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Physiological response of corn and mung beans in intercropping system under eucalyptus stands on application of biochar and urea fertilizer ratio

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ABSTRACT

An agroforestry system that integrates eucalyptus trees with agricultural crops is a promising approach to sustainable land management, combining timber production with food security objectives. The study was conducted on dry land between eucalyptus stands to increase food crop productivity. The experiment using rice husk biochar, urea fertilizer and manure aimed to maintain water and nutrient availability. This study used a three-factor complete randomized block design. The first factor was without biochar and rice husk biochar, the second factor was the ratio of urea and manure fertilizer doses (150:0, 75:10, 0:20 kg ha^{-1}:ton ha^{-1}), and the third factor was the corn monoculture planting pattern, corn + mung bean intercropping, and mung bean monoculture. The analysis was carried out using ANOVA, DMRT, and T test. This study showed that the combination of rice husk biochar, as well as the ratio of urea and manure fertilizer doses (75 kg ha^{-1}:10 ton ha^{-1}) in the monoculture planting pattern can increase chlorophyll A content and the number of corn stomata, and the monoculture planting system can increase the width of corn stomatal aperture. The combination of the ratio of urea and manure fertilizer doses (75 kg ha^{-1}:10 ton ha^{-1}) and the monoculture planting system can increase the total chlorophyll content in mung beans.

Keywords: ameliorant, crops, intercropping, physiological, shade.

INTRODUCTION

Corn and mung beans are seasonal crops that support and complement staple foods in Indonesia. Increasing food crop production in Indonesia currently faces complex challenges. Meeting food needs is essential and strategic for achieving food security. An agroforestry system that integrates eucalyptus trees with agricultural crops is a promising approach to sustainable land management, combining timber production with food security objectives. This system faces unique challenges in nutrient management, particularly nitrogen (N) dynamics, due to complex interactions between tree and crop components (Lan et al., 2023). The integration of corn (*Zea mays* L.) and mung bean (*Vigna radiata* L) in eucalyptus plantations offers complementary benefits: corn provides high biomass production and carbohydrate yields, while mung beans contribute to biological nitrogen fixation and soil improvement through their legume properties.

However, this system faces resource competition constraints, particularly water and nutrients, between annual crops and eucalyptus stands. Eucalyptus is known to have an intensive root system and allelopathy that can inhibit the growth of understory plants (Li et al., 2023). Limited water and nutrients, especially nitrogen (N), affect the physiological response of plants, such as stomatal closure, decreased photosynthesis rate, and disrupted chlorophyll synthesis, which ultimately suppress productivity (Mu and Chen, 2021). As a habitat for microorganisms in the soil, biochar is involved in forming stable soil aggregate microorganisms and contributes to increased water retention. Eucalyptus forests, as dry land areas for corn and mung bean cultivation (single or intercropping), require biochar. The application of biochar has emerged as a sustainable soil improvement strategy that influences various physiological processes in cereal and legume crops. The physiological response of corn and mung beans to biochar is influenced by increased soil nutrient retention, water holding capacity, and beneficial microbial interactions (Ren et al., 2024). The combined treatment of biochar and nitrogen fertilizer consistently showed increased leaf photosynthetic efficiency, better nitrogen uptake, and higher dry matter accumulation in corn. Optimal nitrogen use efficiency was typically observed at specific biochar-nitrogen combinations, indicating that these amendments must be carefully balanced to maximize the benefit (Li et al., 2022). Recent research shows that the simultaneous application of biochar and nitrogen fertilizer alters key bacterial taxa in eucalyptus systems, resulting in increased nitrogen uptake by plants, as measured by the N uptake in plant tissues (Ren et al., 2024).

These findings indicate that the benefits of combined biochar-urea application are mediated through complex soil microbial networks, which facilitate nutrient exchange between plants and soil. The mediating role of microbes in fertilization effects is particularly important in eucalyptus agroforestry systems, where interactions between trees, plants, and microbes determine the overall productivity and sustainability of the system. Understanding these interactions is crucial for developing the management strategies that optimize nutrient cycles and minimize environmental losses. Although understanding of the individual effects of biochar and urea on plant physiology continues to evolve, there are still significant knowledge gaps regarding the combined application of both in eucalyptus agroforestry systems. This study aimed to reveal the physiological processes in response to corn and mung beans (monoculture and intercropping) under eucalyptus stands using biochar as well as the ratio of urea and cow manure.

MATERIAL AND METHODS

Research location

The experimental research was conducted from March to July 2023 in the eucalyptus forest in Gunungkidul at coordinates 7.96° 57’35.5 “S and 110.50° 29’47.9” E, with an altitude of 151 meters above sea level. The soil characteristics of the land used is a grumusol have an organic carbon content of 1.56% (low), CEC of 21.60 me% (moderate), and pH of 6.3 (slightly acid). This indicates that the soil has low fertility (Nurmalasari et al., 2025).

Material

The materials used were Bisma corn seeds, Kutilang mung bean seeds from the Food Plant and Horticulture Seed Center of Gunungkidul, Yogyakarta, rice husk biochar, urea fertilizer, manure fertilizer, SP-36 fertilizer, and KCL fertilizer. The chemicals used were acetone and transparent nail polish for taking the stomata. The equipment used included scales, glass objects, microscopes, UV-Vis spectrophotometers, and plant photosynthesis meters.

Experimental design

The experiment used a three-factor randomized block design: 1) biochar (without and 15 ton ha^{-1}), 2) ratio of urea fertilizer doses (kg ha^{-1}) and manure (ton ha^{-1}) (150: 0, 75: 10, and 0: 20), and 3) planting patterns (corn and mung bean monoculture, as well as corn and mung bean intercropping). Plots were made after land preparation, measuring 3.2 × 2.2 m (area 7.04 m^2) with a distance of 0.4 m between each plot. Raised beds 40–50 cm high were made on the plots, and planting holes were made on the raised beds. The planting holes for corn monoculture were spaced 80 × 20 cm apart, while the corn and mung bean intercropping plots were spaced 80 × 20 cm and 20 × 20 cm apart, respectively. The mung bean monoculture plots were spaced 40 × 20 cm apart. Each monoculture plot contained 44 corn plants, each intercropped corn and mung bean plot contained 44 and 66 plants, respectively, and each mung bean monoculture plot contained 88 plants.

Observation parameters

The physiological parameters observed were corn and mung bean biomass by weighing corn and soybean husks without the yield which has been dried in an oven at 60 °C for 48 hours. Stomata observations were conducted by taking stomata prints using nail polish and attaching the prints to glass objects, which were then observed using a microscope with 10x magnification to count the number of stomata and 40x magnification to calculate the width of the stomata openings. Photosynthesis rates were measured using a plant photosynthesis meter between 9:00 a.m. and 12:00 p.m. The chlorophyll content observations were carried out by referring to the Arnon (1949) method, where 1 g of leaves were cut, crushed using a mortar, and added to 10 ml of acetone. The chlorophyll solution was measured for absorbance using a UV spectrophotometer at wavelengths of 645 and 663 nm. The formula for calculating total chlorophyll content according to the Arnon method is:

$$Chlorophyll\ A = (12.7 \times A\ 663 - 2.69 \times A\ 645) \times v/w \times 1000 \quad (1)$$

$$Chlorophyll\ B = (22.9 \times A\ 645 - 4.68 \times 663) \times v/w \times 1000 \quad (2)$$

$$Total\ chlorophyll = (20.2 \times A\ 645) + (8.02 \times A\ 663) \times v/w \times 1000 \quad (3)$$

where: v – supernatant volume, w – leaf weight.

Data analysis

The observation data obtained were analyzed using analysis of variance (ANOVA). If there were significant differences, they were followed up with Duncan's multiple range test (DMRT) at the α 5% level and the T-test.

RESULT AND DISCUSSION

Biomass of corn

Although the application of rice husk biochar did not significantly affect corn biomass, the descriptive trends from Figure 1 show that fertilizer ratios influenced yields. The treatments using full doses of either urea (150 kg ha^{-1}) or manure (20 ton ha^{-1}) resulted in marginally higher average biomass compared to the combined half-dose treatment (75 kg ha^{-1} urea : 10 ton ha^{-1} manure). Corn intercropped with Eucalyptus produced less dry biomass (1860 kg ha^{-1}) than corn grown in monoculture (2555 kg ha^{-1}) (Chavan et al., 2023). The primary constraint on biomass was likely the shaded environment under the eucalyptus trees. As a C4 plant, corn requires high light intensity for photosynthesis (Wasilewska-Dębowska et al., 2022), which was limited in this agroforestry system. This fundamental limitation may have overshadowed the potential benefits of other treatments. This reduced light interception ratio caused a decrease in dry matter production. Furthermore, the effectiveness of the applied fertilizers was likely reduced. Urea was susceptible to loss through volatilization and leaching, especially under the wet conditions of the study period (Shi et al., 2023). Conversely, the manure likely released its nutrients too slowly to meet the immediate demands of corn. Additionally, allelopathic compounds from eucalyptus rainfall leachate, containing phenols, esters, alkanes, and alcohols (Song et al., 2019), may have further inhibited nutrient uptake and reduced fertilizer efficiency. The absence of significant biochar effects suggests that its capacity to ameliorate soil properties was insufficient to overcome the dominant stress factors within the experimental timeframe. This may be attributed to the marginal soil conditions at the study site, whereby biochar application did not manifest positive effects within a single growing season. The beneficial impacts of biochar are more likely to be observed in subsequent planting seasons, as biochar requires a period of aging and stabilization within the soil matrix to exert its full ameliorative potential (Joseph et al., 2021; Tsolis and Barouchas, 2023)

Chlorophyll of corn

On the basis of Table 1, the application of biochar, fertilizer, and planting system did not affect the chlorophyll content in corn. Corn with a urea and manure fertilizer ratio of 75 kg ha^{-1}: 10 tons ha^{-1} achieved the highest total chlorophyll content, namely 0.42 mg g^{-1}, compared to urea and manure fertilizer ratios of 150 kg ha^{-1}: 0 tons ha^{-1} and 0 kg ha^{-1}: 20 tons ha^{-1}, which had an average of 0.38 mg g^{-1} each. The total chlorophyll content of this corn is still low compared to other studies of 1.04 mg g^{-1} (Pan et al. 2024), 2.3 mg g^{-1} (Moeinnamini et al., 2024), and 1.10 mg g^{-1} (Ahmed et al., 2020). This indicates that the use of biochar, urea fertilizer, and manure, both in monoculture and intercropping systems, has not been able to increase the chlorophyll content of corn under eucalyptus shade. Environmental factors may play a major role in this. Eucalyptus shade can reduce light intensity for chlorophyll synthesis, while nitrogen from urea cannot be utilized optimally. Biochar and manure, which are

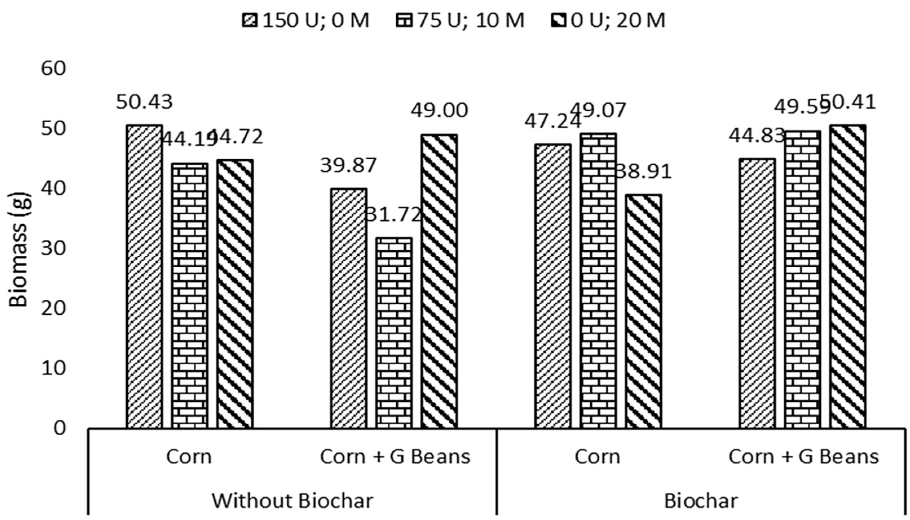

Figure 1. Biomass of corn. U: Urea; M: Manure

slow-release fertilizers, cause insufficient nutrient supply. Allelopathic compounds from eucalyptus, such as cineol, pinene, and limonene, can inhibit chlorophyll synthesis. These compounds can inhibit the formation of porphyrin, a precursor of chlorophyll, and damage the thylakoid membrane (Kong et al., 2021). This is also supported by the fact that 20% *Eucalyptus camadulensis* leaf extract reduces chlorophyll fluorescence in three wheat cultivars (Andualem et al., 2024). Although biochar can absorb allelochemicals from some plants, this study found the negative effects from the use of biochar. The negative effects can include the binding and deactivation of organic matter in the soil, as well as the release of allelochemicals bound by biochar.

However, the interaction between biochar, urea fertilizer, and fertilizer in monoculture and intercropping systems affects the chlorophyll A content of corn. A significant three-way interaction between biochar, fertilizer, and planting system was observed for chlorophyll A content (Figure 2). The combination of biochar with the fertilizer (urea 75 kg ha^{-1}: manure 10 ton ha^{-1}) had contrasting effects depending on the cropping system. In the monoculture system, biochar application increased the chlorophyll A content under the fertilizer (urea 75 kg ha^{-1}: manure 10 ton ha^{-1}). However, in the intercropping system, biochar application led to a decrease in chlorophyll A for the same fertilizer (urea 75 kg ha^{-1}: manure 10 ton ha^{-1}) compared to the plots without biochar. This inconsistency

Table 1. Chlorophyll content of corn (mg g^{-1})

Treatment	Chlorophyll A of corn (mg g^{-1})	Chlorophyll B of corn (mg g^{-1})	Chlorophyll total of corn (mg g^{-1})
Biochar (ton ha^{-1})			
0	0.19	0.20	0.39
15	0.20	0.20	0.40
Fertilizer ratio			
Urea 150 kg ha^{-1} : manure 0 ton ha^{-1}	0.18	0.20	0.38
Urea 75 kg ha^{-1} : manure 10 ton ha^{-1}	0.21	0.22	0.42
Urea 0 kg ha^{-1} : manure 20 ton ha^{-1}	0.20	0.18	0.37
Planting pattern			
Monoculture	0.20	0.21	0.41
Intercropping	0.19	0.19	0.39
Interaction	+	-	-

Note: Numbers accompanied by letter are significantly different to the Duncan multiple range test and T test with $\alpha = 5\%$.

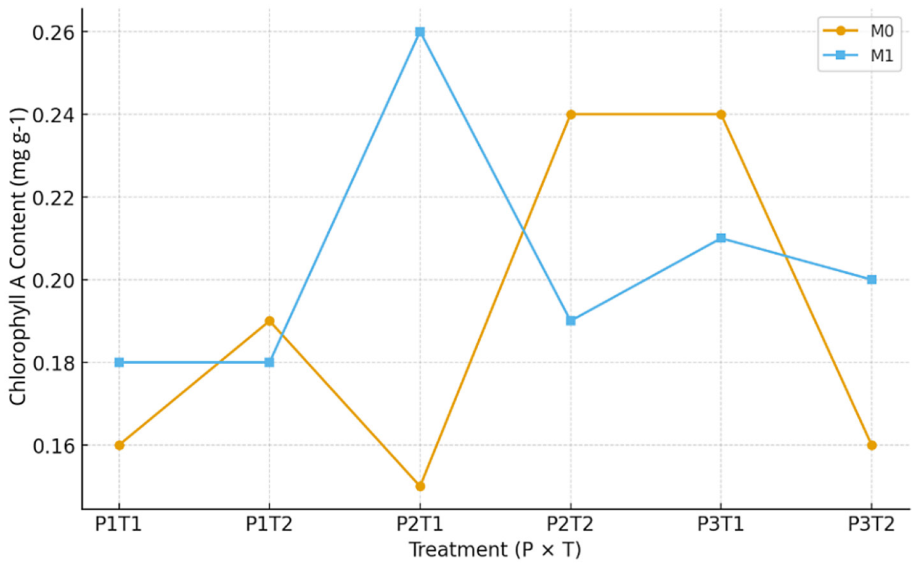

Figure 2. Interaction between biochar, fertilizer, and planting system on corn chlorophyll A content, M0: without biochar; M1: biochar 15 tons ha^{-1}; P1: urea 150 kg ha^{-1} : manure 0 tons ha^{-1}; P2: urea 75 kg ha^{-1} : manure 10 tons ha^{-1}; P3: urea 0 kg ha^{-1} : manure 20 tons ha^{-1}; T1: monoculture, T2: intercropping

demonstrates that the effect of biochar is not universal but is highly context-dependent, influenced by both nutrient management and interspecies competition. This could mean that in monoculture, biochar may enhance the availability of nitrogen from the combined fertilizer, promoting corn growth. In intercropping, however, biochar might improve the growing conditions for the mung beans as well, increasing their competitive pressure on the corn for light, water, and nutrients, ultimately leading to greater stress and reduced chlorophyll synthesis in the corn.

Number of corn stomata, width aperture of corn stomata, and corn photosynthesis rate

Figure 3 shows significant cross-interactions, indicating that the effect of biochar on stomatal number depends on the fertilizer regimen and cropping system. The most pronounced changes occurred in the mixed cropping system. Treatment with high urea doses (150 kg ha^{-1}: 0 tons ha^{-1} manure) caused a drastic decrease in stomatal number in the absence of biochar. This suggests ammonia toxicity or root stress due to concentrated mineral fertilizers, exacerbated by competition in the intercropping system. Biochar likely mitigates these effects by absorbing excess nutrients and improving soil conditions. Conversely, the full manure treatment (0 kg ha^{-1}: 20 tons ha^{-1}) without biochar increased the number of stomata. In this case, the slow and stable release of nutrients from manure provides a non-toxic nutrient source that supports corn growth even under competitive conditions. The balanced fertilizer comparison (75 kg ha^{-1}: 10 tons ha^{-1}) resulted in stable stomatal numbers regardless of the biochar presence, indicating that this combination provides sufficient and balanced nutrient supply that prevents extreme stress.

According to this explanation, biochar is known to increase the availability of essential nutrients, such as nitrogen and phosphorus (Oyeyiola et al., 2024), which are crucial for cell division and leaf development, including stomata formation. Additionally, manure increases the soil moisture content (Korslinggo et al., 2020), supporting better plant water status and physiological function. Overall, these results highlight that optimal stomatal development, a key factor for photosynthesis, depends on a careful balance between fertilizer type, soil amendments, and agricultural systems (Figure 3).

On the basis of Table 2, the application of biochar and fertilizer had no effect on the stomatal aperture width of corn under eucalyptus trees. However, monoculture cropping systems produced higher stomatal aperture widths (6.78 mm) than intercropping (6.12 mm). This may be because the mechanism of stomatal opening and closing is regulated by water status and environmental conditions. Stomatal opening is induced by red and blue light through two different pathways (Driesen et al., 2020). Therefore, the application of biochar

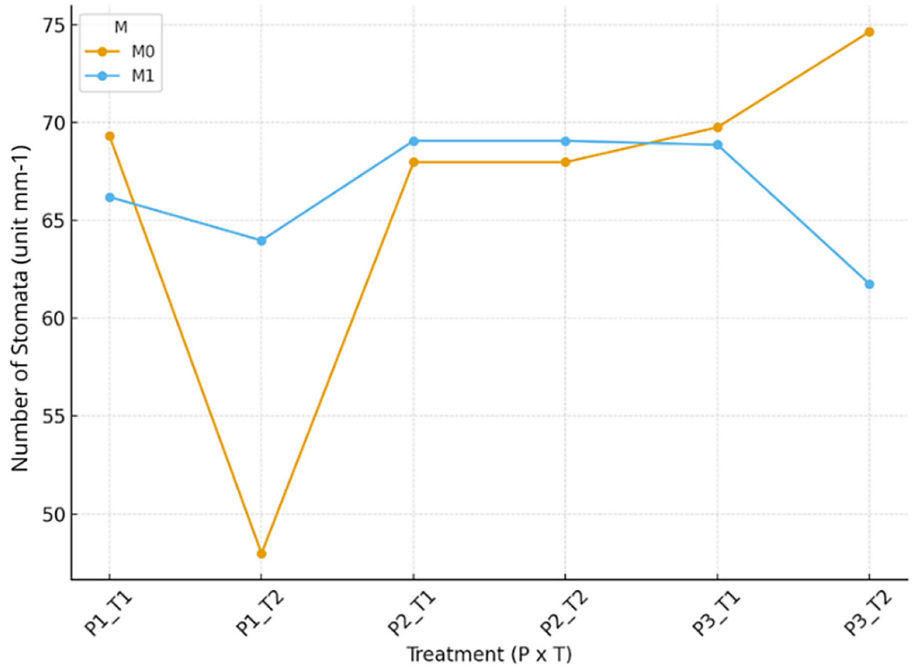

Figure 3. Interaction between biochar, fertilizer, and planting system on number of corn stomata, M0: without biochar; M1: biochar 15 tons ha^{-1}; P1: urea 150 kg ha^{-1} : manure 0 tons ha^{-1}; P2: urea 75 kg ha^{-1} : manure 10 tons ha^{-1}; P3: urea 0 kg ha^{-1} : manure 20 tons ha^{-1}; T1: monoculture, T2: intercropping

and fertilizer had no direct effect and did not cause any signals to change the stomatal activity. The monoculture corn planting system will change the activity of the stomata to open wider than the intercropping system. This is related to the suboptimal environment for corn in terms of light and water requirements. As a form of adaptation to water and light stress conditions, corn plants respond by narrowing or closing their stomatal openings. Light stress can reduce the number of stomata by 15.6% and the width of the opening by 73.1% (Sun et al., 2023). The use of intercropping systems increases competition among corn plants for water, causing the width of stomatal openings to narrow. Water deficiency can cause stomatal closure, reducing CO_2 absorption and disrupting photosynthetic efficiency (Budiastuti et al., 2025). Table 2 also explains that the application of biochar, fertilizer, and planting systems did not affect the photosynthesis rate of corn under eucalyptus trees. The photosynthesis rate ranged from 1.6 to 1.7 μmol m^{-2} s^{-1}. This occurs because the environmental pressure from eucalyptus stands, particularly intense competition for water and light, becomes such a dominant limiting factor that corn plants experience severe water stress and respond by closing their stomata to reduce transpiration. These closed stomata directly cut off the supply of carbon dioxide (CO_2), which is the main raw material for photosynthesis. This is consistent with the fact that solar radiation transmittance and corn productivity are influenced by eucalyptus stand thinning (Aragão et al., 2023). Under the conditions where water, CO_2, and light supplies are severely limited, additional inputs such as biochar (which serves to increase water and nutrient retention) and fertilizer (which provides nutrients) become ineffective.

Biomass of mung bean

Mung bean biomass shows that the provision of biochar, the ratio of urea and manure fertilizers, and planting patterns have no significant interaction. Mung beans with a ratio of urea and manure fertilizer doses (75 kg ha^{-1}: 10 ton ha^{-1}) produced the highest average biomass of 6.41 grams, compared to the ratio of urea and manure fertilizer doses (150 kg ha^{-1}: 0 ton ha^{-1}) producing an average of 6.17 grams. This is because the main limiting factor is not only nutrient availability, but also environmental conditions under the canopy. The shade of eucalyptus trees reduces light intensity, resulting in suboptimal photosynthesis in mung beans, even though nutrients are available. Legume productivity in agroforestry

Table 2. Width aperture of corn stomata (mm) and photosynthesis rate (μmol m^{-2} s^{-1})

Treatment	Width aperture of corn stomata (mm)	Photosynthesis rate (μmol m^{-2} s^{-1})
Biochar (ton ha^{-1})		
0	6.55	1.706
15	6.53	1.705
Fertilizer ratio		
Urea 150 kg ha^{-1} : manure 0 ton ha^{-1}	6.47	1.793
Urea 75 kg ha^{-1} : manure 10 ton ha^{-1}	6.31	1.704
Urea 0 kg ha^{-1} : manure 20 ton ha^{-1}	6.57	1.619
Planting pattern		
Monoculture	6.78 a	1.644
Intercropping	6.12 b	1.767
Interaction	-	-

Note: Numbers accompanied by letter are significantly different to the Duncan multiple range test and T test with α = 5%.

systems is mainly influenced by the competition for light. The competition for light is more limiting than the competition for water (Tramacere et al., 2024). In alfalfa plants, the decrease in biomass was caused by limited light due to their location between rows of trees (Mantino et al., 2021). This also occurred in the mung beans grown under eucalyptus trees in India, where there was a 7% decrease in mung bean biomass grown in intercropping (Chavan et al., 2023). In addition, the competition for water also inhibited the growth of mung beans. The shallow roots of mung beans could not compete with corn or eucalyptus. Biochar and manure tend to release nutrients slowly, while urea fertilizer is easily lost through volatilization, so the contribution to biomass increase was not apparent during the study period. Thus, the increase in mung bean biomass is not only determined by fertilizer input, but also highly dependent on light availability, plant interactions, and ecosystem conditions under eucalyptus trees. The marginal soil conditions also explain why biochar application combined with fertilization failed to significantly enhance the mung bean biomass during the present study. Multiple growing seasons are required for the positive effects of biochar to become evident. This is consistent with the findings indicating that plant responses to biochar amendment are more strongly influenced by climatic zones and soil type than by fertilizer application regimes. These observations are supported by Ye et al. (2020), who reported that biochar application at a rate of 10 tons per hectare does not contribute to significant yield improvements in the short term (Figure 4).

Total chlorophyll is the sum of chlorophyll a and b as an indicator of a plant's photosynthetic activity level. The higher the chlorophyll content, the higher the rate of photosynthesis. In Table 3, total chlorophyll shows that the provision of biochar, the ratio of urea and manure fertilizers, and the planting pattern of interaction are not significant. In contrast, the ratio of urea and manure fertilizer doses with planting patterns has a significant effect on the total chlorophyll content of mung beans. The chlorophyll content of mung beans in the seed portion is 0.70 mg g^{-1} (Huang et al. 2022), 2–3 mg g^{-1} (Islam et al., 2023). The insignificant effect of the treatment is due to the dominance of abiotic stress from eucalyptus, which creates the conditions where additional inputs are ineffective. mung beans experience complex physiological barriers in nutrient absorption and chlorophyll synthesis due to unbalanced resource competition. As a result, nitrogen is also not well absorbed by mung beans. Nitrogen is responsible for chlorophyll production, which increases the rate of photosynthesis, gives plants greening, as well as increases crop quality and yield (Farhan et al. 2024). The shade from eucalyptus and corn limits the growth of mung beans (Gong et al., 2022).

Although the application of biochar, fertilizer, and cropping systems did not have a singular effect, there was an interaction between fertilizer use and cropping systems. In monoculture cropping systems, reducing urea fertilizer and increasing manure fertilizer increased total chlorophyll content, but in intercropping systems, it decreased total chlorophyll content. Although the

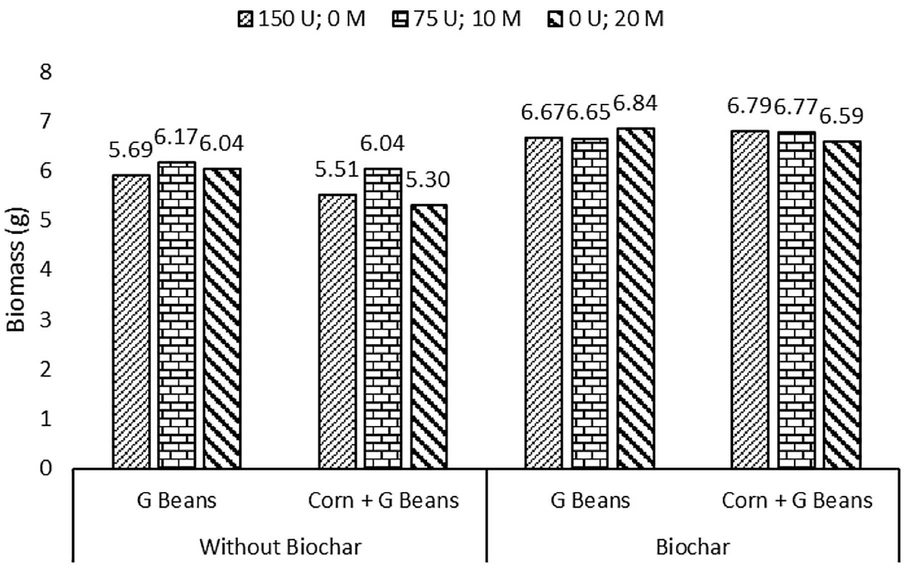

Figure 4. Biomass of mung bean. U: urea; M: manure

Table 3. Chlorophyll content of mung bean (mg g^{-1})

Treatment	Chlorophyll A of mung bean (mg g^{-1})	Chlorophyll B of mung bean (mg g^{-1})	Chlorophyll total of mung bean (mg g^{-1})
Biochar (ton ha $^{-1}$)			
0 t	0.13	0.17	0.30
15	0.14	0.18	0.32
Fertilizer ratio			
Urea 150 kg ha^{-1} : manure 0 ton ha^{-1}	0.13	0.16	0.29
Urea 75 kg ha^{-1} : manure 10 ton ha^{-1}	0.13	0.18	0.31
Urea 0 kg ha^{-1} : manure 20 ton ha^{-1}	0.13	0.19	0.33
Planting pattern			
Monoculture	0.13	0.19	0.32
Intercropping	0.13	0.17	0.30
Interaction	-	-	+

Note: Numbers accompanied by letter are significantly different to the Duncan multiple range test and T test with α = 5%.

application of biochar, urea fertilizer, and manure fertilizer individually did not have a significant effect on corn chlorophyll content, there was an interaction between fertilizer type and cropping system. In a monoculture system, reducing the urea fertilizer dose and increasing manure fertilizer content increased total chlorophyll content. This is due to the availability of sufficient nitrogen, reduced nutrient loss through volatilization, and the supply of micro nutrients (Mg and Fe) from manure, which are important in chlorophyll synthesis. It was found that 100% urea and a combination of goat manure + 50% urea + 50% urea often produce much higher chlorophyll content than 100% goat manure, especially for rice husk biochar (Rahayu et al., 2025). However, in intercropping systems under eucalyptus trees, the same pattern actually reduces total chlorophyll content. This condition is caused by limited light due to shade, competition for water and nutrients with eucalyptus trees, and the possible effect of allelopathy, which inhibits the mineralization of manure. These environmental factors are more dominant in determining chlorophyll formation than nutrient availability, so additional fertilizer cannot significantly increase chlorophyll. These results are in line with the findings that the productivity of understory plants in agroforestry systems is more limited by light availability than by soil nutrients (Chen et al., 2020) (Figure 5).

Number of mung bean stomata, width aperture of mung bean stomata, and mung bean photosynthesis rate

On the basis of Table 4, the treatment did not affect the number of stomata, stomatal aperture width, and photosynthetic rate of mung beans under whitewood trees. The presence of whitewood trees created different environmental conditions, such as reduced light intensity and increased air humidity, which are major factors affecting plant physiology. Stomata, which function as gas exchange gates, tended not to show significant changes in number or aperture width. As a result, the rate of photosynthesis, which is highly dependent on the availability of carbon dioxide entering through the stomata, remained relatively stable because the main input for photosynthesis, namely light, was limited by shade (J. Li et al., 2023). Although stomata play a role in gas exchange, their response to environmental factors such as light is more dominant than nutrient availability (Li et al., 2022). With little light available for photosynthesis, plants do not need to open their stomata wide or have a large number of stomata to absorb CO_2. Shading on eucalyptus also increases water competition, which also plays a role in stomatal activity and photosynthesis. Eucalyptus is considered a plant that can deplete soil water and dry out the soil without considering the water area (Medeiros et al., 2025).

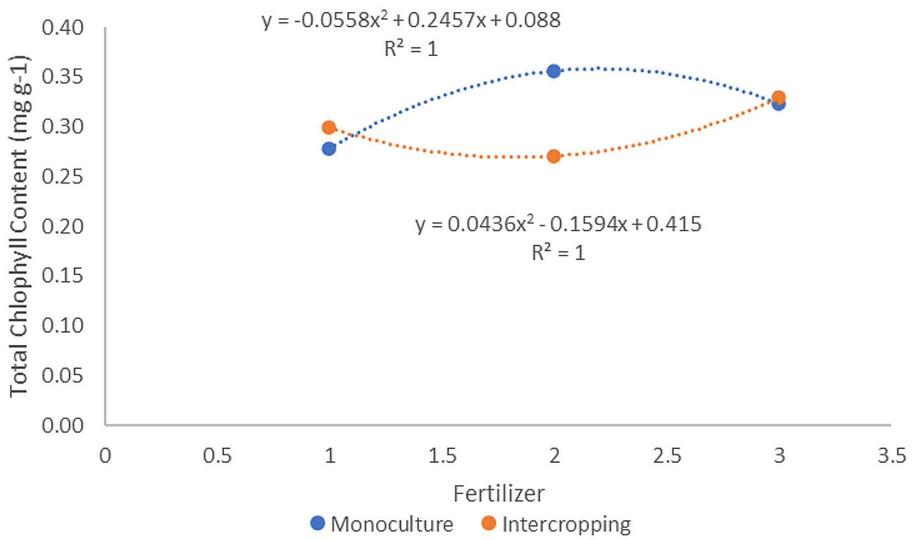

Figure 5. The interaction between fertilizer application and planting system on the chlorophyll content of mung bean

Table 4. Number of stomata (unit mm^{-1}), width aperture of mung bean stomata (mm), and photosynthetic rate of mung beans ($\mu mol\ m^{-2}\ s^{-1}$)

Treatment	Number of mung bean stomata (unit mm^{-1})	Mung bean stomatal aperture width (mm)	Photosynthetic rate of mung beans ($\mu mol\ m^{-2}\ s^{-1}$)
Biochar (ton ha^{-1})			
0	168.81	4.55	2.29
15	177.10	4.27	2.71
Fertilizer ratio			
Urea 150 kg ha^{-1} : manure 0 ton ha^{-1}	176.25	4.36	2.38
Urea 75 kg ha^{-1} : manure 10 ton ha^{-1}	169.70	4.40	2.35
Urea 0 kg ha^{-1} : manure 20 ton ha^{-1}	172.92	4.48	2.77
Planting pattern			
Monoculture	174.07	4.49	2.38
Intercropping	171.85	4.34	2.62
Interaction	-	-	-

Note: Numbers accompanied by letter are significantly different to the Duncan multiple range test and T test with α = 5%.

CONCLUSIONS

On the basis of the results of the study, it can be concluded that the combination of rice husk biochar, biochar, and the ratio of urea and manure fertilizer doses (75 kg ha^{-1} : 10 ton ha^{-1}) in the monoculture planting pattern can increase the chlorophyll A content and the number of corn stomata, and the monoculture planting system can increase the width of corn stomatal openings. The combination of the ratio of urea and manure fertilizer doses (75 kg ha^{-1} : 10 ton ha^{-1}) and the monoculture planting system can increase the total chlorophyll content in mung beans.

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