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ORIGINAL PAPER

Role of *arbuscular mycorrhizal fungi* and *Pongamia pinnata* for revegetation of tropical open-pit coal mining soils

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Abstract
Open-pit coal mining activities may cause forest and environmental degradation. Thus, forests need to be reclaimed and revegetated after coal mining. This study aimed to determine the combined effects of *arbuscular mycorrhizal fungi* (AMF) inoculation on the revegetation of postcoal-mining lands with *Pongamia pinnata*. This completely randomized study was conducted for 6 months in a greenhouse. The first factor consisted of four different levels based on soil medium type: forest soil, mined-out soil, overburdened soil, and landfill soil. The second factor consisted of three levels based on three different dosages of AMF: control, 2 g of AMF, and 4 g of AMF. Open-pit coal mining activities in East Kalimantan caused serious land degradation in tropical ecosystem. Revegetation with *P. pinnata* accelerated land reclamation by passing the land preparation stage and decreased the costs of land preparation. Forest soil was the optimal medium for the growth of *P. pinnata* seedlings. However, when the seedlings were planted in degraded soil of mining, their average height, diameter, and total biomass decreased drastically. The inoculation of 2 g AMF colonized the root and therefore improved growth of seedling. This result may reduce cost of chemical fertilizer. AMF inoculation improved Fe absorption by 11.7% that was higher than that under control, whereas 90.4% of the assimilated Fe was retained in plant roots. Revegetation by exotic fast-growing pioneer legume species of *P. pinnata* and application of AMF drastically improved some chemical soil properties that suitable for rehabilitation program in tropical post-mining areas.

Keywords Bioremediation · Exotic fast-growing species · Open-pit coal mining · Reclamation · Tropical ecosystem

Introduction

The overexploitation of the tropical forest ecosystem has caused severe land degradation and damage (Agus et al. 2004, 2011, 2016, 2017; Agus 2013, 2018). In Indonesia, extensive open-pit coal mining has exposed the environment and humans to heavy metals that were previously buried. Thus, open-pit coal mining has become a major cause of land degradation and severe local–global environmental damages. In 2015, the thermal coal industry in Indonesia contributed 36% to the international market share. In the same year, Indonesia's coal production reached 392 million tons, 81.6% of which (approximately 319.87 million tons) was exported to India, China, Japan, and South Korea. Meanwhile, domestic coal consumption reached 18.4% (72.13 million tons) and increased by 9.38% over the period of January 2015 to September 2015 (Anonymous 2015). Coal is expected to become one of the major energy sources that will be exploited to meet the high energy consumption of Indonesia over the long term. The increased use of coal, in turn, will potentially intensify coal mining activities in Indonesia.

Open-pit mining activities in East Kalimantan are predicted to cause serious long-term environmental damage. Anonymous (2015) stated that 7.2 million ha of land in East Kalimantan has been allocated to mining activities. This

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acreage is equivalent to a quarter of the total area of the whole province. Extensive mining activities in East Kalimantan will drastically degrade the forests in the areas. The most significant damage caused by open-pit mining operations is acid mine drainage (AMD), which results from the oxidation of sulfuric minerals. AMD causes the acidification of the soil, the imbalance of soil nutrients, and the accumulation of microelement solutions that contain phytotoxic metal elements (Havlin et al. 1999).

The productivity of former mining lands may be improved through revegetation with the appropriate plant species (Katoria et al. 2013; Cahyanti and Agus 2017). The cultivation of fast-growing pioneer legumes increases the soil N supply by as much as 9–27 times and produces humus layers that accelerate the carbon cycle (Agus et al. 2003, 2004; Agus 2018). *Pongamia pinnata* is a leguminous member of the *Papilionoideae* family. *P. pinnata* originated from India and is found naturally from Pakistan, India, and Sri Lanka as well as throughout Southeast Asia including Indonesia to northeastern Australia, Fiji, and Japan, as well as scattered in Egypt and the USA (Orwa et al. 2009). This plant can grow at an altitude of 0–1200 m AMSL, can withstand temperatures above 50 °C, and can tolerate acid to alkaline soil (Bohre et al. 2014). *P. pinnata* is used as a pioneer vegetation in environmental restoration (Datar et al. 2011), and their seed oil can be processed to produce biofuel (Kazakoff et al. 2010; Scott et al. 2008).

Pongamia pinnata is associated with *Rhizobium* bacteria, which help retain the nitrogen necessary for plant growth and biomass production (Bohre et al. 2014). *P. pinnata* may also associate symbiotically with AMF. This association may produce higher numbers of productive spores and colonies when associated with *P. pinnata* than when associated with other plant species, such as *Acacia catechu, Acacia nilotica, Acacia indica,* and *Leucaena leucocephala* (Kumar et al. 2010; Venkatesh et al. 2009). The use of AMF is crucial in the reclamation of former mining areas because the soil has been severely deprived of nitrogen and phosphorus (Bucking and Shachar-Hill 2005). The revegetation of postcoal-mining sites with AMF can improve the absorption of soil nutrients, particularly phosphorus, support the growth of plants, and enhance the productivity of the sites (Suhardi et al. 2006). Therefore, initial research must be conducted on *P. pinnata* plants at the nursery stage to improve the current understanding of the growth and AMF interaction of this plant in the reclamation of abandoned coal mining sites.

Materials and methods

This research was conducted in the greenhouse of the Faculty of Forestry, Universitas Gadjah Mada, over the period of August 2015 to April 2016. Soil analysis was performed at the SEAMEO BIOTROP Laboratory, Bogor. This research applied a completely randomized design with two factors, namely the type of soil media and the dosages of *Glomus clarum,* a type of *arbuscular mycorrhizal fungi* (AMF). The soil media used for plant growth were divided into four levels on the basis of the types of former coal mine areas or sites from which the soil media were obtained: forest soil (M1), mined-out soil (M2), overburdened soil (M3), and ready-for-revegetation landfill soil (M4). AMF inoculum approximately contained 66 spores/gram and applied into three levels of dosages: (1) no inoculation or control (E0), (2) inoculation at the rate of 2 g/plant or with approximately 132 spores (E1), and (3) inoculation at the rate of 4 g/plant or with approximately 264 spores (E2).

Soil media were sterilized through the full sun drying or solarization method by turned over the soil every day for 5 days. Wild *P. pinnata* seedlings were transported from Banyuwangi after adaptation to ordinary soil for 2 months. This adaptation process was carried out until the seedlings had 2 new leaves at an average height of 15.47 ± 2.58 cm and diameter of 3.70 ± 0.63 mm. AMF of *G. clarum* was collected from coal mining areas in Berau Coal Company, Berau District, East Kalimantan, Indonesia, and used as an inoculant. The inoculant of AMF was propagated in sterilized zeolite media with sorghum as the surrogate plant for 3 months in the greenhouse of SEAMEO BIOTROP Bogor (Wulandari et al. 2014). AMF inoculation in the nursery was performed during the weaning period of the *P. pinnata* seedlings. Each *P. pinnata* seedling was grown on each polybag (18 cm × 20 cm) that filled with soil, and placed in a green house. Water was applied everyday and seedlings were allowed to grow at room temperature of 28–36 °C, relative humidity 55–80%, and relative light intensity 19.64% under greenhouse condition. Each polybag was perforated with a 10-cm hole with a diameter of 2 cm; AMF inoculum was inserted through the hole to create direct contact with the roots of the plant as proposed by Schenck (1982) and Kurniaty and Damayanti (2011). *P. pinnata* seedlings without inoculation of AMF was prepared as control. Soil nutrients and plant tissues taken from the roots and from the top of the plants were analyzed before and after treatment. Plant growth for 6 months was observed on the basis of the percentage of surviving seedlings; the height, diameter, and biomass volume of the seedlings; top–root ratio (T/R); and the quality index of the seedlings. The chlorophyll A and B content was measured by Wintermans and De Mots method (Nio and Banyo 2011) with Konica Minolta SPAD 502 Plus Chlorophyll Meter at a wavelength of 645 nm and 663 nm, from 3 leaf samples taken from the bottom, middle, and top of each sample seedling after extracted by acetone. AMF infection rate was assessed through Giovanneti and Mosse's slide method (Schenck 1982). Research data were analyzed through the analysis of variance method, followed by the

F-test with alpha 0.05 and Duncan's test by using SAS program version 9.0.

Results and discussion

The texture of the soil samples can be categorized as clayey with a sand content of 24.7–37.3%, silt content of 28.8–36.5%, and clay content of 32.4–39.4%. The composition of the soil samples used in this study is consistent with that of the soil sample in the study performed by Agus et al. (2016) in the same region.

The pH of H_2O of forest soil had a value of 5.1, whereas that of the soil from abandoned coal mines fell in the range of 2.4–3.8 that can be categorized as very acid (Table 1). The pH value of KCl indicates a more potentially acidic pH level until 2.3 to 3.4. Mined-out soil was the most acidic soil, and its acidity ranged from very acid to slightly acid (Djaenudin et al. 2003; Agus et al. 2016). The acidity of soils collected from mining sites may have resulted from coal, which mostly contains sulfate. The reaction of coal with rainwater could produce Fe, Al, and several other oxidized metals that cause high acidity (Agus et al. 2016).

The organic C level of forest soil was 1.88% (low), whereas that of former mine soil fell in the range of 0.42–2.2% (very low to medium). Agus et al. (2016) stated that the top soil layers of former open-pit coal mining sites are heterogeneous. Mined-out soil showed a higher organic C content than forest soil because the former may still contain high coal content. Nufus (2016) asserted that the high level of organic C of soils collected from former coal mine sites indicates a high degree of soil fertility because mined-out soil collected from undeveloped coal mines may contain a high level of organic C with the highest C/N ratio of 18 (high category).

The total N content of forest soil was 0.22% and was categorized as moderate, whereas that of mined-out soil was categorized as low and ranged from 0.11 to 0.12%. Labile organic substances have a high N content but low C/N ratio, whereas recalcitrant organic substances have a low N content but a high C/N ratio (Havlin et al. 1999). Substances with excessively high C/N ratios deprive microbes of N, which is necessary for protein synthesis. Thus, N deprivation will decelerate decomposition (Havlin et al. 1999).

The total Fe concentration was approximately 0.8–3.91%, which was considered as very high. Under high Fe concentrations, phosphate will complex with Fe minerals and form an insoluble compound that decreases the amount of plant-available phosphate (Havlin et al. 1999). Although the Al content of forest soil was low (0.1 me/100 g), that of mined-out soil was excessively high and

Table 1 Soil physical and chemical properties of forest and postcoal-mining soils

No.	Parameter	Unit	Soil media							
			Forest soil (M1)		Mined-out soil (M2)		Overburdened soil (M3)		Landfill soil (M4)	
			Value	Criteria[a]	Value	Criteria[a]	Value	Criteria[a]	Value	Criteria[a]
1	pH H_2O		5.1	Acid	2.4	Very acid	2.5	Very acid	3.8	Very acid
2	pH KCL		4.1	Neutral	2.3	Very acid	2.5	Acid	3.4	Acid
3	C-organic	%	1.88	Low	2.2	Medium	1.62	Low	0.42	Very low
4	N-total	%	0.22	Medium	0.12	Low	0.12	Low	0.11	Low
5	C/N ratio		9	Low	18	High	14	Medium	4	Very low
6	P_2O_5	ppm	10.4	Medium	6.6	Low	9.9	Medium	1.9	Very low
7	Ca	cmol/kg	4.27	Low	0.93	Very Low	1.76	Very low	4.58	Low
8	Mg	cmol/kg	3.95	High	0.62	Low	8.37	Very high	7.43	High
9	Na	cmol/kg	5.84	Very high	2.08	Very high	2.21	Very high	2.46	Very high
9	K	cmol/kg	1.8	Very high	0.51	Medium	0.47	Medium	0.88	High
10	CEC	cmol/kg	12.89	Low	19.9	Medium	18.24	Medium	10.1	Low
11	BS	%	100	Very high	20.8	Low	70.19	Very high	100	Very high
12	Al^{3+} H_{dd}	me/100 g	0.1	Low	6.61	Very high	29.28	Very high	4.5	Very high
13	Texture									
	Sand	%	28.6	Clay	37.3	Clay	35.9	Clay	24.7	Clay
	Silt	%	32		30.3		28.8		36.5	
	Clay	%	39.4		32.4		35.3		38.8	
14	Fe-total	%	2.88	Very high	0.8	Very high	3.39	Very high	3.91	Very high
15	S-total	ppm	42	Medium	1980	Very high	6190	Very high	573	Very high

[a] Soil Research Center Indonesia (2009)

ranged from 4.5 to 29.29 me/100 g. Al and Fe intoxication leads to abnormal plant growth or stunting (Taylor et al. 2010).

The total S concentration of forest soil was moderate at 42 ppm, whereas that of mined-out soil was high and ranged from 573 to 61.897 ppm. Coal consists of carbon, hydrogen, oxygen, nitrogen, sulfur, and other mineral compounds (Katoria et al. 2013); overburdened soil exhibited the highest sulfur content in this research (Table 1). Sulfur will bind with metals to form sulfuric complexes that may oxidize when exposed to open air and may produce acidic water when leached out by surface water or groundwater. The passage of acidic water through matter will corrode metallic components and produce dangerous toxic substances that may pollute the environment (Katoria et al. 2013).

AMF can infect *P. pinnata* seedlings through root vesicles and arbuscules. However, in this study, root arbuscules were not easily identified. This result is similar to that reported by Wulandari et al. (2014) who found more vesicles than arbuscules. Soil media and AMF dosage exhibit a significant interaction that affected the AMF infection rate of *P. pinnata* roots.

The soil media significantly influenced the infection rate of AMF (Table 2). Forest soil produced the best result with an average infection rate of 50.7%, followed by landfill soil with 34.4%, mined-out soil with 25.6%, and overburdened soil with 18.5%. Forest soil had the highest infection rate because it has not yet been affected by coal mining activity. Thus, its physical and chemical traits have remained undisturbed. The infection rate of AMF in mined-out soil was lower because it had been contaminated by heavy metal substances. Contamination by heavy metals decreases soil pH to extremely acidic levels and provides a non-ideal environment for the growth of microorganisms, including AMF (Suhardi et al. 2006). The lowest infection rate was found in overburdened soil because of its high sulfur content of 61.897 ppm, which is 1.474 times higher than the sulfur content of forest soil which is only 42 ppm (Table 1). Nevertheless, AMF can survive, adapt, and infect *P. pinnata* roots in the extreme environmental conditions provided by coal mine soil (Wulandari et al. 2014).

The lone factor of AMF dosage significantly influenced the infection rate of roots. The average infection rate under control was 0%; that under 2 g of AMF was 50%; and that under 4 g of AMF was 46.94%. No AMF was found in these media because no AMF inoculant was present in marginal soil. Alternatively, soil sterilization may have resulted in the absence of microorganisms that could infect *P. pinnata* roots (Nufus 2016). By inoculating mined-out soil with AMF, the infection rate of the roots increased. The best combination treatment was M1E1, which yielded an average infection rate of 78.9% (very high). By contrast, the treatment combination of M3E2 only yielded an average infection rate of 21.1% (low).

Table 2 Duncan analysis of AMF infection in *P. pinnata* roots

Treatment	Percentage of AMF infection (%)[a]	Criteria[b]
Soil media		
M1	50.74^{a}	High
M2	25.56^{bc}	Medium
M3	18.52^{c}	Low
M4	34.44^{b}	Medium
AMF		
E0	0.00^{b}	Very low
E1	50.00^{a}	Medium
E2	46.94^{a}	Medium
Soil media × AMF		
M1E0	0.00^{f}	Very low
M1E1	78.89^{a}	Very high
M1E2	73.33^{ab}	High
M2E0	0.00^{f}	Very low
M2E1	30.00^{de}	Medium
M2E2	46.67^{cd}	Medium
M3E0	0.00^{f}	Very low
M3E1	34.44^{de}	Medium
M3E2	21.11^{e}	Low
M4E0	0.00^{f}	Very low
M4E1	56.67^{bc}	High
M4E2	46.67^{cd}	Medium

[a] Numbers followed by the same letter in the same column are not significantly different at the test level of 5%

[b] Based on criteria of effectiveness of degree of infection of AMF (Schenck 1982)

The *P. pinnata* seedlings exhibited 100% survival rate under all treatments. Similarly, Casuarina (2014) reported that the application of AMF *G. agregatum* in a *P. pinnata* nursery yielded a 100% survival rate. This result shows that *P. pinnata* seedlings could adapt and grow optimally as a pioneer plant in marginal soil (Casuarina 2014) and thus proves that revegetation with *P. pinnata* seedlings is an appropriate step in rehabilitating former coal mine lands.

The lone factor of soil media had a significant influence on plant height, diameter, total biomass, canopy biomass, T/R ratio, and leaf chlorophyll content. The lone factor of AMF dosage and the combination of AMF dosage and soil media did not exert significant effects on all growth parameters (Table 3). Forest soil produced significantly different results from with other types of soil taken from abandoned coal mine sites (Fig. 1a). The height of *P. pinnata* seedlings grown on mined-out soil was significantly different from that of seedlings grown on overburdened and landfill soil. The best average height was exhibited by seedlings grown on forest soil (43.6 cm), followed by that of seedlings grown on

Table 3 Analysis of variance of all growth variables of *Pongamia pinnata* seedlings

Source of variant	*DF*	*Pr>F*						
		Height	Diameter	Biomass total	Biomass aboveground	Biomass root	T/R ratio	Chlorophyll
Soil media	3	<0.0001*	0.0016*	0.0502*	0.027*	0.2653	0.0056*	<0.0001*
AMF dosage	2	0.0613	0.0721	0.7036	0.5912	0.8792	0.2074	0.3689
Media × AMF	6	0.7618	0.9336	0.8861	0.8427	0.8902	0.3462	0.9284

Remarks: *Significant at 5% probability level

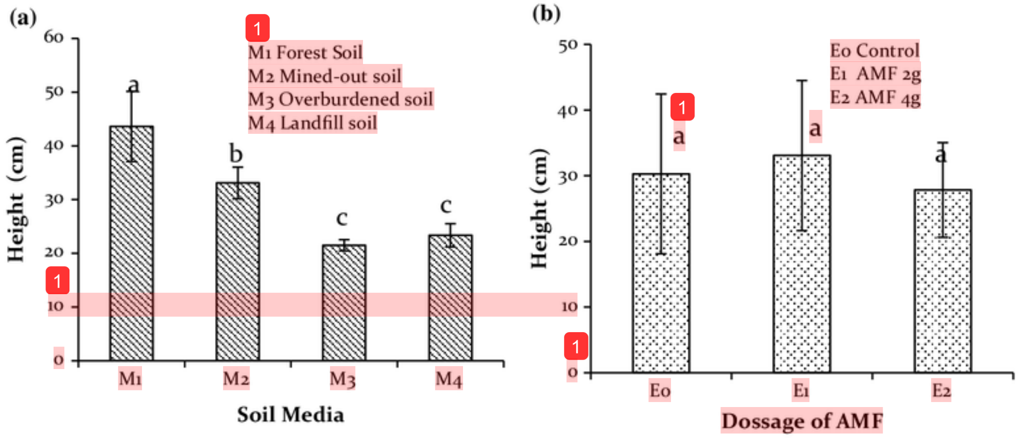

Fig. 1 Effect of soil media (**a**) and AMF dosage (**b**) on the average height of *P. pinnata* seedlings. Values followed by the same letter in the same diagram are not significantly different at the 5% level of significance

mined-out soil (33.1 cm), that of seedlings grown on landfill soil (23.4 cm), and that of seedlings grown on overburdened soil (21.5 cm) (Fig. 1a). The higher level of soil fertility in forest soil, especially soil pH, CEC, N, P, K, Ca, and Mg, and lower levels of Fe and S toxicity (Table 1) have led to better growth of *P. pinnata* seedling than those grown on post-soil media. Hall et al. (2003) reported that on naturally occurring forest soils, *Entandrophragma cylindricum* and *E. utile* exhibited increased relative growth rate (RGR) and decreased root mass ratio (RMR) with an increase in soil fertility while *E. angolense* and *E. candollei* did not.

The growth rate of *P. pinnata* seedlings grown on mined-out soil was better than that of seedlings grown on landfill soil and that of seedlings planted in mined-out soil closely matched that of seedlings planted in forest soil. These results may be attributed to the characteristics of mined-out soil. Specifically, although mined-out soil was collected from former coal mines, it has the highest organic C content of 2.2% and CEC of 19.9 cmol/kg among all the soil media tested in this study. Furthermore, it is highly porous given that it is composed of large sand particles with clay content. Highly porous soils facilitate the growth of seedling roots. Mined-out soil also has the lowest Fe content of 0.8% (Table 1). Scott et al. (2008)) concluded that *kranji* or *P. pinnata* seedlings could grow optimally in mining land, which contains high amounts of sulfur. Dewi (2016) reported that the growth of *kranji* or *P. pinnata* in landfill soil contained in organic pots can closely match that of *P. pinnata* grown on forest soil. Therefore, the use of organic pots in the revegetation of former coal mine sites is recommended. Veronika (2016) found that organic pots made from chicken manure more effectively support the growth of *kranji* or *P. pinnata* seedlings in soils taken from abandoned coal mine sites than those made from cow manure.

Although the lone factor of AMF dosage had no statistically significant effect (Fig. 1b), the average height of *P. pinnata* seedlings under treatment with different AMF dosages differed. The application of 2 g AMF led the highest seedling height of 33.08 cm that was higher than the value of 30.27 cm obtained under control. However, under 4 g of AMF, average height decreased to 27.83 cm. The additional dose of AMF did not effectively support the growth of *P. pinnata* seedlings. The nonsignificant result between inoculated and non-inoculated seedlings could be due to the type of AMF colonization that was dominated by vesicle while arbuscular was absent (data not shown). It is well known that the function of arbuscular is carbon nutrient exchange between fungi and host plant. Therefore, *arbuscular* is used to determine the AMF symbiosis. Meanwhile, *vesicle* is used as carbon storage for fungi (Smith and Read, 2008). This result was inline with Kurniaty and Damayanti (2011) that reported the inoculation rate of 2 g mycorrhiza spores/plant is effective but does not effectively support the growth of 5-month-old *calliandra* seedlings.

Klironomosi (2003) reported that plant growth responses to mycorrhizal inoculation within an ecosystem can range from highly parasitic to highly mutualistic. The direction and magnitude of the response depended on the combination of plant and fungal species. The range of responses is greatest when using native plants and local fungi, whereas the range of responses was significantly reduced, as was the relative frequency of positive responses (Klironomosi 2003).

Exotic species of *P. pinnata* inoculated by native AMF of *Glomus clarum* are likely to require longer adaptation and tolerance and thus have not shown significant increases in plant growth. This variation in plant growth response may be a large contributor to plant species coexistence and the structure of plant communities. Besides, the lack of effectiveness of inoculation of mycorrhizae can be caused by failed colonization of AM, water logged, high soil fertility, improper host plant, old inoculums, low number of inoculums, and too short planting periods. Furthermore, Tuheteru et al. (2017) reported that proper colonization *A. tuberculata* and *Glomus sp*. significantly increased dry weight of root and shoot, with different ability on metal uptake.

The diameters of plants grown on forest soil significantly differed from those of plants grown on other types of soil. However, the diameters of plants grown on soils collected from former mine sites did not show significant differences. *Pongamia* seedlings grown on forest soil showed the highest growth rate with an average diameter of 4.87 mm, followed by those grown on mined-out, overburdened, and landfill soil media, which exhibited diameters of 4.09, 3.5, and 3.5 mm, respectively (Fig. 2a). Although the lone factor of AMF dosage produced no statistically significant effect, the average diameter of *P. pinnata* seedlings differed under treatment with different dosages of AMF. The highest diameter of 4.32 mm was observed under 2 g of AMF and was larger than those under control (3.86 mm). Plant diameter further decreased to 3.76 mm under 2 g of AMF. Apparently, the lone factor of AMF resulted in an average root infection rate of 48.47%. Wulandari et al. (2014) stated that the standard infection rate by mycorrhiza should exceed 70% to promote plant growth.

Leaf chlorophyll content may also be a sensitive indicator of the physiological condition of plants. Insufficient chlorophyll levels will affect photosynthesis, which produces the carbohydrates necessary for plant growth (Nio and Banyo 2011). The chlorophyll content of *P. pinnata* in this research ranged from 0.98 to 1.09 mg/g (Fig. 3). Subjecting the chlorophyll content of *P. pinnata* leaves to variance analysis showed that the lone factor of soil media had a significant effect but that the lone factor of AMF dosage and the interaction between soil media and AMF dosage did not yield different results (Table 4). Figure 3 shows that plants grown on overburdened soil had significantly different leaf chlorophyll contents. However, no significant difference was found under forest, mined-out, and landfill soil. The lowest chlorophyll content was found in the *P. pinnata* grown on overburdened soil, which might contain the highest concentration of Al, Fe, and S among all the tested soil media (Table 1).

The lone factor of soil media resulted in significant differences in canopy biomass or aboveground biomass, total biomass, and T/R ratio but not root biomass (Table 4).

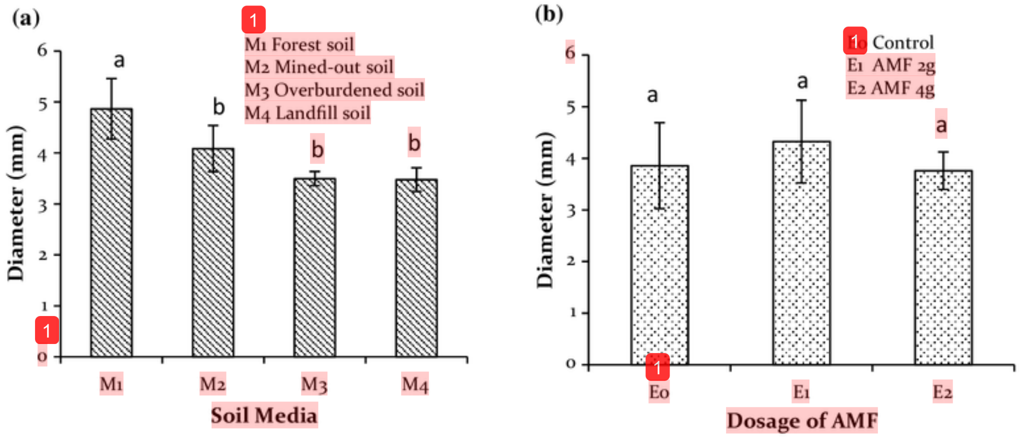

Fig. 2 Effect of soil media (**a**) and AMF dosage (**b**) on the average diameter of *P. pinnata* seedlings. Values followed by the same letter in the same diagram are not significantly different at the 5% level of significance

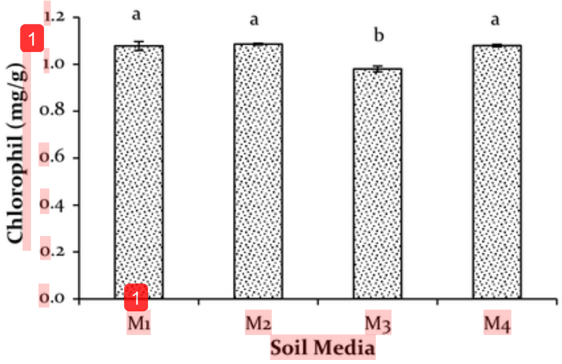

Fig. 3 Effect of soil medium type on the chlorophyll content of *P. pinnata* seedlings. Values followed by the same letter in the same diagram are not significantly different at the 5% level of significance

Table 4 Effect of soil media and AMF dosage on the biomass of *P. pinnata* seedlings

Treatment	Above-ground biomass (g)	Root biomass (g)	Total biomass (g)	T/R ratio
Media				
M1	26.15^{a}	16.57^{a}	42.72^{a}	1.58^{a}
M2	24.57^{ab}	16.13^{a}	40.70^{ab}	1.52^{a}
M3	20.28^{b}	15.06^{a}	35.34^{b}	1.35^{b}
M4	21.19^{b}	15.33^{a}	36.52^{b}	1.38^{b}
AMF				
E0	22.56^{a}	15.56^{a}	38.12^{a}	1.45^{a}
E1	24.11^{a}	15.90^{a}	40.01^{a}	1.51^{a}
E2	22.48^{a}	15.85^{a}	38.33^{a}	1.41^{a}

Values followed by the same letter in the same diagram are not significantly different at the 5% level of significance

Aboveground biomass accounted for the majority of total biomass with an average contribution of 59.3%. The average belowground biomass of *P. pinnata* seedlings contributed approximately 40.7% to the total biomass. Thus, a T/R ratio of 1.5 was obtained in this study. Suhardi et al. (2006) concluded that seedlings are considered of good quality if the ratio of the aboveground and the belowground parts of the seedlings is within the range of 1–3.

The homeostasis of the canopy and the root (T/R ratio) reflects the effort of plants to maintain physiological balance to ensure normal organ function. T/R ratio is genetically controlled but can also be influenced by environmental factors (Suhardi et al. 2006). The highest average T/R ratio of 1.58 was found under forest soil followed by the T/R value of 1.52 under mined-out soil. These values were significantly different from those observed under overburdened soil (1.38) and under landfill soil (1.35) (Table 4). In line with Dewi (2016), the T/R ratio of the *P. pinnata* planted in former coal mine soil fell in the range of 1.2–1.6.

Soil media did not significantly affect root biomass because all soil media used in this research had a clayey texture (Table 1). Taylor et al. (2010) stated that root growth is highly influenced by soil porosity, and poor porosity would restrict oxygen supply because the roots cannot adequately penetrate the soil. Thus, plants would grow poorly in soils with low porosity despite the nutrient content of the soil. The best biomass was exhibited by plants grown on forest soil, followed by that exhibited by plants grown on mined-out, landfill, and overburdened soil.

Seedling quality greatly affects the success of land rehabilitation and reforestation programs because good-quality seedlings provide highly productive vegetation cover (Kurniaty and Damayanti 2011). Seedling quality can be determined on the basis of the seedling quality index (SQI), which is derived by using the weighted average of three key parameters that represent the height, diameter, and total dry weight of the plant. These three parameters reflect the physiological state of the plant.

M1E1 yielded the highest SQI of 25, and M3E2 and M3E0 provided the same SQI of 8, which is the lowest value (Table 5). These results can be attributed to the highly acidic pH and high S, Al, and Fe content of M3 relative to those of other soil media. Low SQIs were obtained from these latter combinations despite the increased AMF inoculation rate.

AMF inoculation improved the SQI of seedlings planted in forest soil. Seedlings that have mycorrhiza-infected roots can better withstand drought, easily absorb nutrients, and resist pathogenic attacks (Wulandari et al. 2014). The SQIs of the *P. pinnata* in this research are consistent with those reported by Casuarina (2014), who stated that

Table 5 Effect of treatment on the seedling quality index of *P. pinnata*

Treatment	Value				Rating
	Height	Diameter	Biomass	Total SQI	
M1E0	9	9	6	24	2
M1E1	9	9	7	25	1^{a}
M1E2	6	6	5	17	4
M2E0	5	4	4	13	6
M2E1	6	7	6	19	3
M2E2	5	5	6	16	5
M3E0	2	3	4	9	10
M3E1	3	4	3	10	8
M3E2	2	3	3	8	12^{b}
M4E0	2	3	3	8	11
M4E1	4	4	4	12	7
M4E2	3	3	3	9	9

^{a}The best growth; ^{b}the worst growth the inoculation with *G. aggregatum* yields good-quality *P. pinnata* seedlings with an SQI of 30, whereas treatment without AMF produces poor-quality seedlings with an SQI of 4. Inoculation with AMF is one method to reinforce and support the growth of seedlings in *P. pinnata* nurseries (Scott et al. 2008).

Inoculation with AMF can facilitate nutrient absorption by phytoremediators grown on former coal mine sites given that nitrogen and phosphorus supplies are limited in this type of sites (Bucking and Shachar-Hill 2005). AMF inoculation significantly increased the pH level, organic C, total N, and available P levels of the soil (Fig. 4). In general, the pH–H_2O level of forest soil increased from 5.1 (acid) to 6.1 (slightly acid). Meanwhile, the average pH level of soils taken from former coal mines increased from 2.9 to 3.3 (very acidic) (Fig. 4a).

The organic C content of the soil increased by 41.5% after treatment. The highest increase in organic C content of 131.85% was found in revegetation land, followed by that in mined-out soil (43.2%) and that in overburdened soil (17.3%). By contrast, the organic C content of forest soil decreased by 26.2% (Fig. 4b). Total N increased by an average of 49.3% after treatment. The highest increase in N of 231% was found in mined-out soil. N did not increase and instead decreased in other soil media (Fig. 4c). Available P increased by 189% in all soil samples after treatment. The highest increase of 384% was found in landfill soil, followed by that in forest soil (260%) and by that in mined-out soil (124%); however, the available P of overburdened soil decreased (Fig. 4d).

P absorption by *P. pinnata* seedlings exhibited the highest increment. This result can be attributed to the AMF treatment, which facilitated the absorption of soil nutrients,

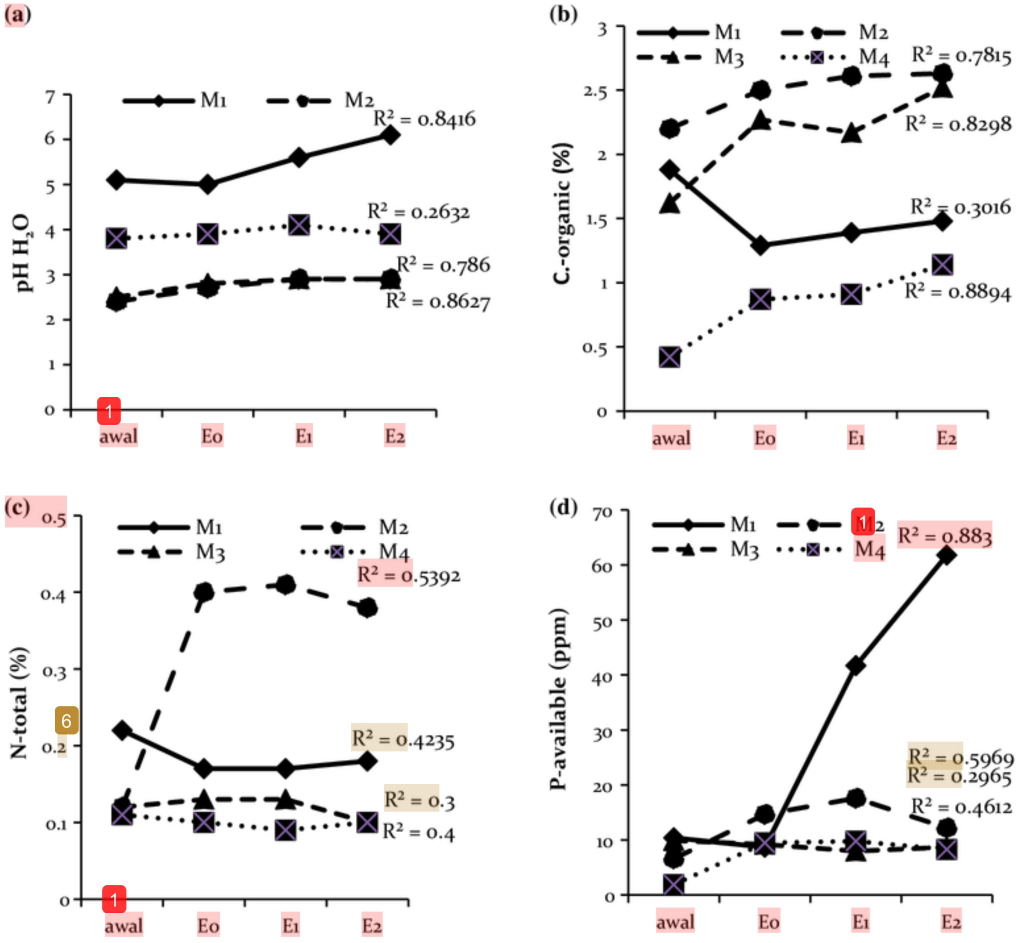

Fig. 4 Trend of soil nutrient (**a**) pH–H_2O (**b**) organic C (**c**) total N (**d**) available P before and after treatment

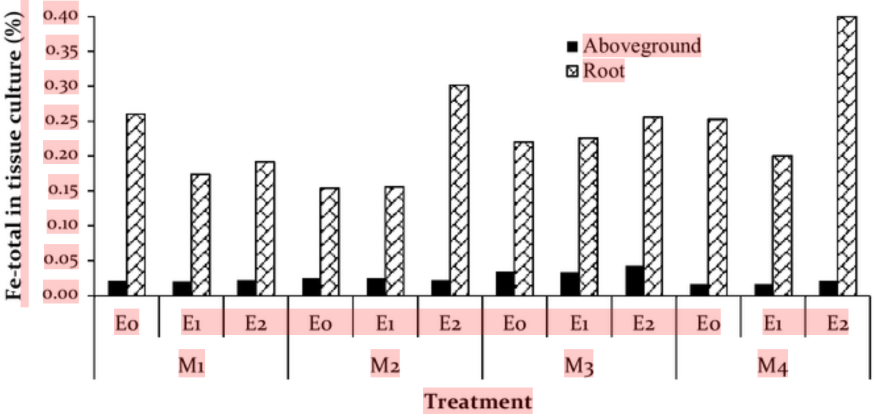

Fig. 5 Total Fe concentration of the aboveground tissues (leaf and stem) and roots of *P. pinnata* seedlings

particularly the absorption of available P. Macronutrients are crucial for plant growth (Taylor et al. 2010). The use of *P. pinnata* plants as phytoremediators can decrease the pollutant loads of soil, air, and water to attenuate the harmful effects of pollutants on the biological elements of an ecosystem (Salt et al. 1998). Compared with that under the control treatment, *P. pinnata* seedlings can absorb and retain Fe by 11.7% under AMF inoculation. Approximately 90.4% of Fe was accumulated in the roots, and only 9.6% of the absorbed Fe was transferred to the leaves (Fig. 5). Similarly, Nufus (2016) reported that the seedlings of *gempol* (*Nauclea orientalis)* can absorb and retain heavy metal elements, such as Fe, from soils collected from former coal mine sites. Kharathanasis and Thompson (1995) reported that Al and Fe are retained in plant roots. The highest average increase in Fe absorption under AMF inoculation was found in *P. pinnata* plants grown on mined-out soil. Fe absorption by these plants reached 48.47%. The average Fe absorption rate of plants grown on revegetation land increased by 18.8% and that of plants grown on overburdened soil increased by 9.27%. However, the lone factor of AMF dosage did not significantly increase the absorption of Fe by plants grown on forest soil. This result can be attributed to the increased Fe tolerance of *P. pinnata* seedlings grown on forest soil, which has a slightly acidic pH.

Conclusion

Open-pit coal mining activities in East Kalimantan caused serious land degradation in tropical ecosystem. Revegetation by fast-growing pioneer legume species of *P. pinnata* and application of *arbuscular mycorrhizal fungi* (AMF) could improve some chemical soil properties drastically that suitable for rehabilitation program in post-mining areas. The pH of forest soil increased from 5.1 (acid) to 6.1 (moderately acid), whereas that of former coal mine soils increased from 2.9 to 3.3 (very acid). Soil organic C, total soil N, and plant-available P contents increased by 41.49%, 49.31%, and 188.9%, respectively.

Pongamia seedlings can adapt and grow optimally in soils taken from former coal mines with a 100% survival rate. Revegetation with *P. pinnata* can accelerate land reclamation by passing the land preparation stage and can decrease the costs of land preparation. Forest soil is the optimal medium for the growth of *P. pinnata* seedlings. Seedlings grown on forest soil after 6 months exhibit an average height of 43.6 cm, diameter of 4.87 mm, and total biomass production of 42.72 g. However, when the seedlings were planted in soil degraded by mining activities, their average height, diameter, and total biomass drastically dropped to 25.98 cm, 3.69 mm, and 37.52 g, respectively. The inoculation rate of 2 g AMF/plant is sufficient for infecting the roots of *P. pinnata* of seedlings and should be adapted to reduce the cost of AMF inoculum propagation. Inoculation of 2 g AMF into seedling grow in forest soil resulted on high AMF colonization and had highest growth compare to other 3 mined soil media. AMF inoculation improved Fe absorption by 11.7% that was higher than that under control, whereas 90.4% of the assimilated Fe was retained in plant roots.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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