

Effects of Nitrogen-Fixing Tree Species *Acacia mangium* on Soil Aggregate Size Distribution and Stability in *Eucalyptus grandis* × *E. urophylla* Plantations (Postprint)

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Abstract

Soil aggregate stability is an important indicator for evaluating soil structure and soil fertility. To investigate the effects of the nitrogen-fixing tree species *Acacia mangium* on soil aggregate size distribution and stability in *Eucalyptus grandis* × *Eucalyptus urophylla* plantations, this study examined 17-year-old pure *Eucalyptus grandis* × *Eucalyptus urophylla* plantations (PP) and mixed plantations of *Eucalyptus grandis* × *Eucalyptus urophylla*/*Acacia mangium* (MP). Dry and wet sieving methods were used to determine aggregate size distribution and stability indices—including mean weight diameter (MWD), geometric mean diameter (GMD), fractal dimension (Dm), water-stable aggregate content (WSA), aggregate disruption rate (PAD), and aggregate stability index (ASI)—in 0–10 cm and 10–20 cm soil layers. The results showed: (1) Compared with PP, MP exhibited varying degrees of improvement in soil physicochemical properties, most notably in soil pH, organic carbon (SOC), and total nitrogen (TN). (2) MP demonstrated superior soil aggregate size distribution compared to PP, with differences primarily observed in the >2 mm and <0.25 mm fractions, both dominated by macroaggregates (>0.25 mm). The mechanical stability of soil aggregates in MP was significantly enhanced only in the 0–10 cm layer, whereas water stability was significantly improved in both 0–10 cm and 10–20 cm layers. (3) Mantel analysis revealed that aggregate stability was most strongly correlated with TN, and RDA analysis further identified TN as the key factor driving variation in aggregate stability. In conclusion, the nitrogen-fixing species *Acacia mangium* significantly improves soil aggregate stability in *Eucalyptus grandis* × *Eucalyptus urophylla* plantations, providing a scientific basis for soil and water conservation, nutrient management, and sustainable management of South

subtropical *Eucalyptus* plantations.

Full Text

Effects of the Nitrogen-Fixing Tree Species *Acacia mangium* on Soil Aggregate Size Distribution and Stability in *Eucalyptus urophylla* Plantations

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Abstract

Soil aggregate stability is a crucial indicator for evaluating soil structure and fertility. To investigate the effects of the nitrogen-fixing tree species *Acacia mangium* on soil aggregate size distribution and stability in *Eucalyptus urophylla* plantations, we examined 17-year-old pure *E. urophylla* plantations (PP) and mixed *E. urophylla/A. mangium* plantations (MP). Using dry and wet sieving methods, we measured aggregate size distribution and stability indicators—including mean weight diameter (MWD), geometric mean diameter (GMD), fractal dimension (Dm), water-stable aggregate content (WSA), percentage of aggregate destruction (PAD), and aggregate stability index (ASI)—in 0–10 cm and 10–20 cm soil layers. The results showed: (1) Compared with PP, MP exhibited improved soil physicochemical properties, with significant increases in soil pH, organic carbon (SOC), and total nitrogen (TN). (2) MP demonstrated superior soil aggregate size distribution, with differences primarily in the >2 mm and <0.25 mm fractions, and both plantations were dominated by macroaggregates (>0.25 mm). The mechanical stability of soil aggregates in MP was significantly higher only in the 0–10 cm layer, whereas water stability was significantly improved in both 0–10 cm and 10–20 cm layers compared with PP. (3) Mantel analysis revealed that aggregate stability correlated most strongly with TN, and redundancy analysis (RDA) further identified TN as the key driver of variation in aggregate stability. In conclusion, the nitrogen-fixing species *A. mangium* significantly improved soil aggregate stability in *E. urophylla* plantations, providing a scientific basis for soil and water conservation, nutrient management, and sustainable management of eucalyptus plantations in subtropical southern China.

Keywords: soil aggregates, mechanical stability, water stability, *Eucalyptus* plantations, nitrogen-fixing tree species

Introduction

Soil aggregates are the fundamental units of soil structure, playing vital roles in coordinating soil water, nutrients, air, and heat, influencing soil enzyme types and activities, and maintaining the soil's loose, mellow layer (Lu & Li, 2002; Six et al., 2004). Generally, the content of >0.25 mm water-stable aggregates is considered a key indicator of soil fertility, reflecting nutrient supply, water-holding capacity, aeration, and determining soil productivity and erosion resistance (Cai et al., 2008; Delelegn et al., 2017).

Soil aggregate stability is a critical factor affecting soil structure and a key indicator of soil fertility and quality (Six et al., 2000; Bronick & Lal, 2005). As a soil physical property, improved aggregate stability helps resist soil degradation and maintain specific structures under destructive physical stresses such as rainfall and surface runoff (Besalatpour et al., 2013; Li et al., 2013). Enhancing aggregate stability can substantially improve soil structure and fertility, preventing soil erosion and other environmental problems caused by soil degradation (Zhu et al., 2017). Aggregate stability is closely related to soil organic matter content (Bronick & Lal, 2005), soil microbial quantity and activity (Lin et al., 2019), land use patterns, management practices, climatic conditions, and vegetation types (Dong, 2020). Research on aggregate stability has focused on quantification theories and methods (Ding & Zhang, 2016; Aksakal et al., 2020) and influencing factors and mechanisms (Dong, 2020) across various ecosystems including farmland, wetlands, grasslands, and forests (Liu et al., 2022). Common measurement methods include dry sieving for mechanical stability, wet sieving for water stability, and Le Bissonnais method for investigating aggregate breakdown mechanisms (Dong, 2020). The proportion of macroaggregates obtained by wet sieving is typically lower than that by dry sieving, with differences in aggregate size distribution primarily attributed to varying energy inputs (Zhu et al., 2021) and different aggregate breakdown modes (Wang et al., 2011).

Eucalyptus species, characterized by wide adaptability, strong stress resistance, and rapid growth, are widely planted in coastal provinces such as Guangxi, Guangdong, Hainan, and Fujian, generating substantial economic benefits (Wen et al., 2018). However, continuous development of eucalyptus plantations has revealed various ecological problems, including soil degradation, declining site productivity, and reduced understory plant diversity resulting from unsustainable management practices such as short rotation cycles, high-generation pure stand continuous planting, heavy fertilization, and herbicide use (Huang & Zhao, 2014; Wen et al., 2018), which severely constrain plantation development. Consequently, improving the ecological environment, mitigating soil degradation, and maintaining or enhancing soil nutrient content in eucalyptus plantations have become research priorities. Previous studies on eucalyptus plantation soils have focused primarily on soil nutrient cycling and regulation mechanisms (Huang et al., 2017; Tang et al., 2021; Shao et al., 2022), while in-depth research on aggregate size distribution and stability mechanisms remains lacking. Investigating soil aggregate characteristics is crucial for maintaining and restoring soil

fertility in eucalyptus plantations.

Recent research by Wang et al. (2022) found that continuous eucalyptus planting exacerbates soil degradation, reduces erosion resistance, and decreases aggregate stability. Lin et al. (2020) compared aggregate stability among five typical plantations in subtropical southern China and found that eucalyptus plantations had relatively poor soil structure and the lowest aggregate stability. Therefore, identifying silvicultural measures to improve aggregate stability in eucalyptus plantations is essential. Studies have shown that introducing nitrogen-fixing species such as *Alnus nepalensis*, *Acricarpus fraxinifolius*, and *Dalbergia odorifera* into degraded forests can significantly enhance soil organic matter, total nitrogen, and phosphorus availability, effectively improving soil fertility (Li et al., 2022a; Li et al., 2022b). Mo et al. (2022) found that introducing nitrogen-fixing species into eucalyptus plantations improved enzyme activities and stoichiometric ratios in soil aggregates, alleviating soil N and P limitations. Huang et al. (2017) reported that nitrogen-fixing species improved soil microbial community structure and extracellular enzyme activity, thereby increasing soil carbon storage and recalcitrant carbon content. However, whether nitrogen-fixing species can improve aggregate stability in eucalyptus plantations, how this relates to soil physicochemical properties, and what mechanisms and key driving factors are involved remain poorly understood. This study addresses these knowledge gaps by examining pure *Eucalyptus urophylla* plantations (PP) and mixed *E. urophylla*/*Acacia mangium* plantations (MP) at the Experimental Center of Tropical Forestry, Chinese Academy of Forestry, using combined dry and wet sieving methods to comprehensively analyze aggregate size distribution and stability characteristics, elucidate the mechanisms through which *A. mangium* influences aggregate stability, and identify key driving factors to provide a theoretical foundation for soil nutrient management and sustainable plantation management.

Materials and Methods

1.1 Study Area The study site is located at the Experimental Center of Tropical Forestry, Chinese Academy of Forestry, in Pingxiang City, Guangxi (106°56 E, 22°03 N). Pingxiang features a hilly mountainous landscape with a subtropical monsoon climate characterized by abundant solar radiation and water-heat resources, distinct wet and dry seasons, an average annual temperature of 21 °C, annual precipitation of 1400 mm, and a frost-free period exceeding 340 days. The soil type is primarily red soil derived from granite weathering, with acidic pH, moderate to low organic matter and total nitrogen content, and limited phosphorus, potassium, zinc, boron, and molybdenum availability.

We selected 17-year-old pure *E. urophylla* plantations (PP) and mixed *E. urophylla*/*A. mangium* plantations (MP) as study objects, establishing five independent 20 m × 20 m plots in each plantation type. The mixed plantation consisted of *E. urophylla* and *A. mangium* of the same age at a 1:1 mixing ratio in alternating rows. Both plantations were established in 2004 after clear-

cutting and slash-burning of a *Pinus massoniana* forest planted in 1977, and were managed similarly throughout the experiment. Before planting, 500 g of base fertilizer was applied per tree, with manual weeding and fertilization conducted semi-annually for the first two years, totaling 200 kg N · ha⁻², 150 kg P · ha⁻², and 100 kg K · ha⁻². Basic plot characteristics are shown in Table 1 .

1.2 Sample Collection Based on plant growth characteristics, soil samples were collected in early August 2021 during the peak growing season. Starting from 0°, directional lines were established every 45°, with a sampling point set 5 m from the plot center along each line. After removing surface litter, animal and plant residues, and stones, undisturbed soil was collected from each point at two depths: 0–10 cm and 10–20 cm. Soils from the eight sampling points were mixed and stored in rigid plastic boxes to prevent structural damage during transport. Additionally, soil cores (100 cm³) were collected at each depth using cutting rings to determine bulk density (BD) and soil porosity (SP). In the laboratory, samples were cleaned of gravel and residues; one portion was air-dried to 20% field moisture content and gently broken into ~1 cm fragments along natural cleavage planes for aggregate analysis, while another portion was ground and sieved for physicochemical property determination.

1.3 Measurement Methods (1) Dry Sieving Method

Following Lin et al. (2020), 500 g of soil was passed through nested sieves with 2 mm, 1 mm, 0.5 mm, and 0.25 mm apertures to determine the mass of aggregates in size fractions >2 mm, 1–2 mm, 0.5–1 mm, 0.25–0.5 mm, and <0.25 mm, calculating percentage content and mechanical stability indices.

(2) Wet Sieving Method

Following Elliott (1986), 50 g of soil was prepared according to the proportions obtained from dry sieving. Aggregates were immersed in deionized water for 30 minutes before wet sieving with the same aperture sizes using a particle analyzer at 38 mm amplitude and 30 vibrations · min⁻¹ for 30 minutes. After sieving, aggregates were transferred to aluminum boxes, oven-dried at 105 °C, weighed, and used to calculate percentage content and water stability indices.

(3) Soil Physicochemical Properties

Soil properties were determined following *Soil Agrochemical Analysis* (Bao, 2000). Soil pH was measured using a pH meter (1:2.5 soil:water ratio); BD and SP were determined by the cutting ring method; soil organic carbon (SOC) was measured by potassium dichromate oxidation; total nitrogen (TN) by Kjeldahl digestion; and total phosphorus (TP) by molybdenum-antimony colorimetry.

1.4 Data Analysis Since single indicators cannot comprehensively reflect aggregate stability, we used multiple indices: mean weight diameter (MWD) (Van Bavel, 1950), geometric mean diameter (GMD) (Mazurak, 1950), mass fractal dimension (Dm) (Tyler & Wheatcraft, 1992; Yang et al., 1993), water-stable aggregates (WSA) (Leng et al., 2021), percentage of aggregate destruction

(PAD) (Wei et al., 2022), and aggregate stability index (ASI) (Shi, 2006).

MWD and GMD characterize aggregate size composition, with larger values indicating greater stability. WSA represents water-stable aggregate content, with higher values indicating stronger water stability. Dm indicates aggregate uniformity, with smaller values suggesting higher macroaggregate proportions and better stability. PAD, combining dry and wet sieving, represents the proportion of mechanically stable macroaggregates (>0.25 mm) that break down into microaggregates (<0.25 mm) after wet sieving, with lower values indicating greater stability. ASI, also combining both methods, represents the probability of mechanically stable aggregates remaining in their original size fraction after wet sieving, serving as a comprehensive stability indicator where larger values indicate greater stability.

Calculation Formulas:

(1) Mean Weight Diameter (MWD, mm) and Geometric Mean Diameter (GMD, mm)

$$MWD = \sum_{i=1}^n x_i w_i$$

$$GMD = \exp \left(\sum_{i=1}^n w_i \ln x_i \right)$$

where x_i is the mean diameter (mm) of aggregates in size fraction i , and w_i is the mass percentage (%) of aggregates in size fraction i .

(2) Fractal Dimension (Dm)

Following Tyler et al. (1992) and Yang et al. (1993):

$$\frac{M(r < \bar{x}_i)}{M_T} = \left(\frac{\bar{x}_i}{\bar{x}_{\max}} \right)^{3-D_m}$$

where M_T is total aggregate mass (g), \bar{x}_i is the mean diameter (mm) of aggregates in size fraction i , \bar{x}_{\max} is the mean diameter of the largest aggregates (mm), $M(r < \bar{x}_i)$ is the mass of aggregates smaller than size fraction i , and D_m is the mass fractal dimension.

(3) Water-Stable Aggregates (WSA) and Percentage of Aggregate Destruction (PAD)

$$WSA = \frac{M_{>0.25}^{wet}}{M_T^{wet}} \times 100\%$$

$$PAD = \frac{M_{>0.25}^{dry} - M_{>0.25}^{wet}}{M_{>0.25}^{dry}} \times 100\%$$

where $M_{>0.25}^{wet}$ is the mass of >0.25 mm aggregates after wet sieving (g), M_T^{wet} is total mass after wet sieving (g), and $M_{>0.25}^{dry}$ is the mass of >0.25 mm aggregates after dry sieving (g).

(4) Aggregate Stability Index (ASI)

Following Shi (2006), we used a transition matrix approach to fully utilize aggregate analysis information by calculating the retention probability of each size fraction during conversion from mechanical to water stability. Assuming the percentage of mechanically stable aggregates in i size fractions forms matrix M_i , and the corresponding water-stable aggregate percentage after wet sieving forms matrix N_i , with X_1, X_2, \dots, X_i representing the probability of each size fraction remaining unchanged, we obtain $MX = N$. ASI is the sum of retention probabilities across all size fractions:

$$ASI = X_1 + X_2 + X_3 + \dots + X_i$$

where X represents the retention probability for each size fraction. Since <0.25 mm is the smallest fraction and cannot break down further during wet sieving, its retention rate is 1.

Data were analyzed using Excel 2019 and SPSS 25. Independent samples t -tests compared soil physicochemical properties, aggregate size distribution, and stability characteristics between plantation types at the same depth ($P < 0.05$). Mantel tests were performed using the mantel function in R 4.0.3's vegan package to analyze correlations between soil physicochemical properties and aggregate stability ($P < 0.05$). Redundancy analysis (RDA) was conducted using Canoco 5, with aggregate stability characteristics as response variables and soil physicochemical properties as explanatory variables. Origin Pro was used for figure preparation.

Results

2.1 Soil Physicochemical Properties As shown in Table 2, in the 0-10 cm layer, MP exhibited significant increases of 18.93%, 63.17%, 88.70%, and 11.63% in pH, SOC, TN, and SP, respectively, compared with PP ($P < 0.05$). In the 10-20 cm layer, MP showed significant increases of 19.71%, 40.16%, and 60.24% in pH, SOC, and TN ($P < 0.05$), while TP and BD decreased significantly by 31.25% and 9.52%, respectively ($P < 0.05$).

2.2 Soil Aggregate Size Distribution Characteristics Under different sieving methods, aggregate size distribution varied between plantation types, with both dominated by macroaggregates (>0.25 mm) (Figure 1 [Figure 1: see original paper]).

Under dry sieving, both PP and MP were dominated by >2 mm aggregates, accounting for 68.04%-75.66% of total aggregates in both 0-10 cm and 10-20

cm layers. In the 0-10 cm layer, MP had significantly higher >2 mm aggregates than PP ($P < 0.05$) but significantly lower 0.5-1 mm aggregates ($P < 0.05$). In the 10-20 cm layer, MP showed significantly lower 0.5-1 mm and 0.25-0.5 mm aggregates compared with PP ($P < 0.05$) (Table 3).

Under wet sieving, aggregate size distribution in the 0-10 cm layer followed the order >2 mm, <0.25 mm, 0.5-1 mm, 1-2 mm, and 0.25-0.5 mm for both plantations. Compared with PP, MP had significantly higher >2 mm and 0.25-0.5 mm aggregates but significantly lower <0.25 mm aggregates ($P < 0.05$) (Table 4). In the 10-20 cm layer, distribution patterns differed: PP was dominated by <0.25 mm aggregates, while MP was dominated by >2 mm aggregates. MP showed significantly higher >2 mm and 0.25-0.5 mm aggregates but significantly lower <0.25 mm aggregates compared with PP ($P < 0.05$) (Table 4).

2.3 Soil Aggregate Stability Characteristics Under dry sieving, MWD and GMD in MP were significantly higher than in PP in the 0-10 cm layer ($P < 0.05$) (Figure 2 [Figure 2: see original paper]: A, B). Under wet sieving, MWD, GMD, and WSA in MP were significantly higher than in PP in both layers ($P < 0.05$), while fractal dimension D_m was significantly lower ($P < 0.05$) (Figure 2, Figure 3 [Figure 3: see original paper]: A). PAD in MP was significantly lower than in PP in both layers ($P < 0.05$) (Figure 3: B), while ASI was significantly higher ($P < 0.05$) (Figure 3: C), indicating that MP had significantly better overall aggregate stability.

2.4 Correlation Analysis Between Aggregate Stability and Physicochemical Properties Mantel tests revealed varying degrees of correlation between aggregate stability and pH, SOC, TN, TP, BD, and SP (Figure 4 [Figure 4: see original paper]). TN showed the strongest correlation with aggregate stability, being significantly correlated with all stability indicators except PAD ($P > 0.05$). TP showed the weakest correlation, being significantly correlated only with WSA ($P < 0.05$).

RDA with aggregate stability indicators as response variables and soil physicochemical properties as explanatory variables showed that the first and second axes explained 92.75% and 5.50% of the variation in aggregate stability, respectively. The first axis clearly separated PP from MP, indicating that introducing *A. mangium* significantly altered aggregate stability (Figure 5 [Figure 5: see original paper]). TN ($F = 16.3$, $P = 0.002$) explained 47.50% of the variation (Table 5), confirming it as the most critical factor driving aggregate stability variation.

Discussion

3.1 Effects of *Acacia mangium* on Soil Aggregate Size Distribution and Stability Soil aggregate structure, with its excellent coordination of water, air, and nutrient storage capacity, represents the ideal soil structure. In this study, dry and wet sieving yielded different results: mechanical stability

differed significantly between plantations only in the 0–10 cm layer, whereas water stability differed significantly in both layers (Figure 2). This suggests that introducing nitrogen-fixing species improved mechanical stability to some extent but primarily promoted the formation of water-stable aggregates with favorable size distribution and stability. PAD and ASI further demonstrated significantly improved overall aggregate stability from the perspective of aggregate breakdown. Additionally, aggregate size distribution and stability decreased with soil depth, consistent with Tong et al. (2022), primarily due to higher organic matter content in surface soils.

Aggregate formation represents a dynamic balance between aggregation of soil particles by cementing agents and aggregate disruption by external forces (Yu et al., 2022). Stand type significantly influences aggregate formation through combined effects of soil fertility, litter, and root systems (Yang et al., 2022). The improved aggregate stability in MP may be attributed to: (1) Higher plant diversity and aboveground biomass in MP providing better interception of rainfall and runoff (Shen et al., 2001), effectively reducing aggregate disruption by raindrop impact (Wei et al., 2022); (2) Higher quality and quantity of litter input increasing soil organic matter (Huang et al., 2014), which serves as an important cementing agent promoting macroaggregate formation and stability (Liu et al., 2022); and (3) Mixed planting with *A. mangium* potentially producing more root exudates and mycorrhizal fungi, facilitating the binding of microaggregates into macroaggregates (Demenois et al., 2018).

3.2 Relationship Between Aggregate Stability and Soil Physicochemical Properties Wang et al. (2022) found that mixed plantations increase litter decomposition rates and nutrient return compared with pure plantations, causing differences in soil physicochemical properties. In this study, mixing *A. mangium* with *E. urophylla* significantly affected pH, SOC, and TN (Table 2), consistent with Wang et al. (2010), who reported that nitrogen-fixing species positively influence soil C and N recovery, more effectively increasing organic matter and total nitrogen than non-fixing species, with *A. mangium* being particularly effective in rehabilitating C and N cycles in degraded lands of southern China. This occurs because nitrogen-fixing species, through symbiosis with nitrogen-fixing bacteria, increase soil N content, promoting vegetation growth, productivity, and litter input, thereby improving soil properties (Kelty, 2006; Huang et al., 2014; Huang et al., 2017), a finding supported by Marron & Epron (2019) through meta-analysis showing 18% higher biomass in nitrogen-fixing mixed plantations globally.

Mantel tests showed strong significant correlations between pH, SOC, TN, BD, and aggregate stability (Figure 4), indicating that changes in soil physicochemical properties induced by *A. mangium* strongly influenced aggregate stability. Increased pH enhances soil aggregation, particularly affecting macroaggregate formation (Xu et al., 2020). BD reflects overall soil structure; higher BD indicates more compact soil with weaker water-holding and aeration capacity,

limiting microbial activity and cementing agent formation (Liu et al., 2022). Soil organic matter, widely recognized as one of the most important factors influencing aggregate stability, promotes aggregate formation while serving as a storage site for organic matter, creating a mutually reinforcing relationship (Lin et al., 2020). However, RDA identified TN as the key environmental factor, explaining 47.50% of stability variation (Figure 5). Since TN does not directly affect aggregate stability, *A. mangium* likely indirectly influences stability by increasing soil N content, promoting SOC accumulation. First, increased TN improves litter quantity and N content, enhancing soil organic matter input and nutrient return (Mo et al., 2022). Second, according to ecological stoichiometry theory (Xing et al., 2015), increased TN alleviates N limitation, particularly in N-limited ecosystems, enhancing microbial biomass and activity and promoting formation of stable soil organic matter during initial decomposition stages (Cotrufo et al., 2013). Furthermore, Huang et al. (2014) reported that nitrogen-fixing species increased microbial biomass carbon, a crucial precursor to SOC formation (Liang et al., 2017) contributing 10%-27% to SOC formation (Fan et al., 2021). Thus, variation in aggregate stability in eucalyptus plantations results from complex interactions among biotic and abiotic factors induced by nitrogen-fixing species.

Conclusion

This study demonstrates that after 17 years of mixing *A. mangium* with *E. urophylla*, soil physicochemical properties including pH, SOC, and TN were significantly improved. Mixed plantations showed significantly enhanced mechanical stability only in the 0-10 cm layer, but significantly improved water stability in both layers, indicating that *A. mangium* has greater effects on water-stable than mechanically stable aggregates. Both mechanical and water stability tended to decrease with soil depth. Aggregate stability was strongly correlated with soil physicochemical properties, with TN identified as the key factor driving stability variation.

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