. The V4-V5 region of the bacterial 16S rRNA gene was amplified by PCR using primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) [30]. The PCR reaction was performed in a 30 μL mixture containing 15 μL of 2 × Phanta master mix, 1 μL of each primer (10 μM), and 20 ng of template DNA. The PCR reaction steps were as follows: pre-denaturation at 95 °C for 5 min, followed by denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and annealing at 72 °C for 45 s. The total time of PCR reactions was 30 min, with a total of 5 min of extension at 72 °C. The total number of PCR reactions was 30 min then 5 min for a total of 30 cycles [31]. Amplicons were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), extracted from 2% agarose gels, and quantified using Qubit® 3.0 (Life Invitrogen, Shanghai, China). The Illumina paired-end library was constructed using pooled DNA products, following Illumina's genomic DNA library preparation procedure. Afterward, the paired-end sequencing (2 × 250) was performed on the Illumina Novaseq 6000 platform (Nanjing Gene Pioneer Co., Ltd., Nanjing, China). Raw reads were submitted to the NCBI Sequence Read Archive (SRA) database under the accession number PRJNA1083629.

2.5. Statistical and Sequence Analysis

Sparsity analysis was employed to compute alpha diversity metrics, such as the Shannon index and observed species count, for evaluating soil microbial diversity under *Eucalyptus* at different stand ages. Moreover, the differences in species complexity of the samples were evaluated through Beta diversity analysis. Principal coordinate analysis (PCoA) was used based on the Bray-Curtis dissimilarity to visualize changes in bacterial community structure between different samples. All indicators in the sample were obtained using QIIME (version 1.9.1). Origin 2021 was used for drawing graphics.

The differences in soil chemical properties, soil enzyme activity, and soil microbial biomass data between treatments were analyzed using IBM-SPSS Statistics (Version 20.0) software (IBM, Armonk, NY, USA). The data were subjected to one-way ANOVA, and the means were compared using Duncan's test ($p < 0.05$) with the three forest ages as the factor, thus evaluating the significant differences in soil physicochemical and biochemical indices of *Eucalyptus* forests at different stand ages. Different lowercase letters indicate significant differences among the different plantation ages in the same soil layer at the 0.05 level. Pearson's correlation analysis was utilized to explore the relationship between the soil physicochemical properties and biochemical properties to visually show the correlation between the properties and the age of planting.

3. Results

3.1. Soil Physicochemical Properties in Eucalyptus Forests

The pH, CEC, SOM, and AP of the *Eucalyptus* plantations displayed a declining trend as the plantation age increased (Table 2). Compared to the 1a plantation, the pH values of the 3a and 5a plantations showed reductions of 0.15 and 0.23 in the 0–20 cm soil layer and 0.09 and 0.19 in the 20–40 cm soil layer, respectively. Furthermore, the SOM within the 0–20 cm soil layer of the 3a and 5a *Eucalyptus* plantations exhibited reductions of 1.2 and 4.9 g kg^{-1}, respectively; simultaneously, in the 20–40 cm soil layer, reductions of 1.5 and 5.1 g kg^{-1} were observed for the same plantations. The available phosphorus content of the 3a and 5a *Eucalyptus* plantations also exhibited reductions of 74.64% and 82.59% in the 0–20 cm soil layer and reductions of 68.50% and 71.93% in the 20–40 cm soil layer, respectively.

Conversely, the alkaline hydrolytic nitrogen content exhibited a contrasting trend, with considerably greater values ($p < 0.05$) in the 3a and 5a plantations compared to the 1a plantation. Alkaline hydrolysis nitrogen increased by 30.47% and 59.94% in layers of 0

to 20 cm and by 29.59% and 49.49% in layers of 20 to 40 cm for the 3a and 5a plantations, respectively. These differences were statistically significant. Additionally, soil pH, CEC, SOM, alkaline hydrolytic nitrogen, available phosphorus, and available potassium content all exhibited a decreasing trend with increasing soil depth within the same plantation age group; soil cation exchange capacity showed a contrasting trend.

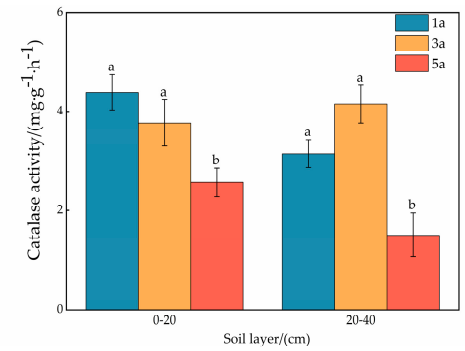
Table 2. Soil properties of *E. urophylla* × *E. grandis* plantations of different plantation ages.

Soil Properties	Soil Layer/cm	Plantation Age/a		
		1	3	5
Bulk density ($g \cdot cm^{-3}$)	0–20 cm	0.983 ± 0.06 a	1.00 ± 0.10 a	1.09 ± 0.12 a
	20–40 cm	1.09 ± 0.05 a	1.21 ± 0.08 a	1.27 ± 0.17 a
pH	0–20 cm	4.86 ± 0.09 a	4.71 ± 0.04 b	4.63 ± 0.04 b
	20–40 cm	4.77 ± 0.11 a	4.68 ± 0.13 b	4.58 ± 0.09 b
Cation exchange capacity ($cmol^{+} \cdot kg^{-1}$)	0–20 cm	5.06 ± 0.11 a	3.73 ± 0.18 b	2.39 ± 0.07 c
	20–40 cm	3.77 ± 0.17 a	3.67 ± 0.31 b	2.04 ± 0.38 c
Soil organic matter ($g \cdot kg^{-1}$)	0–20 cm	19.7 ± 0.67 a	18.5 ± 1.29 a	14.8 ± 1.16 b
	20–40 cm	17.7 ± 1.55 a	16.2 ± 0.94 a	12.6 ± 0.76 b
Alkaline hydrolytic nitrogen ($mg \cdot kg^{-1}$)	0–20 cm	50.4 ± 0.80 c	65.7 ± 0.89 b	80.6 ± 2.29 a
	20–40 cm	48.8 ± 1.36 c	63.3 ± 2.18 b	73.0 ± 4.84 a
Available phosphorus ($mg \cdot kg^{-1}$)	0–20 cm	32.3 ± 3.04 a	8.20 ± 0.54 b	5.63 ± 1.05 b
	20–40 cm	10.2 ± 1.49 a	3.21 ± 0.53 b	2.86 ± 0.46 b
Available potassium ($mg \cdot kg^{-1}$)	0–20 cm	100 ± 6.95 a	79.0 ± 5.67 a	105 ± 5.18 a
	20–40 cm	54.4 ± 6.89 a	47.9 ±3.31 a	57.9 ± 3.67 a

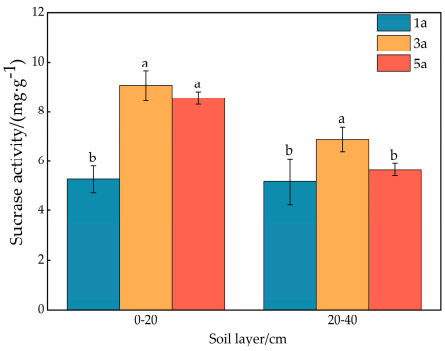
Different lowercase letters in the same row indicate significant differences among the different plantation ages in the same soil layer at the 0.05 level.

3.2. Enzyme Activity in the Soil at Different Ages of the Plantation

The enzyme activities in the soil of the *Eucalyptus* plantations at sites 1a, 3a, and 5a are shown in Figure 2. As shown in Figure 2, soil catalase activity tended to decrease with an increase in plantation age, whereby the soil catalase activity at the 5a plantation decreased significantly ($p < 0.05$) by 41.46% (0–20 cm) and 52.06% (20–40 cm) compared with the 1a plantation. The changes in soil acid phosphatase and sucrase activities from 0 to 20 cm showed contrasting trends, where the soil sucrase activity was notably ($p < 0.05$) higher in the 3a and 5a plantations than in the 1a plantation; simultaneously, there was no significant ($p > 0.05$) variation in acid phosphatase activity in the different soil layers. In addition, soil urease activity decreased and then increased with increasing planting age, with 0–20 cm soil urease activity in the 3a plantation being significantly ($p < 0.05$) lower than that of the 1a and 5a plantations. No significant ($p > 0.05$) difference was observed between levels of soil urease activity at 20–40 cm depth.



(**a**)



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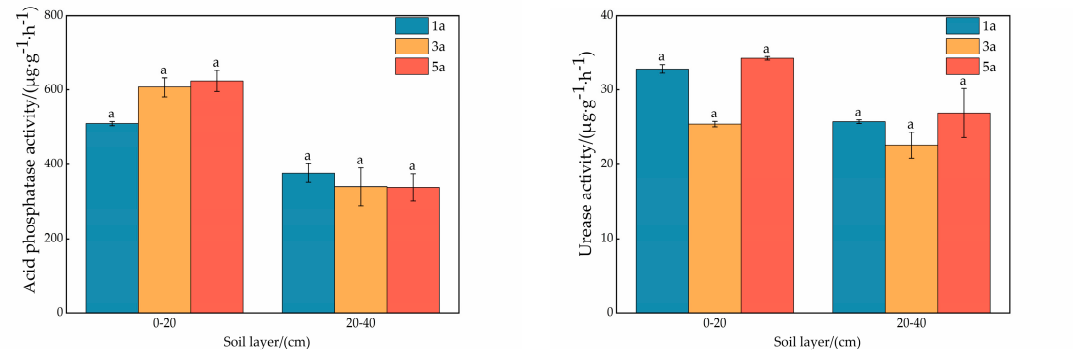


(**c**) (**d**)

Figure 2. Soil enzyme activities of *E. urophylla* × *E. grandis* plantations of different plantation ages. (**a**) Catalase activity; (**b**) sucrase activity; (**c**) acid phosphatase activity; (**d**) urease activity. 1a, 3a, and 5a represent the age of *Eucalyptus* plantations at 1, 3, and 5 years, respectively. Different lowercase letters in the same figure indicate significant differences among the different plantation ages in the same soil layer at the 0.05 level.

3.3. Soil Microbial Carbon and Nitrogen in Eucalyptus Forests of Different Plantation Ages

Soil MBC and MBN increased significantly ($p < 0.05$) from 242.5 and 19.09 mg·kg^{-1} at 1 year of age to 367.4 and 91.51 mg·kg^{-1} at 5 years of age, respectively, in the 0–20 cm layer of *Eucalyptus* soils (Table 3). A similar increase was observed in the 20–40 cm soil layer. Moreover, soil MBC and MBN showed a decrease with increasing soil depth in all of the sample plots of the same plantation age. In addition, the MBC to MBN ratio in *Eucalyptus* soils remained consistent ($p > 0.05$) across varying soil depths, but exhibited a significant decrease ($p < 0.05$) as the planting age increased. Specifically, the ratio at planting ages of 3 and 5 years was markedly lower compared to that at 1 year.

Table 3. Soil properties of *E. urophylla* × *E. grandis* plantations of different plantation ages.

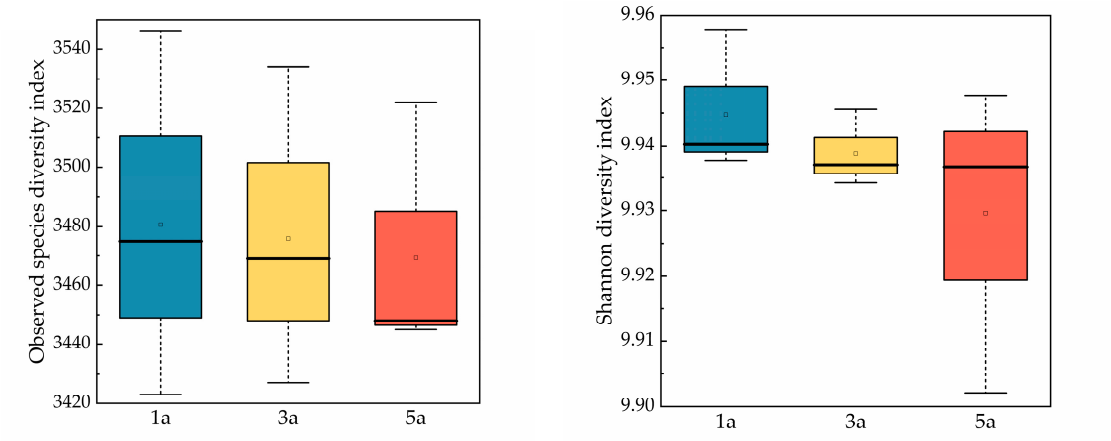
Plantation Age/a	Soil Layer/cm	MBC/(mg·kg^{-1})	MBN/(mg·kg^{-1})	MBC:MBN Ratio
1	0–20 cm	242.5 ± 37.43 c	19.09 ± 1.58 c	12.7 ± 0.76 a
	20–40 cm	168.1 ± 30.84 c	16.25 ± 1.54 c	10.4 ± 0.19 a
3	0–20 cm	295.8 ± 11.09 b	67.85 ± 5.39 b	4.36 ± 0.48 b
	20–40 cm	249.2 ± 10.17 b	57.53 ± 2.92 b	4.33 ± 0.46 b
5	0–20 cm	367.4 ± 18.34 a	91.51 ± 5.59 a	4.02 ± 0.55 b
	20–40 cm	331.9 ± 15.74 a	64.50 ± 5.31 a	5.15 ± 0.29 b

MBC represents microbial biomass carbon, MBN represents microbial biomass nitrogen. a–c Means in the same row with different letters differ significantly, $p < 0.05$.

3.4. Soil Microbial Alpha Diversity and Beta Diversity in Eucalyptus Forests of Different Plantation Ages

The indices of diversity and richness of soil microbes were affected by the age of the plantations. The observed species indices provide information about the diversity and abundance of the soil samples, while the Shannon indices mainly capture the evenness and richness of the specimens. Figure 3 shows the abundance (observed species indices) and diversity (Shannon indices) of the soil samples. The results demonstrate a decline in bacterial species richness and diversity with the increasing plantation ages of *E. urophylla* × *E. grandis*. Specifically, bacterial observed species and Shannon indices exhibited an overall decrease from 3481 and 9.945 (1a) to 3471 and 9.929 (5a), respectively, as the plantation age increased. Notably, there was no significant difference between plantations 1a and 3a.

The UniFrac feature-based weighted principal coordinate analysis (PCoA) revealed differences between the *Eucalyptus* soil samples of different ages. As depicted in Figure 4, PCoA1 and PCoA2 explained 15.03% and 13.34% of the total variations in the bacterial dataset, respectively. The spacing between the three replicates was close, demonstrating that the bacterial communities of the samples from the same years are similar. PCoA1 notably differentiated the microbial communities in the 1a *E. urophylla* × *E. grandis* plantation from those in the 5a samples. Overall, the 1a treatment exhibited lower left distribution, while the 3a and 5a treatments were positioned in the upper left area, suggesting that soil samples from plantation ages 1a and 5a exhibited the strongest microbial community compositions. The impact of plantation age on microbial community structure changed with increasing plantation age.



(**a**) (**b**)

Figure 3. Design and location of the sample in Dongmen town of Chongzuo city, Guangxi, China. (**a**) Observed species diversity; (**b**) Shannon diversity. 1a, 3a, and 5a represent the age of *Eucalyptus* plantations at 1, 3, and 5 years, respectively.

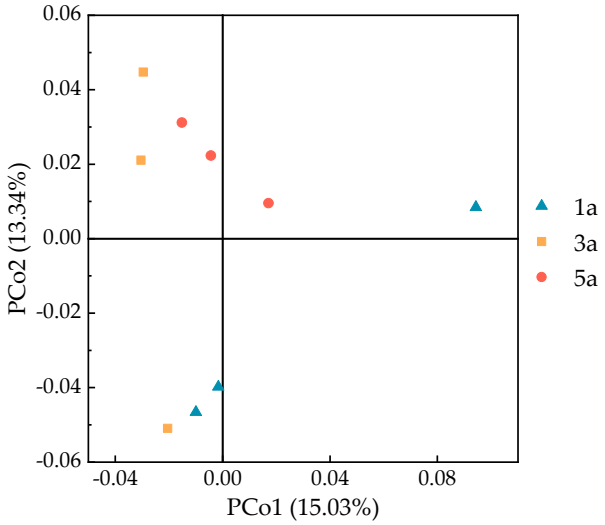
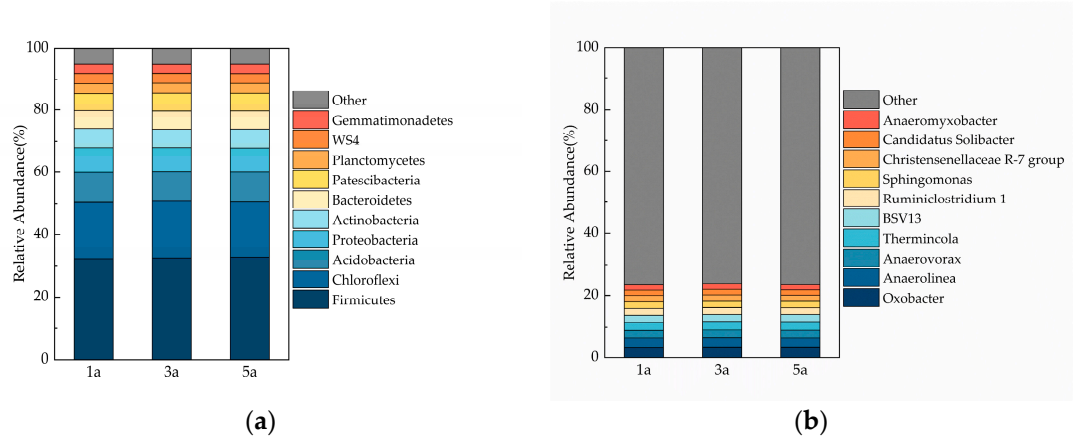


Figure 4. Principal coordinate analysis of microbial community structure (Bray–Curtis distance metrics) among samples from the 1a, 3a, and 5a *E. urophylla* × *E. grandis* plantations at the Guangxi state-owned Dongmen Forest Farm in China. 1a, 3a, and 5a represent the age of *Eucalyptus* plantations at 1, 3, and 5 years, respectively.

3.5. Soil Microbial Community Composition

The obtained ASV sequences were classified and annotated. They belonged to 35 phyla, 87 classes, 161 orders, 216 families, and 344 genera. To generate a column stack diagram of relative species abundance, the 10 most abundant phyla were selected (Figure 5a). *Firmicutes* (32.58%), *Chloroflexi* (18.01%), *Acidobacteria* (9.41%), *Proteobacteria* (7.70%), *Actinobacteria* (6.17%), *Bacteroidetes* (5.79%), and *Patescibacteria* (5.46%) were the dominant bacteria in the soil (with relative abundance > 5%), representing 85.14% of the total bacteria. The abundance of *Firmicutes* increased in samples 3a (0.23%) and 5a (0.47%) compared to 1a. However, there was no statistically significant difference between the three treatments. Additionally, the *Proteobacteria* in plantations 3a (0.12%) and 5a (0.18%) decreased compared with the 1a (7.80%) sample, although this was not to a significant level.

Figure 5b illustrates the relative abundance of the top 10 species at the genus level. The majority of bacteria that remained were of no rank and unexplored. The main genera identified were *Oxobacter*, *Anaerolinea*, *Anaerovorax*, *Thermincola*, *BSV13*, *Ruminiclostridium*, and *Sphingomonas*. Approximately 23.55% of all bacteria were represented by the top 10 bacteria in terms of relative abundance. The abundance of *BSV13* did not change significantly between the three treatment groups, although it increased in sample 5a (0.14%) compared to 1a. In addition, the abundance of *Anaerolinea* decreased in sample 5a (0.08%) compared to sample 1a (3.03%).



(**a**) (**b**)

Figure 5. Relative abundance of dominant bacteria in the *E. urophylla* × *E. grandis* plantations with different stand ages. (**a**) Phylum level; (**b**) Genus level. The graph shows the mean relative abundance in percent of phyla/genera that are present in all samples and account for ≥5% of all phyla and ≥2% of all genera. The relative abundance level after the top 10 phyla/genera were defined as "Others". 1a, 3a, and 5a represent the age of *Eucalyptus* plantations at 1, 3, and 5 years, respectively.

4. Discussion

4.1. Influence of Plantation Age on Nutrient Content of Soil

The physical and chemical properties of soil have been shown to become unbalanced by short-term and continuous planting of *E. urophylla* × *E. grandis* [18]. There was a considerable decrease in soil pH with the number of years of continuous cultivation. This is probably due to increased levels of acidic ions (NO_3^-, SO_4^{2-}, Cl^-, etc.), which gradually acidify the soil [32,33]. The findings of a recent study indicated that the decrease in soil pH may be related to the secretion of more acidic substances by the root system during *Eucalyptus* growth [6]. A statistically significant positive correlation between pH and CEC was found in the correlation analysis of the study ($p < 0.01$) (Figure 6). Soil cation exchange decreases may be due to the lower pH and regular clearance of surface vegetation during forestry operations.

The results of the current research demonstrate a decline in the effective phosphorus content of *Eucalyptus* forest soils with advancing plantation age, potentially attributed to soil erosion resulting from the removal of shrubs during forest conservation efforts,

leading to a reduction in soil phosphorus levels [34]. Research has demonstrated that residue and litter enhance soil humus and boost soil fertility [7]. In spite of this, in contrast to the 1a and 3a plantations, the 5a *Eucalyptus* plantation forests exhibited a notable reduction in SOM, while maintaining consistent levels of alkaline hydrolytic and available potassium. This phenomenon is ascribed to the high nutritional demands of *Eucalyptus* trees for robust growth, coupled with the substantial application of inorganic fertilizers by the plantation forest manager.

Studies indicate that the overuse of inorganic fertilizers over a short period of time results in soil compaction, elevated soil bulk density, diminished soil fertility, and other detrimental consequences [35]. Furthermore, the short-term chemical weed control and clearing of forest vegetation and debris have directly contributed to the decline in soil fertility in the 3a and 5a plantations [36]. Notably, pH was positively and markedly related ($p < 0.01$) to the SOM content in the correlation analysis results of the current research (Figure 6). This reduction in SOM in the *Eucalyptus* plantation may result from the immediate chemical weeding and removal of forest vegetation and litter [34]. Therefore, the practice of short-term management may cause problems with the declining soil fertility of *Eucalyptus* plantations. Supplementary phosphorus and organic fertilizers promote the accumulation of SOM on the forest floor and maintain forest sustainability.

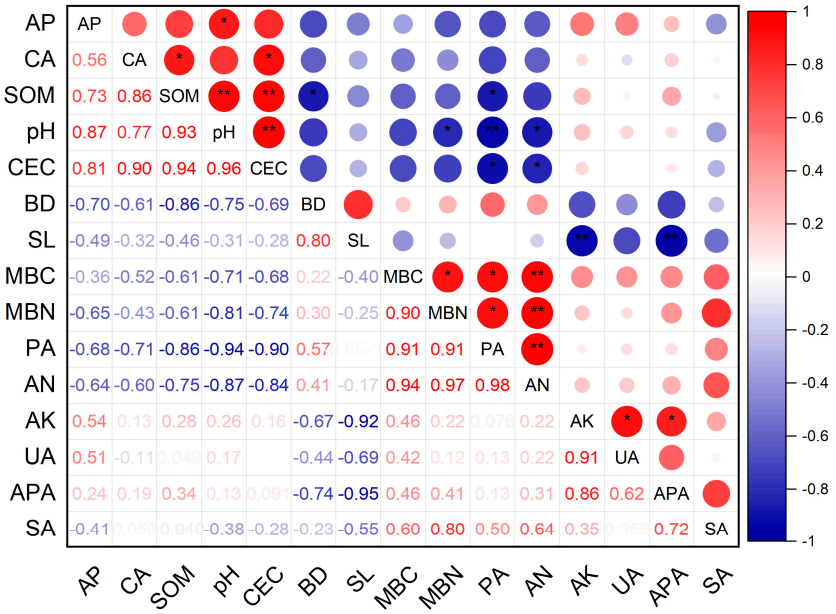


Figure 6. Correlation heatmap illustrating the interrelationships among soil physiochemical properties with distinct letters denoting statistically significant differences. PA, planting age; SL, soil layer; CEC, cation exchange capacity; AN, alkaline hydrolytic nitrogen; AP, available phosphorus; AK, available potassium. SOM, soil organic matter; pH, soil degree of acid or alkali; BD, soil bulk density; UA, urease activity; APA, acid phosphatase activity; CA, catalase activity; SA, sucrase activity; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen. The positive and negative correlations between them are represented by red and blue circles, respectively, and the asterisk indicates significant correlation: * $p \leq 0.05$, ** $p \leq 0.01$.

4.2. Effect of Plantation Age on Soil Enzyme Activity

Research has indicated a substantial relationship between soil enzyme activity and stand nutrient cycling, with enzymes playing a major part in organic matter mineralization [37]. Urease plays a crucial role in the cycling of soil nitrogen by hydrolyzing urea in the soil and converting it into nutrients that can be directly utilized by plants. The activities of urease in soil under different planting ages of *E. urophylla* × *E. grandis* first decreased and then increased. The activities of urease and acid phosphatase were higher in the

plantations of *E. urophylla* × *E. grandis* (5a), but SOM content was lower, contrary to previous findings [9]. Among these, the heightened activity of acid phosphatase was attributed to decreased soil pH [38], while the implications on urease activity remain ambiguous. The results of this research show a positive correlation between the age of the *Eucalyptus* plantation and the activity of soil sucrase, which is consistent with the findings of Wu et al. [39]. Soil catalase protects plants from the phytotoxic effects of hydrogen peroxide by breaking it down [40]. As planting age increases, there was a notable decline in soil catalase activity, potentially attributed to the accumulation of inorganic fertilizers that have adverse effects on the soil ecosystem [41,42]. Changes in soil properties and microbial diversity may explain the increase in soil enzyme activity observed under continuous cropping [43]. In a past study, high levels of acid phosphatase in phosphorus-rich soils were attributed to phosphorus-activated phosphatase [44], which is essential for enhancing soil phosphorus availability in this region [6]. The study results showed a positive correlation between soil physicochemical characteristics, including SOM, CEC, AK, and MBN, and soil enzyme activities. Collectively, these findings indicate that appropriate fertilisation improves the availability of nutrients in the soil by increasing the nutrient content of the soil and the overall activity of the enzymes.

4.3. Influence of Plantation Age on Soil Microbial Biomass and the Diversity of Soil Microbial Community

Soil microbial biomass, a vital element in the nutrient cycle, plays a pivotal role in sustaining essential biological processes including organic matter decomposition [45]. *Eucalyptus* plantations have the potential to significantly affect both the microbial community composition and biomass of soil microorganisms through modifications to biotic and abiotic conditions [46]. In previous research, a considerable accumulation in soil MBC and MBN levels was observed as planting age increased. This may be due to the application of fertilizers and the presence of residual apoplastic substances that offer a diverse array of carbon resources for soil microbes, thereby fostering their colonization [7]. Simultaneously, forestry practices aid the removal of weeds and the removal of some of the remaining apoplectic matter, reinforcing the carbon restriction on soil microbes, and this may be responsible for the decrease in the MBC to MBN ratio [47].

The diversity of soil microflora is associated with the ecological stability and functioning of the ecosystem [48]. The study authors found that the biodiversity of soil bacteria decreased in *Eucalyptus* plantations with increasing planting age. Similar levels of alpha diversity have been noted in comparable plantations [49,50]. In different generations of *Eucalyptus* plantations, different environmental factors, such as changes in the microclimate of the soil, litter production, secretions, and root-associated microorganisms, influence the bacterial diversity of soil communities [51]. In our research, the results showed that MBC and MBN in *Eucalyptus* soils increased simultaneously with the number of years of planting, despite a decrease in soil bacterial diversity. This phenomenon can be attributed to the nutrient-deficient soil prevalent in *Eucalyptus* cultivation settings, which encourages the soil microbial community to engage in SOM decomposition activities, consequently impacting bacterial diversity [52]. Previous research has confirmed that the MBC and MBN are the primary factors affecting bacterial community structure, with other variables potentially playing a role [53].

The prevalent phyla in forest soils, including *Firmicutes*, *Chloroflexi*, *Acidobacteria*, *Proteobacteria*, and *Actinobacteria*, were well-represented [50,54,55] in this study, with stable distribution of the dominant bacteria observed post-*Eucalyptus* plantation in our investigation. This indicates that the short-term planting of *Eucalyptus* has minimal influence on the microbial communities of the soil. Identical findings were noted by Li et al. [9] in the short-term period of five years. The prevalence of *Firmicutes* and *Acidobacteria* in soil samples, as documented in multiple studies [56], implies limited impact from changes in planting ages, potentially explaining the restricted variability. *Firmicutes* are known for their rapid growth and spore production and are typically categorized as copiotrophs [57];

Acidobacteria, in contrast, are often classified as oligotrophs [58]. In this research, *Firmicutes* were more abundant than *Actinobacteria*. This finding suggests that the management practices utilized, such as fertilizer application, could lead to a transition from an oligotrophic bacterial community to a more copiotrophic one.

5. Conclusions

In conclusion, the soil nutrient and microbial characterization of *E. urophylla* × *E. grandis* plantation forests were found to be closely linked to continuous planting age. Variations in soil physicochemical characteristics, soil enzyme activity, and soil microbial diversity were observed among the different planting ages of *Eucalyptus* plantations. The research findings indicate that the soil microbial biomass, sucrase activity, and alkaline hydrolytic nitrogen in *Eucalyptus* plantations increased with planting age; the soil catalase activity, effective phosphorus, and SOM, in contrast, showed a decrease. The consecutive cultivation of *E. urophylla* × *E. grandis* resulted in direct or indirect alterations to the soil environment, which caused significant changes in soil characteristics and the diversity of microbial communities. Therefore, appropriate supplementation with phosphorus and organic and microbial fertilizers during cultivation is recommended to enhance the productivity of *Eucalyptus* plantations, while also aiding in addressing soil quality concerns associated with short-cycle *Eucalyptus* planting and improving economic efficiency. This study will facilitate the sustainable development of *Eucalyptus* plantation forestry in the region by providing practical insights into the cultivation of *Eucalyptus* plantations of different ages in Guangxi, China.

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