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Metabolic and Biochemical Performances of Abaca (*Musa textilis* Née) under Intermediate and Advanced Phase Agroforestry System

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ABSTRACT

Abaca, one of the potential fiber crops with high-quality fiber and promising economic value, is mainly established under the agroforestry system, for it is considered a non-primary crop. The study aims to observe the metabolic and biochemical performance as well as the fiber quality of abaca under the agroforestry system. The experimental design used in this study was nested with two types of agroforestry systems, i.e., intermediate phase (Fase Tengah, FT) and advanced phase (Fase Lanjutan, FL) and was conducted during the rainy season. Parameters observed in this study were divided into edaphic and climatic parameters, oxidative response parameters, foliage macro- and micronutrient, and fiber quality. Despite poor soil quality compared to FL, higher relative humidity (4.35%), lower temperature (2.73%), and lower shading intensity were observed in FT. Improved soil characteristics in FL, *viz.* soil water content (19.64%), organic carbon (72.89%), porosity (4.29%), cation exchange capacity (13.77%), and pH (35.13%), were unable to compensate plant stress induced by the high shading intensity at 83.99%. Consequently, it contributed to higher levels of malondialdehyde, superoxide anion, hydrogen peroxide, superoxide dismutase, peroxidase, and phenol by 0.07%, 1.86%, 32.66%, 0.08%, 14.63%, and 35.08%, respectively, due to shading stress. Nevertheless, ascorbic acid content in FL was lower (18.90%) compared to FT. Higher fiber diameter (23.53%) and tensile strength (18.77%) of abaca in FT were observed compared to

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FL. The improved microclimatic conditions under FT promoted the high adaptability of abaca to poor soil quality. Therefore, it contributed to enhanced growth and fiber quality compared to FL. Pruning is pivotal to managing shading intensity.

Keywords: Abaca, agroforestry, oxidative stress, shading stress

INTRODUCTION

Despite decreasing land productivity topping 1.66 million ha globally, increasing demand for productive agricultural land promotes land use competition between initial cash crops and other crops (Food and Agriculture Organization of the United Nations [FAO], 2022). At the same time, the rise of biomaterial utilization derived from natural fiber in the synthetic industry has been considered to promote a significant impact on people and the planet. However, due to high maintenance and complicated production processes, farmers show little interest in cultivating fiber crops in a monoculture system. Therefore, they only cultivate fiber crops as secondary or supplementary crops (Yokokura, 1992). Abaca is one of the potential fiber crops commonly utilized in textile, electronic, medical, and paper industries. Abaca fiber has better durability than other natural fibers (e.g., sisal). Hence, it is commonly used as currency paper (Franck, 2005). Historically, Indonesia cultivated abaca on a plantation scale under Dutch colonialization, primarily in North Sumatra, Lampung, and East Java (Sudjindro, 2008). In 2009, Indonesia exported 92 tons of abaca fiber, accounting for USD 13,000 (FAO, 2013). Despite its profitable quality, abaca is commonly cultivated in agroforestry systems instead of monoculture because it is not considered profitable as a cash crop.

The agroforestry system is suitable for abaca as it provides shade to avoid the detrimental effects of high light intensity (Siles et al., 2013). Besides, the species diversity contributes to high organic matter and improvement of soil quality (Asigbaase et al., 2020). Abaca belongs to C3 crops, indicating strong high-light sensitivity (Tian et al., 2017); therefore, shading is pivotal. However, growth inhibition induced by shading might lead to crop death, which is determined by shading intensity, shading degree, and shading duration (Y-.B. Wang et al., 2021). Suitable shading intensity for abaca growth ranges from 40-50%, using multi-purpose tree species (MPTS) as shade trees with appropriate planting distance (Petronilo et al., 2016). Light transmission in the abaca-MPTS system could reach 36 and 9% above and under the abaca canopy, respectively. The system showed a more effective growth environment than the abaca-coconut system because of better soil quality; however, shading intensity and species density should be managed through pruning and thinning (Bande et al., 2016). Abaca cultivated under 50% shade produced longer, larger, and heavier pseudostem with higher tensile strength than 0% shade (Bande et al., 2013).

Shading stress can adversely impact stomatal conductivity, which subsequently leads to the production of reactive oxygen species (ROS). Hydrogen peroxide (H_2O_2) and nitric oxide (NO) are signals promoting stomatal closure under darkness. The concentration of H_2O_2 increases due to the activation of respiratory burst oxidase homolog D/F (RBOHD/F), which subsequently induces signaling cascades to produce NO in guard cells, causing stomatal closure (Zhang et al., 2017). Oxidative stress triggers the production of enzymatic and non-enzymatic antioxidants to avoid cell damage. Soybean leaves under partial shading showed higher enzymatic antioxidants (e.g., superoxide dismutase [SOD], catalase [CAT], peroxidase [POD], and ascorbate peroxidase [APX]) at 25, 39, 24, and 18%, respectively, than without shading. Higher enzymatic antioxidant concentration was exhibited under full shading, indicating higher oxidative stress under respective conditions (Raza et al., 2020). To date, scientific study regarding abaca is limited to fiber quality under shading, microclimatic effect on biomass, or nutrient composition in vegetative and generative stages (Armecin, 2008; Armecin & Coseco, 2012; Armecin et al., 2011; Bande et al., 2012, 2016). Therefore, abaca's metabolic and biochemical performances will be investigated to comprehend the adaptability and fiber quality under the agroforestry system.

MATERIALS AND METHODS

Plant Materials and Growth Condition

Three-year-old abaca plants were established under two agroforestry systems in Watualang Village, Pitu Subdistrict, Ngawi, Indonesia in 2020 (Figure 1). Watualang village is located ±150 m above sea level. The experiment was conducted during December 2022–April 2023. An intermediate agroforestry system was established by combining abaca and MPTS (e.g., timber and fruit trees) and was planted in 2020 and 2019, respectively. Meanwhile, under an advanced agroforestry system, abaca was combined with a 20-year-old teak. The abaca plantation belongs to Getas Forest for Specific Purposes (Kawasan Hutan Dengan Tujuan Khusus [KHDTK]) management. It has flat terrain with a 0-8° slope.

Annual precipitation is approximately 2,000–2,400 mm. According to Schmidt-Fergusson, the climate is classified as climate zone D, with a Q value of approximately 60–100% (Chanan, 2019). The total rainfall during the experimental period was 337–751 mm, which is classified as very high according to the Meteorological Climatological and Geophysical Agency (2023). Meanwhile, the duration of daylight in the respective period was approximately 3–5 hr (Figure 2). Based on the International Union of Soil Sciences Working Group (2022) soil analysis, soil type in both agroforestry systems was classified as Pellic Vertisol (from Greek *pellos*: dusty) because the color and chroma of the upper 30 cm soil layer were 3 and 1, respectively, with soil texture classified as clay—silty clay.

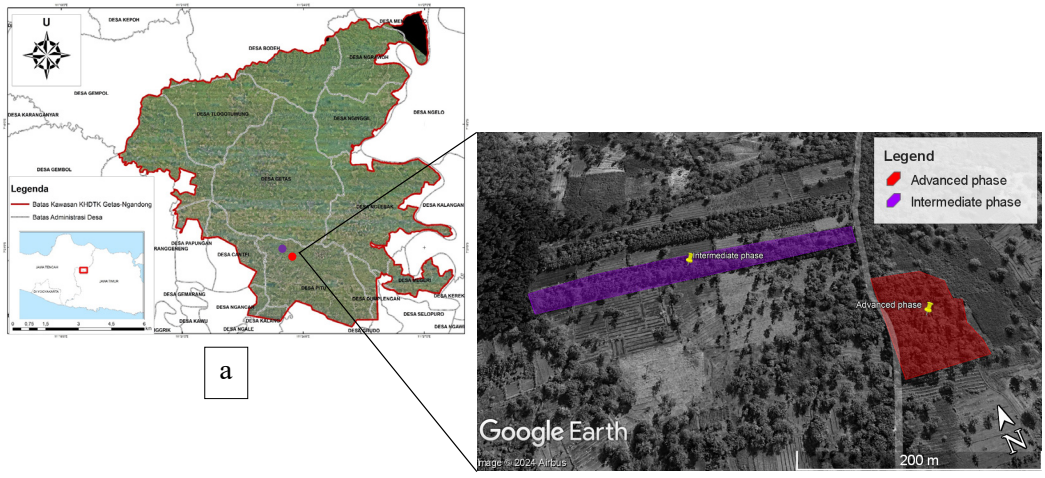

Figure 1. Geographical location of the study area: (a) Modified from Emma (2017); (b) Modified using Google Earth Pro

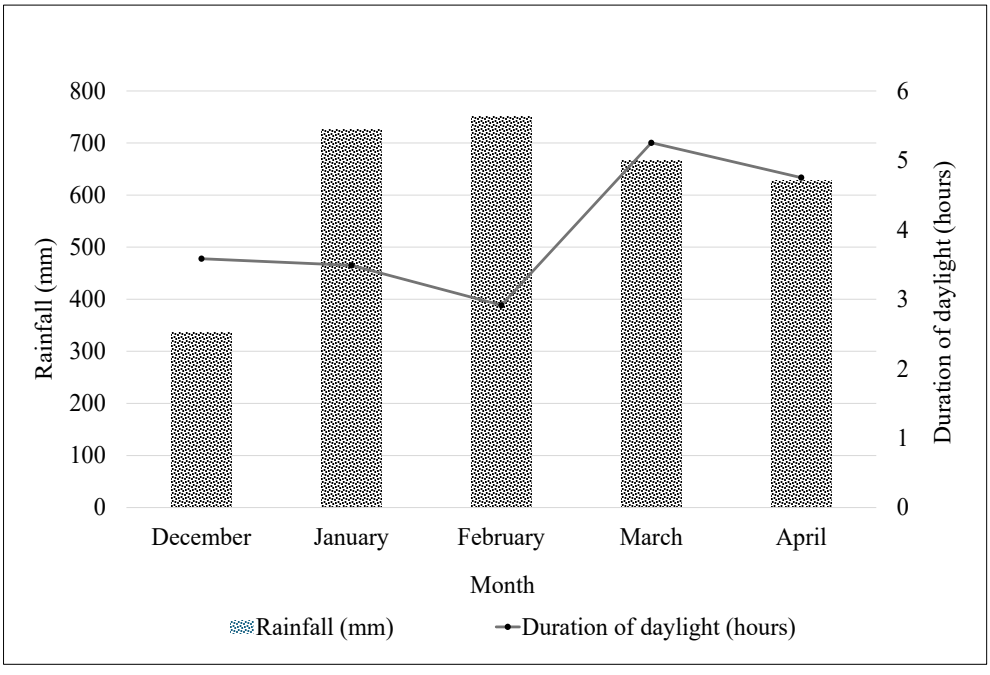

Figure 2. Rainfall intensity and duration of daylight during December 2022–April 2023 in Ngawi

Note. Rainfall range: <50 mm = Low; 50–150 mm = Moderate; 150–300 mm = High; >300 mm = Very high

Experimental Design and Sampling Method

The study was conducted using nested design with two treatments, viz. intermediate phase (Fase Tengah, FT) and advanced phase (Fase Lanjutan, FL) agroforestry, consisting of 5 blocks (12.5 m x 12.5 m) in each system. The planting distance of abaca is 3 m x 3 m, as well as for shading trees. The abaca agroforestry system was established by implementing an alternate cropping system. The agroforestry phase was determined based on Sasaki et al. (2020) and Suryanto et al. (2014), which classified the phase according to shading intensity. Intermediate phase agroforestry was categorized as abaca grown under 50–75% shading intensity; meanwhile, advanced phase was determined by >75% shading intensity. Shading intensity was determined based on canopy projection constructed using SeXI-FS software (ver. 2.1.1). The shading intensity range was adjusted based on the existing abaca cultivation at the location. Sampling locations in this research were determined based on three considerations related to uniformity in abaca age, cultivar, and plant management (*viz.* fertilization) to reduce any variability caused by non-treatment factors.

The growth dimension measured in abaca was diameter in different heights (10 cm, 30 cm, and 1.3 m), which was used in data analysis to estimate nutrient uptake based on an allometric equation for biomass estimation. Meanwhile, the growth dimensions of shading trees were height, diameter at breast height (dbh), branch-free height, outer crown height, and crown width in four directions. The shading intensity in both agroforestry systems was determined based on crown projection illustrated using SeXI-FS (ver. 2.1.1) as the preliminary observation. Canopy closure was determined by calculating intersection spots on the SeXI-FS (ver. 2.1.1) projection. The calculation was modified based on canopy closure measurement using spherical densiometer (Strickler, 1959) and calculated using the equation 1:

$$\text{Shading intensity} = \frac{\text{Shaded intersection spots}}{\text{Total intersection spots}} \text{ x } 100\% \quad [1]$$

Leaf (fully expanded) samples were taken from 3 plants chosen randomly, representing the first, middle, and last rows of the block in each agroforestry system (Figure 3). The part of leaves used as samples was the distal half, away from the middle rib on both surfaces and was chosen from healthy leaves (Ekanayake et al., 1994). The samples were used to measure oxidative stress parameters and macro- and micronutrients. Soil samples taken around abaca plants were conducted in the same manner as leaves and were used for physical and chemical analyses. Pseudostems, 250 in total, were harvested and processed using a decortication machine for fiber quality analysis (physical and mechanical characteristics). The physical and mechanical quality of abaca fiber was fiber diameter and tensile strength, respectively.

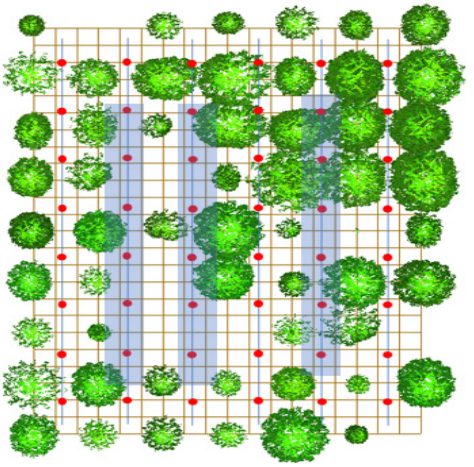

Figure 3. Sampling area of abaca leaves

Note. Red dot = Abaca; Blue blocks = Sampling area; The picture derived from block 1 of intermediate phase is an example of sampling area

ROS and Antioxidant Analyses

Oxidative stress parameters analyzed in the study were malondialdehyde (MDA), superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), superoxide dismutase (SOD), peroxidase (POD), ascorbic acid, and phenol. MDA, $O_2^{\bullet-}$, H_2O_2, SOD, and POD analyses were conducted under low temperatures. The MDA was observed using the thiobarbiturate acid (TBA) method (Senthilkumar et al., 2021). The leaf sample (0.2 g) was homogenized using 3 ml of 0.1% (w/v) trichloroacetic acid (TCA, Merck, Germany) and centrifuged at 13,040 x *g* for 15 min. The supernatant (1 ml) was added into 2 ml of 0.5% TBA (Merck, Germany) in 20% TCA (Merck, Germany), then vortexed and incubated in a water bath at 90°C for 20 min. The mixture is later cooled in cold water for 10 min to stop the reaction. Absorbance was measured at 532 and 600 nm, respectively.

The $O_2^{\bullet-}$ and H_2O_2 were analyzed using the Griess method (Jahan et al., 2020) and the potassium iodide (KI) method (Zhou et al., 2006), respectively. Briefly, the $O_2^{\bullet-}$ analysis was conducted by homogenizing leaf sample (0.2 g) in 2 ml of 50 mM phosphate buffer (pH 7.8, Merck, Germany), then centrifuged at 13,040 x *g* and 4°C for 20 min. The supernatant (0.5 ml) was added into 0.1 ml of 10 mM hydroxylamine hydrochloride (Merck, Germany) and 0.5 ml of 50 mM phosphate buffer (pH 7.8, Merck, Germany). The mixture was incubated at 25°C for 30 min. Absorbance was measured at 540 nm.

The H_2O_2 was analyzed by homogenizing leaf sample (0.5 g) in 3 ml of 0.1% TCA and centrifuged at 13,040 x *g* and 4°C for 15 min. Active charcoal (0.15 g) was added into the supernatant in the new test tube and centrifuged at 5,796 x *g*, 4°C for 1 min. The reaction mixture was made by combining 0.5 ml of 100 mM potassium phosphate buffer (pH 7.4, Merck, Germany) and 2 ml of 1 M potassium iodide (KI, Merck, Germany) reagent, then 1 ml supernatant was added into the mixture and incubated in the dark for 1 hr. Absorbance was measured at 390 nm.

The SOD was analyzed according to pyrogallol autoxidation (Marklund & Marklund, 1974) with modification from Li (2012). The leaf sample (0.5 g) was homogenized using chilled 5 ml of 50 mM phosphate buffer (pH 6.5, Merck,

Germany) and centrifuged at 9,056 x *g*, 4°C for 15 min. The reaction mixture was made of 2.5 ml of 0.05 M Tris-HCl (pH 7.4, Merck, Germany), 100 µl of 1 mM disodium ethyleneiaminetetraacetate dihydrate (Na_2EDTA, Merck, Germany), 100 µl phosphate buffer (50 mM, pH 6.5), 100 µl sample extract, and 200 µl pyrogallol (Merck, Germany). Absorbance was measured kinetically at 325 nm for 3 min at 30-s intervals.

The POD was also analyzed based on pyrogallol autoxidation (Alexander, 1966). The leaf sample was homogenized using chilled 3 ml of 100 mM phosphate buffer (pH 7, Merck, Germany) and centrifuged at 11,866 x *g* and 4°C for 15 min. The reaction mixture was made of 2.1 ml ultrapure water, 0.32 ml of 100 mM phosphate buffer (pH 7, Merck, Germany), 0.16 ml of 0.15% H_2O_2 (Merck, Germany) and 0.32 ml of 5% pyrogallol (Merck, Germany). Absorbance was measured kinetically at 420 nm for 3 min at 30-s intervals.

Non-enzymatic antioxidants consisting of ascorbic acid and phenolic content were analyzed based on chromium reduction (Abera et al., 2020) and gallic acid method (Al-Saeedi & Hossain, 2015), respectively. The ascorbic acid assay was conducted by macerating 1 g leaf sample with 10 ml of 3% metaphosphoric acid (Merck, Germany) in 8% glacial acetic acid (Merck, Germany) and diluting until 100 ml with ultrapure water. The mixture was stirred for 45 min and protected from light. The reaction mixture was made of 2 ml of potassium dichromate ($K_2Cr_2O_7$, Merck, Germany) and manganese(II) chloride ($MnCl_2$, Merck, Germany) (0.335 mM: 0.185 mM) and 1 ml sample extract. Absorbance was measured kinetically at 350 nm for 5 min with 30-s intervals.

The phenolic content was assayed by macerating dried leaf powder (1 g) in 100 ml of 70% methanol (Merck, Germany). The mixture was incubated in the dark for 24 hr. The reaction mixture was made of 200 µl methanol extract (Merck, Germany) and 1.5 ml of 10% Folin-Ciocalteu (Merck, Germany). The mixture was incubated in the dark for 5 min, and later, 1.5 ml of 6% sodium carbonate (Na_2CO_3, Merck, Germany) was added, and it was incubated for 30 min in the dark. Absorbance was measured at 760 nm.

Leaves Nutrient Analysis and Nutrient Uptake based on Allometric Estimation

Macro- and micronutrient analyses were conducted to estimate nutrient uptake in abaca leaves. Dry matter of abaca leaves was used for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, Cl, and B analyses. N and K were assayed based on Kjeldahl and flame photometric analyses. P and B were assayed using a spectrophotometer. Cl was assayed based on the argentometric method. Meanwhile, others were assayed using an atomic absorption spectrophotometer (AAS). Subsequently, the result was used to estimate nutrient uptake based on the foliage biomass (*Y*) allometric equation. The equation was based on 3 different

pseudostem diameters *viz.* diameter at 10 cm (d_{10}), 30 cm (d_{30}), and 1.3 m (d_{13}) height (R^2 = 0.67) (Negash et al., 2013).

$$Y = 3.4 \times 10^{-3}\left(d_{10}^{1.982} d_{30}^{1.154} d_{1.3}^{0.863}\right) \quad [2]$$

Fiber Quality Analysis

The fiber quality analyzed in this study was fiber diameter and tensile strength. Fiber diameter was assayed by macerating abaca fiber in acetic acid (CH_3COOH, Merck, Germany) and H_2O_2 (Merck, Germany). After the fiber swelled, it was soaked and boiled in hot water for 4 hr. Fiber diameter was cut using microtome transversally and observed using a digital microscope Dino-Lite (Taiwan). Subsequently, it was calculated based on the cross-sectional area of the average of five diameters arranged at angle intervals of 36°. Tensile strength was measured based on ASTM D3379-75 (American Society for Testing and Materials [ASTM], 2003) by cutting 50 fiber samples to 90 mm length. The samples were measured using a universal testing machine (Instron 3360c, USA) at the speed of 1 mm/min.

Statistical Analysis

Analysis of variance (ANOVA) and independent *t*-test analysis were conducted using RStudio (ver. 2023.03.0). The relationship between soil, nutrient, and biochemical parameters was analyzed based on Pearson correlation analysis. Stepwise regression analysis forward mode was conducted at α = 0.20 using MINITAB (ver. 21.4) to develop a prediction model on fiber diameter and tensile strength. SeXI-FS software (ver. 2.1.1) was used to construct three-dimensional vegetation coverage.

RESULTS AND DISCUSSION

Climatic and Edaphic Characteristics

Different plant compositions and crown layers in the FT and FL systems promoted specific microclimatic conditions in each agroforestry. Analysis of crown projection showed that shading intensity in FL was 38% higher than in FT (Table 1), which could promote shading stress. Higher relative humidity in the FT system (4.35%; p<0.0001) and lower temperature (2.73%; p<0.0001) compared to FL indicated that the

Table 1
Climatic characteristics in abaca agroforestry

Variables	Agroforestry system		CV (%)
	FT	FL	
Relative humidity (%)***	65.980±0.40[a]	63.233±0.90[b]	0.27
Temperature (°C)***	32.835±0.17[b]	33.757±0.23[a]	0.25
Light interception (%)***	92.776±1.13[b]	95.691±2.10[a]	1.44
Shading intensity (%)*	59.82 (M)	83.99 (H)	-

Note. FT = Intermediate phase agroforestry; FL = Advanced phase agroforestry; *** = p<0.0001; M = Moderate; H = High; * = Based on a modified spherical densiometer calculation (Strickler, 1959); Values (mean ± standard deviation) indicated by the same letters showed no significant difference at α = 0.05

multilayer system in FT (Table 1) promoted a cooling effect, which in turn created specific microclimate conditions. Multilayer systems and various vegetation structures contributed to improving the cooling effect and humidity regulation in a stand (H. Wang et al., 2023). Besides, a multilayer system with various crown architecture in FT could potentially reduce turbulence (mixing air) compared to FL with high branch-free height teak trees. Decreasing turbulence kinetic energy occurred as increasing crown depth (Dupont & Brunet, 2008).

Higher plant density in the FT system (254 shading trees) compared to the FL system (81 teak trees and 66 teak saplings) promoted lower temperature and high relative humidity. The low plant density of cacao-rubber agroforestry contributed to higher temperatures by 3°C compared to higher plant density in the cacao-cabruca agroforestry system in Brazil (Heming et al., 2022). On the other hand, light interception in the FL system (3.14%; $p<0.0001$) was significantly higher than in the FT system (Table 1), indicating higher shading intensity as stated by Delagrange et al. (2006) regarding light interception in broad-leaved forest. Plants exposed to high shading intensity continuously could lead to retarded growth resulting from decreasing physiological activity and activation of certain oxidative stress mechanisms.

Abaca agroforestry systems showed various soil physical and chemical characteristics. FL system, comprised of 20-year-old teak, showed an improvement in soil characteristics compared to the FT system. Soil water content (SWC) value, organic carbon, porosity, CEC, and pH of the FL system were significantly ($p<0.05$) higher than FT by 19.6, 72.7, 4.30, 13.8, and 35.26%, respectively (Table 2). Higher organic carbon in FL contributed to lower bulk density (9.65%) compared to FT. Soil organic carbon negatively correlated (R^2 = -0.83) with bulk density, indicating its importance in determining soil impedance. Agroforestry can provide different organic carbon levels in different soil depths and alleviate soil bulk density (Cherubin et al., 2019). Nevertheless, soil permeability in both systems was classified as very slow (Table 2). High bulk density promotes waterlogging and decreases soil permeability, inhibiting root growth due to soil impedance (Marschner, 2012).

The SWC, organic carbon, CEC, and pH are pivotal factors determining the nutrient uptake ability of plants. Soil organic content contributed to higher CEC and pH, particularly in silty clay texture (Brady, 1984). Soil CEC determined the soil's ability to hold exchangeable cations and release them for plants. Therefore, it contributed significantly to soil classification (Mattila & Rajala, 2022). It was also confirmed by the strong correlation between organic carbon and CEC (R^2 = 0.92) as well as its correlation with pH (R^2 = 0.98) in this research. The high pH value of FL soil promoted high N and Mg availability (Table 3), indicated by the positive correlation between pH and available N (R^2 = 0.83) as well as Mg (R^2 = 0.97). In contrast, the low soil pH of FT contributed to the high

availability of P, Fe, Cu, and Zn, indicated by a negative correlation among them (R^2 P = -0.94; R^2 Fe and Cu = -0.98; R^2 Zn = -0.66). Soil available P, Fe, Zn, and Cu is very low, particularly in alkaline soil (Marschner, 2012). Increasing soil pH at 7 or 8 contributes to decreasing soil availability of Fe and Zn (Brady, 1984).

Table 2
Soil characteristics in abaca agroforestry

Variables	Unit	Agroforestry system		Rating		CV
		FT	FL	FT	FL	
SWC*	%	48.807±3.10[b]	58.393±1.60[a]	Very high	Very high	4.61
Organic C*	%	2.447±0.43[b]	4.230±0.06[a]	Moderate	High	9.21
BD*	g/cm^3	1.143±0.05[a]	1.033±0.03[b]	Low	Low	3.54
Porosity*	%	49.493±1.96[b]	51.620±1.19[a]	Very high	Very high	3.21
CEC*	me%	40.953±2.14[b]	46.593±1.31[a]	Very high	Very high	4.05
Permeability		0.037±0.01[a]	0.067±0.02[a]	Very slow	Very slow	35.34
pH*		5.703±0.24[b]	7.707±0.16[a]	Slightly acidic	Neutral	3.05

Note. FT = Intermediate phase agroforestry; FL = Advanced phase agroforestry; SWC = Soil water content; BD = Bulk density; CEC = Cation exchange capacity; me% = Milliequivalents per 100 g of soil; CV = Coefficient of variation; * = $p<0.05$; Values (mean ± standard deviation) indicated by the same letters showed no significant difference at α = 0.05; Rating was based on Sulaeman et al. (2005) and United States Department of Agriculture (USDA) (1999)

Table 3
Soil available nutrients in the abaca agroforestry system

Soil available nutrients	Unit	Agroforestry system		CV (%)
		FT	FL	
N*	ppm	184.893±20.49[b]	330.117±78.37[a]	22.24
P*	ppm	24.803±4.01[a]	3.270±1.48[b]	21.52
S	ppm	114.197±17.97[a]	104.887±39.65[a]	28.09
K*	me%	0.860±0.07[a]	0.530±0.03[b]	7.75
Mg*	me%	7.080±0.45[b]	10.693±0.50[a]	5.36
Ca*	me%	112.427±8.10[a]	63.407±0.27[b]	6.52
Fe*	ppm	73.503±13.56[a]	21.423±8.66[b]	23.96
Mn*	ppm	71.747±0.65[b]	73.357±0.29[a]	0.69
Cu*	ppm	6.840±0.08[a]	4.887±0.06[b]	1.22
Zn	ppm	1.963±1.07[a]	0.667±0.12[a]	58.22

Note. FT = Intermediate phase agroforestry; FL = Advanced phase agroforestry; CV = Coefficient of variation; me% = Milliequivalents per 100 g of soil; * = $p<0.05$; Values (mean ± standard deviation) indicated by the same letters showed no significant difference at α = 0.05

Foliage Nutrient Uptake

Overall analysis of leaves' macronutrients and micronutrients showed higher nutrient content in abaca leaves of the FL system compared to FT; however, only Ca, Fe, and Mn showed significant differences between both systems (Table 4). Soil CEC and water content of FL were higher than FT; therefore, plants in FL had higher uptake. Besides, higher organic carbon in FL than in FT might increase nutrient mineralization (J. R. Sarker et al., 2017). Ca and Fe content of abaca grown in FL was 81.25% ($p<0.05$) and 23.53% ($p<0.05$) higher than that of FT. Meanwhile, the Mn content of abaca leaves in FL was 78.50% ($p<0.05$) lower than that of FT. Higher Mn content in abaca leaves under FT compared to FL could be associated with lower soil pH in FT. Therefore, it was more suitable for Mn availability (Barrow & Hartemink, 2023) and contributed to higher Mn uptake by plants. High P availability in FT could induce Mn uptake by plants, indicated by the strong positive correlation between these factors ($R^2 = 0.966$). A synergistic relationship between Mn and P was also observed by Barben et al. (2011) in potatoes and by Berríos et al. (2019) in ryegrass. Meanwhile, higher Ca and Fe content in abaca leaves of FL is related to their interaction with other nutrients.

Table 4
Macronutrients and micronutrients of abaca leaves under the agroforestry system

Foliage nutrients	Unit	Agroforestry system		CV (%)
		FT	FL	
N	%	2.557±0.61[a]	2.270±0.14[a]	18.24
P	%	0.253±0.03[a]	0.183±0.05[a]	18.23
K	%	2.510±0.14[a]	2.670±0.07[a]	4.30
S	%	1.593±0.52[a]	1.837±0.31[a]	24.82
Mg	%	0.089±0.00[a]	0.091±0.00[a]	1.70
Ca*	%	0.163±0.01[b]	0.293±0.01[a]	7.37
Fe*	%	0.017±0.00[b]	0.021±0.00[a]	6.03
Mn*	%	0.107±0.01[a]	0.023±0.01[b]	8.88
Cl	%	0.610±0.06[a]	0.552±0.04[a]	8.03
Cu	ppm	12.780±1.70[a]	16.087±3.87[a]	20.71
B	ppm	314.333±43.36[a]	304.333±45.50[a]	14.37
Zn	ppm	22.953±0.40[a]	24.860±3.56[a]	10.60

Note. FT = Intermediate phase agroforestry; FL = Advanced phase agroforestry; CV = Coefficient of variation; * = $p<0.05$; Values (mean ± standard deviation) indicated by the same letters showed no significant difference at $\alpha = 0.05$

Significantly higher N availability in FL (78.55%; $p<0.05$) system compared to FT could induce higher Ca uptake in FL. It could be associated with the activation of transcription factor nitrate transporter 1 (NRT1) when soil nitrate was high. The activation of NRT1 could induce phospholipase C enzyme activity (PLC), subsequently increasing Ca uptake in the cytosol (K.-H. Liu et al., 2020). High Ca uptake could occur under high soil N availability to maintain N balance in leaves (Xing et al., 2022). Meanwhile, higher Fe leaf content in abaca grown in FL was associated with its antagonistic interaction with soil available P (Figure 4). High Fe and P availability in the FT system was associated with declining Fe and increasing P content in leaves. However, high Fe and low P availability in FL could induce declining P and increasing Fe leaf content. Zheng et al. (2009) also observed interaction between Fe and P in rice.

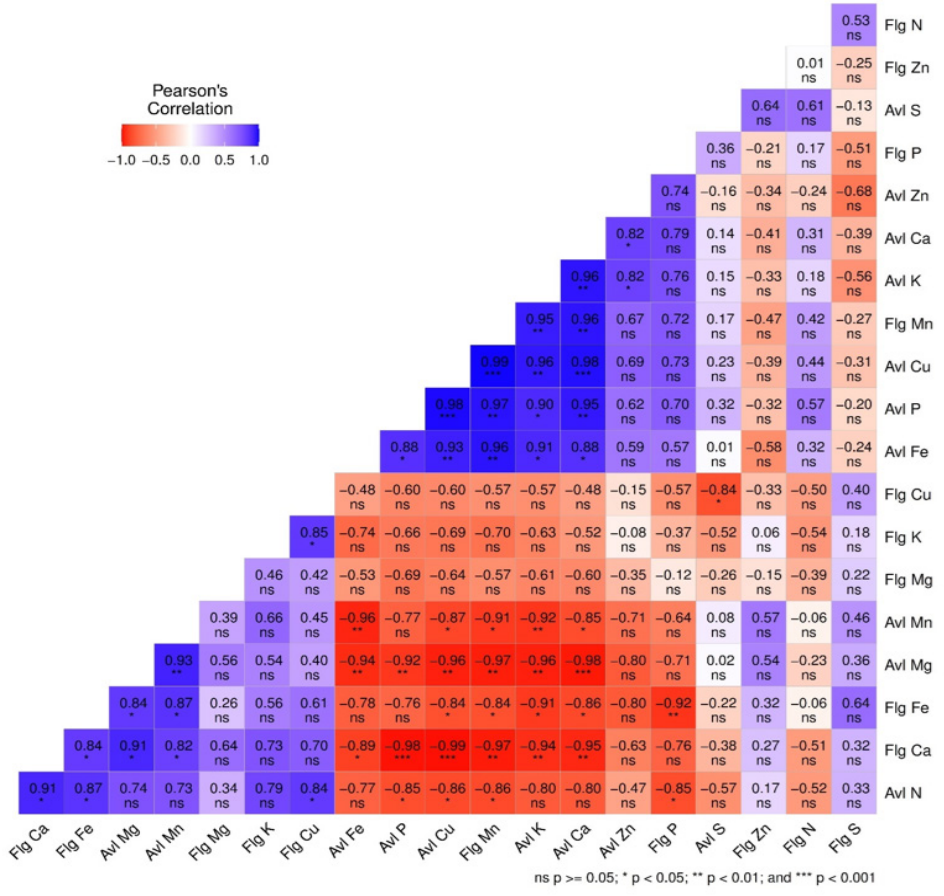

Figure 4. Correlation between soil available nutrients and foliage nutrients

Note. Avl = Available; Flg = Foliage

Nutrient uptake was analyzed based on allometry specifically used for foliage biomass estimation. Macronutrient uptake estimation by abaca in FT and FL, from the largest to the smallest, were N > K > S > P > Ca > Mg and K > N > S > Ca > P > Mg, respectively. Meanwhile, micronutrient uptake in FT and FL were Cl > Mn > B > Fe > Zn > Cu and Cl > B > Mn > Fe > Zn > Cu, respectively (Table 5). Lower nutrient uptake estimation in FL compared to FT was associated with lower biomass estimation of abaca in FL due to lower diameter at 10, 30, and 130 cm height. It indicated that improved soil characteristics in FL could not compensate for growth inhibition caused by shading stress. The high shading intensity in the FL system could hinder abaca growth. Therefore, pruning is essential to compensate for nutrient competition between abaca and shading trees (Raza et al., 2019).

Table 5
Nutrient uptake based on allometry estimation of abaca foliage

Nutrients	Nutrient uptake		CV (%)
	FT	FL	
N* (kg/ha)	107.490±28.07[a]	6.314±0.93[b]	34.90
P* (kg/ha)	10.597±2.11[a]	0.496±0.04[b]	26.94
K* (kg/ha)	104.768±14.37[a]	7.507±1.73[b]	18.22
Ca* (kg/ha)	6.795±0.72[a]	0.828±0.22[b]	14.00
Mg* (kg/ha)	3.691±0.31[a]	0.254±0.05[b]	11.39
S* (kg/ha)	66.528±23.00[a]	5.196±1.57[b]	45.46
Fe* (kg/ha)	0.736±0.08[a]	0.058±0.02[b]	14.99
Mn* (kg/ha)	4.429±0.25[a]	0.064±0.02[b]	7.74
Cl* (kg/ha)	25.512±4.26[a]	1.533±0.21[b]	22.31
Cu* (g/ha)	53.526±11.29[a]	4.622±1.91[b]	27.85
Zn* (g/ha)	95.627±9.58[a]	6.931±1.44[b]	13.35
B* (g/ha)	1317.270±286.43[a]	85.110±19.92[b]	28.95

Note. FT = Intermediate phase agroforestry; FL = Advanced phase agroforestry; CV = Coefficient of variation; * = $p<0.05$; Values (mean±standard deviation) indicated by the same letters showed no significant difference at $\alpha = 0.05$

ROS and Antioxidant Production

Abaca is considered a C3 plant, indicating high sensitivity to photorespiration; therefore, shading trees is required to avoid oxidative damage from high light intensity. However, through specific mechanisms, oxidative damage caused by ROS production is triggered by high light intensity and continuous low light intensity. The oxidative stress response observed in this study was significantly exhibited by abaca grown in the FL system, particularly H_2O_2 and phenolic content. H_2O_2 production in abaca leaves grown in FL was 32.66% ($p<0.001$) higher than that of FT.

Low light intensity could activate RBOHD/F, part of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase in the apoplast, which is involved in ROS production, particularly H_2O_2 (Zhang et al., 2017). Zhang et al. (2017) also observed that H_2O_2 production triggered by low light intensity could lead to NO production through signal cascading, subsequently causing stomatal closure. Low light intensity could trigger ion release from vacuoles in guard cells (Shimazaki et al., 2007), leading to lower carbon assimilation and higher ROS production (Shafiq et al., 2021).

Higher H_2O_2 led to higher phenolic content in abaca leaves of FL by 35.08% (p<0.0001) compared to that of FT (Tables 6 and 7). Phenol is one of the major H_2O_2 scavengers with an aromatic ring structure composed of -OH or -OCH, which plays an important role in trapping free radicals (Dumanović et al., 2021; Sadak et al., 2019; Sadak & Ramadan, 2021). On the other hand, ascorbic acid content was found to be higher (23.31%; p<0.0001) in the abaca of the FT system compared to that of FL (Table 7).

Table 6
Oxidative damage indicators of abaca leaves under agroforestry system

Agroforestry system	Oxidative damage indicators		
	MDA (nmol MDA/g FW)	H_2O_2** (µmol H_2O_2/g FW)	$O_2^{\bullet-}$ (µmol $O_2^{\bullet-}$/g FW)
FT	14.822±4.03[a]	125.000±29.14[b]	17.169±9.86[a]
FL	14.828±3.01[a]	165.830±64.74[a]	17.487±11.98[a]
CV (%)	22.21	18.44	46.86

Note. FT = Intermediate phase agroforestry; FL = Advanced phase agroforestry; MDA = Malondialdehyde; H_2O_2 = Hydrogen peroxide; $O_2^{\bullet-}$ = Superoxide anion; ** = p<0.001; Values (mean ± standard deviation) indicated by the same letters showed no significant difference at α = 0.05

Table 7
Non-enzymatic and enzymatic antioxidants of abaca leaves under agroforestry system

Agroforestry system	Non-enzymatic antioxidants	
	Phenol (mg GAE/g DW) ***	Ascorbic acid (mg AAE/100 g sample) ***
FT	3.045±0.92[b]	216.010±8.05[a]
FL	4.116±0.98[a]	175.180±45.18[b]
CV (%)	5.82	1.33
	Enzymatic antioxidants	
	SOD (U SOD/g FW)	POD (U POD/min g FW)
FT	114.830±16.87[a]	0.412±0.16[a]
FL	114.930±35.21[a]	0.467±0.07[a]
CV (%)	22.58	20.89

Note. FT = Intermediate phase agroforestry; FL = Advanced phase agroforestry; SOD = Superoxide dismutase; POD = Peroxidase; mg GAE/g DW = mg gallic acid equivalent per gram dry weight; mg AAE/100 g sample = mg ascorbic acid equivalent per 100 g sample; U/g FW= unit per gram fresh weight; **= p<0.001; Values (mean±standard deviation) indicated by the same letters showed no significant difference at α = 0.05

Higher ascorbic acid production is associated with activation of L-galLDH as electron transport for ascorbic acid biosynthesis in high light intensity conditions compared to low light intensity (Bartoli et al., 2009). It was also observed in satsuma mandarin oranges by Izumi et al. (1992) and in *Arabidopsis* by Heyneke et al. (2013). Additionally, ascorbic acid biosynthesis in plants mainly occurs through the L-galactose pathway (Smirnoff-wheeler pathway [SW pathway], L-gulose, D-galacturonic, and D-glucuronic, which relies on P content as a major enzyme component involved in ascorbic acid biosynthesis (Chaturvedi et al., 2022). A positive correlation between P leaves content and ascorbic acid ($R^2 = 0.766$) in abaca indicated a strong association between P leaves content and ascorbic acid.

In contrast, other oxidative stress responses, viz. MDA, $O_2^{\bullet-}$, SOD, and POD were observed to be not significantly different between abaca in FT and FL (Tables 6 and 7). However, the overall expression of the respective indicators was higher in FL than in FT. MDA and $O_2^{\bullet}$ of abaca growth in FL were higher by 6.75 and 1.86%, respectively, than in FT. Non-significant MDA and $O_2^{\bullet-}$ differences between the two agroforestry systems might be associated with chlorophyll degradation. Chlorophyll *a* and chlorophyll *b* content, total chlorophyll as well as chlorophyll *a*/*b* of abaca leaves grown in both agroforestry systems showed no significant difference (Table 8). Lipid peroxidation could promote chlorophyll degradation, resulting from oxidated polyunsaturated fatty acid (PUFA) by lipoxygenase (LOX) enzyme activity (Jakhar & Mukherjee, 2014). It can be assumed that no physiological plasticity, particularly chlorophyll degradation, was observed in the abaca of both agroforestry systems; therefore, MDA and $O_2^{\bullet-}$ contents were not significantly different. Other studies by Chaves et al. (2008) and Saleem et al. (2019) also observed similar MDA expression between high and low light intensity. Electron excitation between unshaded and shaded plants might show similar plasticity in transport electron or energy dissipation (Chaves et al., 2008).

The significant differences in SOD and POD between abaca leaves in FT and FL were also absent, despite the higher SOD (8.71%) and POD (14.63%) content

Table 8
Chlorophyll content of abaca leaves under agroforestry system

Parameters	Agroforestry system		CV (%)
	FT	FL	
Chlorophyll *a* (mg/g FW)	0.445±0.09 [a]	0.412±0.06 [a]	15.91
Chlorophyll *b* (mg/g FW)	0.806±0.16 [a]	0.745±0.11 [a]	15.92
Total chlorophyll (mg/g FW)	0.662±0.14 [a]	0.613±0.09 [a]	19.09
Chlorophyll *a*/*b*	0.553±0.00 [a]	0.553±0.00 [a]	0.01

Note. CV = Coefficient of variation; mg/g FW = mg per g fresh weight; Values (mean ± standard deviation) indicated by the same letters showed no significant difference at α = 0.05

of abaca leaves in FL compared to that of FT (Table 7). It might be associated with several nutrients related to the biosynthesis of the respective antioxidants, which were also observed to be not significant, except for POD. Cu and Zn were known to form Cu, Zn-SOD complex with Cu as an active cofactor to directly fix superoxide ions (Alscher et al., 2002). Cu and Zn content in abaca leaves of both agroforestry systems, which were also observed to be not significant (Table 4), might be associated with the absence of significant differences in SOD. A positive correlation between Cu and SOD (R^2 = 0.552) was also observed in this study. Approximately 90% of total SOD in eucaryotic organisms is composed of Cu, Zn-SOD complex rather than Mn-SOD or Fe-SOD (Pelmenschikov & Siegbahn, 2005). Meanwhile, POD might be associated with Fe leaf content, indicated by the positive correlation between POD and Fe (R^2 = 0.546). Despite significant differences between both systems for Fe (Table 4), POD content showed no significant difference. Fe is the major component of the heme complex in POD (Campa, 1990).

Fiber Quality

The fiber quality observed in this study was diameter and tensile strength. Abaca fiber is shaped like an ellipse rather than circular; therefore, a cross-sectional area was used to measure its diameter (K. Liu et al., 2013). Fiber diameter of abaca grown in FT was higher (23.53%; p<0.0001) compared to FL (Table 9). The outer and inner layers of abaca pseudostem grown in FT showed different fiber diameters, ranging from 0.112–0.318 and 0.161–0.287 mm, respectively. Meanwhile, the smallest and largest fiber diameter of the outer layer of abaca pseudostem grown in FL was 0.070–0.240 mm, while the inner layer ranged from 0.066–0.285 mm.

Table 9
Diameter and tensile strength of abaca bast fiber in agroforestry system

Agroforestry system	Fiber diameter (mm)***	Tensile strength (MPa)*	CV (%)
FT	0.208±0.01[a]	379.980±81.07[a]	7.90
FL	0.168±0.02[b]	319.940±107.99[b]	19.01

Note. FT = Intermediate phase agroforestry; FL = Advanced phase agroforestry; *** = p<0.0001; * = p<0.05; Values (mean±standard deviation) indicated by the same letters showed no significant difference at α = 0.05

Significant differences in fiber tensile strength in both systems were also observed. The higher tensile strength of abaca in FT (18.77%; p<0.05) was observed to be significantly different from that of FL (Table 9). The tensile strength of the outer layer of abaca pseudostem grown in FT and FL were 345.78 and 416.06 MPa, respectively. Meanwhile, the tensile strength of the inner layer of abaca pseudostem in FT and FL was 409.41 and 232.45 MPa, respectively. Higher fiber diameter could promote lower tensile strength.

Increasing fiber diameter could adversely affect tensile strength due to increasing defects in cross-sectional area. The fiber diameter and tensile strength of abaca in both agroforestry systems were exponentially distributed (Figure 5). Leaf fiber of abaca showed fiber diameter distribution at 55.6–197.6 µm, with a similar decreasing trend of tensile strength as increasing fiber diameter (Munawar et al., 2007). Decreasing tensile strength and increasing fiber diameter might be associated with alteration of lignocellulose content, microfibril angle, cell volume, and fiber defect density (Lewin, 2007). Despite the positive correlation between higher cellulose and increasing tensile strength in Enset, higher defects due to increasing fiber diameter could decrease tensile strength (Dessie et al., 2023).

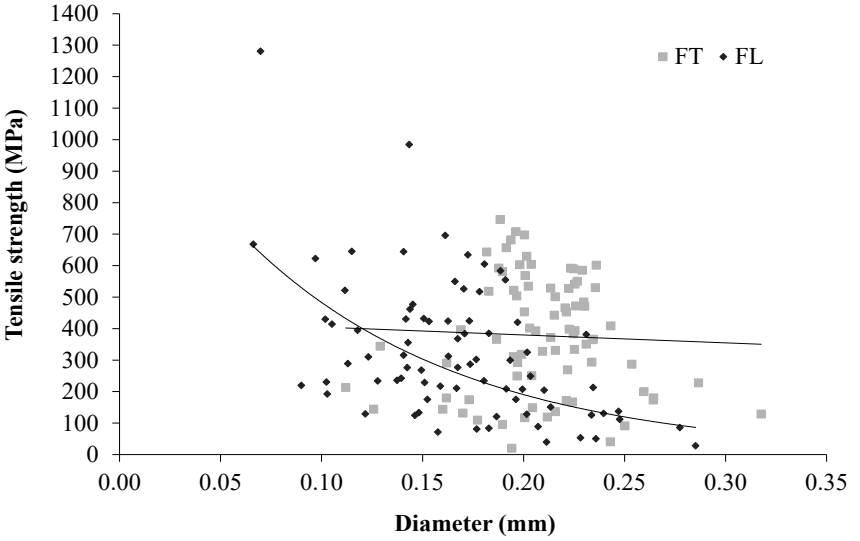

Figure 5. Relationship between diameter and tensile strength of abaca fiber

Note. FT = Intermediate phase agroforestry; FL = Advanced phase agroforestry

A forward stepwise regression analysis was conducted to determine factors that could explain the variable contributing to fiber diameter and tensile strength. Macro- and micronutrient content were used as independent variables in this analysis. The threshold *p* value used in both models was 0.20. The significance level of 0.20 was between the acceptable range (0.15–0.25) suggested by Derksen and Keselman (1992) to allow the authentic variables to be included in the model instead of noise variables. The results showed that Ca, Fe, and K were the variables that explained most of the variance in fiber diameter, indicated by their significant effect on the model (Table 10). These variables exhibited a positive effect on the model. The fourth variable that improved the model is N, which had a negative effect. The positive effect of Ca,

Fe, and K on fiber diameter was assumed to be associated with fiber uniformity. Smaller fiber diameter is attributed to tapered fiber tip morphology, which results in higher fiber uniformity (Graham & Haigler, 2021).

As one of the essential macronutrients, Ca plays an important role in pectin biosynthesis and strengthening cell walls through its bonding with carboxylate ions (de Bang et al., 2021). A positive correlation was observed between Ca content and fiber uniformity in cotton (Fortier et al., 2021). Increasing fiber uniformity of jute (S. R. Sarker et al., 2008) and cotton (Sawan et al., 2008) was attributed to increasing K content. Improved fiber uniformity of 47.5% was observed under 50 kg/ha iron sulphate ($FeSO_4$) fertilization in cotton (Sankaranarayanan et al., 2010). In contrast, higher N content promoted low fiber uniformity in cotton (Hassanzadehdelouei et al., 2022), while N fertilization on *Populus trichocarpa* exhibited no significant effect on fiber diameter (Pitre et al., 2010).

Table 10
Stepwise regression analysis on fiber diameter

		Independent variables					R^2 (%)	Adjusted R^2 (%)
		Constant	Ca	Fe	K	N		
Model 1	Factor	0.117	0.313				57.75	56.25
	SE	0.018	0.073					
	p-value		0.003					
Model 2	Factor	0.028	0.171	6.360			68.70	65.09
	SE	0.016	0.071	0.927				
	p-value		0.025	0.026				
Model 3	Factor	0.105	0.222	5.963	0.031		73.33	69.06
	SE	0.015	0.069	0.920	0.003			
	p-value		0.002	0.003	0.014			
Model 4	Factor	0.107	0.206	6.495	0.033	-0.002	78.06	72.33
	SE	0.014	0.052	0.907	0.001	0.001		
	p-value		0.016	0.017	0.025	0.153		

Note. SE = Standard error

Most variances in tensile strength were attributed to Mg and B content, which indicated a high-significance effect on the model (Table 11). The positive effect of Mg on tensile strength could be associated with its role in cellulose translocation, sucrose loading and unloading through phloem (Ahmed et al., 2020; Pandey, 2018). Increasing tensile strength of cotton by 2.3% was observed under Mg nano-fertilizer (Kanjana, 2020). Meanwhile, the positive effect of B on tensile strength could be associated with its role in cell wall strengthening. As an essential micronutrient, B plays an important role in cell wall structural function through

Table 11
Stepwise regression analysis on tensile strength

		Independent variables			R^2 (%)	Adjusted R^2 (%)
		Constant	Mg	B		
Model 1	Factor	534.1	51.4		81.47	74.41
	SE	45.75	14.20			
	p-value		0.180			
Model 2	Factor	318	53.7	1.103	87.01	78.35
	SE	9.68	13.78	0.119		
	p-value		0.002	0.003		

Note. SE = Standard error

the crosslinking of pectic polysaccharide rhamnogalacturonan II (RG II) (Miwa & Fujiwara, 2010) and resulted in increasing tensile strength in fiber plant (de Souza Júnior et al., 2022).

CONCLUSION

The advanced phase agroforestry system contributed to improved soil characteristics compared to the intermediate phase. However, high shading intensity under the advanced phase could promote abaca growth inhibition and oxidative stress responses. Consequently, it resulted in lower fiber quality compared to intermediate-phase agroforestry. Therefore, pruning management was substantial in improving the growth condition and fiber quality of abaca under agroforestry.

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