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Reginawanti Hindersah
Faculty of Agriculture, Universitas Padjadjaran

Noor Muhammad Mauludy
Faculty of Agriculture, Universitas Padjadjaran

Betty Natalie Fitriatin
Faculty of Agriculture, Universitas Padjadjaran

Toto Sunarto
Faculty of Agriculture, Universitas Padjadjaran

他

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Bacterial Properties, Carbon-Nitrogen Profile, and Plant Growth Potential of Limestone Mining Overburden

Reginawanti Hindersah[1], Noor Muhammad Mauludy[1], Betty Natalie Fitriatin[1], Toto Sunarto[1], Saon Banerjee[2]
[1]Faculty of Agriculture, Universitas Padjadjaran, Indonesia
[2]AIRCP of Agrometeorology, Bidhan Chandra Krishi Viswavidyalaya, India
Corresponding author email: reginawanti@unpad.ac.id

Abstract: Limestone mining generates an overburden that is unsuitable for plant growth. The research analyzed bacterial properties, organic carbon and total nitrogen status, and texture of overburdens compared to topsoil, and the effect of compost on corn growth in the greenhouse. Results showed that the overburdens had lower bacterial populations than the topsoil. 17 isolates revealed diverse colony properties and cell morphologies. Among them, 16 were Gram-negative, and one was Gram-positive bacteria; five rod-shaped and 11 cocci-shaped bacteria were found. Five isolates formed the endospores. Topsoil had higher organic-C and lower total-N, resulting in higher C/N than overburden. The acidity of overburden was slightly higher than topsoil. The topsoil texture was silt, while the overburden was sandy loam. The study indicated that overburdens were less fertile regarding bacterial population and C and N status. However, the high volume of compost amendment enhanced the ability of overburden in supporting plant growth.

Keywords: Bacteria, Carbon, Corn, Nitrogen, Topsoil

1. INTRODUCTION

Limestone, a crucial and irreplaceable component for the cement industry, is a calcareous rock with a calcium carbonate content ($CaCO_3$) of at least 70% of its weight. The unique parent material of limestone, rich in Ca^{2+}, is a defining characteristic of soils in karst landforms [1]. Despite their sensitivity to erosion due to steep slopes and thin soil, these karst areas often serve as vital aquifers due to their permeability. They form underground flows and springs, which are the primary sources of drinking and irrigation water [1,2]. The volume of limestone production in Indonesia, though relatively low compared to metal and sand-gravel-rock aggregate mining, remains a significant resource due to its consistent and indispensable demand in the cement industry. This unique characteristic of limestone and its importance in the cement industry makes the study of limestone mining overburden and its impact on soil properties and plant growth highly relevant and significant.

Opencast limestone mining destroys biodiversity due to land clearing and topsoil removal before the mine is excavated [3]. The process is followed by heaping the overburden, the material that does not meet the cement industry's requirements. Despite limited cement plants in Indonesia, limestone extraction from karst areas poses many problems, including revegetation issues on the overburden. Usually, the topsoil covering the mining area is mixed with overburden during limestone extraction. Topsoil – almost always referred to as O and A horizon - is the most fertile soil needed for plant establishment since it contains more plant nutrients and the population and diversity of organisms than the horizon below [4,5]. The land cover is critical for revegetation and further conservation of degraded and vulnerable areas [6].

Overburden is unsuitable for plant growth due to its low nutrient content, loose, adhered particles, and lack of soil characteristics [7]. However, pioneer and successor plants can grow on the surface of overburden dumping sites and ex-mine land [8,9,10]. The vast growing vegetation in the quarry area of limestone mining has been recorded in spontaneous revegetation rather than reclaimed areas [11]. Accelerating the accessor plant growth by optimizing the plant nutrient availability is required. Once the plants are growing, the soil surrounding and in the vicinity of plant roots, the rhizosphere contains root exudate, including fixed carbon (C) and nitrogen (N), as well as amino and organic acid, which serve as the nutrients for heterotrophic microbes [12]. In addition to the above-said problem, climate change is another menace to soil health. The global warming, which is almost invariable, accelerates the decomposition rate of organic matter, which in turn influences the water storage capacity, nutrient balance, and aggregate stability. Thus, the soil structure, fertility, productivity, and sustainability are threatened [13]. Moreover, climate change contributes to broader socioeconomic and human aspects [14].

Microbial communities in degraded areas are connected to soil properties and are indicators of the plant succession process in the soil. The microbes play an important role in soil functioning and relate to the initial stages of soil revegetation after mining [15]. Soil bacteria are relevant to the nutrient cycle in soil, which provides essential plant nutrients, mainly N and Phosphorus (P), and metabolites for plant growth [16,17]. On the other hand, the C and N content and its ratio determine the growth of bacteria. The C/N ratio of soil microbes is about 3/10 – 10/1, while the C/N ratio of soil is generally 10, in which the N is mobilized and available for plants [18]. They stated that OM with $C/N < 20$ decomposes quickly while OM with $C/N > 20$ demonstrates slowed-down decomposition and requires additional N to speed it up. Immobilization of N takes place when the soil has a high C/N.

Soil bacterial growth and population are related to Soil reaction [19]. The pH for optimal bacterial growth is neutral (7.2-7.6), but polyhydroxybutyrate-producing bacteria reach an accelerated growth phase at a pH of less than five [20]. Even though pH is an essential environmental factor for bacteria, pH alone might not determine soil bacterial diversity [21].

Revegetation of mine sites, including in the overburden area, is complicated and long due to the overburden's chemical and physical properties. Bacteria and other soil microbes are irreplaceable in supporting plant growth and becoming integral to ecosystem services [22,23]. Beneficial bacteria in soil have a prominent function in the nutrient cycle and organic matter regulation in the environment, but their presence and function are influenced by vegetation [24,25]. Organic matter is vital in improving soil porosity, organic-C to total-N ratio, and nutrient availability [26, 27]. Pioneer plant growth might be accelerated by organic matter application.

The cement industry in West Java, Indonesia, depends on limestone mining at the Gunungguruh of Sukabumi Regency. To date, revegetation in the overburdened area has not been conducted. Generally, the focus of revegetation of degraded areas is more on vegetation establishment with a limited explanation of the existence of bacteria in the growth substrates. This pre-revegetation study evaluated the bacterial properties, organic-C and total-N status, and texture of overburdens compared to topsoil near the mining area. Moreover, the study aimed to evaluate the effect of compost on corn growth in the greenhouse.

2. MATERIALS AND METHOD

The study was conducted in the limestone mining area of Tambang Semen Sukabumi (Sukabumi Cement Mine), a company of Siam Cement Group (Figure 1). This research was conducted from September to December 2021 using quantitative-qualitative-descriptive methods. The sampling location was in the Gunungguruh District, approximately 531 m above sea level, with a geographic location of -7.002237 South Latitude and 106.860659 East Longitude. The tropical land used before mining was a forest with natural vegetation of perennial teak (*Tectona grandis*) and pine (*Pinus merkusii*). Sufficient water availability can be indicated by fairly high rainfall in the study area. Rainfall in September, October, November, and December 2021 was 392, 396, 602, and 307 mm 2021, consecutively, and the total rainfall in 2021 was 3690 mm higher than 3240.6 in 2020.

Fig. 1. Limestone quarry of Tambang Semen Sukabumi Co (a) at Gunungguruh District, Sukabumi Regency, West Java (b). Source: a. Tambang Semen Sukabumi Co; b: Wikipedia Commons

2.1 Sample collection

Purposive sampling collected Soil samples from the limestone mining area's overburden and natural vegetation adjacent to the mine. The overburden materials were limestone, which did not meet the cement industry's minimum requirements. The local community cultivates the natural vegetation bordering the limestone quarry to grow vegetables in a limited area under perennial plants; this is such a modest agroforestry. The area of natural vegetation is located on a steeper slope.

Ten soil samples were collected from agroforestry and natural vegetation soil in the natural forest areas close to the overburden heaps area. The distances between the sampling points were approximately 7-10 m apart. The samples were mixed based on the visual characteristics of 0-30 cm deep topsoil and overburden. Before sampling, the soil and overburden surface were cleared of debris and growing plants. The composite method took all samples from 20 cm deep soil or tailing. Five sub-samples of each sampling point were mixed evenly. The weight of each sample was approximately 500 g. The sample was divided into two parts, each for nutritional profile and texture analysis and the other for microbiological analysis; the last was stored in a cool box before being transferred to the laboratory. Finally, four topsoil samples were collected from the natural forest (T1, T2, T3, T4), and another four samples were taken from overburden (O1, O2, O3, O4). The T1 and T2 were collected from annual food crops areas, while the T3 and T3 were taken from natural vegetation.

Before chemical soil properties analysis, all topsoil and overburden samples were mixed evenly. Two composited samples were then picked up from the topsoil and overburden samples for analysis.

2.2 Bacterial Enumeration and Colony Characterization

The bacterial population was counted using the Serial Dilution Plate Method [28] with non-selective media Nutrient Agar (5 g peptone, 3 g beef extract, 0.5 g NaCl, 15 g bacteriological agar, 1,000 mL distillate water, pH 7). The culture was stored at 30^{o}C for two days until bacterial colonies appeared. The enumeration was carried out in triplicate.

The visible colonies, regardless of colony size, were counted. The large colonies (> 2 mm in diameter were differentiated based on their diameter, color, shape, elevation, edge, and opacity. The colony features determination was referred to the Microbiological Society (2024). The colonies in each sample with similar characteristics were regarded as one isolate. The morphological cell purity of all isolates was determined under microscopes after staining with safranin; fresh, pure cultures were subjected to Gram and spore staining.

2.3 Gram and Spore Staining

Gram and Spore staining procedure followed the microbiological standard method [29]. Before staining, a full loop of liquid bacterial pure culture was heat-fixed on the slide. Cell wall morphology was aseptically determined by Gram staining using carbol gentian violet and fuchsin stain. At the same time, the spore staining followed the Wirtz Method with malachite green and safranin stain. Cell morphology was observed using light microscopes with 1,000 magnifications. Gram-positive bacteria will be purple in color, while Gram-negative bacteria will be red. The green color in the red vegetative cell indicated the Spores. The position of the spore in the cell was identified as terminal, subterminal, and central.

2.4 Topsoil and overburden chemical properties determination

The chemical properties of topsoil and overburden, including pH, organic C, and total N, were determined using the Association of Analytical Chemistry proximate analysis [30]. Meanwhile, the composition of their solid fraction (sand, silt, and clay) for texture determination was conducted using the International Soil Reference and Information Centre. All analysis was performed in duplicate. Each property's average and standard deviation were calculated and presented in tabular form.

2.5 Bioassay on corn seedling

Two seedlings of hybrid corn cv Pertiwi were grown in 200 g of overburden mixed with compost in the greenhouse for 21 days. The experiment was set up in a randomized block design with four treatments and six replications. The treatment was a volume ratio of overburden and composts comprised of 5:5, 4:6, and 3:7; the control treatment was overburden without compost. Before the experiment, the overburdens were grounded, sieved using an aluminum filter, and mixed with compost referred to the treatments. Two seedlings of hybrid corn cv Pertiwi were grown in 200 g of overburden mixed with compost in the greenhouse without fertilization. Plant heights were measured 14 and 21 days after sowing, while root heights were analyzed at 21 days. The total bacteria population was counted at 21 days by serial dilution plate method [28].

2.6. Statistical Analysis

The differences in bacterial populations in topsoil and overburden were statistically analyzed using the T-test Pearson at a significance level of 5% using the SPSS application. Data obtained for the corn-seedling bioassay were subjected to analysis of variance (F test; $p < 0.5$) and Duncan Multiple Range Test (DMRT) at $p < 0.5$. The means and standard deviation of data were presented in the histogram.

3. RESULTS AND DISCUSSION

Colony identification revealed that pure culture can be obtained from each soil/overburden sample. The characterization demonstrated that the A1, A2, and A3 samples comprised three pure isolate colonies, while the A4 sample had only one colony. In this study, each colony is suggested as one isolate. The purification of isolated was conducted by streaking a loop full of colonies on NA for three generations.

3.1 Bacterial Population

Table 1 shows the total bacterial count in each sampling point was different. The total bacteria population in the soil reached 10^{11} CFU/mL, while that in the overburden was only 10^{6} CFU/mL.

Table 1. Total bacterial Population in soil and overburden of limestone mining area

Sample Soil	Population (CFU/g)	Sample Overburden	Population (CFU/g)
T1[1]	2.7×10^{11}	O1	2.3×10^{6}
T2[1]	2.8×10^{11}	O2	1.8×10^{6}
T3[2]	3.1×10^{11}	O3	3.8×10^{6}
T4[2]	1.5×10^{11}	O4	1.7×10^{6}

[1]Topoil of vegetable area in the agroforestry; [2]Topoil of the natural vegetation

The average bacterial population in soil and overburden was 2.5 x 10^{11} and 2.4 x 10^{6}, respectively. The T-test ($p<0.05$) verified that the bacterial population of topsoil in the agroforestry and natural vegetation was not significantly different ($p = 1$). However, the bacterial count in the soil of natural vegetation is approximately 45% higher than that in the overburden ($p = 0$).

The soil is commonly rich in nutrients to support and boost bacterial proliferation, including the aerobic heterotrophic bacteria isolated by NA. Table 5 shows that the organic-C in overburden was lower than soil; organic-C is essential for energy production and C metabolisms of heterotroph. Comparing the soil solid fraction, the soil in this study was dominated by silt, but overburden was high in clay. The organic-C was the key factor in distinguishing the population difference since the soil N was quite similar, and their pH supported the growth of bacteria. The results of this study agree with those of research by Bárcenas-Moreno et al. (2011), which obtained bacterial population data in Mediterranean soil of 4.7×10^{7} CFU/g soil, while the bacterial population in Mediterranean soil with land use type for gardens showed data of $6.4\times10^{9} – 7.0\times10^{9}$ CFU/g soil [31].

The abundance of bacteria in soil or overburden cannot explain their function in supporting plant growth. Bacterial isolation used the general media (nutrient agar), which did not distinguish metabolic functions related to plant growth promoters such as N fixation, P solubilization, and phytohormone production.

3.2 Morphological Characteristics of Bacterial Colonies

Based on the morphological features of the colony grown in nutrient agar plates, ten bacterial isolates have been isolated from four natural vegetation soils and seven from overburden samples (Tables 2 and 3). The observations of colony characteristics obtained from soil and overburden samples varied from circular to irregular shapes; some colonies had flat elevations, convex to drop-like. The colony margins were undulate and entire, with a 0.3 mm to 2 mm diameter. The colors of the colonies were clear, yellowish-white to yellow, and had translucent and opaque opacity (Tables 2 and 3).

The colony morphology of each colony was different (Figure 2). The morphology of the Colony is an essential indicator of phenotypic variation; colony shape is a bacterial adaptation process to environmental changes and stress [32]. Bacteria that grow on the surface of agar form colonies, which are important for identifying genera or even species [32]. Recognizing typical colony morphologies for certain genera is crucial due to the diverse media during colony feature identification. For example, a typical *Bacillus subtilis* is a rod in shape and Gram-positive and shows a circular, rough, opaque, fuzzy white or slightly yellow with irregular edges colony on nutrient agar [33].

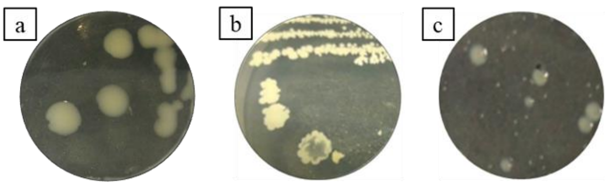


Fig. 2. Colony characteristics based on opacity; a. Isolate O3-2 (Translucent), b. Isolate S4-1 (Opaque), c. Isolate S3-1 (Transparent)

3.3 Morphological Characteristics of Bacterial Cells

The bacteria isolated from topsoil were more heterogeneous compared to overburden. Most isolates were Gram-negative, but S4-1 isolated from overburden was Gram-positive (Table 4; Figure 3). Bacilli and cocci bacteria have been isolated from soil, while the morphology of all bacteria isolated from overburden was cocci (Table 4). All five bacilli-shaped bacteria, including S1-1, S1-2, S2-1, and S3-1, isolated from the topsoil were spore-forming. Meanwhile, all bacteria isolated from overburden were cocci in shape, Gram-negative, and non-spore-forming (Table 4).

Table 2. Colony characteristics of bacteria isolated from the topsoil

Samples Code*	Isolate	Diameter (mm)	Colony Characteristic				
			Form	Elevation	Margin	Color	Opacity
T1	T1-1	2	Irregular	Flat	Undulate	Yellowish white	Translucent
	T1-2	2	Circular	Raised	Entire	Yellow	Opaque
	T1-3	1	Circular	Raised	Entire	Yellowish white	Translucent
T2	T2-1	2	Circular	Convex	Entire	Yellow	Opaque
	T2-2	1	Circular	Raised	Entire	Yellowish white	Translucent
	T2-3	2.5	Irregular	Raised	Entire	Yellowish white	Translucent
T3	T3-1	2	Circular	Raised	Entire	Yellowish white	Transparent
	T3-2	1	Circular	Raised	Entire	Yellowish white	Translucent
	T3-3	5	Irregular	Flat	Undulate	Yellowish white	Opaque
T4	T4-1	1	Circular	Raised	Entire	Yellowish white	Translucent

*T1 and T2: topsoil of vegetable area in the agroforestry; T3 and T4 topsoil of perennial natural vegetation

Table 3. Colony characteristics of bacteria isolated from the overburden

Sample Codes	Isolate	Diameter (mm)	Colony Characteristics				
			Form	Elevation	Margin	Color	Opacity
O1	O1-1	2	Circular	Raised	Entire	Yellowish white	Translucent
	O1-2	3	Circular	Raised	Undulate	Yellowish white	Translucent
O2	O2-1	3	Circular	Raised	Entire	Yellowish white	Translucent
	O2-2	4	Circular	Raised	Undulate	Yellowish white	Translucent
O3	O3-1	4	Circular	Raised	Entire	Yellowish white	Opaque
	O3-2	2	Irregular	Flat	Undulate	Limpid	Translucent
O4	O4-1	3	Circular	Raised	Entire	Yellowish white	Opaque

Table 4. Results of Gram and spore staining of bacterial pure culture isolated from topsoil, and overburdens of limestone mining

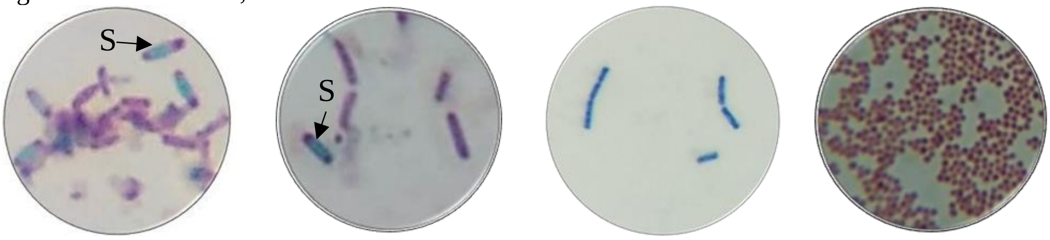
Sample	Isolate	Morphological Properties		
		Gram	Cell form	Spore
T1	T1-1	-	Bacilli	+ (Central)
	T1-2	-	Bacilli	+ (Central)
	T1-3	-	Cocci	-
T2	T2-1	-	Bacilli	+ (Central)
	T2-2	-	Cocci	-
	T2-3	-	Cocci	-
T3	T3-1	-	Bacilli	+ (Central)
	T3-2	-	Cocci	-
	T3-3	-	Cocci	-
T4	T4-1	+	Bacilli	+ (Central)
O1	O1-1	-	Cocci	-
	O1-2	-	Cocci	-
O2	O2-1	-	Cocci	-
	O2-2	-	Cocci	-
O3	O3-1	-	Cocci	-
	O3-2	-	Cocci	-
O4	O4-1	-	Cocci	-

Soil organic-C is the key element for the metabolism of heterotrophic bacteria isolated in this study. The loss of natural vegetation and topsoil replacement from mining areas are associated with the severe reduction of organic matter and usually nutrients from the root zone. Therefore, the topsoil contained a higher bacterial population (Table 1) and more heterogeneous bacteria (Table 2) than the overburdens. The absence of spore-forming isolates in the disturbed overburden might indicate that bacteria's community structure might change due to C and nutrient sufficiency. This research agrees with the abundance of viable bacteria in forest soil in the limestone mine of Siam Cement Group in Lampang Province, northern Thailand [34]; the bacteria richness (OTU; operational taxonomic unit) in the forest area was higher than in the mining area. The gram-positive and cocci-shaped bacterial and gram-negative bacilli cells were found in the Palimanan limestone mining area [35]. Nonetheless, further identification of biochemical and plant-growth-related properties of bacteria is required.

3.4 Soil and Overburden Nutrient Profile

Table 5 demonstrated that the soil reaction was slightly acidic, but the overburden acidity was neutral; the soil's organic C was clearly higher than the overburden's. The organic-C content of soil in agroforestry and natural vegetation were apparently similar, but the total N of soil in natural vegetation was higher, which caused a lower C/N ratio. The soil in the vegetable area had high organic

C and slightly lower total N, resulting in a higher C/N ratio. The intensive use of animal manure might cause higher C/N in agricultural areas; belowground parts of crops contribute to the increase in organic matter in the soil. The C/N ratio of overburden varied from one sample to another due to different content of organic-C. Despite the lower organic matter content, the overburden has a higher total N, resulting in a lower C/N ratio. High rainfall before and during the study might contribute to increased levels of total N in overburdens; moreover, the successor plants grown on the surface of overburdens can supply the N through the root exudation mechanism and mineralization of die roots.



Spore-forming and Gram-negative T2-2 | Spore-forming and Gram-negative T3-1 | Gram-positive S4-1 | Gram-negative O2-2

Fig. 3. Bacterial morphology isolated from the topsoil of annual food crops (T2-1) and natural vegetation area (T3-1 and T4-1); and overburdens of limestone mining (O2-2); S: Spore

Table 5. Acidity, organic carbon, total nitrogen, and texture of soil and overburden in the limestone mining area

Sample	pH	Organic N (%)	Total N (%)	C/N	Texture
Topsoil	6.00 ± 0.003	3.63 ± 0.05	0.26 ± 0.014	14.11 ± 0.57	Silt loam
Overburden	6.72 ± 0.007	1.76 ± 0.02	0.29 ± 0.008	5.97 ± 0.26	Sandy loam

Means ± standard deviation were calculated from four replications.

The C to N ratio is important for growing and establishing heterotrophic microbial communities since they need a good composition of C and N to remain active. The C/N ratio that induces heterotroph activity is 25-30; high C/N leads to long organic matter decomposition and N mineralization, and lower C/N ratio causes N immobilization and N loss from the roots zone [36]. The N availability for root uptake of soil under pine stands differs greatly from agricultural land. However, the N in overburden will be the critical limiting factor for revegetation. Higher C/N in the topsoil of the study area requires additional N to lower the C/N and further induce vast organic matter decomposition without N immobilization. Farmers in this study area used manure to cultivate vegetables in an agroforestry system; the N fertilizer application is suggested to lower to C/N.

The availability of nutrients in the soil, such as organic matter and N, is essential for the growth and development of microorganisms. The slightly acidic to neutral pH in the topsoil ranges from 5.75 – 6.66, while in the overburden, it ranges from 6.46 – 6.86, considered neutral. The pH in topsoil, especially in agricultural land, is lower than in overburden due to fertilizer application by farmers. The NPK fertilizer added to the soil can reduce the soil pH because the concentration of H^+ ions will increase due to the hydrolysis of ammonium in the soil [37].

The solid fraction of topsoil was dominated by silt (Table 5) with a particle size of 0.002-0.05 mm; silty soil is ideal for plant growth, including vegetables. However, erosion might occur mainly in sloped areas where the soil samples were collected. The sand fraction that makes up the overburden also influences the availability of organic material and other nutrients. Physical analysis of the overburden showed that the sand fraction content in the overburden samples averaged 62.1%, the dust fraction was 34.7%, and the clay fraction was 3.2% (Table 5). The high sand fraction causes increased infiltration with the potential for leaching of nutrients due to high porosity. Therefore, the bacterial population in overburden is lower compared to topsoil.

3.5 Effect of overburden-compost Composition on corn growth

The shoot height of corn plantlets grown in overburden with a low compost content (A and B) decreased compared to the control plant, but the growth media composed of high content of compost (C) increased shoot height at 14 days (Figure 4). At 21 days, the increase in shoot height was only shown by corn grown in mixed of overburden and compost with a ratio of 3:7. The root length of corn sown in the overburden mixed contained 60%, and 70% volume of compost (B and C) were significantly higher than the control. Along with shoot height, the root lengths of corn in this experiment were highest in the substrate composed of 30% overburden and 70% compost.

The growth of corn in the overburden during three weeks was remarkably improved due to a higher concentration of compost. The shoot height increment of that treatment was approximately 60% compared to the control and was caused by better root growth. In this study, compost amendment improved the overburden porosity, resulting in better growth of roots, but the effect depended on their amount in the overburden-based substrate. Organic matters have a multi-functioning effect on soil; they increase the C/N of soil, stimulate the N and P mineralization, and further increase their availability [26]. Improved soil porosity related to the root abundance due to organic matter application was reported [27]. The short-time greenhouse experiment verified that organic matter is critical for supporting plant growth. A huge amount of organic matter is needed to increase corn growth.

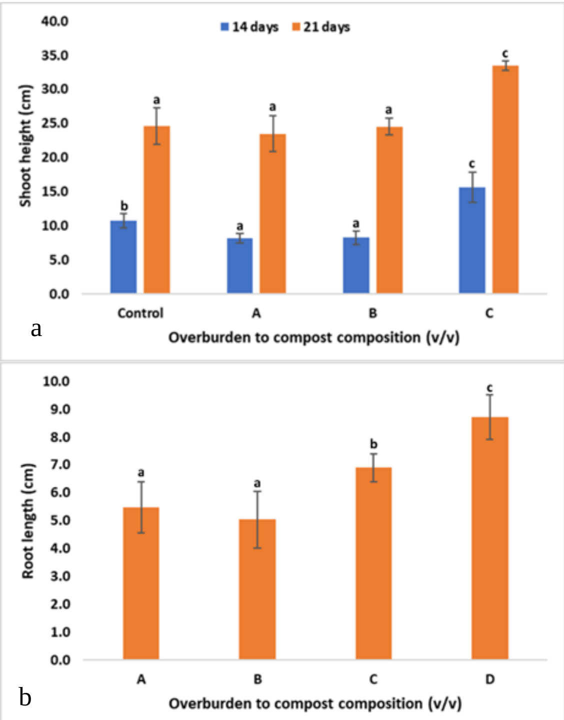


Fig. 4. The shoot height of 14 and 21-day-old corn (a) and root length at 21 days (b) in various volume compositions of overburden (OB) to compost (C). Control: overburden; A: OB/C = 5/5, B: OB/C = 4/6, C: OB/C = 3/7. The values of columns with similar letters above were not significantly different based on DMRT at $p<0.05$

4. CONCLUSION

The population and diversity of soil bacteria in the overburden are lower than in the topsoil because the overburdens have lower organic C. The bacteria in the topsoil were more diverse than the overburden area in terms of their cell wall characteristics and endospore formation. The topsoil and overburden show different organic-C, total-N, and C/N profiles and textures. Overburden has a low C/N due to the lower organic matter and slightly higher N. The solid fraction of topsoil is dominated by silt, but a higher sand fraction occurs in the overburden, resulting in a coarser texture. The acidity of topsoil and overburden were slightly acidic and neutral, respectively. The application of a higher concentration of compost on overburden increased the growth of 21-day-old seedlings in the greenhouse experiment. The study's limitation is that soil and overburden were only collected once, so a long-term assessment should be conducted in both areas. The function and community structure of bacteria have not been analyzed, which is an important focus of further research. Moreover, the bioassay only analyzed the effect of organic matter and limited plant growth; further comprehensive analysis is needed.

5. ACKNOWLEDGMENT

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