Spatial variation of soil bacteria communities and its alpha diversity as a potential bioindicator of land degradation

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Abstract

This study aimed at determining the community structure and diversity of soil bacteria in several land-use changes as an environmental bioindicator. This research was conducted in areas of intensive agriculture (PI), monoculture abandoned old-coffee plantation (KTT), mixed-young coffee plantation (HLS), and secondary forest/reference site (RS) in UB Forest (UBF) area, Malang, Indonesia. Soil samples were taken as a composite at three different points in each area using a soil ring at a depth of 0-20 cm. The 16S rRNA gene was used to determine the community structure, species richness, diversity, and ecological index (Chao1, Shannon, Simpson, ACE) of soil bacteria using the NGS approach. Statistical data were analysed using R and QIIME software. The community structure of soil bacteria at the phylum level displayed the same pattern in all study sites where Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi were the dominant groups. Conversely, the bacterial composition showed differences between study sites at the genus level. Alpha diversity in agricultural areas (PI, KTT, and HLS) was higher than forest area (RS), but it was not followed by bacterial beta diversity. The distinct soil bacteria composition and diversity were influenced by the physicochemical of soil properties in the studied area. Therefore, several bacterial taxa suggested being a potential bioindicator of forest soil degradation due to land-use change in this study. Soil bacterial indicators can be utilized to evaluate or monitor alteration of soil quality in terms of forest restoration or rehabilitation.

Keywords:
alpha diversity
land-use change
soil bacteria community

Introduction

Native tropical forests are regions with a higher degree of biodiversity than other areas of the Earth's surface. However, along with the rising needs of human life, the transformation of forests into the number of land-use changes is also rapidly increasing. Wilcove et al. (2013) stated, this transformation of forests is becoming a major challenge to the survival of Asia’s tropical rainforests and elsewhere. Land-use change is the leading cause of biodiversity loss in tropical areas, either above or below ground biodiversity, which then affects ecosystem functioning due to unsustainable land use (Cai et al., 2018). This situation, therefore, has a detrimental effect on the deterioration of the quality of natural forest ecosystems. Forest conversion is also
the critical factor responsible for the alteration of soil conditions. As is known, the soil is a habitat for different forms of biota, with all its roles supporting ecosystem functions in the forest (Meng et al., 2019).

The soil bacteria are the fundamental pillar of the biosphere and are accountable for the stability of various essential processes in the ecosystem (Hendershot et al., 2017; Liu et al., 2020). Soil bacteria contribute to fundamental ecological processes such as decomposition, greenhouse gas regulation, xenobiotic compound breakdown, biogeochemical nutrient cycles, and plant growth (Sengupta et al., 2020). Soil bacteria are organisms that are susceptible to changing environmental conditions. There is no question that alterations in soil conditions affect microbial communities' composition in the soil. Some studies have been reported by Jangid et al. (2011), Cai et al. (2018), Meng et al. (2019), Wu et al. (2019), and Flores-Renteria et al. (2020) in either tropical or subtropical forest areas. Several former studies have shown that land-use activities can impact soil bacteria populations as a result of alteration in soil characteristics (Tripathi et al., 2012; Rodrigues et al., 2013; Tripathi et al., 2017). Vegetation composition changes can also influence soil bacterial communities through root activity, litter input, and microclimate fluctuations. Leaf litter encourages nitrogen mineralization, and root exudates influence a carbon and nitrogen cycle by nitrifying microorganisms in the rhizosphere, potentially altering the community of microbes in the soil (Haichar et al., 2014; Meng et al., 2019).

The soil bacteria form complex community structures with a very high diversity which demonstrates the resilience of the environment in supporting ecosystem functions. Changes in soil bacteria composition during land modification are closely linked to the changes in the environment's functioning (Li et al., 2018). As a sensitive predictor of soil change and disturbance, the soil bacteria population is highly influenced by edaphic factors (pH, nutrition) (Tripathi et al., 2012), land-use intensity (Jesus et al., 2009), climate (Yao et al., 2017), or changes in vegetation populations (Guo et al., 2016).

A number of studies have been carried out to see how land-use type affects the soil bacteria composition and diversity in the environment. To date, such assessments are carried out with a more sophisticated use of molecular or metagenomic approaches. The use of particular genes, such as 16S ribosomal RNA (rRNA), is a powerful tool for a clear explanation of ecological phenomena. It has been proposed that sequencing of the 16S rRNA gene is a possible approach for discovering the composition and diversity of soil bacteria. This technique has been used to examine soil bacterial communities in many studies (Miyashita et al., 2013; Meng et al., 2019). In prokaryotic cells, 16S ribosomal RNA (rRNA) is a part of the 30S small ribosomal subunit and comprises nine hypervariable regions (V1-V9) of approximately 30-100 base pairs (bp) in length that are involved in the small ribosomal subunit's secondary structure. The conservation degree greatly diverse among hypervariable regions, with more conserved regions attributed to higher-ranking taxonomy and less conserved regions attributed to lower-ranking taxonomy, such as the genus and species. 16S rRNA can also be used for the reconstruction of bacterial phylogenies and classifications. The 16S gene sequence variation is commonly used to classify different microbial communities. For taxonomic classification, the sequence of individual hypervariable regions is appropriate instead of the whole genome. 16S rRNA amplicon sequencing technology is a prevalently beneficial tool for analysing the composition and microbial population structure of environmental samples. Furthermore, conservative regions make it possible to design universal primers. 16S rRNA amplicon sequencing typically chooses one or more hypervariable regions, designs universal primers for PCR amplification in conservative regions, and then conducts sequencing analysis and detection of microorganisms in hypervariable regions (Caporaso et al., 2011; Youssef et al., 2009; Hess et al., 2011).

Soil bacterial populations are responsible for a variety of ecosystem functions. The abundance, richness, and composition of soil bacteria are all affected by land use activities and management (Delgado-Baquerizo et al., 2016). Changes in population or bacterial activity can reflect changes in soil physicochemical properties, providing an early indication of improved soil quality or early warning of soil modification. As potential biological indicators representing environmental conditions, bacterial taxa with different relative abundance trends can be suggested (Lee et al., 2020). Recent research by Hermans et al. (2017) has found that microbial communities from various soil types in New Zealand are vulnerable to evolving soil conditions rather than changes in the climate or increased geographical heterogeneity. The use of the bacterial community's composition or the abundance of individual taxa as indicators of changes in the soil environment has a great promise. Recent advancements in next-generation sequencing (NGS) technologies have made a feasible and appealing research path, leading to the suggestion that bacterial population data can provide alternate environmental health metrics (Hermans et al., 2017). Bacterial communities are progressively being considered as more sensitive and consistent indicators of soil quality alteration than biochemical parameters (Garbisu et al., 2011).

Bacteria are the most ubiquitous and diverse microorganism groups and play a variety of essential roles in soils. Soil bacterial diversity is an essential component that represents the stability and quality of the soil environment and has the ability to regulate
many ecological processes. Therefore, a study on soil bacterial diversity and community structures is critical for evaluating soil quality. This research was conducted to elucidate the land-use change effect on soil bacteria populations in the lower mountainous tropical forest region and identify the correlation between soil bacteria communities and soil environment. In this study, variations in environmental conditions from different areas were also discovered in soil properties. The metagenomic approach was used to identify, analyse the community structure and diversity of soil bacteria, also determine potential bacterial indicators for soil environmental change in the study area.

Materials and Methods

Study area

This study was performed in a special purpose forest area of the Universitas Brawijaya Malang, Indonesia, known as UB Forest (UBF). This location is situated on the slopes of Mount Arjuno, East Java, mainly composed of pine and mahogany forest; other small areas are still covered by natural/protected forests. Local people cultivate coffee, vegetable crops with agroforestry systems under pine or mahogany tree. In this study, the location chosen is a protected forest area that had been fragmented due to land clearing by the local community. The areas used are intensive agriculture (PI), abandoned coffee plantation/old-coffee plantation (KTT), mixed young-coffee plantation (HLS), and secondary forest/reference area (RS) (Figure 1). The PI area is an intensive agricultural land cultivated by local people with vegetable products, such as carrots, mustard greens, and chilies with no other tree stands. The KTT area was an abandoned monoculture coffee plantation of >5 years of age where only one tree was located here, namely trete (Microcos tomentosa) with >90 cm in diameter. The HLS area was a degraded, fragmented forest mixed with coffee plants (Coffea sp.) <5 years of age, serai (Cymbopogon citratus), and several other trees stand in this zone, such as jackfruit (Artocarpus heterophyllus), avocado (Persea americana), gamal (Gliricidia sepium), and bamboo. Meanwhile, the RS was an undisturbed secondary forest in the UBF region which was ecologically better than the other areas. Any forest tree stands can be found here, such as Ficus sp., pasang (Lithocarpus sp.), trete (M. tomentosa), Saurauia (Actinidiaceae), Erythrina sp., and others. These locations were restoration model areas for enrichment planting using various local tree species of natural forest.

![Figure 1. Location of area study in UB Forest (UBF) Malang, East Java. PI = intensive agriculture, KTT = abandoned coffee plantation/old-coffee plantation, HLS = mixed young-coffee plantation, RS = secondary forest/reference area.](image-url)
**Soil sampling**

Information on soil physicochemical properties in the studied area was obtained from our previous study (Yusuf et al., 2020). At each study site, soil samples were obtained from three different points. The soil sampling was conducted in a composite manner using a soil ring at 0-20 cm depth from the soil surface. Soil samples were immediately transported to the laboratory for measurement and other analytical purposes. Subsequently, soil samples were separated into two sections for soil properties and bacteria communities analysis. The soil properties observed included soil bulk density (BDst), soil aggregate (AggrS), water content (Moist), pH, organic matter (SOC), and electrical conductivity (EC). For soil bacteria analysis purposes, soil samples from three different points were then combined so that there was just one sample from each site. A total of 500 g of soil per site was tested in the laboratory for processing. The results of the measurement and calculation of soil properties are shown in Table 1.

| Site | BDst   | AggrS  | Moist  | pH      | EC      | SOC    |
|------|--------|--------|--------|---------|---------|--------|
| PI   | 0.63 ± 0.015<sup>b</sup> | 4.27 ± 1.17<sup>a</sup> | 25.48 ± 7.517<sup>a</sup> | 6.01 ± 0.391<sup>ab</sup> | 0.25 ± 0.172<sup>b</sup> | 6.24 ± 0.368<sup>a</sup> |
| KTT  | 0.61 ± 0.014<sup>b</sup> | 2.93 ± 0.513<sup>a</sup> | 36.23 ± 4.999<sup>a</sup> | 6.24 ± 0.153<sup>b</sup> | 0.14 ± 0.018<sup>b</sup> | 9.06 ± 0.749<sup>b</sup> |
| HLS  | 0.63 ± 0.068<sup>b</sup> | 3.28 ± 0.301<sup>a</sup> | 31.84 ± 25.054<sup>a</sup> | 5.87 ± 0.186<sup>a</sup> | 0.11 ± 0.027<sup>a</sup> | 7.70 ± 2.302<sup>a</sup> |
| RS   | 0.43 ± 0.010<sup>a</sup> | 4.17 ± 0.513<sup>b</sup> | 58.47 ± 9.479<sup>b</sup> | 6.79 ± 0.110<sup>a</sup> | 0.19 ± 0.025<sup>b</sup> | 8.22 ± 0.523<sup>b</sup> |

Note: BDst (bulk density g cm<sup>3</sup>), AggrS (soil aggregate), Moist (soil water content %), pH (soil acidity), EC (soil electrical conductivity), and SOC (soil organic content %). Values indicate mean ± SD, and different superscript letters indicate a significant difference at the p <0.05. PI = intensive agriculture, KTT = abandoned coffee plantation/old-coffee plantation, HLS = mixed young-coffee plantation, RS = secondary forest/reference area.

**Soil bacteria DNA extraction and sequencing**

*Genome DNA extraction.* CTAB/SDS was used to extract the whole genome DNA of soil samples. In 1% agarose gels, the DNA concentration and purity were determined. Using sterile water, DNA was diluted to 1 ng/µL depending on the concentration.

*Amplicon generation.* 16S rRNA/18S rRNA/ITS genes from various regions (16SV4/16SV3-V4/16SV4-V5, 18S V4/18S V9, ITS1/ITS2, Arc V4) were amplified with a particular primer (e.g. 16S V4: 515F-806R, 18S V4: 528F-706R, 18S V9: 1380F-1510R). Then, the PCR reactions were performed with Phusion® High-Fidelity PCR Master Mix (New England Biolabs).

*PCR product quantification and qualification.* The PCR products with the same amount of 1X loading buffer (SYB green) were mixed then run on 2% agarose gel electrophoresis for detection. For further analysis, samples with a bright main strip between 400 and 450 bp were selected.

*PCR products mixing and purification.* To purify the mixed PCR products, the Qiagen Gel Extraction Kit was used. The libraries created by the NEBNext® UltraTM DNA Library Prep Kit for Illumina and quantified by Qubit and Q-PCR would be analysed by the Illumina platform.

**Data analysis**

**Soil bacteria genomic analysis**

There was a certain amount of "dirty data" in the raw data collected by sequencing. In order to make the results of analysis more precise and consistent, the raw data must be combined and filtered for the purpose of obtaining clean data. The clustering of Operational Taxonomic Units (OTUs) was then carried out on the basis of the effective data. According to the OTU clustering findings, a taxonomic annotation was made for each OTU representative sequence to obtain the related tax details and the taxonomic distribution of the abundance. Simultaneously, OTUs were analysed for alpha diversity, beta diversity, Venn diagram to obtain information regarding soil bacteria richness and evenness in the samples, common and specific OTU information among different samples or groups. The complexity of the sample heterogeneity was analysed across five indices to determine alpha diversity, including Observed-Species, Chao1, Shannon, Simpson, and Abundance-based Coverage Estimator (ACE). These indices were measured with QHIME software and shown with the R program. Soil bacteria beta diversity was analysed to determine the variations in the species complex of soil samples. Beta diversity is an intentional comparison of bacterial communities according to bacterial composition. Beta diversity metrics are used to measure variations between bacterial communities.

To compare bacterial communities among each pair of population samples, a distance square or dissimilarity matrix was determined to represent the difference among samples, for instance, Weighted and Unweighted UniFrac distances (Lozupone and Knight, 2005; Lozupone et al., 2007; Lozupone et al., 2011). The UniFrac distance, a web-based tool that helps researchers in answering a number of complex problem about the bacterial species structure and evolution, uses phylogenetic data to assess whether
groups were substantially contrasting and shows different patterns for a variety of environmental samples (Lozupone et al., 2006; Hamady et al., 2010; Lozupone et al., 2011). This method has commonly applied to analyze large sets of data produced by NGS and QIIME analysis (Sun et al., 2020). In this research, beta diversity for both Weighted and Unweighted UniFrac was measured using QIIME software. A principal component analysis (PCA) was also performed using XLSTAT software and used to examine the relationships between edaphic factors and dominant bacterial groups. Analysis of Pearson correlation was also applied to evaluate relationships between bacterial abundance and soil properties. For investigating potential bioindicators of individual taxa, indicator value (IndVal) analysis was conducted in this study. Dufrene and Legendre (1997) and Figuerola et al. (2012) stated that an indicator value could be used for detecting the relationship between biological taxa and land or soil management. The indicator value (IndVal) was calculated by combining the OTUs abundance of OTUs in the target group compared to other groups (called specificity) and their relative frequency of occurrence in that group (called fidelity). The IndVal index was calculated using IndVal function in the ‘labdsv’ package of R software.

Results and Discussion

Spatial variation of soil bacteria community structure

The distribution of soil bacteria populations at the phylum level revealed a similar pattern of spread. Out of 42 bacterial phyla, ten dominant soil bacteria groups were identified, including Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Nitrospirae, Firmicutes, Bacteroides, Latescibacteria, and Verrucomicrobia (total relative abundance >95%). Although the distribution patterns of soil bacteria were identical, the composition of their constituents displayed a different trend, particularly in the ten largest phyla. Phylum Proteobacteria had the highest abundance in each area, particularly in the PI area, with an abundance of up to 50%. Second, Acidobacteria had an overwhelming relative abundance after Proteobacteria at PI and KTT sites with 23% and 32%, respectively. In comparison, a different situation was found in the HLS and RS areas where Actinobacteria had the second-largest abundance after Proteobacteria with 28% and 30%, respectively (Figure 2A).

Proteobacteria is a group of bacteria typically found in soil and have several members. Species of this phylum were known as the main functional bacteria for litter decomposition and transformation (Mander et al., 2012; Huang et al., 2016). Acidobacteria is a group of bacteria commonly present in soils with low acidity (low pH) (Jones et al., 2009). Conversely, the findings of this analysis confirmed a distinctly different matter where the Acidobacteria relative abundance was found to be higher in areas with elevated soil pH (PI and RS). The findings of this study were also similar to those stated by Sengupta et al. (2020) that several Acidobacteria subgroups did not present in soils with low pH caused related to broad metabolic and physiological adaptations. Except for Proteobacteria and Acidobacteria, the relative abundance of Actinobacteria exhibited a significant difference in unmanaged soil (RS) and was lower than the abundance in managed soils in this study. The difference in Actinobacteria abundance between land-use types was related to soil organic content and water content changes. Besides, land-use practices, like soil plowing in PI or KTT area, might be a potential factor that caused decreasing Actinobacteria abundance there.

In line with Sengupta et al. (2020), plowing activities affected low residue input in the soils, decreasing food sources for bacterial growth demands. Actinobacteria are a community of bacteria that play a crucial part in soil litter decomposition (Kopecky et al., 2011), induce plant growth promoters, biogeochemical nutrient cycles, increase abundance of nutrients (Bhatti et al., 2017; Zhang et al., 2019). More than 430 bacterial genera have been identified in this study. Ten dominant bacterial genera had a cumulative relative abundance of <20% at each site, such as Massilia, Methylophilus, Pedomicrobium, Varribacter, Sphingomonas, RB41, Bradyrhizobium, H16, Nocardiooides, and Ramlibacter. However, the soil bacteria community structure showed compositional variations. The PI area had the most different soil bacteria composition relative to other locations (KTT, HLS, and RS). The Massilia, Methylophilus, Sphingomonas, and RB41 genera were co-dominated in the PI area, whereas N-fixing bacteria groups have been more generally found in KTT, HLS, and RS areas such as Pedomicrobium, Varribacter, and Bradyrhizobium. The N-fixing bacteria abundance appeared to increase with less soil disturbance (PI < KTT < HLS < RS) (Figure 2B). The findings indicated that changes in land use could affect soil bacteria composition in the genus-level populations, which was not different from other studies by Vitali et al. (2016), Meng et al. (2019), Sun et al. (2020). As a result of land-use change, a new community would be formed with a different composition from the native states. In Figure 3, the composition of soil bacteria in soil-disturbed areas (PI, KTT, HLS) formed a new trend relative to areas with limited soil disturbance (RS). This was strongly supposed due to differences in soil conditions in these areas, such as soil bulk density, water content, organic content, and pH. Some bacteria groups that are abundantly found in the RS area did not exist in a small number at other areas like genus Pedomicrobium, Varribacter, Bradyrhizobium, and Nocardiooides. While, genus Massilia, Methylophilus, Sphingomonas, and RB41 were mainly found in...
disturbed soil areas. This information indicated that these bacterial groups could act as the differentiator between areas; therefore, this might be potential as bioindicators in the environment due to different land-use types. Environmental conditions at the soil surface critically affected the life of soil bacteria. Changes in vegetation and poor soil management might contribute to changes in the bacterial soil population so as to have an effect on changes in the functioning of ecosystem services.

Figure 2. Relative abundances of soil bacteria in phylum (A) and genus (B) level at each area. PI = intensive agriculture, KTT = abandoned coffee plantation/old-coffee plantation, HLS = mixed young-coffee plantation, RS = secondary forest/reference area.
Soil bacteria diversity

Alpha diversity, including Chao1, Shannon, Simpson, and the effective number of species indices, defines species diversity in a single sample. Neither effective species number and Chao1 indices imply species richness regardless of each species abundance in the population. Index of Shannon and Simpson describe the species richness and evenness in the community. The soil bacteria species richness was found more in the HLS area, followed by KTT and PI, while RS had the lowest soil bacteria species richness. The same trend was also found in the results of the ACE index. The bacteria alpha diversity in RS was the lowest compared to the other three locations, although the diversity of Shannon (richness) was relatively high (>8). The evenness of the bacteria in the sample area was not different; this was demonstrated by the Simpson index value, which was relatively the same (Table 2).

Table 2. Alpha diversity of soil bacteria communities at each area.

| Site | Observed Species | Chao1  | ACE      | Shannon | Simpson |
|------|------------------|--------|----------|---------|---------|
| PI   | 2981             | 3193.588 | 3220.685 | 9.215   | 0.995   |
| KTT  | 3316             | 3494.979 | 3506.519 | 9.612   | 0.996   |
| HLS  | 3451             | 3605.801 | 3634.892 | 9.878   | 0.997   |
| RS   | 2394             | 2424.102 | 2478.877 | 8.833   | 0.993   |

Note: PI = intensive agriculture, KTT = abandoned coffee plantation/old-coffee plantation, HLS = mixed young-coffee plantation, RS = secondary forest/reference area. ACE = Abundance-based Coverage Estimator.

Land-use activities, such as terrace, fertiliser application (Fitria and Kurniawan, 2021), and utilizing machines for crop management could modify the soil physicochemical properties, consequently altered the diversity of bacteria in the studied area. This finding was corresponding with another study conducted by Bissett et al. (2011) and Suleiman et al. (2013). According to Zhang et al. (2016), newly opened areas
would be populated by opportunistic species, accompanied by increased species diversity as cumulative resources rise. As the ecosystem matures, strong competitors might come to dominate, resulting in a reduction in species richness. Anthropogenic interference in land-use management might modify or increase the soil bacteria communities diversity. The soil fertilisation regime in the non-forest areas (PI, KTT, and HLS) was expected to affect the abundance of nutrient resources that supported more microflora growth.

To demonstrate the similarities and differences among the bacterial communities in different areas, we used a Venn diagram with shared and specific OTUs. According to 16S rRNA sequencing data, we found 295, 442, 535, and 243 specific OTUs for the PI, KTT, HLS, RS, respectively; 1423 OTUs were shared within the soil samples (Figure 4). The specific OTUs found provided information on the number of unique soil bacteria in each location. The HLS and KTT areas had the highest bacteria alpha diversity while the forest area (RS) was the lowest; see also Table 2. According to Kumar et al. (2017), the substantial amount of bacterial OTUs shared among the three land-use forms and one forest area indicated the presence of main bacteria resistant to alterations due to land-use changes persistent over time. KTT and HLS were dominated by coffee plants, only differing in the age of the stands.

From these two areas, the diversity of soil bacteria was more diverse in areas with a polyculture (HLS) than monocultures (KTT) or even intensive agriculture (PI) planting systems. Although the soil organic matter content at KTT was as high as the RS (see Table 1), this condition will not last long. Zhao et al. (2018), in the results of their research, reported that the monoculture coffee plantation system in the long term caused soil quality (pH, organic matter, and electrical conductivity) and soil bacterial diversity to decrease. Therefore, the conversion of forests to other forms of monoculture land-use is not the best choice for biodiversity conservation purposes.

Beta diversity defines the diversity of soil bacterial populations between sites based on the composition of the members. The beta diversity of soil bacteria in the study area appeared to vary from one another. Based on Figure 5, the beta diversity value of the two groups compared would be higher (close to 1) if the two communities are unique and vice versa. The beta diversity in the RS site was the largest compared to the other three locations; this suggested that the composition diversity of bacteria in the PI, KTT, and HLS areas varied from that of the RS. KTT and HLS have a similar diversity of bacteria; this was showed by the value of beta diversity, which was not much different.

Figure 5. Beta diversity of soil bacterial communities in each area. Notes: The coefficient of pairwise dissimilarity among pairwise samples is represented by each grid, with the Weighted Unifrac distance displayed above and the Unweighted Unifrac distance displayed below. A high score represents a low similarity between two areas compared, vice versa. The diversity of bacteria in agricultural land (PI) seems to form a different community from other areas. Land-use change activity decreased the level of beta diversity in the converted area, and our study confirmed the other studies in different regions. Rodrigues et al. (2013) stated that the transformation of the Amazon rainforest to agricultural land increased microbial alpha diversity but decreased beta diversity. Besides, most research investigating the effect of forest conversion on the soil bacterial population in the tropical rainforest had shown a similar impact (Rodrigues et al., 2013; Flores-Rentería et al., 2016; 2020). The beta diversity analysis provided information that land-use activities had induced the soil bacterial community changes in the area. It can be caused by modification in the vegetation communities, soil physicochemical properties, and microclimate. As...
a consequence, the composition of soil bacteria varies due to changes in environmental conditions.

**Correlation between soil bacterial community and soil properties**

The PCA was carried out to assess the relationship between the dominant group of bacterial composition and soil properties; the results were summarised in Figure 6. The arrows represented the angles and lengths between the explanatory and response variables, indicating the correlations. The PCA plots were similarly based on ten phyla and dominant genera. Under distinct land-use forms, the general structures of dominant taxa were significantly related to defined soil properties. At the phylum level, the first axis and second axis of eigenvalues were 11.29 and 6.01. The axes accounted for 86.5% of the total bacterial variance (Figure 6A). Analysis of Pearson correlation between dominant bacterial taxa and soil parameters informed that the Proteobacteria abundance was correlated positively with the soil electrical conductivity/EC (coef. = 0.999, p<0.05) and soil aggregate/AggrS (coef. = 0.911, p<0.05).

Figure 6. Ordination plot by PCA informs the relationships between soil bacteria abundance in (A) phylum, (B) genus level, and soil properties. PI = intensive agriculture, KTT = abandoned coffee plantation/old-coffee plantation, HLS = mixed young-coffee plantation, RS = secondary forest/reference area.
Latecibacteria and Verrucomicrobia were positively correlated with soil bulk density/BDst with a correlation coefficient of 0.958 (p<0.05); 0.994 (p<0.05) but were negatively correlated with other soil parameters (Table 3). Bacterial population abundance from the dominant phyla was more present in the PI than in other locations, from the PCA findings. Bacterial communities in the PI and KTT areas appeared to have a similar composition positioned at the same quadrant in the ordination plot.

At the genus level, the first eigenvalue was 11.71, and the second was 6.94. The axes described 93.3% of the overall bacterial variance; thus, the results confirmed that these two axes could represent the effect of soil properties on bacterial population structures (Figure 6B). The relative abundance of *Pedomicrobium* (coef. = -0.971, p <0.05), *Variibacter* (coef. = -0.955, p <0.05), *Nocardioides* (coef. = -0.971, p <0.05) were negatively correlated with soils bulk density, while only *Variibacter* (coef. = 0.960, p <0.05) were positively correlated with soil water content (Table 3). Dominant bacterial communities in all sites indicated variations. The PI and RS locations have different dominant bacteria compositions, whereas the KTT and HLS sites seem identical. The composition of bacteria in the KTT and HLS areas had many similarities so that in the ordination plot, the positions of these two areas were close together. *Variibacter, Pedomicrobium, Nocardioides,* and *Bradyrhizobium* had the greatest abundance in soil conditions with high organic matter content, high humidity, and non-acid soil, as in RS. On the other hand, *Methylotenera, Massilia, Rumibacter, Sphingomonas, H16,* and *RB41* were present in soils that differ from RS characteristics, such as intensive agricultural land.

### Table 3. Pearson correlation coefficients between dominant bacteria groups and soil properties factor.

| Bacterial groups | BDst  | AggrS | Moist | pH    | EC    | SOC   |
|------------------|-------|-------|-------|-------|-------|-------|
| **Phylum**       |       |       |       |       |       |       |
| Proteobacteria   | -0.283| 0.911 | 0.121 | 0.448 | **0.999** | -0.496 |
| Acidobacteria    | 0.698 | -0.533| -0.569| -0.453| -0.154| 0.210 |
| Actinobacteria   | -0.620| 0.090 | 0.583 | 0.323 | -0.295| 0.118 |
| Chloroflexi      | 0.321 | -0.783| -0.213| -0.550| **-0.968**| 0.256 |
| Gemmatimonadetes | 0.672 | -0.128| -0.635| -0.388| 0.248 | -0.140 |
| Nitrospira       | 0.181 | -0.115| -0.084| 0.149 | 0.314 | 0.338 |
| Firmicutes       | 0.562 | -0.123| -0.509| -0.255| 0.281 | -0.020 |
| Bacteroidetes    | 0.885 | -0.076| -0.912| -0.714| 0.152 | -0.514 |
| Latescibacteria  | **0.958**| -0.587| -0.875| -0.840| -0.360| -0.114 |
| Verrucomicrobia  | **0.994**| -0.359| **-0.992**| -0.947| -0.249| -0.463 |
| **Genus**        |       |       |       |       |       |       |
| Massilia         | 0.422 | 0.606 | -0.585| -0.285| 0.702 | -0.843 |
| Methylotenera    | 0.433 | 0.531 | -0.561| -0.229| 0.720 | -0.699 |
| Pedomicrobium    | **-0.971**| 0.361| 0.944 | 0.838 | 0.143 | 0.341 |
| Variibacter      | **-0.955**| 0.227| **0.360**| 0.822 | 0.028 | 0.465 |
| Sphingomonas     | 0.584 | 0.351 | -0.677| -0.366| 0.582 | -0.631 |
| RB41             | 0.782 | -0.054| -0.787| -0.544| 0.254 | -0.367 |
| Bradyrhizobium   | -0.567| -0.034| 0.555 | 0.266 | -0.409| 0.193 |
| H16              | 0.741 | 0.070 | -0.774| -0.507| 0.357 | -0.464 |
| Nocardioides     | **-0.971**| 0.538| 0.902 | 0.853 | 0.317 | 0.176 |
| Rumibacter       | 0.450 | 0.558 | -0.595| -0.281| 0.699 | -0.781 |

Note: Bold values are different from 0 with a significance level alpha of 5%. BDst (bulk density g cm\(^{-3}\)), AggrS (soil aggregate), Moist (soil water content %), pH (soil acidity), EC (soil electrical conductivity), and SOC (soil organic content %).

Seasonality and soil properties in forest soil might have an impact on microbial communities’ phylogetic and functional structure (Preem et al., 2012). Our findings indicated that variations in the soil bacterial community structure were strongly associated with soil properties changes. These variations were related to land-use, and similar environmental gradients could be observed when bacteria and soil properties data were used for biplot analysis (PCA), suggesting that the land-use impact was determined by alteration in the soil. In this study, soil bulk density, water content, and electrical conductivity were the main edaphic factors driving the spatial variability, while soil aggregate, pH, and organic content had a negligible effect on soil bacteria (see Table 3). This result was in accordance with a study carried out by Sun et al. (2020) in the tropical forest soil of China. However, our findings differed from those of most studies, which found that pH was the most important environmental factor influencing bacterial distribution in terms of both
spatial and temporal distribution. Previous work had shown that soil pH was the central aspect that formed the bacterial community pattern (Bárcenas-Moreno, Bååth, and Rousk, 2016; Cho, Kim, and Lee, 2016). This difference might be assigned to the slight soil pH variation with land-use forms in this study (Table 1).

Although land-use change or agricultural land management altered the structure and composition of the soil bacteria population, this condition did not lead to a loss of alpha diversity in the population. The diverse bacteria population in agricultural soil seemed to be more resistant and resilient to stressful conditions and thus more capable of preserving soil functions (Delgado-Baquerizo et al., 2017). The high alpha soil bacteria diversity in the non-forest area of our study was possibly caused by environmental heterogeneity on the local scale. As a consequence of that condition, it would lead to creating various functional traits of soil bacteria followed by its diversity also (Prescott and Grayston, 2013; Cline and Zak, 2015; Cai et al., 2018). Our finding result indicated that the impact of land-use change on soil bacteria communities was not through diversity decrease (or loss), but primarily by imposing a marked shift in the bacterial composition, with uncertain but potentially significant implications for ecological services and functions provided by these communities. Any shift in the bacterial composition may yield significant soil functioning changes, particularly in degraded soil (Verhulst et al., 2010). Different results were reported by some studies which concluded tropical forest conversion to agricultural land had resulted in no variations (Lee-Cruz et al., 2013) or also an improvement in alpha diversity of bacteria (Da et al., 2009; Tripathi et al., 2012).

Based on the results of the IndVal analysis, it showed that Actinobacteria, Chloroflexi, and Firmicutes had higher values compared to other taxa, namely 0.46, 0.45, and 0.40, respectively. Actinobacteria abundance showed differences in each area being compared. Actinobacteria were observed to have high abundance, especially in areas with minimal soil disturbance, such as RS (forest area). Burning crop residues during land clearing or cultivation were thought to cause the low abundance of Actinobacteria in the PI and KTT areas. This statement was in accordance with the research results by Jiménez-Bueno et al. (2016), who revealed that burning crop residues impacted decreasing the abundance of Actinobacteria in the soil. Furthermore, Chloroflexi was observed to have a high abundance in the coffee plantation area (KTT and HLS). According to Lee et al. (2020), Chloroflexi was commonly found in oligotrophic environments with low soil electrical conductivity, where this characteristic was usually observed in agricultural soils. Trivedi et al. (2016), in their research results, stated that in agricultural soils, Chloroflexi abundance was higher than natural soils in the area studied. In addition, the high abundance of Chloroflexi in HLS was thought to be due to the influence of G. sepium litterfall (primarily found in HLS), where this plant species could act as nitrogen sources in the environment (Bah and Rahman, 2001). Chloroflexi played a significant ecophysiological role in the process of the nitrogen cycle in ecosystems, and members of this phylum can act as bioindicators for the application of nitrogen fertilizers to the soil (Jiménez-Bueno et al., 2016). As with Chloroflexi, the phylum Firmicutes was also found in agricultural soils rather than forest soils. This phylum generally had a prevalence in copiotrophic environments, such as agricultural soils experienced with additional nutrient input and intensive management (Trivedi et al., 2016; Lee et al., 2020).

Conclusion
Land degradation due to forest conversion creates a spatial variation in the soil environment. This condition affects the soil bacteria community through alteration in composition and diversity of bacterial taxa members. Soil physicochemical properties are the factor that related to changes in the bacteria community in this study. Soil bacteria community structure in three managed or agricultural areas tend to be different from the natural area. This study found some potential bacterial taxa to be bioindicators in the environment which can indicate any shift in soil quality. As a bioindicator, soil bacteria can be used as an early warning of land degradation and an indication of soil improvement in the land rehabilitation program.

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