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Vertical and island-scale structuring of bacterial and archaeal communities in *Pinus merkusii* forest soils

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Abstract. *Nugroho RA, van Straalen NM, Röling WFM. 2026. Vertical and island-scale structuring of bacterial and archaeal communities in* Pinus merkusii *forest soils. Biodiversitas 27 (3): d270313. https://doi.org/10.13057/biodiv/d270313.* Environmental and spatial processes contribute to the structuring of soil microbial communities, but the relative importance of vertical and horizontal gradients is poorly understood in tropical archipelagic forests. The vertical and spatial distributions of bacterial and archaeal communities were examined in *Pinus merkusii* forest soils across 12 sites on five Indonesian islands (36 composite samples). Litter (L), fragmented litter (F), and mineral (M) soil layers were analyzed using denaturing gradient gel electrophoresis (DGGE) to characterize dominant community profiles. Community dissimilarity was assessed using Mantel and partial Mantel tests, and compositional variance was assessed using PERMANOVA. Bacterial communities were strongly vertically stratified. Mantel tests showed significant correlations between community dissimilarity and soil layer ($r = 0.52$, $p < 0.001$) and geographic distance ($r = 0.60$, $p < 0.001$), which remained significant in partial correlations. PERMANOVA indicated that soil layer explained 43.5% of the variation (pseudo-R^2 = 0.435, $p < 0.001$), whereas island identity explained 15.1% (pseudo-$R^2 = 0.151$, $p = 0.0135$). In contrast, archaeal communities showed no significant association with either geographic distance ($r = -0.03$, $p = 0.68$) or soil layer ($r = -0.04$, $p = 0.80$). In contrast, PERMANOVA revealed strong differentiation among island identity (pseudo-$R^2 = 0.375$, $p = 0.0045$). These findings reveal contrasting domain-specific spatial organization of microbial communities, with bacteria primarily structured by vertical habitat differentiation and distance–decay patterns, whereas archaea communities are mainly differentiated at the island scale. The results highlight distinct spatial scales of organization for major microbial domains in tropical forest soils. However, the absence of environmental physicochemical measurements limits direct identification of the environmental drivers underlying these patterns.

Keywords: DGGE, distance-decay, environmental filtering, microbial biogeography, soil microbial communities

INTRODUCTION

Soil microbial communities are fundamental to the functioning of terrestrial ecosystems through the decomposition of organic matter and the regulation of carbon and nutrient biogeochemical cycles. Bacteria and archaea are dominant members of the soil microbiome and perform many metabolic processes that influence soil fertility and ecosystem productivity. Understanding the drivers of community assembly along environmental and spatial gradients remains a fundamental question in soil ecology (Carvajal et al. 2023; Riddley et al. 2025). Because environmental variables were not measured, this study focuses on spatial and vertical structuring patterns independently of environmental metadata, allowing broad-scale biogeographic signals to be identified that may later be tested using environmental measurements.

Microbial communities are often spatially structured at different scales, which can be interpreted as distance-decay relationships in community similarity (Feng et al. 2019; Liu et al. 2023, 2024; Wu et al. 2024). Such patterns may result from dispersal limitation, historical contingency, or spatially structured environmental variation. Although microbes are often assumed to disperse widely, increasing evidence indicates that geographic distance can influence microbial distributions, particularly at regional scales and across fragmented landscapes (Barbour et al. 2023; Ezzat et al. 2025; Wainwright et al. 2024; Walters et al. 2025).

Most previous studies have focused on temperate or continental systems where spatial gradients are relatively continuous. In contrast, tropical archipelagic landscapes represent a fundamentally different spatial context, where oceanic barriers and discrete island boundaries may restrict dispersal and generate distinct microbial biogeographic patterns. Microbial β-diversity and its drivers are highly scale- and landscape dependent, while tropical island systems remain underrepresented in global analyses (Bahram et al. 2018; Thompson et al. 2017). Understanding microbial community organization in archipelagic ecosystems is therefore important for testing whether patterns observed in continental systems also apply to geographically fragmented tropical landscapes.

Along with horizontal spatial gradients, soil microbial communities are also structured across vertical soil profiles. Soil layers such as litter, fragmented litter, and mineral soil vary substantially in organic matter quality, nutrients availability, aeration, and microhabitat conditions. These vertical gradients can exert intense environmental

filtering, which leads to stratification of microbial community composition along soil profiles (Muneer et al. 2022; He et al. 2023; Hu et al. 2023; Chen et al. 2025).

Spatial isolation and vertical environmental gradients may influence bacterial and archaeal communities differently because of differences in physiology, ecological strategies, and niche widths. Soil bacterial communities are often tightly associated with gradients in substrate availability and soil physicochemical properties, whereas archaeal communities have shown more variable in their responses to environmental factors and geographic distance. These contrasting trends suggest that different community assembly processes may influence bacterial and archaeal distributions in a given ecosystem (Lv et al. 2021; Sun et al. 2025; Yin et al. 2026). However, these trends are context dependent rather than absolute rules, and the relative importance of vertical and horizontal structuring might vary among ecosystems.

Pinus merkusii Jungh. & de Vriese is the only native tropical pine widely distributed in Indonesia and forms extensive forest stands, particularly on Java and Sumatra. These forests play important ecological and economic roles, including timber production, watershed protection, and soil stabilization (Laumonier 1997; Whitten et al. 1996). Forest soils are intensely vertically stratified, creating unique habitats in litter, fragmented litter, and mineral soil layers that shape microbial community structure. Previous studies have shown vertical niche partitioning of microbial communities and horizon-dependent differences in diversity and interactions in forest ecosystems (Jiao et al. 2018; Mundra et al. 2021; Tian et al. 2023).

At the same time, geographical separation across islands introduces a horizontal component of variability that allow evaluation of associations between microbial community dissimilarity and geographic distance. Thus, tropical archipelagic forests provide a natural experiment for comparing vertical habitat differentiation within sites with spatial isolation among islands.

Two main hypotheses were tested in the present study. First, bacterial communities were predicted to be strongly vertically stratified among litter, fragmented litter, and mineral soil layers. Second, archaeal communities were anticipated to exhibit weaker vertical differentiation but stronger island-specific spatial structuring. These hypotheses enable to test the relative contribution of the vertical habitat differentiation and archipelagic spatial isolation to the structuring of the soil microbial communities in a tropical forest ecosystem.

MATERIALS AND METHODS

Sampling site and soil collection

Soil samples were collected from twelve *P. merkusii* forest sites on five islands in Indonesia (Table 1) and consisted of litter (L), fragmented (F) litter, and mineral soil (M) layers. Sites were on Sumatra, Java, Kalimantan, Bali, and Sulawesi and cover a broad geographic gradient across the Indonesia archipelago. Soil layers were operationally defined by morphological characteristics rather than by fixed depth intervals. The litter layer (L) was composed of freshly fallen, undecomposed plant material. The fragmented litter layer (F) is an organic horizon composed of partially decomposed organic matter with apparent plant structures, but it is also visibly altered by fragmentation and humification. The mineral soil layer (M) consisted of the upper 0-10 cm of mineral soil beneath the organic (L and F) layers and was collected in bulk soil. Because litter thickness differed among sites, the thickness of the L and F layers was not standardized by depth. However, it was determined based on the visible organic-horizon boundaries at each site.

Morphological horizons were used because the L and F layers represent ecologically distinct stages of organic matter decay and are functionally relevant to the assembly of microbial communities in forest soils. The layering, as defined by visible organic interfaces, provides a basis for comparing similar substrate types across sites, even when absolute thicknesses differ. Variability in the depth of the organic horizon may affect the total amount of substrate available to a site, but the present investigation was directed at community composition rather than biomass or volumetric abundance; thus, any bias associated with differences in layer depth was minimized.

Table 1. Geographic coordinates and altitude of sampling sites

Island	Sample location	Latitude	Longitude	Altitude (masl)
Sumatra	Mt. Leuser	2°43′46.34″N	98°57′24.78″E	1,148
Java	Mt. Tangkuban Parahu	6°46′53.23″S	107°39′06.91″E	1,373
Java	Gombong	7°32′48.13″S	109°32′51.34″E	117
Java	Mt. Telomoyo	7°21′47.32″S	110°23′09.50″E	1,010
Java	Mt. Merapi	7°33′57.41″S	110°23′32.47″E	942
Java	Mt. Merbabu	7°24′12.42″S	110°25′16.88″E	1,488
Java	Mt. Lawu	7°39′49.40″S	111°10′35.67″E	1,755
Kalimantan	Banjarbaru	3°25′59.16″S	114°50′21.08″E	47
Bali	Mt. Tapak	8°17′02.67″S	115°09′48.07″E	1,351
Sulawesi	Mt. Lompobatang	5°15′10.47″S	119°50′46.71″E	1,011
Sulawesi	Mt. Soputan	1°06′57.05″N	124°48′02.91″E	982
Sulawesi	Linow lake	1°16′14.77″N	124°49′24.35″E	875

Sampling sites were distributed across a wide geographic range and an altitudinal gradient from 47 to 1,755 meters above sea level (masl). The geographic coordinates and altitudes of each sample site are listed in Table 1. At each site, three subsamples were collected within each soil layer (litter, fragmented litter, and mineral soil) and pooled to form one composite sample per layer. To obtain the mineral soil sample, the organic layers were removed, and the sample was collected from a depth of 0-10 cm. Stones and visible roots were removed, and subsamples from the same layer were pooled to produce a single composite sample per layer per site. All composite samples were homogenized and sieved using a 2.0-mm mesh before molecular analyses.

The sampling design (Table 2) was hierarchical, with soil layers (L, F, M) nested within sites and sites within islands. Twelve sites distributed across five islands were sampled, and three composite samples (one per soil layer) were obtained at each site, resulting in 36 composite samples in total. Each composite sample corresponded to a single DGGE lane. Because subsamples were pooled prior to analysis, variability among subsamples within a site was not evaluated. All 36 samples were included in bacterial analyses, whereas archaeal analyses were conducted on 31 samples because five showed insufficient DGGE banding patterns.

DNA extraction

Approximately 1.0 g of homogenized composite sample was used for the DNA extraction using the PowerSoil DNA Kit (MO BIO Laboratories, Solana Beach, CA, USA). Mechanical lysis was performed with a FastPrep Instrument (MP Biomedicals, Santa Ana, CA, USA) using two bead-beating cycles of 20 s at 5.5 m/s. Extracted DNA was visualized on 1% agarose gel and quantified using a Nanodrop® Spectrophotometer (ND-1000).

PCR amplification and DGGE

The 16S rRNA gene fragments of bacteria and archaea were amplified by PCR. A reaction volume of 25 µL was used for each PCR including 0.4 µM of each primer, 0.4 mg/mL bovine serum albumin (BSA, New England Biolabs, Leusden, The Netherlands), 200 nM dNTPs, 1U Taq polymerase (Promega, Southampton, Hampshire, UK), 1× PCR buffer (Promega), 17 µL of DNase- and RNase-free water (MP Biomedicals, Solon, OH), and 1 µL template. Negative controls without template were included for each primer pair to assess contamination of reagents. For bacteria, the primers used were F357 and R518 (Muyzer et al. 1993). The PCR program was: one cycle at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 54°C for 30 s, 72°C for 30 s, and a final cycle at 72°C for 8 min. A second PCR was performed on the PCR product with primers F357-GC and R518 (Muyzer et al. 1993). For archaea, a nested PCR was performed to increase detection sensitivity because archaeal abundance is typically lower in soil samples. The first round used primers PRA46f and Univ0907r, followed by a second amplification with primers PARCH340f-GC and PARCH519r (Ovreås et al. 1997; Vetriani et al. 1999). For both steps, an initial denaturation at 94°C for 4 min was followed by 35 cycles of 94°C for 30 s, 54°C for 1 min and 72°C for 1 min, and a final elongation at 72°C for 5 min. Negative controls were carried through all PCR runs to check for contamination. Each sample underwent a single PCR reaction under standardized conditions, and the reactions were performed simultaneously to avoid inter-run variation.

PCR amplification was performed once per composite sample, and no technical PCR replicates were included. Each DGGE lane, therefore, corresponds to a single amplification product per composite sample. DGGE banding patterns primarily represent dominant members of the microbial community rather than absolute gene copy numbers. Previous studies have shown that major DGGE banding structures are generally reproducible when amplification and electrophoresis conditions are standardized. However, some variability in band intensity may arise from PCR stochasticity.

PCR products eluted from DNA extracted from composite soil samples (each composite resulted from the pooling of three subsamples per site and layer) were subjected to denaturing gradient gel electrophoresis (DGGE). Gels were 8% polyacrylamide gels (37.5:1 acrylamide/Bis) with a denaturant gradient of 30-55% for bacteria and 30-70% for archaea. They were electrophoresed in 1× Tris acetate EDTA buffer using a Bio-Rad DCode Universal Mutation Detection System at 200V and 60°C for 4 h. These conditions were empirically optimized for band separation and resolution.

A DGGE marker containing a mixture of 12 different 16S rRNA gene fragments from bacteria was run on each gel, used only to align lanes and normalize the gels. The marker was used only as a reference standard for migration to adjust for positional variation and was not used for taxonomic identification. The same marker was run consistently on both bacterial and archaeal DGGE gels to ensure comparable positional alignment. Gels were stained with ethidium bromide after electrophoresis, observed under UV light, and recorded digitally.

Table 2. Hierarchical sampling design

Level	Number	Description
Islands	5	Geographic grouping factor
Sites	12	Nested within islands
Soil layers	3 per site	L, F, M
Total composite samples	36	12 sites × 3 layers
DGGE lanes (bacteria)	36	All composite samples analyzed
DGGE lanes (archaea)	31	Five samples excluded due to insufficient banding

To reduce technical variability, samples to be directly compared were run in the same DGGE gel whenever possible. During the image analysis, marker-based normalization was performed uniformly across all gels. Normalization reduces gel-to-gel variation, but a small residual technical variability may remain.

In total, 3 DGGE gels were run to accomodate 36 samples. In each gel, four lanes contained the same molecular marker for inter-gel normalization purposes. From all three gels the banding patterns were analyzed together in GelCompar after alignment and normalization based on the marker. The positions of the markers were used to normalize migration distances across gels, and band alignment was validated by visual inspection prior to dendrogram construction. Band intensity was quantified from densitometric profiles of DGGE lanes and treated as continuous data representing the relative abundance of dominant DGGE bands. Similarity matrices were calculated from densitometric profiles of DGGE lanes, assuming that normalized band positions and intensities provide reproducible representations of dominant community composition across gels. All bacterial samples yielded clear, reproducible DGGE profiles and were considered in further analyses. For archaeal communities, five samples showed very weak or absent DGGE banding patterns and were therefore excluded from the similarity and multivariate analyses. Consequently, archaeal community analyses were conducted on 31 samples rather than on the full set of 36 composite samples.

Data analysis

Analysis of DGGE banding patterns was performed with GelCompar 3.0 (Applied Maths, Kortrijk, Belgium). Lane normalization was applied to account for gel-to-gel variability with an internal marker. Similarities between DGGE profiles were calculated using the Pearson correlation coefficient on densitometric curves of whole lanes. Dendrograms were built by unweighted pair group method with arithmetic mean (UPGMA) based on Pearson similarity values.

To complement hierarchical clustering and distance-based correlation analyses, non-metric multidimensional scaling (NMDS) was performed to reveal emerging patterns of community dissimilarity. Ordinations were calculated using the metaMDS function from the vegan R package from Pearson-derived dissimilarity matrices defined as $1 - (r/100)$, where r is the Pearson similarity coefficient between DGGE densitometric profiles. Two-dimensional solutions ($k = 2$) were generated with up to 200 random starts (trymax = 200). Stress values were used to evaluate the goodness-of-fit of the ordination.

Geographic distances between the sampling sites were calculated on GPS coordinates converted to decimal degree using the great-circle distance. Soil-layer distance matrices were constructed using the ordered vertical distance (0: same layer; 1: adjacent layers, L-F and F-M; 2: two-layer separation, L-M), denoting increasing ecological dissimilarity along the soil profile.

These dissimilarity matrices were used in Mantel and partial Mantel tests to test the associations among microbial community dissimilarity, geographic distance (distance–decay relationships), and soil layer separation. Partial Mantel tests were conducted to assess the effects of geographic distance while controlling for soil layer, and vice versa. All Mantel and partial Mantel analyses were performed in R with the vegan package (Oksanen et al. 2025). Moreover, significance was determined using 9,999 permutations. False discovery rate (FDR) correction was applied to account for multiple testing in the Mantel and partial Mantel tests ($n = 8$).

To evaluate the combined influence of geographic distance and separation between soil layers within a regression framework, a multiple regression on distance matrices (MRM) analysis was conducted in R with the ecodist package. Community dissimilarity matrices were modeled as functions of geographic distance and soil-layer distance using 9,999 permutations. This method accepts multiple distance predictors and yields an inference that complements Mantel-based correlation analyses.

To assess the importance of spatial position and soil layer on community composition, dbRDA analysis was conducted using the capscale function of the vegan package. Dissimilarity matrices of communities were constrained by soil layer (a categorical variable), latitude, and longitude. Model significance was evaluated with permutation tests (9,999 permutations).

To complement Mantel-based analyses and overcome the known limitations of partial Mantel tests, permutational multivariate analysis of variance (PERMANOVA) was conducted with the adonis2 function in the vegan package. Community dissimilarity was modeled as a function of island identity and soil layer: community dissimilarity ~ Island + Layer. The sampling design was hierarchical, with soil layers nested within sites and sites nested within islands. In the PERMANOVA analyses, island identity was included as the spatial factor and soil layer as the vertical habitat factor. Marginal effects were calculated based on 9,999 permutations under unrestricted permutation of residuals which was considered appropriate because island was explicitly defined as a fixed factor and the design was not fully crossed. Homogeneity of multivariate dispersion was evaluated using betadisper to confirm that PERMANOVA outcomes reflected differences in centroid locations, not dispersion.

PERMANOVA was conducted using the adonis2 function in the vegan package with 9,999 permutations. Island identity and soil layer were treated as fixed factors in the model. Sites were considered independent sampling units nested within islands in the sampling design, but were not included as a separate factor in the PERMANOVA model because each site contributed only one composite sample per layer. Therefore, the analysis tested the effects of island-scale spatial grouping and vertical differentiation of soil layers on community composition.

A sensitivity analysis was conducted with a categorical soil-layer distance matrix (0: same layer; 1: different layer) to assess robustness of the ordinal layer coding. The findings were qualitatively consistent across two coding schemes.

RESULTS AND DISCUSSION

DGGE-based patterns of bacterial community structure

Figure 1 shows the UPGMA dendrogram generated from DGGE profiles of bacterial communities along the soil layers. The order of samples reflects the hierarchical clustering based on Pearson similarity coefficients and was not manually rearranged. Bacterial communities were primarily structured by soil layer, with samples clustering into groups corresponding to the L, F, and M layers. Samples from the same soil layer tended to cluster together despite originating from different geographic locations, indicating strong vertical differentiation of community composition.

DGGE-based patterns of archaeal communities

Clustering of archaeal communities is shown in Figure 2. As in Figure 1, the order of the samples follows the result of the hierarchical clustering and was not modified. In contrast to bacterial DGGE profiles, archaeal DGGE profiles exhibited clustering by sampling site rather than soil layer. Samples collected from the same site or from nearby sites tended to cluster together, although soil layers within a site did not always form distinct clusters. This pattern suggests weaker vertical structuring and a stronger spatial structuring of archaeal communities.

NMDS ordination of the Pearson-derived dissimilarity matrices verified the dendrogram clustering patterns (Figure 3). For bacterial communities (n = 36), the samples were largely divided by soil layer, and L, F, and M layers formed well-separated clusters in the ordination space (stress = 0.138), and confidence ellipses corresponding to soil-layer groupings. Conversely, archaeal communities (n = 31) showed clearer separation by island than by soil layers (stress = 0.163), with ellipses representing island identity. Both stress values were below 0.20, indicating that the two-dimensional NMDS solutions adequately represented the multivariate dissimilarity structure.

Spatial and vertical drivers of community dissimilarity

Mantel tests (Table 3) showed that bacterial community dissimilarity was positively correlated with both geographic distance (r = 0.60, p = 0.0001) and soil layers differentiation (r = 0.52, p = 0.0001). These associations remained significant in partial Mantel tests controlling for the alternate factor (r = 0.61 and r = 0.53, respectively) and after FDR correction (q = 0.0002).

In contrast, archaeal community dissimilarity showed no significant association with either geographic distance (r = −0.03, p = 0.68) or soil layer (r = −0.04, p = 0.80). These relationships remained non-significant in partial Mantel tests and after FDR correction ($q \approx 0.80$). These findings suggest pronounced vertical and spatial structuring in bacterial communities, whereas archaeal communities showed no detectable distance–decay or layer-related pattern.

To further evaluate spatial and vertical structuring using a model-based approach, a distance-based redundancy analysis (dbRDA) was performed. The constrained model, including soil layer, latitude, and longitude, explained 5.3% of the total variation in bacterial community composition. However, permutation tests indicated that none of the individual predictors were statistically significant (p > 0.90). These results suggest that although PERMANOVA detected significant compositional differences among soil layers, the proportion of variance that can be linearly explained by the spatial predictors in constrained ordination space is relatively small. This low explained variance likely reflects the effect of other unmeasured environmental variables, historical processes, or stochastic ecological dynamics that were not considered in the present model.

MRM analyses indicated comparable results. For bacteria, both soil-layer separation and geographic distance were significant predictors of community dissimilarity (R^2 = 0.396, p < 0.001). Results were similar when soil layer was treated as an ordered variable (0-1-2) or as a categorical distance (0-1). For archaeal communities, the MRM analysis detected a weak but statistically significant association with geographic distance (R^2 = 0.033, p = 0.014). However, this effect was not detected by Mantel tests, suggesting that the spatial signal for archaea was very weak and sensitive to the analytical framework.

PERMANOVA (Table 4) showed that bacterial community composition was significantly structured by island (pseudo-$F_{5,28}$ = 2.044, pseudo-R^2 = 0.151, p = 0.0135) and by soil layer (pseudo-$F_{2,28}$ = 14.705, pseudo-R^2 = 0.435, p < 0.001). In contrast, archaeal communities were significantly structured by island (pseudo-$F_{4,24}$ = 3.720, pseudo-R^2 = 0.375, p = 0.0045) but not by soil layer (pseudo-$F_{2,24}$ = 0.406, pseudo-R^2 = 0.020, p = 0.754). Tests of homogeneity of multivariate dispersion were non-significant for bacteria ($F_{2,33}$ = 2.10, p = 0.139) and archaea ($F_{2,28}$ = 0.29, p = 0.750), indicating that the differences in dispersion did not drive the results of PERMANOVA.

Table 3. Mantel and partial Mantel test results relating bacterial and archaeal community dissimilarity to geographic distance and soil layer in *P. merkusii* forest soils. Bacterial analyses were based on 36 samples and archaeal analyses on 31 samples

Community	Test type	Predictor	*r*	*p*	FDR *q*
Bacteria	Mantel	Geographic distance	0.60	0.0001	0.0002
Bacteria	Mantel	Soil layer	0.52	0.0001	0.0002
Bacteria	Partial Mantel	Geographic distance (controlling for layer)	0.61	0.0001	0.0002
Bacteria	Partial Mantel	Soil layer (controlling for distance)	0.53	0.0001	0.0002
Archaea	Mantel	Geographic distance	-0.03	0.6827	0.8048
Archaea	Mantel	Soil layer	-0.04	0.8048	0.8048
Archaea	Partial Mantel	Geographic distance (controlling for layer)	-0.02	0.5916	0.8048
Archaea	Partial Mantel	Soil layer (controlling for distance)	-0.03	0.7057	0.8048

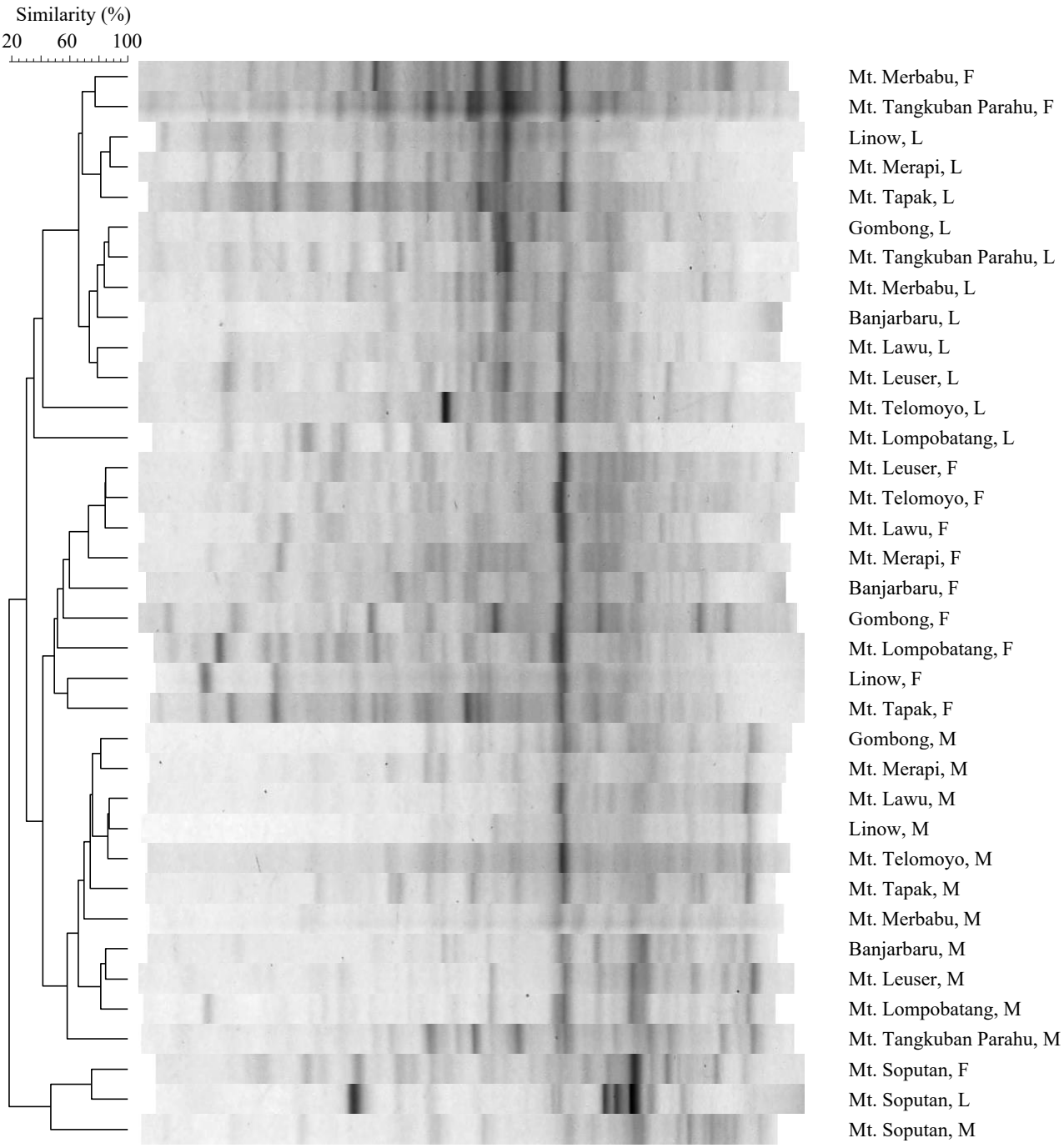

Figure 1. UPGMA cluster analysis of DGGE profiles of bacterial 16S rRNA gene fragments from soils of Indonesian *P. merkusii* forests. Percentage similarity was calculated using the Pearson correlation coefficient of DGGE banding pattern. Each lane is labeled using the format Site, Layer (e.g. Mt. Merbabu, L), indicating sampling location and soil layer. L: litter layer, F: fragmented litter layer, M: mineral layer

Table 4. PERMANOVA results for bacterial and archaeal communities

Community	Factor	Df	Sum of squares	Pseudo-R^2	Pseudo-*F*	*p*-value
Bacteria	Island	5	1.1222	0.151	2.044	0.0135
	Layer	2	3.2294	0.435	14.705	<0.001
	Residual	28	3.0746	0.414		
	Total	35	7.4262	1.000		
Archaea	Island	4	1.9186	0.375	3.720	0.0045
	Layer	2	0.1047	0.020	0.406	0.754
	Residual	24	3.0949	0.605		
	Total	30	5.1182	1.000		

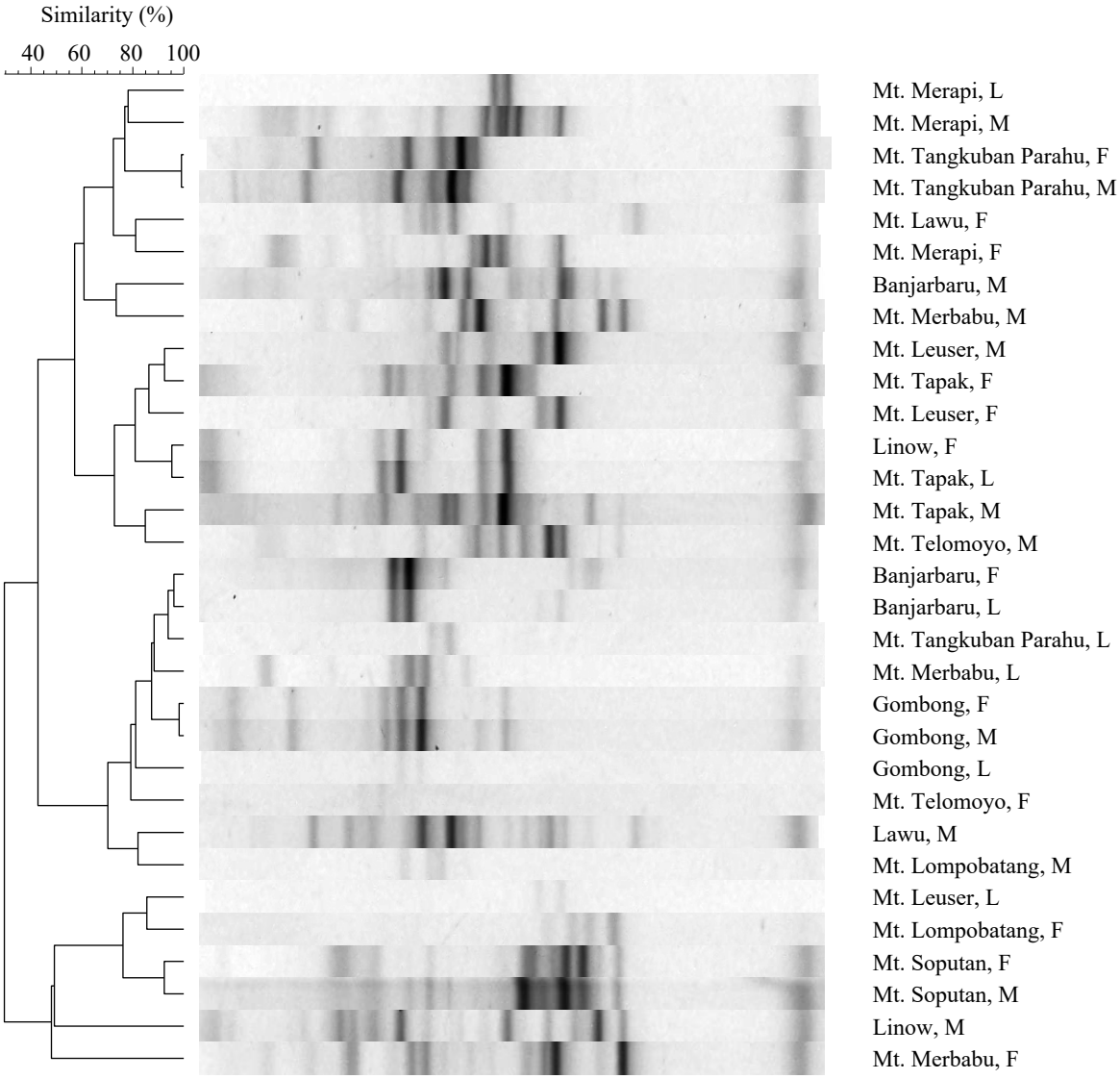

Figure 2. UPGMA cluster analysis of DGGE profiles of archaea 16S rRNA gene fragments from soils of Indonesian *P. merkusii* forests. Percentage similarity was determined using the Pearson correlation coefficient for the DGGE banding pattern. Each lane is labeled using the format Site, Layer (e.g. Mt. Merbabu, L), indicating sampling location and soil layer. L: litter layer, F: fragmentation layer, M: mineral layer

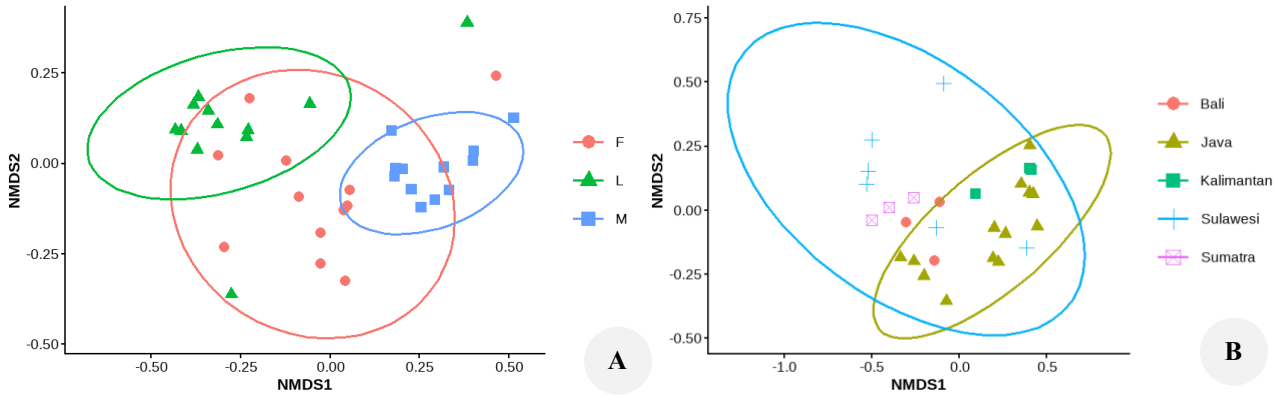

Figure 3. NMDS ordination of microbial community dissimilarity (1 − Pearson *r*). A. Bacterial communities ($n = 36$; stress = 0.138). B. Archaeal communities ($n = 31$; stress = 0.163). Points represent composite samples and ellipses indicate 95% confidence intervals of group centroids (soil layer for bacteria; island identity for archaea)

Discussion

This study showed contrasting vertical and spatial structuring of bacterial and archaeal communities in *P. merkusii* forest soils across the Indonesian islands. Bacterial communities showed strong vertical structuring among litter (L), fragmented litter (F) and mineral (M) layers. In contrast, archaeal communities showed little vertical segregation but were mainly structured at the island scale. Synthesis of various statistical methods uniformly confirmed this domain-specific scaling, suggesting stark contrasts between the sensitivities of bacterial and archaeal communities to spatial and vertical gradients.

Distinct vertical structuring of bacterial communities suggests strong habitat differentiation within soil profiles. Forest soil layers differ in organic matter characteristics and microenvironmental conditions (He et al. 2023; Hu et al. 2023; Zheng et al. 2024). Litter on the surface generally contains more recently deposited and potentially labile substrates, whereas mineral soils contain more processed organic matter and exhibit different physicochemical properties (Fanin et al. 2012; Qiao et al. 2025). These vertical gradients are commonly associated with habitat filtering in forest soils. In the present study, bacterial communities differed strongly among the L, F, and M layers, indicating pronounced vertical habitat differentiation within the soil profile. This stratification implies that microbial communities are strongly influenced by the differing resource availability and microenvironmental conditions within soil layers.

However, because physicochemical soil properties were not measured in this study, the mechanisms underlying this vertical differentiation cannot be directly identified. Consequently, the interpretation of environmental filtering should be considered tentative and limited to the detection of vertical community patterns rather than confirmation of specific environmental drivers. The proposed mechanisms therefore remain hypothetical and should be tested in future studies that integrate microbial community analyses with detailed soil physicochemical measurements. Previous studies combining microbial community profiling with environmental measurements have shown that variables such as soil pH, carbon quality, and nutrient availability can influence vertical microbial turnover in forest soils (Bahram et al. 2018; Delgado-Baquerizo et al. 2018; Tripathi et al. 2018). Future studies integrating detailed soil physicochemical measurements with microbial community analyses will be required to determine whether similar mechanisms operate in tropical *P. merkusii* forest soils.

In addition to vertical differentiation, bacterial communities were also spatially structured among islands following a distance-decay pattern. While geographic distance was correlated with community dissimilarity, its effect size was moderate, suggesting that spatial separation explains some but not all of the turnover in bacteria. As environmental factors and dispersal processes were not directly measured, the spatial pattern observed should therefore be interpreted as evidence of broad-scale spatial structuring rather than direct identification of dispersal barriers. The increase in the Mantel correlation after controlling for soil layer indicates that strong vertical differentiation partly masked the spatial signal in the simple Mantel test. After controlling for the layer-associated variation, the residual dissimilarity matrix showed a stronger geographic signal, consistent with a suppression effect. This interpretation was further supported by dbRDA and multivariate regression approaches, which separated compositional variance into vertical and spatial fractions and revealed that layer-related differentiation explained a large proportion of bacterial turnover independent of geographic distance. Sensitivity analyses confirmed that the results are robust to changes in distance metrics, reinforcing the conclusion that vertical structuring is not an artifact of methodological choice. The low proportion of variance explained by the dbRDA model (5.3%) further indicates that although compositional differences among layers are statistically detectable, much of the variation in microbial community composition likely reflects additional environmental, historical, or stochastic processes that were not measured in the present study.

In contrast, the archaeal communities exhibited only weak vertical differentiation but strong separation at the island level. PERMANOVA showed that the variation in archaeal community was mostly associated with island identity, and to a lesser extent with soil layer, suggesting that archipelagos are the primary scale of archaeal assemblage structuring. However, the observed island effect should not be interpreted solely as evidence of dispersal limitation, because island identity may also capture unmeasured environmental differences among locations, such as variation in soil chemistry, climate, or vegetation structure. Regional climate, vegetation types, and historical biogeographic processes may also affect the island-scale environmental variation in a way that may govern assembly of archaeal community.

The statistical frameworks employed in this study quantify the community patterns in distinct ways, potentially accounting for the conflicting results among Mantel, MRM, and PERMANOVA outputs, particularly in the case of archaeal communities. Mantel tests assess correlations between distance matrices and therefore capture simple distance–decay relationships. In contrast, MRM evaluates multiple spatial predictors simultaneously and may detect weak spatial signals, whereas PERMANOVA tests differences among predefined categorical groups such as islands. The combined results therefore suggest that archaeal communities may be structured by island-scale factors that are not strictly expressed as simple geographic distance relationships.

In line with this interpretation, the archaeal community did not show a significant distance-decay pattern across the sampled islands. Similar broad-scale patterns of archaeal community have been observed in studies including environmental factors, with community composition correlating with regional gradients such as pH, nutrient level, or climate (Zhang et al. 2024; Yin et al. 2026). The present study cannot identify the specific drivers due to the lack of environmental metadata, therefore the island effect should be interpreted as an empirical spatial pattern rather than an indication of specific ecological strategies. The more pronounced island-level structuring of archaea than

bacteria may therefore imply differences in dispersal limitation, niche breadth, or historical contingency, as recently acknowledged in microbial biogeography (Thompson et al. 2017; Gao et al. 2020).

The contrasting responses of bacteria and archaea highlight the need to consider the microbial domains independently in soil biogeography. Bacteria seemed more responsive to fine-scale vertical heterogeneity within sites, whereas archaea showed stronger differentiation at coarser spatial scales. This domain-specific scaling challenges the premise that microbial communities are homogenously affected by island biogeographic gradients, and instead lends support to recent findings that at least partially distinct assembly regimes may govern bacteria and archaea (Logares et al. 2018; Ning et al. 2019). In tropical island systems, where environmental heterogeneity and dispersal routes converge at multiple spatial scales, such taxon-dependent responses may indeed be a generalizable feature rather than an exception.

Several methodological limitations should be considered. DGGE mainly detects dominant taxa and provides lower taxonomic resolution than high-throughput sequencing (Muyzer et al. 1993; Muyzer and Smalla 1998). Analyses of similarity based on DGGE band intensities correspond to relative, not absolute, abundances and are therefore dependent on normalized densitometric profiles, which might not capture all quantitative differences in microbial populations. Furthermore, PCR amplification was conducted once per sample without technical replicates, which could cause uncertainty as to amplification bias, nested amplification for archaea, and gel-to-gel variation. Hence, the patterns revealed in the present study are to be viewed as large-scale shifts in the dominant fractions of the microbial communities rather than the entire coverage of total diversity. Five archaeal samples yielded poor DGGE profiles and were therefore excluded from the analyses, thus potentially reducing the ability to detect weaker patterns. In spite of these caveats, the agreement in NMDS, PERMANOVA, Mantel, and dispersion analyses indicates that the observed structuring does indeed correspond to substantial differences in the dominant fractions of the communities. Future studies using high-throughput sequencing together with detailed measurements of soil physicochemical properties and functional assays would allow more explicit testing of environmental filtering, dispersal limitation, and stochastic assembly processes. In particular, the use of sequencing in combination with phylogenetic null models (e.g., βNTI frameworks) and multivariate regression analyses might allow for a more explicit separation of deterministic and spatial processes (Ning et al. 2019).

In conclusion, bacterial communities were structured primarily by vertical habitat differentiation with additional spatial influence, whereas archaeal communities were mainly differentiated among islands. These results demonstrate domain-specific spatial structuring in tropical forest soils and indicate that microbial biogeographic scaling in island systems may differ between major microbial domains. By revealing contrasting vertical and archipelagic organization within a single ecosystem, this study contributes empirical evidence for scale-dependent microbial community structuring in tropical landscapes. However, the absence of environmental physicochemical measurements and the limited taxonomic resolution of DGGE constrain the identification of specific ecological mechanisms underlying the observed patterns. Future studies integrating high-throughput sequencing and detailed soil physicochemical measurements will be required to clarify the environmental drivers underlying these patterns.

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