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The role of soil ameliorants and arbuscular mycorrhizal fungi in improving the growth of *Samanea saman* (Jacq.) Merr seedlings in coal mine post-mining media

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ABSTRACT

Improvement of coal post-mining soils is necessary to increase the success of reclamation activities. Organic and inorganic materials are often used as soil improvers (ameliorants), and are usually combined with microorganisms, one of which is arbuscular mycorrhiza fungi (AMF), to support and improve the success of revegetation activities on post-mining land. This study aims to evaluate the application of soil ameliorants and AMF in increasing the success of revegetation in coal post-mining media. The study used a completely randomized design (CRD) with a split plot design consisting of three treatments, namely: AMF, soil ameliorants: compost, lime and fly ash and bottom ash (FABA), exposure to Al and Fe, and the plants used were *Samanea saman*. The results showed that AMF treatment combined with compost and lime ameliorants, as well as exposure to Al and Fe were able to increase the growth of *S. saman* seedlings. In addition, the combination of AMF and ameliorants was also able to increase the uptake of N and P nutrients. The provision of FABA ameliorants was able to increase soil pH from slightly acidic to slightly alkaline, and caused the unavailability of P-available elements. *S. saman* is very suitable for planting in revegetation of post-mining land, with compost and FABA ameliorants. FABA has the potential to be used as a substitute for lime.

Keyword: ameliorant, arbuscular mycorrhiza fungi, fly ash and bottom ash, revegetaion, Samanea saman.

INTRODUCTION

Generally, coal mining activities are carried out in forest areas (Mishra et al., 2022), especially in Indonesia, this is because coal reserves are under the cover of forest areas (Ahirwal et al., 2022). Coal mining activities in forest areas have an impact on changes in the characteristics of forest areas and surrounding ecosystems (Wang et al., 2022). Generally, coal mining activities are carried out by open pit mining (Woodbury et al., 2020), which removes significant vegetation cover, top and subsoil, and overburden. vegetation cover, top and subsoil, and overburden that significantly significant negative impacts on changing ecosystem functions, changes to the surface landscape, damage to the surrounding environment, reduction in biodiversity and affecting biodiversity and affecting the resilience of the surrounding ecology (Prosekov 2021; Ranjan et al., 2021; Guo et al., 2024). This open-pit mining activity can remove topsoil, damage soil components (horizon and soil structure) soil profile, reduce soil microbial population and disrupt nutrient cycles (Zhang et al., 2019; Wang et al., 2022). This This causes post-coal mining land to be classified as marginal land, the characterized by the destruction of soil characteristics (physical, chemical, and biological), nutrient-poor, making it unfavorable for plant growth and land productivity (Pihlap et al., 2019; Mao et al., 2024).

Every company that holds a mining business license (IUP) has an obligation to reclaim ex-mining lands. This has been regulated in Government Regulation of the Republic of Indonesia Number 26 of 2020 concerning Forest Rehabilitation and Reclamation. Forest reclamation is an effort to restore damaged forest ecosystems so that they can function again in accordance with their designation (Dallaire and Skousen 2019). Reclamation activities can improve the soil fertility of post-mining land and become an effective measure in restoring the ecological environment due to coal mining activities (Zhang et al., 2019). One of the reclamation activities is revegetation, which aims to restore damaged vegetation by replanting on post-mining land. Revegetation activities are a promising ecological aspect to maintain environmental stability, increase biodiversity, improve soil quality, increase carbon sequestration and offset greenhouse gas emissions in post-mining areas (Dallaire and Skousen, 2019; Yang et al., 2019).

Soil improvement or soil amendment after coal mining is needed to support the success of reclamation activities. Soil improvement or soil amendment aims to build and increase soil organic matter (SOM) which can later restore the physical, chemical and biological functions of the soil, can support plant growth, and a greater impact can affect soil microclimate, biomass turnover and nutrient mineralization (Amoah-Antwi et al., 2020). Soil management by keeping the pH of the soil less extreme determines the availability of nutrients in the soil, which is one aspect that can support the success of revegetation activities on post-mining land (Woodbury et al., 2020). Drastic pH depletion and nutrient-poor soils are among the barriers to revegetating post-mining lands in humid tropical regions, such as Indonesia (Sheoran et al., 2010; Woodbury et al., 2020). Soil amendments using organic matter have been widely practiced to restore and improve soil fertility and support plant growth on post-mining lands (Feng et al., 2019; Worlanyo and Jiangfeng 2021). Several soil amendment techniques have been widely used to

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improve post-mining soil conditions such as using green manure (Pietrzykowski et al., 2017), addition of compost from oil palm bunches and/ or municipal solid waste (Miller et al., 2019), and several other organic materials.

FABA are waste products of the coal combustion process and are mostly generated from coalfired power plants (Ahmad et al., 2021; Lu et al., 2023). The accumulation and disposal of large amounts of FABA as coal combustion waste is considered to be a serious environmental problem (Finkelman et al., 2021; Hanum et al., 2023). This shows that there is a need to utilize FABA to reduce the accumulation and volume of FABA. The utilization of FABA has been widely used in various aspects, one of which is in agricultural or forestry activities, namely as a soil amendment material, especially on degraded soils, one of which is post-mining land (Akinyemi et al., 2020; Bucka et al., 2021). FABA contains macro and micro elements that are generally needed to improve soil fertility and plant growth, (Ahmad et al., 2021), and is an alternative material that can be added to neutralize soil acidity (soil pH) on coal post-mining land (Pandey at al. 2019). These have become the basis that FABA can be used as an alternative soil ameliorant material in reclamation activities, especially revegetation of coal post-mining lands.

In addition to the addition of soil ameliorants, the application of soil microorganisms, one of which is the AMF is very important in supporting the success of reclamation activities on coal post-mining land (Salim et al., 2020a; Mao et al., 2024). AMF has an important role in various ecosystems and contributes to the restoration of soil ecological functions and can facilitate revegetation activities on degraded lands (Bi et al., 2021; Zhang et al., 2021). Soil, plants and AMF have a mutualistic relationship and are essential for the recovery of ecological functions and processes in ecosystems on post-mining land (Krüger et al., 2017; Zhang et al., 2020). The mutualistic relationship between AMF and plants, where plants serve as hosts and carbon sources, while AMF can help plants absorb water and essential nutrients (Mekkaoui et al., 2024). The results of research by Wulandari et al., (2024) reported that AMF application in coal mine reclamation activities was able to significantly improve plant adaptation. In addition, AMF application has a significant effect on the success of rehabilitation activities in damaged areas, such as coal post-mining land (Prematuri et al., 2020).

The selection of plant species and other revegetation practices are important aspects to accelerate the recovery process of post-mining lands (Pihlap et al., 2019). One of the potential fast-growing species used in post-mining land reclamation activities is rain tree (Samanea saman). S. saman is able to grow in soil with nutrient-poor characteristics (Setyaningsih et al., 2024). In addition, S. saman is widely planted as a shade tree along roads and parks, and contains bioactive compounds that have medicinal properties (Vinodhini et al., 2018; Jacob et al., 2022). Research related to the combination of AMF, ameliorant materials, especially FABA and exposure to Al and Fe in coal post-mining soil has not been widely conducted. Therefore, the purpose of this study is to evaluate the application of soil ameliorants and AMF in increasing the success of revegetation on coal post-mining media.

MATERIAN AND METHOD

Soil sampling and preparation of planting media

Coal post-mining soil was taken directly from the former coal mining area with initial soil characteristics shown in Table 1. The soil was sterilized in an autoclave at 121 °C and 1 atm pressure, for 15 minutes. After that, the soil was put into polybags as much as 600 g/polybag, with a polybag size of 15×20 cm. Soil ameliorants and aluminum (Al) and iron (Fe) treatments were applied 1 week before weaning. The soil ameliorants used were compost, lime and faba. Meanwhile, Al and Fe treatment was done by dissolving AlC₃ and FeSO₄.7H₂O in distilled water according to the predetermined concentrations.

Seed germination and AMF preparation

The plant used in this study was *S. saman*. The seeds of *S. saman* were germinated by sowing them in a sprout tub containing zeolite media and maintained by watering the media twice a day by paying attention to the humidity of the sprout media. Meanwhile, AMF preparation was carried out by collecting AMF inoculum that had previously been maintained and propagated in the greenhouse. The AMF inoculum used was indigenous and taken directly from coal postmining land. The AMF used was a mixture of various types of AMF from the genus Glomus, Acaulospora, and Gigaspora.

Seedling weaning and AMF inoculation

Weaning is done when the seedlings are ready to wean (2–3 leaves have appeared). AMF inoculation was done at the same time as weaning the seedlings and the inoculum was tried to be given close to the seedling roots. After that, the plants were maintained in the greenhouse (with the daily temperature of 29–35 °C and relative humidity of 60–90%) for 4 months and maintenance included: watering the plants twice a day (depending on whether the media was still moist or not) and preventing pests and diseases.

Parameters observed

Data collected during the observation included: growth (height, diameter and biomass). Seedling quality index (*SQI*) using the equation from Dickson et al., 1960 as follows:

SQI =	
Dry weight of plant (g)	(1)
Height of plant (cm) + dry weight of shoot (g)	(1)
Diameter of plant (mm) dry weight of root (g)	

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ParameterS	value	Criteria	Parameters	value	Criteria
pH H ₂ O	5.40	Acid	Mg (cmol ⁽⁺⁾ /kg)	3.54	High
C-org (%)	3.70	High	Kapasitas tukar kation (cmol ⁽⁺⁾ /kg)	19.62	Medium
N-total (%)	0.25	Medium	AI (cmol ⁽⁺⁾ /kg)	0.75	Very low
P-tersedia (ppm)	6.00	Low	Fe (%)	2.29	Very high
P-total (ppm)	70.00	High	Pasir (%)	27.00	
K (cmol ⁽⁺⁾ /kg)	0.13	Low	Debu (%)	32.00	
Ca (cmol ⁽⁺⁾ /kg)	8.81	Medium	Liat (%)	41.00	

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Table 1. Characteristics of coal post-mining media

Note: Criteria by Eviati and Sulaeman (2009).

Chlorophyll content of SPAD was determined using a SPAD chlorophyll meter. The formula for converting SPAD chlorophyll values to μ g·cm⁻², by following the equation from Cerovic et al. (2012):

$$Chlorophyll = (99 \times SPAD)/(144-SPAD) \quad (2)$$

Photosynthesis rate was measured using a portable photosynthesis system analysis tool (LI-COR model LI-6400). Measurements were taken on the third leaf sample of each seedling. Soil analysis (pH, N, P, K, Al and Fe) and plant tissue nutrient analysis (N, P, K, Al and Fe) were conducted at the end of observation.

Root colonization analysis

The technique of determining the percentage of root colonization uses the method of Clapp et al., (1996) with modifications. Roots were washed using water until clean, then soaked with 20% KOH for 48 hours. After that, the roots were washed again using water, then soaked with 0.1 M HCl for 15 minutes. The roots were again soaked with trypan blue dye for 2 days. after that, the roots were washed again using water and soaked again in destaining solution for 24 hours. Preparation of root preparations begins with cutting the roots approximately 1 cm and placed parallel to the object preparation. Each object preparation has 10 pieces of roots and every five pieces of roots are covered using a cover slip. Root colonization can be calculated based on the appearance of AMF intraradical structures (hyphae, arbuscules, vesicles, and spores). Determination of root colonization percentage was calculated using the formula of Giovannetti and Moose (1980). The following is the formula used in calculating root colonization:

$$\frac{Colonization \ percentage =}{\frac{Total \ root \ field \ infected}{Total \ root \ field \ observed \times 100\%} \times 100\%$$
(3)

Percent colonization is then classified based on O'Cannor et al., (2001), namely:

- Not colonized: colonization value 0%,
- Low: colonization value < 10%,
- Medium: colonization value 10–30%,
- High: colonization value > 30%.

Research design and data analysis

This study used a CRD with a split plot design consisting of three treatments, namely: AMF, soil ameliorants (compost, lime and faba) and exposure to Al and Fe. AMF consisted of 2 levels

(M0: without AMF, M1: with AMF), soil ameliorants consisted of 5 levels (A0: 100% soil + 0% compost + 0% lime + 0% faba; A1: 95% soil + 0% compost + 0% lime + 5% faba; A2: 90% soil + 0% compost + 0% lime + 10% faba; A3: 9. 45% soil + 5% compost + 0.5% lime + 0% faba;A4: 89.5% soil + 5% compost + 0.5% lime + 5% faba; A5: 84.5% soil + 5% compost + 0.5% lime + 10% faba), and A1 and Fe exposure consisted of 3 levels (C0: 0 mM Al + 0 mM Fe; C1: 2 mM Al + 0.25 mM Fe; and C2: 6 mM Al + 1.25 mM Fe). Data analysis used anova test with 95% confidence level ($\alpha = 5$). When the anova test showed a significant effect, then continued with the Duncan multiple's ranget test (DMRT) at the 95% confidence level ($\alpha = 5$).

RESULT

Seedling growth on post-coal mining media

Each treatment gave varied responses to the growth of height and diameter of S. saman seedlings (Fig. 1a and 1b). The treatment with AMF showed the best height growth compared to the treatment without AMF (Fig. 1a). Meanwhile, the provision of ameliorants is also able to have a positive effect on increasing the height growth of S. saman seedlings. The treatment of M1A3C2 gave the best height growth of 24.34 cm. This shows that the combination of AMF treatment and ameliorants, especially compost and lime, as well as the provision of Al and Fe exposure can stimulate the growth of S. saman seedlings. Meanwhile, the application of 5% faba combined with AMF, compost and lime with exposure to 6 mM Al and 1.25 mM Fe (M1A4C2) was also sufficient to increase the growth of S. saman seedlings to 23.38 cm (Fig. 1a). The treatment of AMF was also able to stimulate the growth of diameter and height. The AMF treatment combined with compost and lime (M1A3C0) was the best treatment (2.88 mm) compared to other parameters (Fig. 2).

AMF treatment also had a significant impact on increasing biomass in some treatments (Table 1). Treatment M1A3C2 gave the highest biomass of 7.907 g, but the treatment did not show significant differences with treatments M1A3C0 (7.390 g) and M1A3C1 (7.030 g) (Table 2). Even in the treatment without AMF, the treatments of compost and lime as well as exposure to Al and Fe, namely M0A3C0 (1.470 g), M0A3C1 (1.680 g) and M0A3C2 (1.547



Figure 1. Growth of height (a) and diameter (b) of *S. saman* seedlings in various treatments. M0: without AMF, M1: with AMF, A0: 100% soil + 0% compost + 0% lime + 0% faba, A1: 95% soil + 0% compost + 0% lime + 5% faba, A2: 90% soil + 0% compost + 0% lime + 10% faba, A3: 94. 5% soil + 5% compost + 0.5% lime + 0% faba, A4: 89.5% soil + 5% compost + 0.5% lime + 5% faba, A5: 84.5% soil + 5% compost + 0.5% lime + 10% faba, C0: 0 mM A1 and 0 mM Fe, C1: 2 mM A1 and 0.25 mM Fe, C2: 6 mM A1 and 1.25 mM Fe



Figure 2. Chlorophyll content of SPAD (a) and photosynthetic rate (b) of *S. saman* seedlings in various treatments. M0: without AMF, M1: with AMF, A0: 100% soil + 0% compost + 0% lime + 0% faba, A1: 95% soil + 0% compost + 0% lime + 5% faba, A2: 90% soil + 0% compost + 0% lime + 10% faba, A3: 94. 5% soil + 5% compost + 0.5% lime + 0% faba, A4: 89.5% soil + 5% compost + 0.5% lime + 5% faba, A5: 84.5% soil + 5% compost + 0.5% lime + 10% faba, C0: 0 mM A1 and 0 mM Fe, C1: 2 mM A1 and 0.25 mM Fe, C2: 6 mM A1 and 1.25 mM Fe

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Treatment	Biomass (g)	Treatment	Biomass (g)
M0A0C0	0.707 ± 0.192 ^{gh}	M1M0C0	2.537 ± 0.354 ^{bcdef}
M0A0C1	$0.930 \pm 0.049^{\text{fgh}}$	M1M0C1	3.030 ± 0.607^{bcd}
M0A0C2	0.980 ± 0.046 ^{fgh}	M1M0C2	2.847 ± 0.271 ^{bcde}
M0A1C0	0.377 ± 0.124 ^h	M1M1C0	2.337 ± 0.262 ^{cdefg}
M0A1C1	0.363 ± 0.082 ^h	M1M1C1	2.440 ± 0.271 ^{bcdefgh}
M0A1C2	0.453 ± 0.107 ^h	M1M1C2	1.843 ± 1.275 ^{cdefgh}
M0A2C0	0.363 ± 0.180 ^h	M1A2C0	1.677 ± 0.589 ^{cdefgh}
M0A2C1	0.330 ± 0.063 ^h	M1A2C1	1.693 ± 0.529 ^{cdefgh}
M0A2C2	0.387 ± 0.126 ^h	M1A2C2	2.150 ± 1.780 ^{cdefgh}
M0A3C0	1.470 ± 0.686 ^{cdefgh}	M1A3C0	7.390 ± 1.986ª
M0A3C1	1.680 ± 0.200 ^{cdefgh}	M1A3C1	7.030 ± 0.945ª
M0A3C2	1.547 ± 0.159 ^{cdefgh}	M1A3C2	7.907 ± 2.627ª
M0A4C0	0.423 ± 0.250^{h}	M1A4C0	4.093 ± 2.362 ^b
M0A4C1	0.667 ± 0.162 ^{gh}	M1A4C1	2.633 ± 1.363 ^{bcdef}
M0A4C2	0.413 ± 0.023^{h}	M1A4C2	3.167 ± 0.340 ^{bc}
M0A5C0	0.497 ± 0.298^{h}	M1A5C0	1.570 ± 0.819 ^{cdefgh}
M0A5C1	0.403 ± 0.160^{h}	M1A5C1	1.187 ± 0.978 ^{efgh}
M0A5C2	0.480 ± 0.024 ^h	M1A5C2	1.257 ± 0.396 ^{efgh}

Table 2. Biomass of S. saman seedlings in each treatment

Note: Mean \pm standar deviation. M0: without AMF, M1: with AMF, A0: 100% soil + 0% compost + 0% lime + 0% faba, A1: 95% soil + 0% compost + 0% lime + 5% faba, A2: 90% soil + 0% compost + 0% lime + 10% faba, A3: 94. 5% soil + 5% compost + 0.5% lime + 0% faba, A4: 89.5% soil + 5% compost + 0.5% lime + 5% faba, A5: 84.5% soil + 5% compost + 0.5% lime + 10% faba, C0: 0 mM Al and 0 mM Fe, C1: 2 mM Al and 0.25 mM Fe, C2: 6 mM Al and 1.25 mM Fe.

g) were able to increase biomass compared to other treatments without AMF (Table 2). The results of *S. saman* seedling growth on coal post-mining media show that AMF and compost play a very important role, coupled with the provision of lime which helps plant growth to be optimal.

Seedling quality index (SQI)

SQI is one of the parameters used to assess the quality of forest plant seedlings that will be planted into the field. Based on the results of the analysis, the M1A3C0 (0.867), M1A3C1 (0.767) and M1A3C2 (0.837) treatments provide significant differences from the other treatments, and are the treatments with the highest SQI values compared to the other treatments (Table 2). SQI values that are close to one indicate the best value, and the higher the SQI value of a species indicates that the quality of the seedlings is very good. Seedlings that are ready to be planted in the field have an SQI value of at least 0.09 (Lackey and Alm 1982; Sudomo and Santoso 2011). All treatments given were able to produce SQI values > 0.09, this mandates that the treatment given was able to provide a fairly good SQI value

and the *S. saman* species was ready to be planted in the field. In fact, the AMF treatment was able to provide a high SQI value compared to those that were not given AMF (Table 3).

SPAD chlorophyll and photosynthesis rate

Chlorophyll SPAD shows the concentration of chlorophyll contained in the leaves of a plant. The measurement results showed that some treatments showed significant differences in SPAD values (Fig. 2a). The presence of AMF treatment was able to increase the chlorophyll value of SPAD and was very clear in all AMF treatments that had higher values compared to those without AMF (Figure 2a). Treatment M1A0C1 (45.38 µg cm⁻²) gave the highest SPAD value compared to other treatments. Meanwhile, the value of photosynthesis rate was quite fluctuating and varied, and only in some treatments showed significant differences (Fig. 2b). The treatment of AMF was also able to increase the rate of photosynthesis, although not consistently. Treatment M1A0C2 (18.53 µmol CO_2 m⁻²·s⁻¹) gave the highest photosynthesis rate compared to other treatments.

Treatment	SQI	Treatment	SQI
M0A0C0	0.107 ± 0.038 ^{bcd}	M1M0C0	0.280 ± 0.046^{bcd}
M0A0C1	0.147 ± 0.015^{bcd}	M1M0C1	0.337 ± 0.055 ^{bc}
M0A0C2	0.163 ± 0.031^{bcd}	M1M0C2	0.247 ± 0.038^{bcd}
M0A1C0	0.053 ± 0.025 ^d	M1M1C0	0.193 ± 0.0015^{bcd}
M0A1C1	0.057 ± 0.023 ^d	M1M1C1	0.203 ± 0.049^{bcd}
M0A1C2	0.073 ± 0.025^{cd}	M1M1C2	0.147 ± 0.107^{bcd}
M0A2C0	0.050 ± 0.06^{d}	M1A2C0	0.153 ± 0.047^{bcd}
M0A2C1	0.050 ± 0.017 ^d	M1A2C1	0.160 ± 0.060^{bcd}
M0A2C2	0.057 ± 0.015 ^d	M1A2C2	0.253 ± 0.232^{bcd}
M0A3C0	0.233 ± 0.122 ^{bcd}	M1A3C0	0.867 ± 0.571ª
M0A3C1	0.260 ± 0.062^{bcd}	M1A3C1	0.767 ± 0.185ª
M0A3C2	0.237 ± 0.042^{bcd}	M1A3C2	0.837 ± 0.329ª
M0A4C0	0.053 ± 0.035^{d}	M1A4C0	0.370 ± 0.223 ^{bc}
M0A4C1	0.107 ± 0.023 ^{bcd}	M1A4C1	0.217 ± 0.095^{bcd}
M0A4C2	0.057 ± 0.006 ^d	M1A4C2	0.230 ± 0.026^{bcd}
M0A5C0	0.070 ± 0.050^{d}	M1A5C0	0.140 ± 0.061^{bcd}
M0A5C1	0.063 ± 0.040^{d}	M1A5C1	0.100 ± 0.085^{bcd}
M0A5C2	0.073 ± 0.006 ^d	M1A5C2	0.110 ± 0.026 ^{bcd}

Table 3. Seedling quality index (SQI) S. saman in each treatment

Note: Mean \pm standar deviation. M0: without AMF, M1: With AMF, A0: 100% soil + 0% compost + 0% lime + 0% faba, A1: 95% soil + 0% compost + 0% lime + 5% faba, A2: 90% soil + 0% compost + 0% lime + 10% faba, A3: 94. 5% soil + 5% compost + 0.5% lime + 0% faba, A4: 89.5% soil + 5% compost + 0.5% lime + 5% faba, A5: 84.5% soil + 5% compost + 0.5% lime + 10% faba, C0: 0 mM Al and 0 mM Fe, C1: 2 mM Al and 0.25 mM Fe, C2: 6 mM Al and 1.25 mM Fe.

Root colonization

Root colonization is a condition where plant roots have been infected by AMF. The association of AMF with plant roots is the success of AMF inoculation which is characterized by the presence of AMF structures such as hyphae, vesicles, arbuscules and spores that enter the roots. The results of the analysis showed that the treatment of AMF gave significant differences only in some treatments, and the value of root colonization in the treatment of AMF was quite varied (Table 4). The AMF treatment had high colonization criteria based on O'connor et al., (2001) with a value range of 60–100%. Treatment M1A5C2 has the highest root colonization value of 100% (Table 4). Meanwhile, the treatment without AMF had 0% colonization (low).

Nutrient uptake

Nutrient uptake shows how much nutrients are absorbed by plants from the soil or media. The analysis showed that the AMF treatment was able to increase the uptake of nitrogen (N) and phosphorus (P) nutrients (Figure 3a and 3b). This shows that AMF treatment is very effective in increasing the uptake of N and P nutrients in *S. saman* species planted in coal post-mining media. N uptake by *S. saman* reached 1.26–3.61% and P uptake between 0.03–0.23%. Meanwhile, K uptake was quite varied between the presence of AMF treatment and without AMF, K uptake reached 0.58–1.52%. Of the three macro-nutrients analyzed (N, P and K) showed that N uptake was the highest compared to P and K. Fe and A1 uptake were quite varied among the treatments given. Fe uptake in *S. saman* reached 1.25–10.53 ppm with treatment M0A1C2 (10.53 ppm) alue.

Effect of treatment on soil fertility

The provision of soil ameliorant treatments (compost, lime, and faba) can affect soil fertility in coal post-mining media. Soil ameliorant treatments (compost, lime, and faba) are able to increase soil pH from acidic to normal to slightly alkaline (Table 5). The application of 5% and 10% faba was able to increase soil pH to slightly alkaline category 7.99 (5% faba) and 8.08 (10% faba)). Meanwhile, the treatment of 5% compost

Treatment	Colonization (%)	Criteria	Treatment	Colonization (%)	Criteria
M0M0C0	0.00 ± 0.00^{f}	Low	M1A0C0	83.33 ± 11.55 ^{abcd}	High
M0M0C1	0.00 ± 0.00^{f}	Low	M1A0C1	86.67 ± 23.09 ^{abcd}	High
M0M0C2	0.00 ± 0.00^{f}	Low	M1A0C2	93.33 ± 11.55 ^{ab}	High
M0M1C0	0.00 ± 0.00^{f}	Low	M1A1C0	73.33 ± 15.28 ^{bcde}	High
M0M1C1	0.00 ± 0.00^{f}	Low	M1A1C1	73.33 ± 37.86 ^{bcde}	High
M0M1C2	0.00 ± 0.00^{f}	Low	M1A1C2	80.00 ± 17.32 ^{abcde}	High
M0A2C0	0.00 ± 0.00^{f}	Low	M1A2C0	60.00 ± 10.00°	High
M0A2C1	0.00 ± 0.00^{f}	Low	M1A2C1	66.67 ± 15.28 ^{de}	High
M0A2C2	0.00 ± 0.00^{f}	Low	M1A2C2	70.00 ± 20.00 ^{cde}	High
M0A3C0	0.00 ± 0.00^{f}	Low	M1A3C0	73.33 ± 5.77 ^{bcde}	High
M0A3C1	0.00 ± 0.00^{f}	Low	M1A3C1	90.00 ± 17.32 ^{abc}	High
M0A3C2	0.00 ± 0.00^{f}	Low	M1A3C2	83.33 ± 11.55 ^{abcd}	High
M0A4C0	0.00 ± 0.00^{f}	Low	M1A4C0	73.33 ± 5.77 ^{bcde}	High
M0A4C1	0.00 ± 0.00^{f}	Low	M1A4C1	90.00 ± 0.00^{abc}	High
M0A4C2	0.00 ± 0.00^{f}	Low	M1A4C2	73.33 ± 15.28 ^{bcde}	High
M0A5C0	0.00 ± 0.00^{f}	Low	M1A5C0	$80.00 \pm 0.00^{\text{abcde}}$	High
M0A5C1	0.00 ± 0.00^{f}	Low	M1A5C1	96.67 ± 5.77ª	High
M0A5C2	0.00 ± 0.00^{f}	Low	M1A5C2	100 ± 0.00ª	High

Table 4. Root colonzation S. saman seedlings in each treatment

Note: Mean \pm standar deviation. M0: without AMF, M1: With AMF, A0: 100% soil + 0% compost + 0% lime + 0% faba, A1: 95% soil + 0% compost + 0% lime + 5% faba, A2: 90% soil + 0% compost + 0% lime + 10% faba, A3: 94. 5% soil + 5% compost + 0.5% lime + 0% faba, A4: 89.5% soil + 5% compost + 0.5% lime + 5% faba, A5: 84.5% soil + 5% compost + 0.5% lime + 10% faba, C0: 0 mM Al and 0 mM Fe, C1: 2 mM Al and 0.25 mM Fe, C2: 6 mM Al and 1.25 mM Fe.

and 0.5% lime was able to increase soil pH to 7.34 (neutral). This shows that the application of faba is proven to be able to increase soil pH, and can be used as an alternative to lime in improving or increasing soil pH. However, the high pH (slightly alkaline) causes the element P-available in the soil to be unavailable, as evidenced by only the treatment with low pH (without ameliorant application) which has P-available content, even then with a low category (5.00 ppm (M0A0C0) and 1.70 ppm (M1A0C0)). N-total content was low in almost all treatments, only in two treatments which were classified as very low. This shows that the provision of soil ameliorants and AMF has not been able to increase the N-total content. In addition, ameliorants were able to increase K-available although not consistently. Fe content showed a decrease with the provision of soil ameliorants. The treatment without soil amelioration (A0) had a higher Fe content compared to the treatment with soil amelioration. This shows that the application of soil ameliorants is able to reduce the Fe content. The provision of Fe exposure was not able to increase the Fe content in the soil (media). Meanwhile, Al content was only measured in the treatment without ameliant

and AMF (M0A0C0, M0A0C1, and M0A0C2) with the same value of 0.09 cmol⁽⁺⁾/kg. Soil ameliorant, AMF and Al addition treatments did not increase the Al content in the soil (media).

DISCUSSION

The application of AMF combined with ameliorants can support the growth of height and diameter of S. saman seedlings (Fig. 1a and 1b). This shows that the combined application of AMF with other organic materials provides greater benefits than a single application (Zhao and Naeth 2022). The provision of soil ameliorants in the form of compost can enrich the growing medium with nutrients, and the presence of AMF can facilitate the absorption of these nutrients (Anguiby et al., 2020). The results of research by Bekti et al. (2022) reported that Mycosilvi inoculation (AMF inoculum enriched with mycorrhizal helper bacteria (MHBs)) combined with soil ameliorants (compost and lime) was significantly able to increase the growth and survival of F. moluccana plants on former silica sand mining land. The combination of AMF and compost is one of the promising approaches to improve plant



Figure 3. Nutrient uptake of *S. saman* in various treatments, (a) N: nitrogen; (b) P: phospor, (c) K: pottasium; M0: without AMF, M1: With AMF, A0: 100% soil + 0% compost + 0% lime + 0% faba, A1: 95% soil + 0% compost + 0% lime + 5% faba, A2: 90% soil + 0% compost + 0% lime + 10% faba, A3: 94. 5% soil + 5% compost + 0.5% lime + 0% faba, A4: 89.5% soil + 5% compost + 0.5% lime + 5% faba, A5: 84.5% soil + 5% compost + 0.5% lime + 10% faba, C0: 0 mM Al and 0 mM Fe, C1: 2 mM Al and 0.25 mM Fe, C2: 6 mM Al and 1.25 mM Fe

adaptation and tolerance to unfavorable environmental conditions (Soussani et al., 2023), including post-coal mining land. In this case, AMF can stimulate plant growth by increasing nutrient and water uptake (Tang et al., 2023), protecting plants from a wide range of soil pathogens and abiotic stress (drought, salinity, etc.) (Li et al., 2025).

Compost application aims to restore the physical, chemical and biological conditions of the soil which will improve and maintain the fertility of postcoal mining soil (Wiskandar and Ajidirman 2024). Meanwhile, lime application aims to increase and neutralize soil pH (Mahmud and Chong 2022). Soil improvement using lime and compost is needed to improve soil fertility and accelerate the revegetation process (Iskandar et al., 2022a). Compost can increase soil organic matter content and improve soil quality (Miller et al., 2019). The application of compost and lime without AMF (M0A3C0, M0A3C1 and M0A3C2) also provided quite good growth compared to the combination of AMF, although it did not provide the best growth (Figures 1a, 1b and Table 2). In fact, the biomass of *S. saman* seedlings with M0A3C0, M0A3C1 and M0A3C2 treatments was the highest compared to other treatments without AMF (Table 2). The results of research by

Treatment	рН	N-total (%)	P-availlabel (ppm)	K (ppm)	AI (cmol ⁽⁺⁾ /kg)	Fe (ppm)
M0A0C0	5.36 a	0.10	5.00 I	21.60 I	0.09 vl	113.20 vh
M0A0C1	5.33 a	0.13	2.80 vl	58.60 h	0.09 vl	112.90 vh
M0A0C2	5.30 a	0.11	2.60 vl	53.50 h	0.09 vl	110.60 vh
M0A1C0	7.99 sa	0.08	-	26.80 m	nm	46.10 vh
M0A1C1	8.35 sa	0.11	-	60.10 h	nm	45.00 h
M0A1C2	8.34 sa	0.16 I	-	61.00 h	nm	39.20 h
M0A2C0	8.08 sa	0.07	-	48.10 h	nm	42.90 h
M0A2C1	7.89 sa	0.11	-	16.00 I	nm	49.70 h
M0A2C2	7.93 sa	0.08	-	26.80 m	nm	38.60 h
M0A3C0	7.34 n	0.11	-	38.10 m	nm	48.70 h
M0A3C1	7.35 n	0.13	-	37.60 m	nm	46.20 h
M0A3C2	7.16 n	0.13	-	58.90 h	nm	50.80 h
M0A4C0	8.11 sa	0.10	-	58.70 h	nm	56.80 vh
M0A4C1	7.99 sa	0.13	-	48.10 h	nm	48.90 h
M0A4C2	8.13 sa	0.14	-	72.20 vh	nm	43.50 h
M0A5C0	8.28 sa	0.14	-	48.30 h	nm	49.40 h
M0A5C1	8.32 sa	0.07	-	80.60 vh	nm	51.40 h
M0A5C2	8.3 sa	0.08	-	134.30 vh	nm	45.60 h
M1A0C0	5.57 a	0.10	1.70 vl	12.60 I	nm	112.40 vh
M1A0C1	5.55 a	0.08	2.40 vl	13.80 I	nm	130.10 vh
M1A0C2	5.47 a	0.081	2.20 vl	12.80 I	nm	136.00 vh
M1A1C0	7.70 sa	0.07	-	187.10 vh	nm	56.20 vh
M1A1C1	7.88 sa	0.18	-	58.90 h	nm	54.80 vh
M1A1C2	7.91 sa	0.14	-	17.10	nm	54.00 vh
M1A2C0	8.20 sa	0.06 vl	-	12.80 I	nm	48.50 h
M1A2C1	8.27 sa	0.10	-	23.40 m	nm	50.80 h
M1A2C2	8.24 sa	0.10	-	37.30 m	nm	49.40 h
M1A3C0	7.22 n	0.11	-	48.30 h	nm	57.10 vh
M1A3C1	7.37 n	0.11	-	13.90 I	nm	60.30 vh
M1A3C2	7.21 n	0.10	-	18.20	nm	54.60 vh
M1A4C0	8.01 s	0.17	-	37.60 m	nm	48.50 h
M1A4C1	8.12 sa	0.06 vl	-	37.50 m	nm	52.20 vh
M1A4C2	8.09 sa	0.10	-	26.90 m	nm	55.00 vh
M1A5C0	8.18 sa	0.07	-	58.80 h	nm	56.80 vh
M1A5C1	8.32 sa	0.10 I	-	48.70 h	nm	55.70 vh
M1A5C2	8.32 sa	0.08 vl	-	58.60 h	nm	62.20 vh

Table 5. The content of some elements in the planting media after treatment

Note: sa: slightly alkaline, a: acid, n: neutral; l: low; vl: very lowly, h: highly, m: moderately; vr: very highly; N: nitrogen; nm: not measured; P: phospor, K: pottasium; Al: aluminum, Fe: iron; M0: without AMF, M1: With AMF, A0: 100% soil + 0% compost + 0% lime + 0% faba, A1: 95% soil + 0% compost + 0% lime + 5% faba, A2: 90% soil + 0% compost + 0% lime + 10% faba, A3: 94. 5% soil + 5% compost + 0.5% lime + 0% faba, A4: 89.5% soil + 5% compost + 0.5% lime + 5% faba, A5: 84.5% soil + 5% compost + 0.5% lime + 10% faba, C0: 0 mM Al and 0 mM Fe, C1: 2 mM Al and 0.25 mM Fe, C2: 6 /mM Al and 1.25 mM Fe.

Spargo and Doley (2016) reported that the application of compost as much as $100 \text{ t} \cdot \text{ha}^{-1}$ applied to the top layer of coal post-mining soil was able to significantly increase the biomass of grassland species. The addition of Al and Fe exposure in combination with AMF and ameliorant materials also supports the increased growth of *S. saman* seedlings, although the effect is not as great as that of AMF and soil ameliorants. Fe is an essential micronutrient that plants need in low amounts or concentrations for plant growth and development (Salim et al., 2021b; Zhang et al., 2023). Fe plays an important role in plant physiological processes, such as photosynthesis, respiration, protein synthesis and nitrogen metabolism (Liang 2022; Mahawar et al., 2022). Meanwhile, Al although not an essential element, but in low concentrations can be beneficial to plant growth (Salim et al., 2021c; Ofoe et al., 2023). Al in the soil plays a role in increasing P availability, reducing the toxicity of H⁺, Fe and Mn, and activating genes related to abiotic stress tolerance (Muhammad et al., 2019).

The high biomass in the AMF treatment indicates that S. saman used in this study is highly dependent on mycorrhiza (Anguiby et al., 2020). AMF can increase the accumulation of aboveground biomass more than 3 times compared to non-mycorrhizal plants (Basyal and Walker 2023), and increase root and shoot biomass of Commiphora myrrha seedlings under drought conditions (Birhane et al., 2023). This is thought to be because AMF is able to access additional photosynthate from the host plant, even under stress conditions (He et al., 2021). AMF inoculation was able to increase the biomass of S. bicolor seedlings compared to the control (Wulandari et al., 2024) and significantly increased the biomass of maize plants grown on clay-associated mine soil (Song et al., 2020). Sun et al., (2021) also reported that inoculation of AMF type Funneliformis mosseae was able to increase the dry weight of corn plants compared with no AMF application.

The treatment of FABA is less able to support the growth of S. saman seedlings. The high level of FABA is a factor that causes non-optimal growth of S. saman. The amount and dose of FABA given will have a negative impact because it increases micronutrients, while micronutrients are needed by plants in low conditions (Padhy et al., 2016). This shows that the provision of FABA amendments must be given in the right amount, so that later it will have a positive influence on the physicochemical and biological properties of the soil, and later can support optimal plant growth (Taupedi and Ultra 2022). The results of research by Ahmad et al. (2021) reported that the application of 10-30% fly ash was able to increase the growth and yield of Cucurbita moschata plants, while the application of 40-50% fly ash was able to have a negative effect on the growth and yield of pumpkin plants. The results of Yu et al. (2019) also

reported that the application of fly ash or FABA less than 25% was able to increase growth and yield, and was able to avoid high meta(loid) accumulation. In addition, the results of research by Putri et al., (2023) reported that FABA application did not increase heavy metal content in vegetables.

The seedling quality index (SQI) describes the quality of seedlings assessed based on morphological attributes, such as height, diameter and dry period, in the nursery and in the field (Gallegos-Cedillo et al., 2021; Guimarães et al., 2024). SQI has been widely used to evaluate forestry crops before planting in the field (Yücedağ et al., 2019). The results showed that the SQI value in all treatments given reached > 0.09. Higher SQI values indicate vigorous plants with an optimal balance between shoot and root biomass, and result in increased vigor and high field performance (Lin et al., 2019). S. saman species meet the criteria for field planting, as seedlings that are ready to be planted in the field have an SQI value of at least 0.09 (Lackey and Alm 1982; Sudomo and Santoso 2011). The combination treatment of AMF and compost was able to increase the SQI value (Table 3). This is in line with the best growth results produced in the combination treatment between AMF and compost, and has an impact on improving the quality of seedlings. Prayudianingsih and Sari (2016) reported that the combination of AMF and compost was able to produce SQI values > 0.09, indicating that both treatments were able to improve the quality of teak seedlings. AMF combined with compost can produce quality seedlings quickly (Anguiby et al., 2020).

The AMF treatment was able to increase the chlorophyll value of SPAD (Figure 2a). The increase in chlorophyll content is thought to be due to more optimal magnesium (Mg) uptake in AMFinoculated plants compared to those not inoculated (Mathur et al., 2018). In addition, the increase in chlorophyll content in the AMF treatment is thought to be due to an increase in N absorption by S. saman seedlings (Table 5), where N is one of the main molecules of chlorophyll (Mathur et al., 2019). AMF is able to produce hormones, one of which is cycloninin, which plays a role in increasing chlorophyll development and increasing photosynthesis (Fan et al., 2024). The inoculation of AMF can have a significant effect on chlorophyll content (SPAD value) in pea leaves (Parihar et al., 2020). Research by Peng et al., (2024) also reported that AMF inoculation showed an increase in chlorophyll content in cotton plants under saline-alkali stress conditions. Research by Vallejos-Terros et al., (2023) also reported that the application of compost and AMF was able to increase the growth and chlorophyll content of *Oryza sativa*.

The AMF treatment was also able to increase the photosynthesis rate of S. saman seedlings although not consistently (Figure 2b). The association of AMF with plant roots can regulate the ability and photosynthetic efficiency of host plants (Wahab et al., 2023). AMF is able to increase the levels of essential elements for chlorophyll synthesis, which in turn supports photosynthetic efficiency (Ma et al., 2022). This AMF phenomenon is thought to be related to the presence of proline in cells that play a role in facilitating chlorophyll synthesis (Peng et al., 2024). The results of research by Mekkaoui et al., (2024) reported that the application of AMF and compost has a positive effect on photosynthetic pigments, and the presence of compost can play a beneficial role in increasing photosynthetic efficiency. This indicates that the treatment of AMF either singly or combined with compost ameliorants or vice versa is able to improve the physiological state with the plants tested in the coal post-mining media. In addition, Anli et al., (2020) hypothesized that the combination of AMF treatment and organic amendments can prevent abiotic stress that can disrupt the photosynthesis process by increasing chlorophyll pigment synthesis. The results of Alotaibi et al., (2021) reported that AMF was able to increase photosynthesis of lotus plants by 18% under Al exposure conditions, and Peng et al. (2024) reported that AMF inoculation in cotton plants was able to increase photosynthetic rates and overall growth under saline-alkali stress levels.

The association of AMF with plant roots is the success of the AMF inoculation process which is marked by the colonization of roots by AMF. Root colonization by AMF is characterized by the presence of AMF structures such as hyphae, vesicles, arbuscules and spores that enter the roots. The research by Ma et al. (2022) also reported that AMF colonization status in Gleditsia sinensis plants was indicated by the presence of hyphae, vesicles, spores and arbuscules. The benefits of AMF for these plants are highly dependent on the level of root colonization or the number of extra-radical hyphae produced in the soil (Basyal and Walker, 2023). The inoculation of AMF can increase the rate of mycorrhizal colonization of plants and increase the density of mycelium around the rhizosphere (Zhang et al., 2020). This is in accordance with this study, where the AMF treatment was able to colonize the roots of S. saman seedlings with values and categories from medium to high (10-100%). The AMF treatment gave significant differences only in some treatments in all types tested (Table 4). The level of root colonization by AMF is influenced by several factors, such as the environment and the host plant (Owiny and Dusengemungu 2024). This also caused some differences between root colonization in each treatment. AMF is able to maintain colonization levels despite unfavorable growing media conditions (Basyal and Walker 2023). This can be seen from several treatments such as M1A5C0, M1A5C1 and M1A5C2 which produced less optimal growth compared to other AMF treatments, but had high colonization values. The application of compost and FABA in some treatments did not support the optimal root colonization by AMF (Table 4). This is thought to be due to byproducts of organic decomposition from compost containing several substances that can cause root colonization by AMF (Mekkaoui et al., 2024).

The AMF treatment was able to increase the absorption of N and P elements (Figure 3a and 3b). This can be seen in the treatment with AMF which shows greater N and P uptake than without AMF (Figure 3a and 3b). This condition is one of the supporting factors for the growth of S. saman seedlings to be more optimal than without AMF. Almost 50% of Nitrogen (N) and 90% of phosphorus (P) transport from soil to host plants is assisted by mycorrhizal associations (Smith and Smith 2011; Nouri et al., 2015). Meanwhile, the absorption of K varied significantly between those treated with and without AMF. The results of research by Ma et al., (2022) reported that AMF inoculation was able to regulate the absorption of nitorgen and phosphorus in the roots and improve the nutritional status of plants. AMF is able to produce the enzyme posfatase which can increase the transformation of insoluble phosphorus in the soil into available for plants (Anguiby et al., 2020).

The improvement of physicochemical characteristics can be done by applying soil ameliorants such as lime, phosphate rock and compost to improve soil fertility and revegetation success quickly (Iskandar et al., 2022b). The results showed that the provision of ameliorants and AMF was able to renew some nutrient content in the media soil (coal post-mining soil) (Table 5). The provision of soil ameliorants in the form of FABA was able to increase soil pH from acidic to slightly alkaline, while the provision of lime and compost increased soil pH from acidic to normal and the provision of AMF showed no change in pH category (still classified as acidic). The research by Du et al., (2020) reported that fly ash can cause certain changes in the physical and chemical properties of the substrate. FABA is one of the best soil improvers and can significantly improve soil physico-chemical properties, especially soil pH (Nugraha et al., 2024). Taupedi and Ultra (2022) also reported that 5%, 10% and 15% fly ash application was able to increase the pH to 8.23, 8.4, and 8.17 respectively after 8 weeks of application. The increase in soil pH after FABA application is thought to be due to the rapid release of Ca, Na, Al and OH ions from Faba (Wong and Wong 1990; Pandey and Singh 2010). FABA has the potential to replace lime in increasing soil pH in agriculture which in turn results in a net CO₂ reduction and thus reduces the effects of global warming (Jambhulkar et al., 2018).

N-total content is low in almost all treatments given, only three treatments have a very low category, while for P-available is only detected in the treatment without ameliorants, and with low to very low categories. Similar results were reported by Tuheteru at al. (2023) that the soil with a pH classified as alkaline (slightly alkaline) contains low total N and P₂O₅. The provision of FABA was not able to increase N in the media. This is because FABA does not contain humus and N caused by the oxidation of C and N during coal combustion (Yao et al., 2015). The results of research by Priyadi et al., (2023) also reported that the application of fly ash did not show a significant N increase. In addition, the P-availability that was only detected in the treatment without ameliorant material was due to the soil pH in the treatment with ameliorant application was quite high (normal to slightly alkaline category). In soils with alkaline pH as in the results of the study, phosphorus is thought to be mostly fixed by calcium (Penn and Camberato, 2019; Hartemink and Barrow, 2023). When the pH is high (> 8), it causes limitations on P uptake because it is bound by other minerals and thus unavailable to plants or microbes (Barrow et al., 2020).

Potassium (K) content in various treatments was classified as low to very high (Figure 3c). The treatment of faba and lime can increase the K content in the media. Lime is a rich source of potassium (K) and is widely used to increase soil pH (Yang et al., 2020; Gondal et al., 2021). The results of research by Privadi et al., (2023) reported that the application of fly ash was able to increase the total K content from 16.19 to 25.75 mg/100 g. Fly ash contains high K content so it has a high potential to supply K for plants (Wang et al., 2008). FABA contains macro nutrients, one of which is K and micro nutrients, one of which is Fe, which plays a role in supporting plant growth (Jarosz-Kremińska and Poluszyńska, 2020). This also has an impact on the Fe content classified as high to very high in the treatment given. The results of research by Kumari et al., (2013) reported that the application of fly ash 25-100% was able to increase the Fe content in the media compared to the control. Tuheteru et al., (2023) also reported that soil with a slightly alkaline pH contained higher Fe. Meanwhile, Al content was only measured in the control treatment and without the application of AMF and soil ameliorants, with a very low category. This is due to the alkaline nature of FABA, so, Al is only available in the control treatment (acidic media pH) (Table 5). When alkalinity increases, Al ions will precipitate from aqueous solutions at pH levels of 6 to 8, while other ions can precipitate at higher pH values (Nfissi et al., 2017), making Al unavailable. The treatment of Fe and Al solution also has no effect on the content of Al and Fe in the media. This is probably because the concentration of both elements is not too high.

CONCLUSIONS

The application of soil ameliorants, especially compost and lime combined with AMF and the addition of Al and Fe exposure, had a positive effect on the growth of S. saman seedlings. The combination of compost, lime and AMF ameliorants, as well as exposure to Al and Fe is also able to improve the quality of S. saman seedlings as indicated by the high SQI value of the combination treatment. The combination of AMF and ameliorants was also able to increase the chlorophyll content of SPAD, while in the photosynthesis rate parameter the increase was only in some treatments. The treatment of AMF has a high root colonization value (60-100%). The treatment of AMF also had a positive impact on nutrient absorption, especially N and P. The application of FABA ameliorants was able to increase soil pH from acidic to slightly alkaline, while the application of lime and compost increased soil pH from

acidic to normal. FABA can be an alternative material used in increasing soil pH, but with the appropriate amount and dose, so that later it will have a positive impact on plant growth. The combination of compost and AMF ameliorants can be an alternative in supporting the success of revegetation activities of coal post-mining land, and the use of lime can be replaced with FABA, but with the right amount and dose, by implementing fast-growing species, one of which is *S. saman*.

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