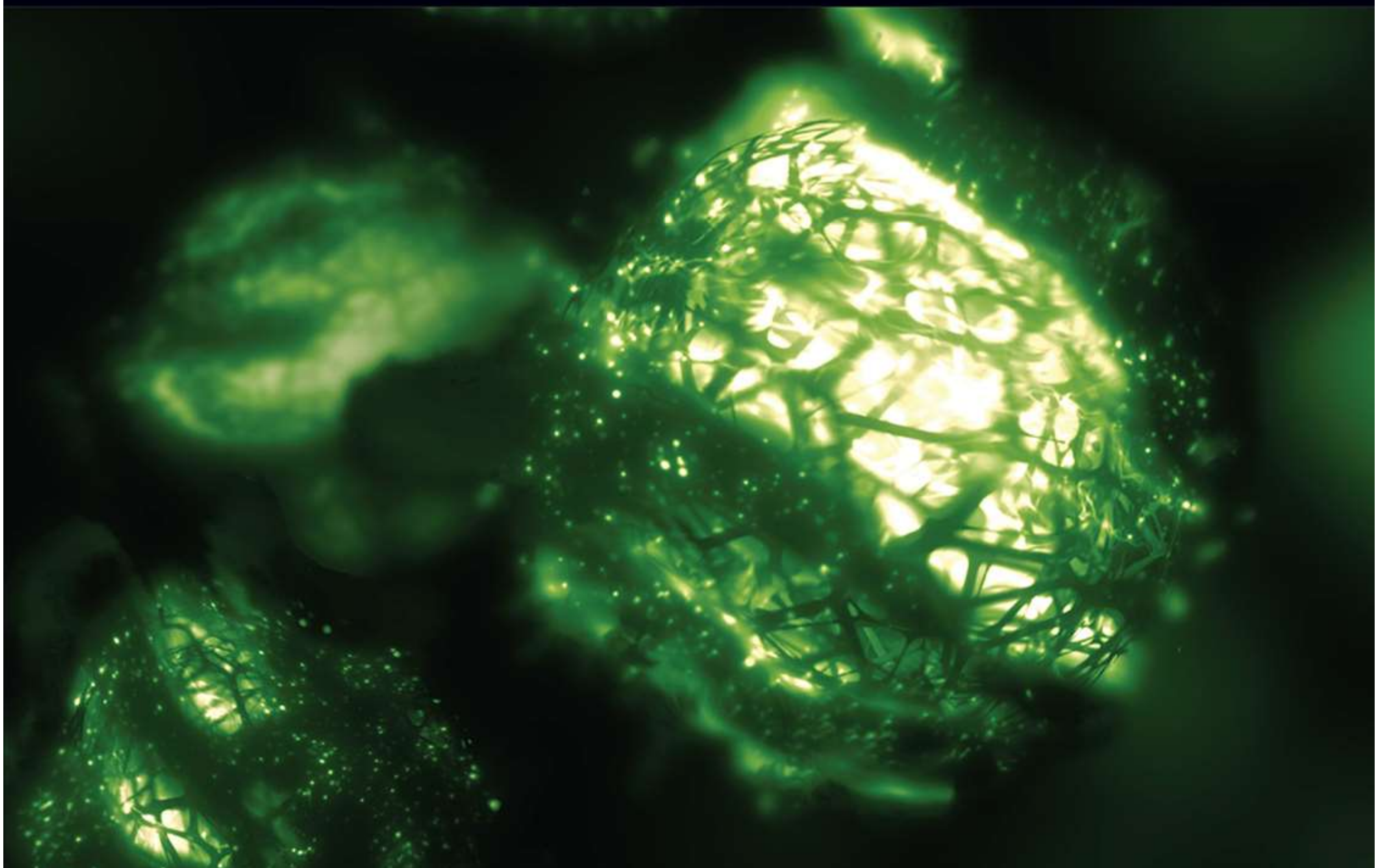
MICROBIOME RESEARCH IN PLANTS AND SOIL



MICROBIOME DRIVERS OF ECOSYSTEM FUNCTION

Edited by
Javid A. Parray, Nowsheen Shameem,
Dilfuza Egamberdieva

Microbiome Drivers of Ecosystem Function

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Preface

The environment is a strong driver of most ecosystem processes, and microbial community structure adds to our understanding of processes under certain circumstances. In addition, the key address will be to establish new insights or advancements about the relationship between microbial community structure and a variety of ecosystem processes on ecological, biogeochemical, and phylogenetic trends.

Plant responses to various environmental or climatic stresses are complex and implicate changes at the transcriptome, cellular, and physiochemical levels, consequently hindering crop growth and yield quantity and quality. The beneficial microbial communities belowground represent a novel and promising solution for agro-environmental sustainability through biofertilizers, bioprotectants, and biostimulants. Plant growth—promoting soil organisms increase net crop uptake of soil nutrients, resulting in larger crops and higher yields of harvested food. The many symbiotic associations between plants and microbes can ultimately be exploited for more outstanding food production necessary to feed the expanding human populace, in addition to safer farming techniques for the sake of minimizing ecological disruption. Therefore the understanding of various ecosystem processes, environmental factors, microbial community structure, and biogeochemical cycling will be helpful to assess the importance of microorganisms.

Microorganisms play a pivotal role in the functioning of ecosystems and harbor keystone taxa, which drive community composition and function irrespective of their abundance. The microbial consortium with multifunctional plant growth—promoting attributes vis-à-vis the beneficial plant microbiome's harnessing is the pillar of a sustainable agriculture and other intensification measures. There is an excellent potential for near future enhancements in the use of plant growth—promoting microorganisms in world agriculture and other valuable plants. A paramount need is to bridge the gap between fundamental, applied science and agriculture practices. The earliest possible transition of knowledge to the farmers as end-users of innovative products and biotechnologies can ensure efficient commercialization of scientific results to achieve more sustainable use of natural resources and more efficient production of biological or ecological plants. This book addresses the critical issues of how ecosystem functions are correlated with microbial diversity, besides what aspects of microbial biodiversity influence ecosystem function. The plant root contains dense microbial communities vital for host health, providing nutrients, protecting from pathogens, and promoting immune system development. The dynamic relationships between microbial community structure and ecosystem function are essential for improving predictions of ecosystem process rates.

The book gives detailed information about the understanding of microbial diversity and function in soil from genes to ecosystems, microbes as engines of ecosystem function, diversity of various symbiotic associations between microbes and host plants, molecular ecological network analyses, and ecosystem processes to unravel multispecies microbial community functioning, functional metagenomic profiling of different ecosystems, and biotechnological developments including futuristic trends.

Javid A. Parray

CHAPTER

10 Indigenous symbiotic soil microbes and native tree species for revegetation of nickel postmining area in Indonesia

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10.1 Introduction

Nickel mining is a sector that generates extensive foreign exchange for the country. Globally, Indonesia is ranked fourth in the world as a nickel producer. The potential of nickel resources in Indonesia is estimated at 1,878,550,000 tons, with an average nickel content of 1.45%. Nickel reserves in the form of mineral deposits that can be extracted (ore) are 577,000,000 tons. They are spread over Sulawesi, Kalimantan, Maluku, and Papua. Southeast Sulawesi is one of the areas with high nickel mining activity. Several mining industries operate in the area. Since 2013 nickel ore production in Southeast Sulawesi has reached 29,431,004 tons with a production value of Rp. 8,387,836,000,000, ferrous nickel production reached 15,535 tones with a production value of Rp. 2,563,275,000,000. However, the mining industry, especially with the open-pit method, causes severe environmental damage in forest areas.

Natural recovery through natural succession can occur on land that has been disturbed. Krebs (1972) states that succession is a process of changing biotic communities that replace each other, and the physical environment changes over time after disturbance. The damage that occurs due to nickel mining causes the land left to be open and very poor in organic matter, so the natural succession process that occurs in the nickel postmining area is considered primary succession. According to Kimmins (1997), primary succession occurs in areas with open

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surfaces and a lack of organic matter and has not been changed by the activity of organisms. Therefore natural recovery through the natural succession process is running slowly, especially with the character of the nickel postmining land being very extreme and not supporting the development of plants/biotic communities that colonize the land.

Efforts to accelerate the natural succession process in nickel postmining areas through technological input are needed for the Rehabilitation to succeed. According to Weissenhorn et al. (1995), using local microbes and plant species that are proven suitable for land conditions is a challenge that must be answered in rehabilitating ex-mining areas so that natural succession can be accelerated. Furthermore, according to Smith and Read (2008), the success of land rehabilitation depends on an understanding of plant succession, which is influenced by dynamic soil components, such as organic matter produced by vegetation and microbial communities. Therefore utilizing soil microbes, especially those in symbiosis with local plants and species, is essential in rehabilitating nickel fields. Technology to produce indigenous symbiotic soil microbial isolates, determination of potential local plant species, and mastery of cultivation techniques are needed to achieve this goal.

10.2 Nickel mining and problems with postmining land

Nickel mining in Indonesia uses the open-pit mining method. The method is done by digging a hole/making a pit. The mining method includes several stages, namely:

1. Land clearing. This activity aims to remove vegetation, both trees and shrubs, on the land to be mined.
2. Stripping/stripping. This activity aims to strip/remove the soil layer above the layer containing nickel ore. The top layer of soil removed is the topsoil layer, also known as iron capping. This layer usually has humus, plant roots, and other organic debris. The iron content in this layer is high, but the nickel content is so low that it is not extracted for mining. The topsoil that has been peeled off is then transported and placed in a waste dump and will then be used for reclamation of ex-mining land. After the topsoil has been removed, the next layer to peel is overburden or limonite. Plant roots can still be found in this layer but in minimal quantities. Lateritic soil and clay are the main constituents of this layer. Like topsoil, this layer also has a shallow nickel content ($<1.4\%$), so it is not economical and qualifies as a mining material (Putri, 2016). After being excavated and peeled off, the overburdened soil is transported and stored in a waste dump to be reused in ex-mining land reclamation activities.

3. Ore digging. The layer below the overburden is a saprolite layer with high nickel content, so it has economic value to be mined as nickel ore. Nickel ore excavation is carried out to prepare ore to be produced.
4. Ore hauling. The excavated nickel ore is then transported and stored in the stockyard area and grouped according to the grade/classification of the nickel ore grade. The grouping is usually based on the color that shows the level of nickel content, namely, green 2.00%, blue 1.8%−1.9%, yellow 1.5%−1.6%, orange 1.1%−1,4% (Putri, 2016). The next stage is the processing of nickel ore in the factory to produce nickel in the form of nickel matte. This form of nickel matte is then marketed.

Nickel ore mining activities with the open-pit mining method change the overall form of the land. This mining method will leave holes excavated for mining materials. Moreover, the soil's physical, chemical, and biological properties have also decreased drastically. Physically, the site does not have a clear soil layer/horizon, the soil structure is damaged, and compaction occurs. Judging from the chemical aspect, there is a shortage of organic matter and essential nutrients. Meanwhile, from the natural part, there was a decline in population and uneven distribution of soil microbes.

Soil organic matter content can change due to the conversion process of forests into human-managed land, including mechanical disturbances from mining activities (Sabaruddin et al., 2009). The content of primary macronutrients such as nitrogen, phosphorus, and potassium was also found to be very low in the ground cover of ex-mining land (Sheoran et al., 2010). Widiatmaka et al. (2010) stated that the decline in the chemical properties of nickel postmining areas was characterized by low organic matter content, shallow available P content, low cation-exchange capacity, but relatively high Mg content. High levels of Mg cause nutrient imbalances in the nickel postmining soil.

The decrease in soil chemical properties due to nickel mining was also reported by Prayudyaningsih et al. (2019), which stated the availability of C-org, P, N, and low cation-exchange capacity even on revegetated land (Table 10.1). Meanwhile, the decrease in the physical properties of the ex-nickel mine soil is indicated by high bulk density, low water availability, low porosity, and low soil permeability (Widiatmaka et al., 2010). In addition, Dalimunthe et al. (2007) mentioned that the vegetation clearing and stripping of topsoil in the mining process, such as nickel mining, causes the population of soil microbes on the surface to decrease.

Another problem encountered in nickel postmining areas is the high content of heavy metals. Widiatmaka et al. (2010) stated that nickel ex-mining soils have high levels of heavy metals, especially nickel (Ni) and chromium (Cr). However, even the levels of both types of metals are at the toxicity threshold for plant growth.

The return of topsoil to ex-mining land as part of the rehabilitation also does not have an optimal impact on plant growth. Topsoil that is reused covers the

Table 10.1 Content of C-org, N, P available, and cation-exchange capacity in nickel postmining soil.

Areas	C-org (%)	N content (%)	P available (ppm)	CEC (cmol/kg)
NTNR	0.27	0.11	2.33	5.99
NTR	0.80	0.10	4.67	5.85
TNR	0.89	0.08	4.33	6.86
TR	2.97	0.09	6.50	6.97

NTNR, *The postmining area already stockpiled with overburden, not followed by topsoil spreading and revegetation,* NTR, *postmining area already accumulated with overburden, not followed by topsoil spreading but revegetated;* TNR, *postmining area stockpiled with overburden soil followed by topsoil spreading but no revegetation activities;* TR, *postmining area accumulated with overburden + topsoil + revegetated.*
Data from Prayudyaningsih, R., Sari, R., & Mangopang, A. D. (2019). Isolation of indigenous arbuscular mycorrhizal fungi (AMF) to support revegetation on the nickel post-mining land. IOP Conference Series: Earth and Environmental Science, 308, *012038.*

overburden layer; if it is stored for a long time, it will experience a decrease in organic matter content. According to Sheoran et al. (2010), the properties of the stockpiled soil will continue to deteriorate and eventually become biologically unproductive if not properly conserved. Meanwhile, Yassir and Omon (2003) stated that the content of organic matter in the soil could also affect the return of vegetation diversity as part of the succession process. The relationship between soil organic matter and the variety of plant species around the nickel mining area shows that if there is an increase in organic matter in the soil, the diversity of plants at the level of seedlings, saplings, poles, and trees will increase. The content of organic matter affects the diversity of seedlings level by 77.1%, saplings by 80.1%, poles by 84.8%, and tree-level plants by 82% (Mangopang & Prayudyaningsih, 2016).

10.3 Potential utilization of symbiotic soil microbes for rehabilitation of nickel postmining

According to Killham in Ervayenri (2005), local inoculum production, soil properties, climate, and competition for several other microbes in the environment influence the successful use of microbial inoculum. Furthermore, Killham in Ervayenri (2005) also mentions that the effectiveness of the symbiosis between microbiomes and their hosts is influenced by climate and soil factors in their environment. Based on this, indigenous microbes are thought to be more effective in symbiosis because they are adaptive to environmental conditions. Therefore this is by the statement of Pfleger et al. (1994), the indigenous mycorrhizal arbuscula (FMA) is the best candidate for inoculum for reinoculation in the rehabilitation of ex-mining land, as well as indigenous rhizobia.

10.3.1 Arbuscula mycorrhizal fungi for rehabilitation of ex-nickel mines

AMF are the most common group of mycorrhizae. Arbuscular mycorrhizae (AM) show a symbiotic relationship between the plant and fungal levels. Nearly 90% of plant species (Smith & Read, 2008) exhibit mycorrhizal relationships, of which the mycorrhizal fungal mycelia relationship with plant roots is one of the most prominent structures of this association. Fungi are related to root plants; symbiosis is created with plants because assimilation is sent to the root system. Therefore plants provide constant and direct access to carbohydrates, such as glucose and sucrose, for fungi. In return, plants benefit from the absorption of water and minerals. The fungus causes a large surface area of fungal hyphae, which mobilizes soil minerals unavailable to plant roots. The effect is the plant's ability to absorb minerals and air. According to Bussaard et al. (2007), various mycorrhizae contribute positively to nutrition and efficiency of air utilization.

The diversity of mycorrhizal fungi as part of creating forest soil microbial communities has decreased when mining activities are carried out. Prayudyaningsih et al. (2019) stated that the AMF population in ex-nickel mining areas was meager, namely, 7—83 spores per 100 g of soil sample. Further, Prayudyaningsih et al. (2019) also stated that *Glomus* is the genus that dominates the ex-nickel mining area compared to two other genera, Acaulospora and *Gigaspora* (Fig. 10.1). Slightly different results were obtained by Akib et al. (2018), which state that the genus *Acaulospora* dominates ex-nickel mining areas compared to *Glomus* and *Gigaspora*.

AM, as a symbiosis between plant roots and soil fungi, can increase the supply of water and nutrients to the host plant, thereby helping the growth of the host plant (Smith & Read, 2008). Furthermore, Abbaspour et al. (2012), Birhane et al. (2015), and Cavagnaro et al. (2015) also reported that the association of AM has a vital role in improving plant survival, growth, and performance through increased absorption of nutrients and water under environmental stress conditions. Prayudyaningsih et al. (2019) stated that 16 types of plants that grow naturally in nickel postmining areas are associated with AMF in their root system. Further explained by Prayudyaningsih et al. (2019) that the rate of AMF colonization on the roots of pioneer plants growing in nickel postmining areas varied widely, between 1% and 50% (Table 10.2).

The seedling phase is highly dependent on mycorrhizae (Mosse et al., 1981). Planting mycorrhizal seeds for revegetation of nickel postmining is expected to produce fast-growing crops and have a facilitative impact on improving soil properties. The presence of AMF improves plant viability on disturbed lands, such as former nickel mines. Mycorrhizal fungal communities can be important drivers of pedogenesis, are involved in the N cycle, increase P availability, and play a role in nutrient cycling. Therefore their presence can improve soil fertility (Barea et al., 2011; Veresoglou et al., 2012; Cavagnaro et al., 2012; Dickie et al., 2013).

FIGURE 10.1

Various AMF species on the nickel postmining land. (A) Spore of *Acaulospora* in Meltzer's solution. (B) Spore of *Acaulospora* in a PVLG solution. (C) *Gigaspora* spore in Meltzer's solution. (D) *Gigaspora* spore in a PVLG solution. (E, G, I) *Glomus* spore in Meltzer's solution. (F, H, J) *Glomus* spores in a PVLG solution. *AMF*, Arbuscular mycorrhizal fungi; *PVLG*, polyvinyl-lactoglycerol.

Reproduced with permission from Prayudyaningsih, R., Sari, R., & Mangopang, A. D. (2019). Isolation of indigenous arbuscular mycorrhizal fungi (AMF) to support revegetation on the nickel post-mining land. IOP Conference Series: Earth and Environmental Science, 308, *012038.*

AMF inoculation on plants growing on nickel postmining soil was proven to increase growth, as indicated by the increasing biomass and absorption of P elements. Moreover, AMF inoculation also increased plant tolerance to nickel toxicity. For example, Amir et al. (2013) reported that two endemic plant species in New Caledonia, *Alphitonia neocaledonica* and *Cloezia artensis* inoculated with FMA Glomus etunicatum isolate, showed a more substantial positive effect on biomass and phosphorus uptake, and a more significant reduction in toxicity symptoms and Ni concentration in roots and shoots. Similar results were also reported by Husna et al. (2017), which stated that AMF inoculation increased the growth, biomass, and nutrient uptake (especially P) of an accumulator and nickel-sensitive plants.

The application of compost and the addition of AMF is also known to increase the growth of *Nauclea orientalis* plants and reduce heavy metal levels in the soil (Ekawati et al., 2016). Other evidence of the effect of inoculation on plant tolerance to high levels of Ni has also been demonstrated by Jamal et al. (2002), which stated that mycorrhizal soybean tissue had a higher Ni content than

Table 10.2 Colonization of AMF (arbuscular mycorrhizal fungi) in pioneer plant roots that grow spontaneously in each type of area on nickel postmining land.

Area	Species	Life form	AMF colonization level ± SD	AMF colonization structure
NTNR	*Machaerina glomerata* (Gaudich)	Grass	26.11 ± 12.62	Hypha
	Scleria lithospherma (Linnaeus) Swartz	Grass	10.56 ± 3.47	Hypha, microsclerotia
	Trema orientalis	Tree	0.00	–
NTR	*Casuarina sumatrana*	Tree	0.83 ± 0.91	Hypha
	Cynodon dactylon (L.) Pers.	Grass	2.22 ± 2.54	Hypha, spore
	M. glomerata (Gaudich)	Grass	4.00 ± 5.60	Hypha, vesicle
	Palaquium luzoniense Vid.	Tree	7.78 ± 6.94	Hypha
	Paraserianthes falcataria	Tree	18.89 ± 25.62	Hypha, spore, vesicle
TNR	*Digitaria sanguinalis*	Grass	0.00	–
	Dracaena sp	Grass	21.67 ± 28.28	Hypha, vesicle
	M. glomerata (Gaudich)	Grass	5.00	Hypha
	Melastoma malabatrichum	Shurb	8.19 ± 4.58	Hypha, spore, vesicle, microsclerotia
	Paspalum scrobiculatum	Grass	21.94 ± 9.03	Hypha, spore, vesicle, microsclerotia, arbuscular, Hypha coil
	Sarcotheca celebica	Tree	1.67	Hypha, vesicle
	Scleria lithosperma (Linnaeus) Swartz	Grass	29.44 ± 9.81	Hypha
	Spermacoce sp	Herb	1.11 ± 0.96	Hypha
	T. Orientalis	Tree	10.93 ± 6.93	Hypha, spore, vesicle
	Trichospermum kjellbergii Burret	Tree	8.89 ± 2.54	Hypha, spore
TR	*Callicarpa pachyclada*	Herb	25.00 ± 7.07	Hypha, spore, vesicle
	Centrosema pubescens	Herb	28.33	Hypha, vesicle
	C. dactylon (L.) Pers.	Grass	10.56 ± 7.72	Hypha, vesicle

(*Continued*)

Table 10.2 Colonization of AMF (arbuscular mycorrhizal fungi) in pioneer plant roots that grow spontaneously in each type of area on nickel postmining land. *Continued*

Area	Species	Life form	AMF colonization level ± SD	AMF colonization structure
	Fimbristylis sp	Grass	4.44 ± 4.19	Hypha
	M. glomerata (Gaudich)	Grass	11.67 ± 10.38	Hypha, vesicle
	M. malabatrichum	Share	15.33 ± 20.76	Hypha, spore, vesicle
	P. falcataria	Tree	26.25 ± 28.01	Hypha, spore, vesicle, microsclerotia
	P. scrobiculatum	Grass	30.56 ± 15.44	Hypha, spore, vesicle
	S. lithosperma (Linnaeus) Swartz	Grass	11.11 ± 6.12	Hypha
	Scleria sp.	Grass	50.00	Hypha
	T. orientalis	Tree	22.96 ± 15.92	Hypha, spore, vesicle

NTNR, *The postmining area already stockpiled with overburden, not followed by topsoil spreading and revegetation,* NTR, *postmining area already stockpiled with overburden, not followed by topsoil spreading but revegetated;* TNR, *postmining area stockpiled with overburden soil followed by topsoil spreading but no revegetation activities;* TR, *postmining area stockpiled with overburden + topsoil + revegetated.*
Data from Prayudyaningsih, R., Sari, R., & Mangopang, A. D. (2019). Isolation of indigenous arbuscular mycorrhizal fungi (AMF) to support revegetation on the nickel post-mining land. IOP Conference Series: Earth and Environmental Science, 308*, 012038.*

nonmycorrhizal plants. These results showed that mycorrhizal associations were able to increase plant tolerance to heavy metal toxicity (especially Ni) and reduce heavy metal contamination in the soil. Therefore the use of AMF in the revegetation of ex-mining land has an essential value because it not only increases plant growth but can also improve the quality of ex-mining land.

10.3.2 Rhizobia as biofertilizer in the rehabilitation of ex-nickel mines

Plant growth—promoting rhizobacteria (PGPR) are a group of rhizosphere bacteria (living around roots) that can stimulate plant growth and are biological control agents that are beneficial for plants (Istiqomah et al., 2018). One type of PGPR, nitrogen-fixing bacteria, is the most significant nitrogen contributor that plants can use directly. Rubio and Ludden (2008) stated about 2/3 of global nitrogen fixation. Rhizobia bacteria are the largest group of rhizobacteria capable of colonizing legume roots and converting free nitrogen into ammonia available to plants (Ahemad & Kibret, 2013). Sari and Prayudyaningsih (2015) added that mutualism symbiosis occurs when rhizobia bind free nitrogen in the atmosphere and convert it into ammonia which is needed by plants in the process of growth and development, while plants provide carbohydrates that are a source of energy for rhizobia.

PGPR microbial groups, especially rhizobia bacteria, can be applied to increase plant growth, including on critical land. Ex-mining land has conditions that damage the soil's physical, chemical, and biological properties. Poor soil quality can reduce soil fertility. Microbes with good infectivity and effectiveness can help restore soil quickly, safely, and relatively cheaply so the land can eventually be reused (Rezkikasari et al., 2018). Even though it has degraded soil characteristics, ex-nickel mining areas still have the opportunity to contain potential microbial isolates, especially rhizobia bacteria, to be developed as biological fertilizers. Indigenous bacterial isolates have advantages when used as biological fertilizers to rehabilitate ex-nickel mines. Nurmas et al. (2014) stated that indigenous isolates could adapt to their new environment, especially if introduced to land similar to their original habitat.

Indigenous rhizobia bacteria were isolated by inoculating soil samples diluted in YEMA (Yeast Extract Mannitol Agar) media. YEMA media is a selective medium for rhizobia bacteria. Sari and Prayudyaningsih (2016) isolated rhizobia bacteria from former nickel mining areas, which were divided into four areas, areas that had experienced natural succession, areas that had been filled with overburden material (backfilling), areas that had been stockpiled with overburden material and sown topsoil and continued with revegetation, and areas that have been revegetated without first sowing topsoil. Sari and Prayudyaningsih (2016) reported that the bacterial density in each area was 3.9×105 CFU/g for natural succession areas, 2.5×105 CFU/g in the backfilling regions, 3.6×105 CFU/g in revegetation areas with topsoil, and 3.5×105 CFU/g in the revegetation area without topsoil. Furthermore, Sari and Prayudyaningsih (2016) stated that the density of rhizobia bacteria in the Lalindu protected forest, especially in the regions that had experienced fires, was 4.4×105 CFU/g, while the unspoiled area contained 4.6×105 CFU/g. The former nickel mining area and the protected forest around the mining area have a low density of rhizobia bacteria. Following Purwaningsih's (2009) statement, the indicator of fertile soil is if it contains more than 100 million (10^8) microbes/gram of soil.

When grown on agar media, several types of rhizobia bacteria have different colony morphological characteristics. Bhargava et al. (2016) stated that rhizobia had circular and irregular (irregular) colony shapes, flat elevation (flat), raised (raised) to convex (convex), lobate (uneven), and entire (flat) edges. Some rhizobia isolates obtained from nickel postmining areas have mucus on their cell surfaces called extracellular polysaccharides (Sari & Prayudyaningsih, 2017b). This compound is essential in developing nodules, releasing bacteria from infection, developing bacteroids, and protecting against plant antimicrobial compounds (Hidayat, 2013). The provision of indicator solutions, Congo red (CR) and bromthymol blue (BTB), can show the character of each type of bacterial isolate. Several rhizobia isolated from nickel postmining areas and protected forests in nickel mining areas were unable or only to absorb a small amount of the CR indicator solution. This can be seen from the rhizobia colony isolates, which were white or pink when grown on YEMA + CR media. Rhizobia isolates that were able to change the color of YEMA media to which BTB had been added to yellow were acidic, while the medium with alkaline isolates remained blue (Sari & Prayudyaningsih, 2016; Sari & dan Prayudyaningsih, 2017a).

Sari and Prayudyaningsih (2019) stated that in addition to fixing nitrogen, rhizobia isolates from former nickel mining areas were able to produce indole acetic acid (IAA) compounds. IAA can affect plant cell division, root cell elongation and differentiation, stimulate seed germination, initiation of lateral root formation, and biosynthesis of various metabolites (Spaepen & Vanderleyden, 2011).

Rhizobia inoculation on *Paraserianthes falcataria* (L) Nielsen seedlings using nickel-mined soil did not show a significant effect, except for stem diameter and seedling biomass (Sari & Prayudyaningsih, 2019). Furthermore, Sari and Prayudyaningsih (2019) stated that although it had no significant effect on seedling growth, treatment with the addition of rhizobia inoculum gave better results than the control. The development of *P. falcataria* (L) Nielsen inoculated with rhizobia isolates can be seen in Fig. 10.3. However, the low quality of the growing media, especially the essential P nutrients, made the bacteria ineffective in infecting plant roots.

Devi (2016) stated that combining nitrogen-fixing bacteria and phosphate-solubilizing bacteria was more effective than single inoculation. Therefore double inoculation using AMF, phosphate-solubilizing bacteria, or input of organic fertilizer at certain levels is thought to increase the potential of rhizobium bacteria as a biofertilizer in plants, especially on critical land.

10.4 Selection of native tree species for rehabilitation of nickel postmining

One of the activities in the rehabilitation of nickel postmining areas is revegetation. The tree species used in revegetation is one of the crucial factors that influence the success of the rehabilitation of nickel postmining. Selection of the appropriate tree species can affect the acceleration of the succession process to support the success of rehabilitation. The rehabilitation concept currently being developed is more directed towards using species that are easy to grow and quickly colonize ex-mining areas. The tree species are usually nonlocal (exotic) introductions considered pioneers and fast-growing. However, the use of introduced species from outside has weaknesses. The species finds difficult to adapt to local soil and climatic conditions and tends not to be resistant to disease (Setiadi, 2012). Revegetation using plants that are not local species can change the ecosystem so that it is feared to cause a reduction or loss of flora and fauna biodiversity (Withrow & Johnson, 2006).

The government recommends the use of local species for the rehabilitation of ex-mining land. The recommendation is contained in the Minister of Forestry Regulation No. P. 4/Menhut-II of 2011 concerning guidelines for forest rehabilitation. The selected plant species are directed at planting native plants that have adapted to the local climate and soil conditions. Article 25 of the Minister of Forestry emphasizes that species selection needs to pay attention to several things, including the plant species used are local. Local species are plant species that previously grew naturally in the area to be rehabilitated or grew in the surrounding area (Elliott et al., 2002). According to Mansourian et al. (2005), preference should always be given to using local species, and alternative species introduced from outside are used only when no local species are available. Local species are more adaptive to local environmental conditions, are catalytic, have a more robust genetic composition, maintain the purity of biodiversity, the possibility of seeds being more commonly available, and local people are more familiar with these local species. In addition, local species, especially pioneer species, grow relatively quickly, do not require intensive care, and have low nutrient requirements. Local species also produce a litter that is easily decomposed naturally, improves soil character and increases the thickness of topsoil, and is a conducive medium for colonization of other plants (Withrow & Johnson, 2006; Mansur, 2013; Setiadi, 2012). Another function of local plant species is as nurse plants that can help facilitate the growth of other species and have better adaptation and resistance to climate change (Ren et al., 2008; Heidelberg et al., 2011).

Information on local plant species that can be used for revegetation after nickel mining is not widely available. Pitopang et al. (2009) found several native plant species growing and distributed on ultramafic soils in Sulawesi, including *Sarcotheca celebica*, *Knema celebica*, *Deplanchea* banana, *Dysoxylum nutans*, Gironniera subaequalis, *Macadamia hildebrandii*, *Manilkara fasciculata*, and *Terminalia supitiana*. These species are proven to adapt well to ultramafic soils but have not been widely used for rehabilitating nickel postmining areas.

A native plant species selection scheme is needed to identify and select the appropriate species for rehabilitating nickel ex-mining areas. The selected local species should be able to reestablish the natural mechanism of forest regeneration and further catalyze the natural succession process. Identifying plant species around the nickel mining area needs to be done to determine the potential local species that will be used in the rehabilitation. Identification is conducted by taking an inventory of native plant species that grow naturally in the nickel mining area. After knowing what species grow around the nickel mining area, the next step is determining which local species will be used. Several considerations when determining suitable local species for rehabilitation of ex-nickel mining land, namely: (1) having the ability to grow in nutrient-poor soils and dry soils, high survival rates (high growth), and (2) good canopy. Spread and density, (3) as a biotic agent for wild animals (feed plants or nests), (4) availability of propagation material, (5) symbiotic with mycorrhizal fungi and nitrogen-fixing rhizobium bacteria to increase the supply of nutrients, establishment/development of vegetation cover, fast biomass accumulation, and good soil binding ability to prevent erosion, and (6) knowledge of plant propagation techniques (Rahmawaty, 2002; Elliott et al., 2006; Singh et al., 2002).

Selecting priority local species that are the most likely for nickel mining land rehabilitation can be done by collecting data in the form of tree species, the number of individuals for each species, and the classification of growth rates (seedlings, saplings, poles, and trees). The vegetation analysis data were then analyzed quantitatively to obtain the Important Value Index (INP). A high INP value illustrates that this species is adaptive to local site conditions (Adman et al., 2012). Vegetation analysis also provides an overview of what types grow in natural forests around ex-nickel mining and ex-nickel mining areas. The results of identifying these species are then selected to obtain the most suitable species for rehabilitating ex-nickel mines. Some of the criteria considered when selecting local species for rehabilitation of ex-nickel mining areas are paying attention to the habitus, habitat, ecological value, symbiosis with microorganisms, regeneration ability, and the ability to act as a nurse plant of the local species.

Habitus is the shape or stature of a plant species such as herbs, shrubs, or trees. The plant habitus used is shrubs and trees to rehabilitate the nickel postmining area. Some species of shrubs and trees have shorter life cycles and grow in open areas to be used as pioneer plants (Fig. 10.2). Meanwhile, tree species found in natural forests around nickel postmining areas have a longer life cycle, can grow under the shade (tolerant), and have the potential to be a climax species.

The condition of the habitat is also essential to know because it can be used as an indicator of the ability of a species to grow and develop according to the conditions of its site. Native plant habitats can be categorized into primary and secondary forests. The potential species used as climax tree species can be determined based on the species that grow in the primary forest, while pioneer tree species can be determined based on the species in the secondary forest. Tree species that grow first in the secondary forest can be indicated as potential pioneer species (intolerant). Identification of plants that naturally grow in the nickel mining areas in South Sulawesi and Southeast Sulawesi showed *Trema orientalis*, *T. kjellbergii* Burret, and *Callicarpa pachyclada* are the potential pioneer trees for the rehabilitation of nickel postmining areas. These species grow and colonize secondary forests (burned), fragmented open forest areas, and postmining areas that have undergone a natural succession process. Several species that grow and regenerate well in natural forests are *Podocarpus neriifolius*, *Metrosideros* cf. *petiolata*, *Canarium* sp., *Metrosideros* spp., *Ochrosia acuminata* Trimen, and *Elaeocarpus ganitrus*. These species can grow under shade (tolerant); hence they can be used as climax plants (Fig. 10.3).

One of the concerns in choosing local species for the rehabilitation of postmining land is the ability of a plant species to support and support the life of fauna or wildlife as the ecological value. Plants can have ecological importance as a source of food or a place to live, or a nest for fauna (host plant). For example, *P. neriifolius*, *Canarium* sp., and *O. acuminata* that are known to grow well in natural forests

FIGURE 10.2

The pioneer species in the nickel postmining area in North Konawe, Southeast Sulawesi. *Trema orientalis* (A), *Trichospermum kjellbergii* Burret (B), *Callicarpa pachyclada* (C), and *Casuarina sumatrana* (D).

From Mangopang, A. D. (2017). Jenis Pohon Lokal Potensialuntuk Revegetasi Lahan Bekas Tambang Nikel Di Konawe Utara, Sulawesi Tenggara. Prosiding Seminar Nasional Silvikultur V & Kongres Masyarakat Silvikultur Indonesia IV. Banjarmasin: Lambung Mangkurat University Press.

around nickel mining have ecological value as food sources for forest fauna. The ripe fruit of *P. neriifolius* (dark purple) is very popular with birds. Field observations found bite marks on the fruits of *Canarium* sp., *O. acuminata*, *Garcinia* sp, and *Baccaurea* sp. that fall on the forest floor and piles of seeds that have no flesh (Mangopang, 2017).

Another important criterion considered in the selection of local species is the ability of plants to symbiotically with microorganisms such as mycorrhizae and rhizobia. Research conducted by Prayudyaningsih et al. (2019) in the ex-mining area of PT. Stargate Pasific Resources, North Konawe, Southeast Sulawesi, found several pioneer species in the former nickel mine area colonized by the AMF, namely, *T. orientalis*, *Melastoma malabatrichum*, *Casuarina* sp, and *Sarchoteca celebica*. Meanwhile, from the former nickel mining soil in the same location, 12 rhizobium isolates were obtained (Sari & Prayudyaningsih, 2016). Singh et al. (2002) reported that the use of local legume species showed a more significant increase in soil fertility compared to legume species introduced from outside.

FIGURE 10.3

Climax tree species grow in the primary forest near nickel postmining in North Konawe, Southeast Sulawesi. *Podocarpus neriifolius* (A), *Canarium* sp. (B), *Elaeocarpus ganitrus* (C), and *Ochrosia acuminata* Trimen (D).

From Mangopang, A. D. (2017). Jenis Pohon Lokal Potensialuntuk Revegetasi Lahan Bekas Tambang Nikel Di Konawe Utara, Sulawesi Tenggara. Prosiding Seminar Nasional Silvikultur V & Kongres Masyarakat Silvikultur Indonesia IV. Banjarmasin: Lambung Mangkurat University Press.

The ability to regenerate is an integral part of the plant life cycle to maintain its existence. Better regeneration shows that a plant species can adapt to its environment. Conversely, environmental conditions can support its life cycle, starting from seedlings, becoming trees, flowering, fruiting, producing seeds, and growing back into seedlings. Plant regeneration ability can be determined by its growth rate (seedlings, saplings, poles, and trees). The more complete the growth rate, it can be said that the regeneration of the plant is going well. The total regeneration rate can give an idea of the more potent natural sources of seed or seedlings. In the North Konawe Lalindu Forest, South Sulawesi, it is known that there are five tree species with complete regeneration rates and the highest IVI, *P. neriifolius*, *Metrosideros* spp, *Calophyllum* sp, Hopea gregaria, and *G. subaequalis* Planch (Mangopang, 2017).

A nurse plant is the ability of a plant to facilitate or assist the growth of other plants. Indigenous species are the best nurse plant species in promoting the development of different species and have a better adaptation or resistance to climate change (Ren et al., 2008; Heidelberg et al., 2011). Local plants are also the best nurse plants in a degraded environment. Nurse plants work by creating a microhabitat that supports the growth of other plants, such as buffering extreme temperatures. The shading effect can avoid high temperatures, retain humidity, and control the transpiration of the target species. In addition, nurse plants can increase the availability of water, increase the availability of soil nutrients, and protect target species from herbivores so that, in the end, it can increase the survival of the target species (Ren et al., 2008).

The above criteria can be considered when selecting local species for rehabilitating nickel postmining areas. This method has been applied to determine species for the rehabilitation of nickel postmines in the Lalindu Forest, North Konawe Regency, Southeast Sulawesi. From the results of the research, it is known that 15 plant species have the potential for revegetation of nickel postmining areas, including *P. neriifolius*, *Casuarina sumatrana*, *Metrosideros* cf. petiolate, *Canarium* sp., *Metrosideros* spp., *O. acuminata* Trimen, *E. ganitrus*, *Buchanania arborescens*, Santeria celebica H.J.L., *Palaquium obovatum*, *Gnetum gnemon*, *Litsea acuminata* (Blume), *Trichospermum* cf. *monosperm* (Mangopang, 2017). The cultivation techniques of these local species need to be known to meet the availability of seeds for the rehabilitation of nickel postmining areas.

10.5 Conclusion

Rehabilitation of nickel mines through revegetation requires technology that can accelerate natural processes, especially natural succession. The applied technology inputs should use local components or resources so that, in the end, they can have an autonomous impact on the development of natural vegetation. The reintroduction of indigenous symbiotic soil microbes and the use of native plant species are expected to accelerate the growth of plants planted in revegetation for nickel mine rehabilitation and accelerate the establishment of a safe site. The assembling of nickel mine rehabilitation technology includes bioprospecting indigenous symbiotic soil microbes to obtain proven effective isolates for increasing plant growth and identification of potential local plant species for rehabilitation, as well as mastery of local plant species nursery techniques.

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