

## Postprint: Characteristics of Soil Microbial Quantity and Enzyme Activity in Eucalyptus Plantations of Different Stand Ages in Northern Guangxi

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**Date:** 2019-08-27T00:00:00+00:00

### Abstract

To investigate the response characteristics of soil microbial activity in eucalyptus plantations at different stand ages, the space-time substitution method was adopted, and 1-5 year-old eucalyptus plantations in the low mountain and hilly region of northern Guangxi were selected as the research object to analyze the variation patterns of soil microorganisms and enzyme activities in different soil layers and seasons. The results showed that: (1) Soil microbial and enzyme activities exhibited obvious vertical distribution characteristics in soil layers, both tending to decrease with increasing soil depth, and the differences among soil layers were significant. (2) The seasonal order of bacteria and actinomycetes numbers was autumn > summer > spring > winter, the variation pattern of fungi was spring > summer > autumn > winter, while enzyme activity showed seasonal variation with higher activity in summer and autumn, and lower activity in spring and winter. (3) Bacteria, fungi, urease, and catalase showed a trend of first decreasing then increasing with increasing stand age, actinomycetes showed a trend of first decreasing, then increasing, then decreasing again, while sucrose and acid phosphatase tended to increase with increasing stand age. (4) There was an extremely significant positive correlation between the three major microbial groups and four soil enzymes in forest soil, indicating that soil microorganisms and soil enzyme activities mutually influence each other, the relationship between them is close, and they jointly affect soil quality. The seasonal response characteristics of soil microorganisms and enzyme activities in different soil layers varied considerably, being overall lowest in winter, which was mainly related to influences of temperature, moisture conditions, and litter nutrient return. The interactions among different seasons, soil layers, and stand ages had significant effects on soil microorganisms and enzyme activities.

## Full Text

### Study on Soil Microbe Quantity and Enzyme Activity Characteristics in Eucalyptus Plantations of Different Ages in Northern Guangxi

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**Abstract:** To investigate the response characteristics of soil microbial activity under different stand ages of Eucalyptus, we employed a space-for-time substitution approach to examine variations in soil microbes and enzyme activities across different soil layers and seasons in 1- to 5-year-old Eucalyptus plantations in the low hilly areas of northern Guangxi. The results revealed: (1) Soil microorganisms and enzyme activities exhibited distinct vertical distribution patterns in the soil profile, decreasing significantly with soil depth. (2) Bacterial and actinomycete quantities followed the seasonal order of autumn > summer > spring > winter, while fungi showed a pattern of spring > summer > autumn > winter. Enzyme activities were generally higher in summer and autumn, and lower in spring and winter. (3) Bacteria, fungi, urease, and catalase displayed a trend of decreasing first and then increasing with stand age, whereas actinomycetes showed a pattern of decreasing, increasing, and then decreasing again. Invertase and acid phosphatase activities tended to increase with stand age. (4) Highly significant positive correlations existed among the three major microbial groups and four soil enzymes, indicating that soil microbes and enzyme activities mutually influence each other and jointly affect soil quality. The seasonal response characteristics of soil microbes and enzyme activities varied substantially across different soil layers, with overall lowest values in winter, primarily related to temperature, moisture conditions, and litter nutrient return. Interactive effects among season, soil layer, and stand age significantly influenced soil microbial and enzyme activities.

**Keywords:** Eucalyptus plantations, soil microorganisms, soil enzyme activity, stand age, season

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## Introduction

Eucalyptus, belonging to the Myrtaceae family, is widely adapted and serves as an important fast-growing and high-yield timber species in southern China (including Hainan, Guangdong, Guangxi, and Fujian). In recent years, Guangxi has led the nation in both the expansion rate and total area of Eucalyptus plantations. While intensive management practices have increased timber production and contributed significantly to China's wood supply, unreasonable cultivation methods aimed at accelerating growth or shortening rotation periods have caused increasingly prominent ecological problems, including soil quality degradation and reduced biodiversity. Soil quality degradation manifests primarily as declining soil fertility, particularly altering soil nutrient characteristics, microorganisms, and enzymes. Soil microbes and enzymes respond more sensitively to environmental changes than organic matter and other nutrients, with minor variations capable of altering microbial community structure and activity. Therefore, studying changes in soil microbial quantity and enzyme activity is crucial for the sustainable development of Eucalyptus plantations.

Soil microorganisms are essential participants in biogeochemical cycles and energy flow, as well as indispensable decomposers in forest ecosystems that influence material and nutrient cycling. Soil enzymes play key roles in various biochemical processes and material cycles, with their activity serving as an important indicator for evaluating soil quality. China's Eucalyptus plantation area exceeds 4.5 million hectares, with Guangxi accounting for approximately half of the national total. In the low hilly regions of northern Guangxi, collective and private management is common, characterized by intensive but extensive practices such as heavy fertilization and herbicide application, with some stands harvested as early as 3-4 years. The seasonal dynamics of soil microbial quantity and enzyme activity under different stand ages remain unclear, particularly during the rapid growth phase from 1 to 5 years when stand development is most dynamic. Using a space-for-time substitution approach, this study selected 1- to 5-year-old Eucalyptus plantations in Huangmian Forest Farm of northern Guangxi, with adjacent *Pinus massoniana* stands (10 years) as controls, to investigate soil microbial quantity characteristics, seasonal variations in enzyme activities, and their correlations, providing scientific guidance for efficient management and quality improvement of Eucalyptus plantations.

## 1. Study Area Description

The study was conducted at Huangmian Forest Farm, located at the junction of Luzhai County (Liuzhou City) and Yongfu County (Guilin City) in Guangxi (109°43'46" E, 24°37'25" N). The region has a

mid-subtropical climate with long frost-free periods and concurrent rainfall and heat. The mean annual temperature is 19 °C, with average annual precipitation of 1,750–2,000 mm concentrated from April to August. Heat is abundant, with annual evaporation of 1,426–1,650 mm. The terrain consists primarily of low mountains and hills with steep slopes. Soils are mainly red soil and mountainous yellow-red soil developed from sandstone and sandy shale parent materials.

## 2. Methods

**2.1 Soil Sampling** In April 2013 (spring), July 2013 (summer), October 2013 (autumn), and January 2014 (winter), we conducted detailed field surveys using a space-for-time substitution method. We selected 1-, 2-, 3-, 4-, and 5-year-old Eucalyptus plantations (*Eucalyptus urophylla* × *E. grandis*) converted from *Pinus massoniana* secondary forests with consistent site history. In different compartments with similar parent material, elevation, slope aspect, and management practices, we established three replicate plots (20 m × 20 m) spaced 60–80 m apart for each stand age. Adjacent *Pinus massoniana* stands (10 years) that had not been converted served as controls, with three 20 m × 20 m standard plots. After removing surface litter, we collected soil samples using five representative sampling points per plot. A soil corer (5 cm diameter) was used at 10-cm intervals to a depth of 40 cm, with four layers (0–10, 10–20, 20–30, and 30–40 cm) sampled separately. Soils from the same layer were mixed to form one composite sample per plot. Samples were stored at 4 °C for microbial analysis, while air-dried samples were used for enzyme activity measurements.

**2.2 Analysis of Soil Microbial Quantity and Enzyme Activity** Soil microbial quantities were analyzed using the dilution plate counting method described by Xu and Zheng (1986), with detailed procedures provided in Zhou et al. (2017). Soil enzyme activities were determined using methods from Guan (1986), including phenol sodium colorimetry, 3,5-dinitrosalicylic acid colorimetry, and disodium phenyl phosphate colorimetry, with detailed protocols described in Xu et al. (2014).

**2.3 Data Processing** Data were processed and tables created using Excel 2010 and SPSS 23.0. Statistical analyses included one-way ANOVA, multi-way ANOVA, and LSD multiple comparisons ( $\alpha = 0.05$ ).

## 3. Results and Analysis

**3.1 Changes in Soil Microbial Quantity Across Different Stand Ages** Soil bacterial quantities in Eucalyptus plantations showed distinct vertical distribution patterns, with the highest numbers in the surface layer and significant decreases with depth in all seasons. Bacterial quantities exhibited a decreasing-then-increasing trend with stand age. Within the same soil layer and season, bacterial quantities differed significantly between Eucalyptus stands of different ages and the *Pinus* control ( $P < 0.05$ ), though differences diminished with

depth. Seasonal patterns showed autumn > summer > spring > winter across soil layers. In contrast, the *Pinus* control showed winter > spring > autumn > summer, with significant seasonal differences ( $P < 0.05$ ).

Actinomycete quantities in both Eucalyptus plantations and the *Pinus* control decreased with soil depth. Within the same layer and season, actinomycetes in Eucalyptus plantations showed a decreasing-increasing-decreasing pattern with stand age, with significant differences among ages ( $P < 0.05$ ). Seasonal variation was consistent: autumn > summer > spring > winter. In Eucalyptus plantations, significant seasonal differences occurred in 0–30 cm layers ( $P < 0.05$ ), but in the 30–40 cm layer, differences were not significant between spring and winter for 1–5-year stands, or between summer and autumn for 3- and 4-year stands ( $P > 0.05$ ), indicating diminishing variation with depth. The *Pinus* control showed significant seasonal differences in all layers ( $P < 0.05$ ).

Fungal quantities in Eucalyptus plantations followed the seasonal pattern spring > summer > autumn > winter, while the *Pinus* control showed autumn > summer > spring > winter. In Eucalyptus plantations, seasonal differences were not significant between autumn and winter for 3-year stands in 0–20 cm layers, or between summer and autumn for 4-year stands ( $P > 0.05$ ). In 30–40 cm layers, no significant differences occurred between summer and autumn for any stand age ( $P > 0.05$ ). Within each season, fungal quantities decreased with soil depth. Across seasons within the same layer, quantities showed a decreasing-then-increasing trend with stand age.

### 3.2 Changes in Soil Enzyme Activities Across Different Stand Ages

Both Eucalyptus plantations and the *Pinus* control showed clear vertical distribution of invertase activity, with higher activity in surface layers decreasing significantly with depth. Seasonal variation in invertase within the same layer followed autumn > summer > spring > winter, with significant differences among seasons ( $P < 0.05$ ). Across seasons and layers, activity increased with stand age in the order: control (10 years) > 5 years > 4 years > 3 years > 2 years > 1 year, approaching control values in older stands.

Urease activity showed seasonal patterns of summer > autumn > spring > winter within the same layer, with most seasonal differences being significant. Within the same season and stand age, urease activity decreased with soil depth. Across stand ages within the same layer and season, urease activity decreased from 1 to 3 years, then increased substantially. The *Pinus* control exhibited much higher urease activity than 1–5-year Eucalyptus plantations.

Acid phosphatase showed no consistent pattern across stand ages, with *Pinus* control values greater than 1–4-year Eucalyptus but less than 5-year stands. Seasonal patterns within the same layer and age followed summer > autumn > spring > winter, with significant seasonal differences ( $P < 0.05$ ). Activity decreased with soil depth.

Catalase activity was significantly higher in surface layers, decreasing with depth.

Seasonal patterns within the same layer and age showed summer > autumn > spring > winter . Catalase activity decreased then increased with stand age, with 2-year stands showing the lowest activity. *Pinus* control values fell between 4- and 5-year Eucalyptus stands, though some differences were not significant (e.g., in 30–40 cm layers between 1- and 2-year stands in spring, or between 3- and 4-year stands).

### 3.3 Correlations Between Soil Microbes and Enzyme Activities

Highly significant positive correlations ( $P < 0.01$ ) existed among bacteria, actinomycetes, fungi, invertase, urease, acid phosphatase, and catalase . The correlation coefficient between bacteria and actinomycetes reached 0.794, while those between catalase and invertase, urease, and acid phosphatase were 0.887, 0.817, and 0.904, respectively. These relationships indicate that the three major microbial groups and four enzyme activities are closely interconnected, jointly influencing soil quality. Season, soil layer, and stand age, along with their interactions, significantly affected soil microbial groups and enzyme activities ( $P < 0.05$ ) .

## 4. Discussion

**4.1 Changes in Soil Microbial Quantity** Soil microbial quantities in forest ecosystems are influenced by climate conditions, vegetation type, stand composition, and soil organic carbon content. This study found that bacteria were most abundant, followed by actinomycetes, with fungi being least abundant in northern Guangxi Eucalyptus plantations, consistent with findings by Xie (2005) and Chen et al. (2008). However, Ji (2015) reported higher fungal than actinomycete abundance, possibly due to different soil pH. The subtropical monsoon climate of southern China, characterized by high temperature and humidity, facilitates litter decomposition and creates favorable conditions for microbial growth. Different vegetation types and stand structures create distinct ecological conditions, providing varied food sources and habitats that indirectly cause differences in microbial quantities.

Soil bacteria, actinomycetes, and fungi in Eucalyptus plantations showed clear vertical distribution patterns, decreasing significantly with soil depth—a result consistent with numerous studies. Significant differences occurred among soil layers across all stand ages and seasons. As Eucalyptus is a shallow-rooted species, most nutrients derive from surface litter, and root exudates promote microbial activity in surface layers. Myers et al. (2001) identified soil temperature, moisture, rainfall, physicochemical properties, and species characteristics as primary regulators of seasonal microbial dynamics in specific forest ecosystems. Wang et al. (2008) found that soil microbes exhibit obvious seasonal fluctuations under the combined effects of multiple ecological factors. In this study, bacterial and actinomycete quantities followed autumn > summer > spring > winter, similar to Xie (2005), with higher autumn abundance due to abundant litter and suitable soil temperature promoting decomposition. Fungi showed

spring > summer > autumn > winter. Microbial distribution is influenced by vegetation type, stand composition, and soil physicochemical properties, with stand age being particularly important.

**4.2 Changes in Soil Enzyme Activities** Soil invertase, urease, acid phosphatase, and catalase activities in Eucalyptus plantations showed vertical distribution patterns consistent with microbial quantities, a finding supported by multiple studies. This indicates that microbial quantities are sensitive to stand age changes, with minor variations directly affecting enzyme activities and reflecting their close relationship. Niu et al. (2015) found that soil microbial quantity, enzyme activity, and nutrient content were higher in spring and autumn than summer in Japanese larch plantations, with soil fertility declining during stand development. He et al. (2015) reported that soil nutrients and enzyme activity increased with stand age in Taiwan plantations, improving soil fertility. Other studies have shown seasonal patterns of enzyme activity increasing then decreasing, with higher activities in summer and autumn. In this study, invertase followed autumn > summer > spring > winter, while urease, acid phosphatase, and catalase showed summer > autumn > spring > winter, with *Pinus* controls exhibiting the same patterns. These results differ from Hu and Wang (2015), who found that phosphatase, polyphenol oxidase, and catalase activities decreased significantly with planting duration, with 3-5-year plantations showing lower activity than 1-year stands. They also differ from Liu (2013), who attributed higher summer-autumn enzyme activity to elevated temperatures and rainfall promoting microbial activity and respiration. These discrepancies likely relate to differences in study region, stand type, site conditions, management practices, and environments. For example, Hu and Wang (2015) studied plantations converted from farmland, while Liu (2013) focused on different planting generations with shrub-grass slopes as controls. Vegetation community and seasonal variation are also important factors affecting soil enzyme activity.

The *Pinus* control generally showed higher enzyme activity than Eucalyptus plantations. Soil invertase and acid phosphatase increased with Eucalyptus stand age, while urease and catalase decreased then increased. As a fast-growing species with short rotation, Eucalyptus harvest removes approximately 80% of nutrient stocks. Zhou et al. (2009) found higher urease activity in *Pinus* than Eucalyptus plantations, while Tan et al. (2014) reported significantly lower protease and phosphatase activities in Eucalyptus compared to *Pinus* and natural secondary forests. Zhang et al. (2015) found that converting *Pinus* to Eucalyptus significantly decreased soil enzyme activity. In northern Guangxi's low hills, the short rotation of Eucalyptus plantations compared to longer-rotation *Pinus* stands may cause substantial nutrient export, reducing soil nutrients, microbes, and enzyme activities. Therefore, we recommend reasonable management practices, including reducing intensive site preparation during planting and harvest periods to avoid destroying litter layers, applying organic fertilizer, and extending rotation periods to improve soil quality in Eucalyptus plantation

ecosystems.

## 5. Conclusions

1. The three major microbial groups in Eucalyptus plantations ranked as bacteria > actinomycetes > fungi. Both microbial quantity and enzyme activity showed vertical distribution patterns, decreasing with soil depth.
2. Microbes and enzymes exhibited seasonal variation with distinct patterns. Seasonal response characteristics differed across soil layers but were consistently lowest in winter, primarily related to temperature, moisture, and litter nutrient return.
3. Stand age significantly affected soil microbial quantity and enzyme activity. Highly significant correlations existed between microbes and enzymes, with their interactions jointly indicating soil fertility trends in Eucalyptus plantations.

**Acknowledgments:** We thank Cheng Guixia, Liu Jianchun, Jiang Yulong, Li Cuiling, and others for assistance with sample analysis.

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