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Bacterial diversity in the western slopes of Mount Lawu, Karanganyar, Indonesia

RETNO ROSARIASTUTI[1,♥], SUTAMI[1], SETYA NUGRAHA[2,3], BAMBANG SIGIT AMANTO[1,3]

[1]Faculty of Agriculture, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia.
Tel./Fax.: +62-271-632477, ♥email: retnobs@staff.uns.ac.id
[2]Faculty of Geography, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia
[3]Environmental Research Center, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia

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Abstract. *Rosariastuti R, Sutami, Nugraha S, Amanto BS. 2023. Bacterial diversity in the western slopes of Mount Lawu, Karanganyar, Indonesia. Biodiversitas 24: 2125-2133.* Bacteria are an essential ecosystem component that is critical in nutrient recycling. Mount Lawu is an ecosystem with various habitats supporting bacterial growth. This region contains a diverse range of bacteria that has received little attention. This study aimed to investigate the bacterial biodiversity on Mount Lawu's western slopes of Karanganyar District, Central Java Province, Indonesia based on land use differences. There are five land uses including vegetable plantations, paddy fields, residential, mixed land, and forests. This study is exploratory-descriptive; the survey method and exploration technique are employed. Then, the Next Generation Sequencing (NGS) approach was administered to identify the type of bacteria. This research revealed more than 10 phylum and 28 species of bacteria. The diversity index (H') value was 8.80 for Vegetable plantations, 8.50 for paddy fields, 8.26 for mixed land, 8.10 for residential, and 7.66 for forests, with the highest bacterial diversity index in vegetable plantations. The bacterial diversity incorporates the phylum Proteobacteria, Methylomirabillota, Firmicutes, Actinobacteria, Verrucomicrobiota, Acidobacteriota, Bacteroidota, Myxococcota, Chloroflexi, and others. Phylum Proteobacteria was dominant in each land use, followed by Phylum Actinobacteria. The bacterial diversity on Mount Lawu's western slope was high. This study's findings are useful as a database (information) on microbial biodiversity in Central Java.

Keywords: Biodiversity, bacteria, Mount Lawu

INTRODUCTION

Biodiversity is a significant study as it is closely associated with human life as a part of the living system (Frac et al. 2018). In the study of biodiversity, Indonesia is frequently incorporated in the country being discussed since it is a country that is tremendously rich in biological resources (Keong 2015). Indonesia is assumed as one of the largest archipelagic countries; hence it has become a significantly important country in terms of biodiversity in the world (Duha and Saputro 2022). Indonesia possesses the second highest biodiversity wealth after Brazil (Rohman et al. 2019). Furthermore, Indonesia also owns more than 38,000 species (Arifin and Nakagoshi 2011).

Regarding species diversity, Indonesia has the world's most diverse palm species, with over 400 species of Dipterocarpaceae wood (the entire commercial wood species in Southeast Asia), approximately 25,000 flowering plants, and various Indonesian fauna that ranks first in the world. Mammal species richness (515 species, 36% of which are endemic) and swiftlet butterfly species richness (121 species, 44% of which are endemic) are among the highest rank. In addition, the diversity of reptile species is ranked third (more than 600 species). Indonesia is also ranked fourth in bird species richness (1,519 species, 28% of which are endemic), fifth in amphibian species richness (over 270 species), and seventh in flowering flora (Keong 2015). Therefore, biodiversity information is required in natural resource management to maintain the sustainability of species use, explore biological potentials, monitor species and their ecology, make policies, and develop biotechnology innovations (Angelina et al. 2018).

Biodiversity is vital in ecosystem stability, encompassing humans as an ecosystem component. Therefore, biological resources must be utilized wisely (Tilman et al. 2014; Rosariastuti et al. 2022). The higher the level of biodiversity, the more stable the ecosystem was. However, the research on diversity is still deficient, particularly in the diversity of microorganisms (especially bacteria). Soil microorganisms play an essential role in enhancing the chemical and biological conditions of the soil, specifically soil structure and soil fertility in general, as well as other processes supporting human life to provide a better quality of life (Singh et al. 2011). The above-mentioned functions, services, and products of biodiversity, as well as the magnitude of the resulting economic value, will not be acquired sustainably unless the resources themselves are managed sustainably (Bennett et al. 2015). For example, the natural forests surrounding Mount Lawu have a low plant species diversity but a high density. This condition causes the environment in that location to become wet and humid, making it ideal for the growth of various organisms, including bacteria.

A moist tropical forest is a place rich in a diversity of bacteria and fungi. Therefore, the development of the National Park at Mount Lawu must be enhanced by

generating biodiversity conservation centers to become an integrated biodiversity conservation area (Setyawan 2001). In addition, this research is exploratory-descriptive research. Therefore, the survey method is employed in this study, along with technique exploration. This exploratory, descriptive research type aims to collect specimens, describe, identify, classify, and inventory the overall data on bacterial diversity obtained in this study. The western slope of Mount Lawu is divided into vegetable plantations, paddy fields, mixed land, residential, and forests. Purposive random sampling was employed in selecting sample points for each land cover. Therefore, this study is a useful database (information) of microbial biodiversity in the Greater Solo Region.

MATERIALS AND METHODS

Sample collection

This exploratory, descriptive study was carried out in 2021. The survey method was implemented in this study, along with the exploration technique. This survey focused on five land uses on Mount Lawu's West Slope, located in the Samin watershed of Karanganyar Regency, Central Java Province, Indonesia (7º 39" 17,793 South Latitude and 111º 03" 31,149 East Longitude). Vegetable plantations, paddy fields, mixed land, residential, and forests are the study sites. Purposive random sampling was employed to determine sample points for each land cover. This method was utilized to identify sample points for each land use based on an overlay of a map of soil type distribution, a map of slopes, and a map of land use. Moreover, random sampling was carried out during this study.

Sampling

Soil samples were collected on April 2021 and it was conducted following the research sample point. After cleaning the soil surface, a soil tester was used to measure pH and moisture. Meanwhile, a soil drill was utilized to collect soil samples from the rhizospheric soil at a depth of 20 cm. Soil samples were placed in sterile containers, stirred with a spatula, and kept in a cooler place. The samples were obtained under sanitary conditions and brought to the laboratory.

Extraction of genome DNA

Total genome DNA from samples was extracted using CTAB/SDS method. DNA concentration and its purity were monitored on 1% agarose gel. Therefore, following the concentration, DNA was diluted to 1ng/μL using sterile water.

Amplicon generation

ITS genes of distinct regions (ITS1/ITS2, Arc V4) were amplified using a specific primer with the barcode. All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs).

PCR products quantification and qualification

Mix the same volume of 1X loading buffer (containing SYB green) with PCR products and conduct electrophoresis on 2% agarose gel for detection. Samples with the bright main strip between 400-450bp were selected for further experiments.

PCR products mixing and purification

PCR products were mixed in equidensity ratios. Then, the mixture of PCR products was purified with Qiagen Gel Extraction Kit (Qiagen, Germany).

Library preparation and sequencing

Sequencing libraries were generated using the NEBNext Ultra DNA Library Pre ® Kit for Illumina, following the manufacturer's recommendations and adding the index codes. Next, the library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina platform, and 250 bp paired-end reads were generated.

Data analysis

The paired-end reading was performed based on its unique barcode, and the barcode and primary sequence were then cut. FLASH (V1.2.7) was utilized to perform paired-end reads; this is an accurate analytical tool in paired-end readings when multiple reads overlap with reads resulting from opposite ends of DNA with the same fragment, and the splicing sequences are identified as raw tags. According to Qiime, quality screening of raw tags was conducted under special screening conditions to obtain high-quality clean tags (V1.7.0). Tags were compared to the reference-based SILVA database to detect chimera sequences using the UCHIME algorithm. The chimera sequence was then removed, leaving only the effective tag. The Uparse software was employed for sequence analysis with all effective tags. If a sequence has 97% similarity, the same OTU is assigned. Each OTU was subjected to representative screening to obtain a more detailed explanation. The Qiime in Mothur method was applied to the SSUrRNA SILVA database for species annotations at each taxonomic rank in regular series (kingdom, phylum, class, order, family, genus, species). This method could quickly compare multiple rows to obtain phylogenetic relationships for all OTU representative sequences. Operational Taxonomical Units (OTU) normalization was conducted using a standard sequence number corresponding to the minor order sample. Furthermore, alpha diversity was analyzed to determine the complexity of biodiversity in the sample through the Shannon index. The diversity index in the model was calculated by QIIME (ver 1.7.0).

Bacterial diversity index

The species diversity index was calculated by the Shannon of General Diversity formula (Odum 1993; Rahim et al. 2023):

Species diversity index
(H') = - (ni N) log (ni N)
Where:
Ni = Significance index of type i
N = Total value of the significant index

The amount of the species diversity index following Shannon-Wiener was defined as follows (Fachrul 2007):

1. If H> 3 indicates a high level of bacterial diversity.
2. If 1 H 3 indicates a moderate level of bacterial diversity
3. If H< 1 indicates that the level of bacterial diversity is low

RESULTS AND DISCUSSION

Relative abundance of bacteria in various land layers

The relative abundance of soil bacteria in Mount Lawu was studied on five land uses: vegetable plantation, paddy fields, residential, mixed land, and forest, divided into upper and lower slopes and upper and lower layers. Figure 1 depicts the relative abundance of soil bacteria in Mount Lawu.

Diversity based on bacterial phylum revealed differences in the number of phylum and the relative abundance of each phylum in various land uses (Figure 1). The diversity of bacterial phylum obtained from the results of NGS analysis on the western slopes of Mount Lawu is more than 10 phylum, including Proteobacteria, Methylomirabilota, Firmicutes, Actinobacteria, Verrucomicrobia, Acidobacteriota, Bacteroidota, Myxococcota, Chloroflexi, and others. The top 9 genera found were *Streptomyces*, *Bacillus*, *Rokubacteriales*, *Bradyrhizobium*, *Methylotenera*, *Massilia*, *Acinetobacter*, *Lysobacter*, and *Candidatus Udaeobacter*. The dominant species were *Bacillus funiculus*, *Bradyrhizobium elkanii*, and *Lysobacter dokdonensis*, with a relative abundance of 2.70%, 1.23%, and 0,66%, respectively. The proteobacteria phylum has the highest relative density. Proteobacteria are bacteria that come in various shapes (Šimek et al. 2005). Proteobacteria are divided into chemoautotrophic purple bacteria, chemoautotrophic Proteobacteria, and chemoheterotrophic Proteobacteria. Chemoautotrophic purple bacteria, as the name implies, are typically purple, red, brown, or orange (Hanada 2003). Purple bacteria are photoautotrophic organisms with chlorophyll and carotenoids. Thus they can produce food through photosynthesis (Mirkovic et al. 2017). Chemoautotroph Proteobacteria are bacteria that can produce food using the energy from chemical reactions. Some chemoheterotrophic proteobacteria are anaerobic. They are also facultative bacteria that can live in the absence or presence of oxygen. Some of these bacteria are dangerous such as *Salmonella* is an example of a harmful bacteria that can cause food poisoning. Proteobacteria predominate in all land uses. The population of Proteobacteria is substantially greater in the surface layer of soils and gradually decreases with depth; individual Proteobacteria strains can be discovered in all soil layers. Proteobacteria are numerous, widespread in soil, and abundant compared to bacteria. As a first responder, Proteobacteria is expected to be sensitive to environmental factors (Shin et al. 2015).

The most extensively administered index in determining species diversity is the Shanon wiener index (H'). A better H′ value indicates a higher species diversity. The Shanon wiener index value follows the number of individuals in the bacterial species. Based on the diversity index, the variety on the western slope of Mount Lawu is classified as high. The diversity index is demonstrated in Table 1.

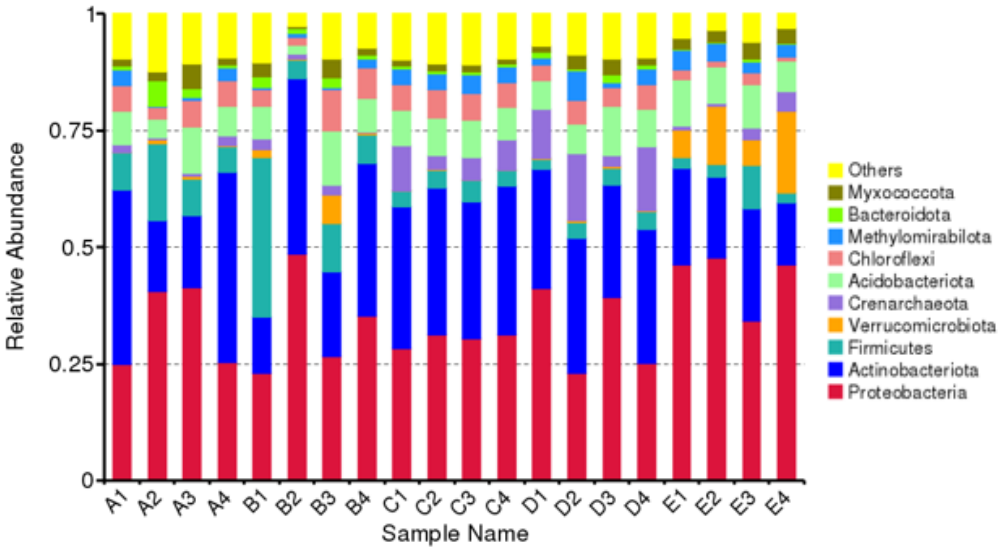

Figure 1. Relative density of soil bacteria in various land layers, (A) Vegetable plantation; (B) Paddy fields (C) Residential; (D) Mixed land; (E) Forest; 1: Top slope, the top layer; 2: Top slope, the bottom layer; 3: Bottom slope, the top layer; 4: Bottom slope, the bottom layer

Table 1. The index diversity of soil bacteria in various land layers

Group	Index diversity	Category
A1	8.19	High
A2	8.95	High
A3	10.33	High
A4	7.71	High
B1	9.59	High
B2	7.00	High
B3	9.74	High
B4	7.68	High
C1	7.98	High
C2	8.14	High
C3	8.29	High
C4	8.02	High
D1	7.90	High
D2	7.61	High
D3	9.67	High
D4	7.89	High
E1	7.77	High
E2	7.22	High
E3	8.91	High
E4	6.77	High

Notes: (A) Vegetable plantation; (B) Paddy fields (C) Residential; (D) Mixed land; (E) Forest; 1: Top slope, the top layer; 2: Top slope, the bottom layer; 3: Bottom slope, the top layer; 4: Bottom slope, the bottom layer

The diversity of bacteria on the western slope of Mount Lawu is illustrated in Table 1 by the H' value, which is in the high category. The more valuable a variety, the more types are produced, which is highly dependent on the total value of the individuals of each type or genera. When all individuals come from different genera or species, diversity (H') has the highest value. In contrast, the value is the smallest of all individuals from the same genus or species. If the H' value is greater than 3.0, the sample has increased diversity and very high productivity, indicating heavy pressure, and the ecosystem is stable, according to Fachrul (2007). The results of this study confirm that the environmental conditions on the western slope of Mount Lawu are tremendously stable.

Relative abundance of bacteria in various land uses

The diversity of phylum based on Phylum Taxa shows differences in the number of phylum and the relative abundance of each phylum at the five land uses. Figure 2 depicts the relative abundance of taxa within phylum.

Based on Figure 2, it can be seen that the diversity of phylum in each land use is more than 10 phylum. For example, the diversity of phylum in Vegetable plantation (A) in the order of greatest to smallest relative abundance includes Proteobacteria (0.330%), Actinobacteriota (0.272%), Firmicutes (0.093%), Acidobacteriota (0.069%), Chloroflexi (0.047%), Myxococcota (0.025%), Bacteroidota (0.022%), Methylomirabilota (0.017%), Verrucomicrobiota (0.004%), and others (0.026%). In Paddy fields (B), including Proteobacteria (0.333%), Actinobacteriota (0.251%), Firmicutes (0.136%), Acidobacteriota (0.069%), Chloroflexi (0.051%), Myxococcota (0.021%), Verrucomicrobiota (0.021%), Bacteroidota (0.015%), Methylomirabilota (0.009%), and others (0.015%). Residential (C) includes Actinobacteriota (0.307%), Proteobacteria (0.303%), Acidobacteriota (0.076%), Chloroflexi (0.056%), Firmicutes (0.038%), Methylomirabilota (0.034%), Myxococcota (0.013%), Bacteroidota (0.006%), Verrucomicrobiota (0.001%), and others (0.015%). In Mixed land (D), they include Proteobacteria (0.321%), Actinobacteriota (0.268%), Acidobacteriota (0.076%), Chloroflexi (0.044%), Firmicutes (0.031%), Methylomirabilota (0.031%), Myxococcota (0.022%), Bacteroidota (0.009%), Verrucomicrobiota (0.003%), and others (0.011%). In Forest (E), include Proteobacteria (0.436%), Actinobacteriota (0.189%), Verrucomicrobiota (0.103%), Acidobacteriota (0.083%), Firmicutes (0.040%), Methylomirabilota (0.032%), Myxococcota (0.029%), Chloroflexi (0.017%), Bacteroidota (0.003%), and others (0.007%).

Moreover, the phylum Proteobacteria dominates in every land use except Residential. Proteobacteria share the trait of being Gram-negative bacteria with lipopoly-saccharides in their outer membrane. Proteobacteria are the most numerous phylum in the bacterial domain. Many Proteobacteria use flagella to move, but some are immobile or rely on bacterial glide (Cavalier-Smith 2006). The phylum Proteobacteria was divided into six classes (previously considered a subclass) based on phylogenetic analysis of the 16S rRNA gene: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteo-bacteria, Epsilonproteobacteria, and Zetaproteobacteria. Many common pathogens are discovered in the phylum Proteobacteria: for example, the *Brucella* and *Rickettsia* genera belong to the Alphaproteobacteria class, *Bordetella* and *Neisseria* to the Betaproteobacteria class, *Escherichia*, *Shigella*, *Salmonella*, and *Yersinia* to the Gammaproteobacteria class, and *Helicobacter* to the Epsilobacteria class (Rizzatti et al. 2017). Based on Figure 3, Proteobacteria revealed on the western slopes of Mount Lawu are the class Alphaproteobacteria with the genus *Bradyrhizobium* as much as 1.23% and Gammaproteobacteria 52.82% with the division of the genus *Methylotenera* 1.69%, *Massilia* 20.33%, *Acinetobacter* 0.07%, *Lysobacter* 30.72%.

Actinobacteria is the most abundant in residential land use and the second most in any land use. Most Gram-positive bacteria belong to the Actinobacteria phylum. They can be either terrestrial or aquatic in the world. They are economically significant to humans because agriculture and forests rely on their contribution to the soil system. They help decompose dead organisms' organic matter in the soil, allowing plants to retrieve molecules (Barka et al. 2016). Some soil actinobacteria (such as Frankia) live symbiosis with plants whose roots seep into the soil. They could bind nitrogen to plants instead of access to some plant saccharides (Santi et al. 2013). Other *Mycobacterium* species, like many others in the genus, are pathogens. Many species of actinomycete bacteria exist, including *Streptomyces sp*. and *Kitasatospora sp*. A *Streptomyces* genus was revealed in this study; Rice growth and yield can be increased by *Streptomyces* sp. These bacteria were

found to have the hormone IAA, which promotes cell extension or division (Abd-Alla et al. 2013). This bacterial group can also produce antibiotic compounds such as polyketides, β-lactams, and peptides. These antibiotics have antifungal, antitumor, and immunosuppressive properties (Adegboye and Babalola 2013). The antibiotic Kasugamycin produced by *Streptomyces kasugaensis* encompasses bactericidal and fungicidal (Gohain et al. 2020). In addition, Firmicutes is one of the edges in which bacteria are classified. This phylum comprises three classes (Bacilli, Clostridia, and Erysipelotrichia), 26 families, and 223 genera, making it the main bacterial phylum (Galperin et al. 2022). These bacteria are classified in this phylum due to sharing common evolutionary history. All possess rigid cell walls, from which Firmicutes derives (in Latin, Firmus means firm and skin see skin or cell wall). The genus found in this phylum is *Bacillus*.

Based on its distribution Figure 4, Proteobacteria are extensively dominated in paddy fields> mixed land > forest > residential > vegetable plantations. Methylomirabilota is discovered in mixed land > forest> residential > vegetable plantations > paddy fields. Firmicutes are employed in paddy fields > forests >vegetable plantations > residential> mixed land. Actinobacteria have been utilized on mixed land > vegetable plantations > forest > paddy fields. Verrucomicrobiota is used for forest > paddy fields > mixed land. Paddy fields are a focal environment in which redox conditions are determined by the balance between rice roots' reduction and oxidation capacity and the production of C root compound compounds, which provide an energy source for microbial growth (Wei et al. 2019). The inundation of paddy fields alters the microflora in the soil. In some countries, bacteria predominate in inundated soils. At the same time, fungi and actinomycetes predominate in dry land.

Shanon Weiner Diversity Index on various land uses

The results of the Shanon Weiner Diversity Index research on the western slopes of Mount Lawu, conducted on five land uses, encompassing Vegetable plantations, Paddy fields, Residential, Mixed land, and Forest, are demonstrated in Figure 5.

The diversity of bacteria on the western slope of Mount Lawu is high. The Shannon-Wiener diversity index analysis reveals that if bacterial species have a greater number of individuals, with a total of all individuals proportional to the number of individuals of each species, the diversity value will be higher. The Shannon-wiener diversity index (H') ranges between 7.66 - 8.80, classified as high. The diversity index (H') value is 8.80 for vegetable plantations, 8.50 for paddy fields, 8.26 for mixed land, 8.10 for residential, and 7.66 for the forest, with the vegetable plantation having the highest bacterial diversity index (Figure 5). The higher the diversity value of an area, the more stable the community (Ouyang et al. 2021). The diversity of a species can change rapidly in an ecosystem. The high diversity of species shows the balance of the ecosystem; conversely, low species diversity indicates that the ecosystem is experiencing stress or pressure. This study revealed it appears that there is a tendency that land use that has undergone land use change to have decreased diversity, especially in forest land use.

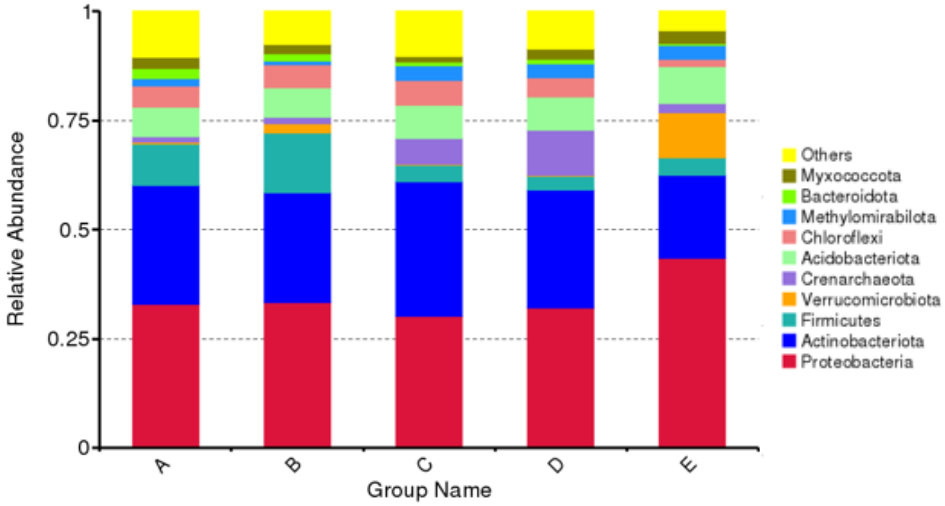

Figure 2. Histogram of the relative abundance of soil bacteria in various land uses, (A) Vegetable plantation; (B) Paddy fields (C) Residential; (D) Mixed land; (E) Forest

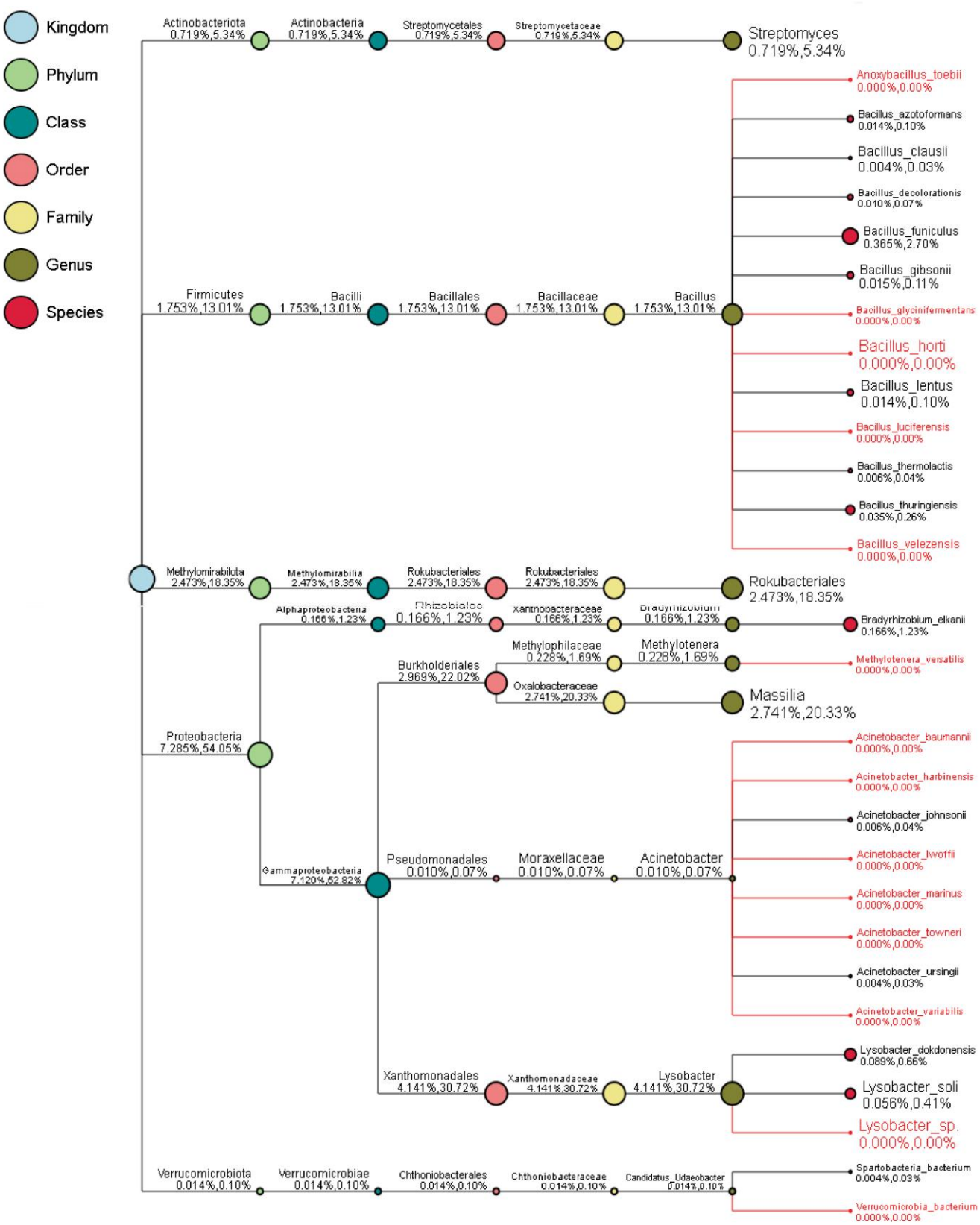

Figure 3. Taxonomy tree of big five phylum, (A) Vegetable plantation; (B) Paddy fields (C) Residential; (D) Mixed land; (E) Forest

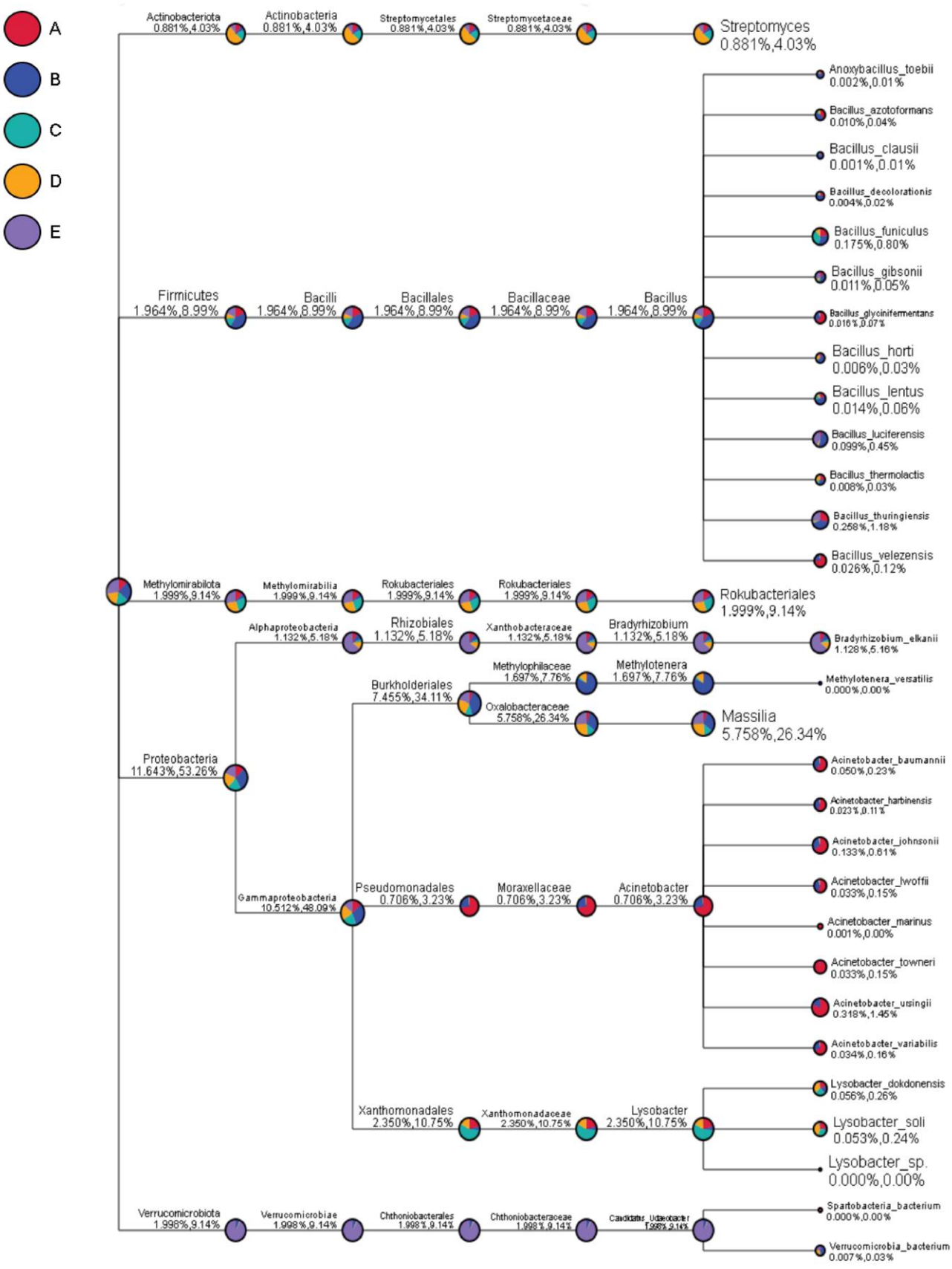

Figure 4. Taxonomy tree of the group, (A) Vegetable plantation; (B) Paddy fields (C) Residential; (D) Mixed land; (E) Forest

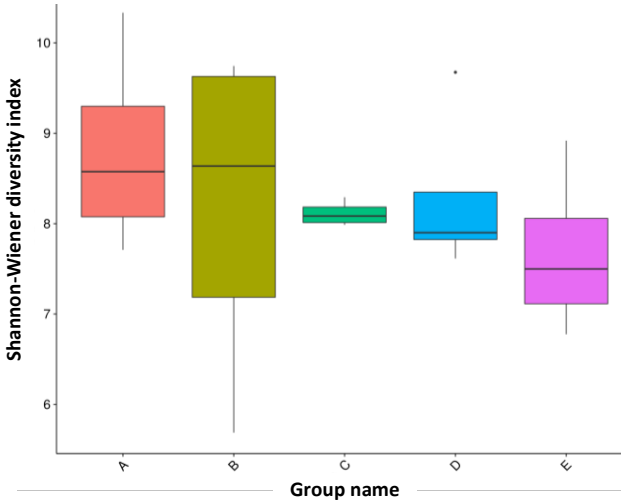

Figure 5. The value of the Shannon-Wiener diversity index (H') on various land uses, (A) Vegetable plantation; (B) Paddy fields (C) Residential; (D) Mixed land; (E) Forest

In conclusion, the diversity of bacteria on Mount Lawu's western slope is classified as high. The diversity index value (H') for vegetable plantations was 8.80, 8.50 for paddy fields, 8.26 for mixed land, 8.10 for residential, and 7.66 for forests, with vegetable plantations having the highest bacterial diversity index. According to these results, there are more than ten phylum and 28 species of bacteria. Proteobacteria, Methylomirabillota, Firmicutes, Actinobacteria, Verrucomicrobiota, Acidobacteriota, Bacteroidota, Myxococcota, Chloroflexi, and other phylum are among the bacteria. The phylum Proteobacteria was dominant in each land use, followed by the phylum Actinobacteria. The findings of this study may be utilized to create a database (information) about microbial biodiversity in the Solo area.

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