Changes in Essential Soil Nutrients and Soil Disturbance Directly Affected Soil Microbial Community Structure: A Metagenomic Approach

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ABSTRACT

Soil environment, both biotic (e.g., microbial community) and abiotic (e.g., nutrients and water availability) factors determine soil fertility and health and are directly affected by soil management systems. However, only limited studies evaluate the combined effect of nutrients availability and soil disturbance on the soil bacteria community structure, especially in conventional agricultural practices, on the forests converted to agricultural land. This study aimed to provide a viewpoint of the effect of different soil management systems, i.e., forest soil (natural process) and tilled land, on soil bacteria community structure on forest converted to agricultural land, according to a metagenomics approach. Moreover, each land use was sampled to identify the bacterial community using 16S gene as a biomarker. The sequencing was performed using MinION (Oxford Nanopore Technologies) to read the DNA sequence from each soil sample. Principle Component Analysis (PCA) was performed to comprehend the relationship between availability of nutrients and bacterial diversity. The results revealed that the concentrations of soil micronutrients, such as iron, zinc, and magnesium, were significantly higher in forest soil than in tilled land. According to diversity indices, soil bacteria were more diverse in forest soil than in tilled land. Forest soil had more distinctive taxa than tilled land. Several species comprised the most abundant taxa, such as *Candidatus Koribacter versatilis*, *Candidatus Solibacter usiatus*, *Rhodoplanes* sp., *Luteitalea pratensis*, and *Betaproteobacteria bacterium,* were more scarce in tilled land. On the distinctive taxa in each soil sample, *Anseongella ginsenosidimutans* and *Janthinobacterium* sp. were the most abundant species in forest and tilled land, respectively. According to PCA analysis, soil management system affected the soil micro-and macronutrients also microbial community structure between forest and tilled land. In conclusion, soil management influences the essential nutrient content and bacterial community structure of soil. Better management should be adopted to maintain soil quality near forest soil.

Keywords: forest soil, metagenome, soil bacteria, soil nutrients, tilled land.

INTRODUCTION

Soil fertility is highly influenced by biotic and abiotic factors (Fortier et al., 2019). However, anthropological disturbances, such as improper soil management affect the interaction between these factors, resulting in inadequate soil ability to support plant growth and production and provide ecosystem services (Wang et al., 2020). Soil mismanagement, such as intensive soil tillage, influences physicochemical properties and microbial structure of soil in terms of diversity and density (Willy et al., 2019; Durrer et al., 2021). Several studies have reported that the microbial community structure of soil has a significant correlation with soil properties (Koorem et al., 2014; Jacoby

et al., 2017; Bargaz et al., 2018). Disturbance in the physicochemical properties of soil reduces the number of beneficial bacteria, which is valuable for plant growth due to the soil microbial roles in nutrient cycling (Koorem et al., 2014; Jacoby et al., 2017; Bargaz et al., 2018). Currently, assessing the status of essential nutrients of soil has been a crucial strategy for monitoring soil fertility and health that is important for maintaining the microbial activity of soil, furthermore assisting plant growth for high yield of plant production (Lehmann et al., 2020; Jin et al., 2021).

As a crucial part of ecosystem services, forest soil is an undisturbed ecosystem (natural ecosystem) with low essential nutrients loss through leaching and evaporation (Allen et al., 2016; Kurniawan et al., 2018). However, land conversion from forest to agricultural purposes has altered the biological properties of soil, precisely forest soil function in provisioning ecosystem services. A previous study reported that land conversion from natural forest to agricultural land, within ten decades, has declined total organic carbon and soil essential nutrients (such as magnesium, boron, nitrogen, and iron) by 72% and 65%, respectively (Willy et al., 2019). The decline in soil essential nutrients affects plants as well as the microbial growth and development of soil because the nutrients are crucial factors for them (Singh & Gupta, 2018; Oliveira et al., 2022). Moreover, altering the microbial (e.g., soil bacteria) growth and developments of soil, mainly due to abiotic disturbance reducing the microbial biomass of soil (Holden & Treseder, 2013). A previous study revealed that abiotic disturbance in forests reduces soil bacteria and microbial biomass by 15.3% and 29.4%, respectively (Holden & Treseder, 2013).

Many studies have reported that soil microbiome is a critical indicator of soil health (Dubey et al., 2019; Saleem et al., 2019) because it is a key ecological driver in ecosystem services, such as maintaining soil sustainability, supporting agricultural production and environmental regeneration, conserving soil health under changing climate, and rehabilitation of degraded lands by supporting plant growth and biomass production (Singh & Gupta, 2018; Ustiatik et al., 2020, 2022; Oliveira et al., 2022). Monitoring soil microbiome will have a significant role in sustainable land management (Allen et al., 2016; Saleem et al., 2019). However, only limited studies evaluate the combined effects of soil disturbance and availability of nutrients on microbial community structure between forest and tilled land that was previously converted from forest to agricultural land which is less than 10 years old. This study aimed to analyze the effect of essential soil nutrients (micro-and macronutrients) on soil microbiome under different soil managements, i.e., undisturbed (forest) and disturbed soil (tilled land) on the forest that was converted to agricultural land. Thus, this study will be beneficial for evaluating the difference between soil management (soil tillage and nutrients availability) on the microbial community, both density and diversity, to address the direct impact of soil management on soil microbial communities and nutrients status.

MATERIAL AND METHOD

Study site and sample collection

This study was conducted at the education forest of Brawijaya University (UB Forest), located in Karangploso Sub-regency, Malang Regency, East Java, Indonesia (Figure 1A). The soil samples were collected from two different soil management systems, i.e., undisturbed soil (forest soil; 7°49'39.8" S 112°34'43.9" E; Figure 1B) and disturbed soil (tilled soil; 7°49'28.1" S 112°34'46.6" E; Figure 1C).

Previously, the location was a natural forest that was converted to agricultural land in 2015. The soil sample was collected from a plot (50×30 m). The sample point was determined using a diagonal method (soil sample was collected from each corner and center of the plot; 5 points in each sampling location) within 30 cm soil depth. The distance between undisturbed soil (forest soil) and disturbed soil (agricultural land) plot was >50 m. The soil samples were kept in a cooling box and transported to the laboratory for genomic DNA extraction.

Soil chemical analysis

The essential macronutrients of soil were analyzed, namely: total nitrogen (N) was analyzed using the Kjeldahl method; available phosphorus (P) was analyzed using the Olsen method (soil pH >5.5); available potassium (K) and magnesium (Mg) were analyzed using the ammonium acetate extraction method, then the extracted solutions were measured using flame photometry (for available-K) and Atomic Absorption Spectroscopy (AAS) for Mg. Moreover, soil essential micronutrients were analyzed, namely: copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn) using the diethylenetriamine pentaacetate (DTPA) method; thus, the extracted solutions were measured using AAS.
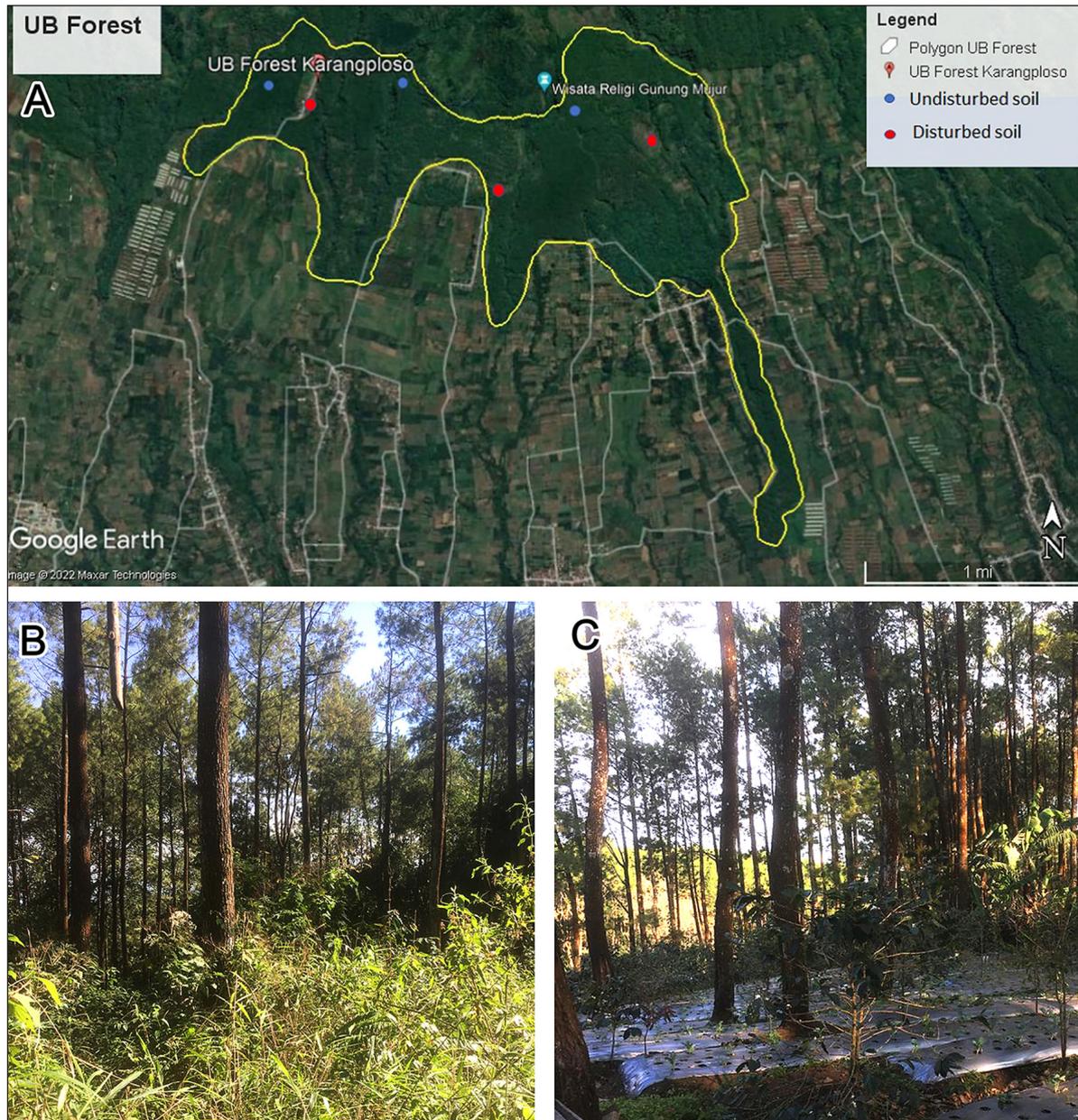
Figure 1. Study site: map of sampling location (A); sampling point on forest (B) and tilled land (C)

DNA extraction and sequencing

Genomic DNA extraction was performed using PowerSoil DNA Isolation Kit (Mo Bio Laboratories), then quantified using Nanodrop Spectrophotometer and Qubit Fluorometer to assess the DNA concentration and purities. DNA was sequenced using MinION (Oxford Nanopore Technologies) provided by Genetika Science Laboratory (Tangerang, Indonesia) with the kits provided by the manufacturer for library preparation. GridION sequencing was operated by MinKNOW software version 20.06.9. 16S gene (V1-V9) region was used as a taxonomic marker. The sequence of 16S primers was as follows:

- 27F–5'–AGAGTTTGATCMTGGCTCAG–3' (forward)
- 1492R – 5' – TACGGYTACCTTGTTAC-GACTT–3' (reverse)

Raw sequence processing, taxonomic assignment and diversity analysis

Upon sequencing, base calling was performed using Guppy version 4.0.11 with high accurate mode (Wick et al., 2019). Nanoplot was used to visualize the obtained FASTQ sequence quality (De Coster et al., 2018). The filtered sequences were then assigned to Operational Taxonomic Units (OTU) with

a Centrifuge classifier (Kim et al., 2016). Relative abundance of OTU was visualized in a heatmap with Graphpad Prism 8. Diversity indices were calculated using Pavian (Breitwieser & Salzberg, 2020).

Data analysis

The soil micro and macronutrients between forest and tilled land were analyzed using t-independent test with p<0.05 determined as significantly different in SPSS version 22 (IBM Statistics). To investigate the interaction among soil chemical properties with microbial diversity indices, multivariate ordination analysis with correlation matrix was performed and visualized in PCA plot using PAST version 4.09.

RESULT AND DISCUSSION

Soil micro and macronutrient status

Undisturbed soil (forest soil) had significantly higher Fe and Zn concentrations than disturbed soil (tilled soil in agricultural land) (p<0.01 and p<0.05, respectively). However, there was no difference in the Cu and Mn concentration between forest and tilled land (Figure 2A). This result indicates that soil micronutrients are sensitive to soil management (such as soil tillage), even though the situation cannot be applied to all measured soil micronutrient parameters; in this study, it was applied only for Fe and Zn. The result is the opposite of a previous study (de Santiago et al., 2008) who reported that Cu, Mn, Fe, and Zn in vertisol are significantly affected by soil tillage. However, in this study, only Fe and Zn are more sensitive to soil management (soil tillage) than Mn and Cu. The difference might be due to different soil types. Soil order in UB Forest is classified as Inceptisol or young soil (Kurniawan et al., 2019).

Nevertheless, the result aligns with an earlier study which reported that soil micronutrients decline under long-term soil tillage (Shiwakoti et al., 2019). In the study site, specifically tilled land, soil tillage is a part of agriculture activity that is difficult to avoid, because farmers tend to carry out soil tillage for weeds control and prepare the land for seeding and applying fertilizer. Meanwhile, soil tillage has been well known to disturb the physical and chemical properties of soil (Issaka et al., 2019). On the contrary, no-tillage and minimum tillage secure these properties, especially at 0-20 soil depth (Issaka et al., 2019). Moreover, the soil N and P losses from water runoff and drainage can be minimized (Issaka et al., 2019).

Soil disturbance, such as tillage, is considered a core influence affecting the communities of soil organisms (micro-and macro-organisms) due to the disturbance affecting organic matter supply as nutrient sources for soil organisms' biological activities; moreover, different soil organisms feed on varying organic substrates (Madegwa & Uchida, 2021). The organisms contribute to various processes, e.g., decomposition, which release and mineralize nutrients through the breakdown of organic matter. Mineralization by soil microorganisms plays a vital role in the environment, releasing trapped mineral nutrients, such as phosphorus (Koshila Ravi et al., 2019), and making the nutrient available for plants.

For macronutrients, there were no differences in the concentration of soil macronutrients (p>0.05), except the Mg concentration (p<0.05), which was higher in the forest than in the tilled land (Figure 2B). The result might be due to the supply of macronutrients from fertilizers applied by farmers (such as NPK fertilizers) and insufficient Mg supply from the applied fertilizer in tilled land. Contrary to forest soil, the supply of soil nutrients comes from tree litterfalls as a source of organic matter that decomposes and mineralizes as available nutrients for plants (Giweta, 2020).
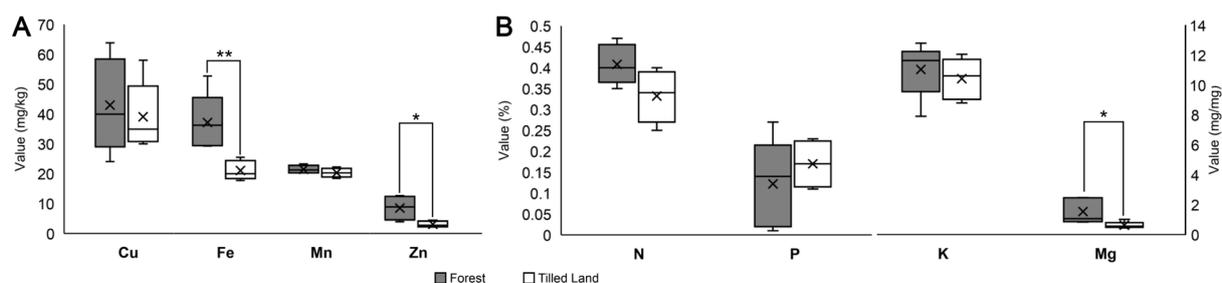

Figure 2. Soil micro- (A) and macronutrients (B) concentration of forest and tilled land. The boxplot was constructed with n=5 for each nutrient with asterisk (*) symbol representing a significant difference (* = p<0.05 and ** = p<0.01)

Microbial diversity

Microbial diversity analysis highlighted that forest soil contains higher diversity than tilled land. Forest soil has higher taxa richness with better evenness, represented by Simpson index. Additionally, Shannon and Chao1 described higher diversity than tilled land. All diversity measures described that forest soil serves a better environment for soil bacterial growth (Table 1). A previous study reported that the diversities of bacteria, Acidobacteria, and fungi are affected by land use change (Sui et al., 2019). A richer place indicates a favorable place for microbial growth and development. The finding aligns with the previous study which reported that forests serve a better condition for microbial diversity and structure richness due to optimal availability of microbial growth development factors, i.e., optimal soil nutrient availability and oxygen levels (Sui et al., 2019). In contrast, anthropogenic activities in managing land (e.g., land use change) alter soil microbial community structures, due to the activity significantly affecting soil pH, phosphorus, soil nitrogen, and total organic carbon (Sui et al., 2019; Dang et al., 2021). It is well-known that soil organic carbon is a nutrient source and an important component in conserving soil microbial diversity and density (Page et al., 2020; Szostek et al., 2022).

The data from the number of obtained sequences revealed that forest soil had greater microbial frequency than tilled land (Figure 3A). In addition, forest soil also had more unique taxa than the tilled land, starting from the phylum, family, and species level (Figure 3B). At the phylum level, Proteobacteria was the most abundant phylum in both soil samples, followed by Acidobacteria, Firmicutes, Bacteroidetes, Planctomycetes, and Actinobacteria. However, several phyla appeared to have decreasing or increasing frequency, suggesting the occurrence of population shift in each sample (Figure 3C). In line with the data at the phylum level, Acidobacteriaceae became the most abundant family in both soils. Interestingly, there was a significant

Table 1. Diversity measures of bacterial population in forest and tilled land

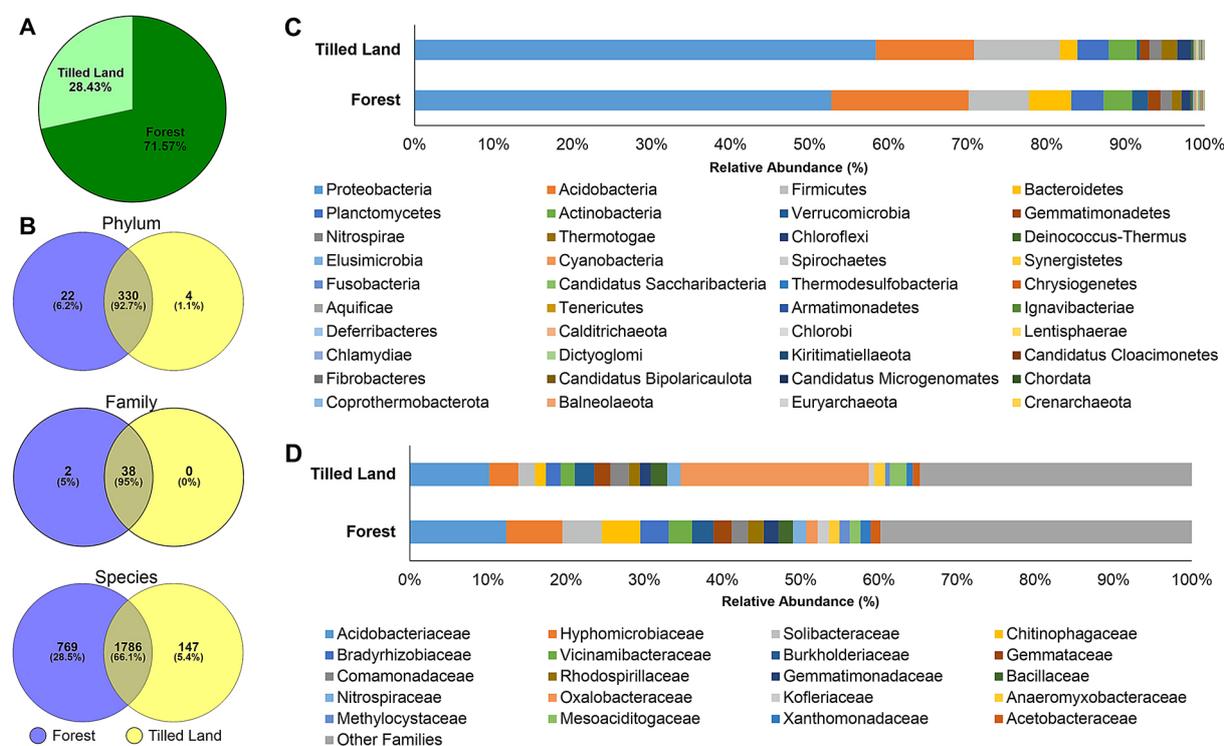| Soil type	Richness	Simpson	Shannon	Chao1
Forest	2,682	0.0157	5.59	3,190.2
Tilled Land	2,011	0.0326	5.15	2,525.2

Figure 3. The abundance of bacterial frequency according to the number of obtained sequence (A), the number of observed taxa at the phylum, family, and species level (B), and the relative abundance of bacterial phylum (C) and family (D) in forest and tilled land

increase of Oxalobacteraceae in tilled land, accompanied by attenuation of the abundance of several families, such as Acidobacteriaceae, Hyphomicrobiaceae, Solibacteraceae, Chitinophagaceae, and Bradyrhizobiaceae (Figure 3D).

Taxonomic assignment at the species level discovered that *Candidatus Koribacter versatilis, Candidatus Solibacter usiatus, Rhodoplanes* sp., *Luteitalea pratensis,* and *Betaproteobacteria bacterium* were the most abundant taxa in both samples. Nonetheless, the percentage of abundance decreased in tilled land. Top 30 species with the highest abundance indicated a decreasing number of individual bacteria in tilled land relative to the forest soil (Figure 4A). Moreover, 769 taxa found in forest soil differed from tilled land. On the other hand, 147 taxa were only found in tilled land (Figure 3B). Among these spesies, *Anseongella ginsenosidimutans* was the most abundant species in forest soil, while *Janthinobacterium* sp. was the most abundant distinctive species in tilled land. Since the frequency was higher in forest soil than tilled land, the distinctive taxa from tilled land have lower relative abundance than forest soil (Figure 4B).

Interaction among soil nutrients and microbial diversity

The analysis of the PCA plot revealed that forest and tilled land had different nutrients and microbial diversity interactions. The micro- and macronutrient parameters of soil affected bacterial diversity, mainly N, P, K, and Fe, positively correlated with the diversity indices (Figure 5). A positive correlation between soil nutrients with diversity indices indicated that the available soil nutrients influence microbial diversity and structure. Thus, optimal availability of micro- and macronutrients will directly affect bacterial diversity, with forest soil serving a better environment for bacterial growth and development than tilled land. Agricultural cultivation activities, i.e., soil tillage, change the availability of soil micro- and macronutrients nutrients. Many studies reported that the activity reduces the availability of nutrients (de Santiago et al., 2008; Issaka et al., 2019; Kurniawan et al., 2019). However, in a particular case, the activity has a more beneficial effect on improving oxygen content in wetlands, thus preserving high microbial diversity (Sui et al., 2019). In this study, soil tillage alters microbial diversity and structure by reducing soil nutrients (Mg, Fe and Zn) sensitive to soil disturbance. Moreover, a previous study revealed that soil tillage, such as deep plow, decreases soil water content (Alvarez & Steinbach, 2009), thus reducing the bacterial population due to water availability as a source of energy (Ustiatik et al., 2021). For long-term and intensive tillage without conservation measures, soil will face significant degradation such as soil erosion and compaction that trigger soil biodiversity loss (Busari et al., 2015; Tibbett et al., 2020).
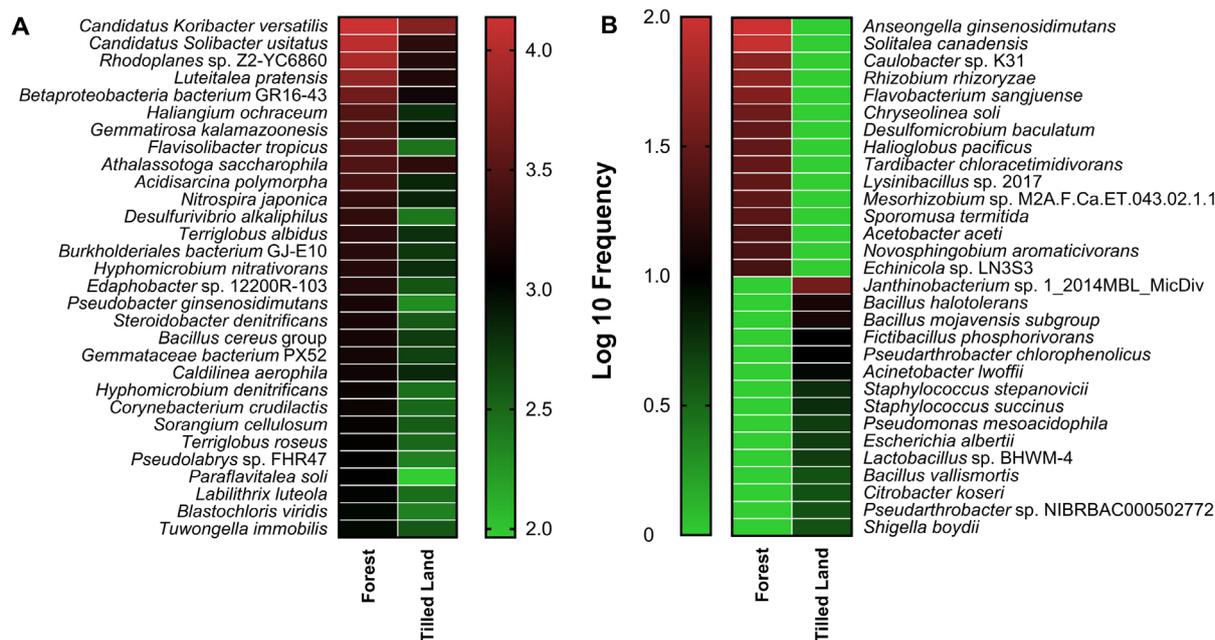

Figure 4. Top 30 most abundand species (A) and top 15 distinctive species (B) in forest and tilled land. The data was sorted relative to the forest sample
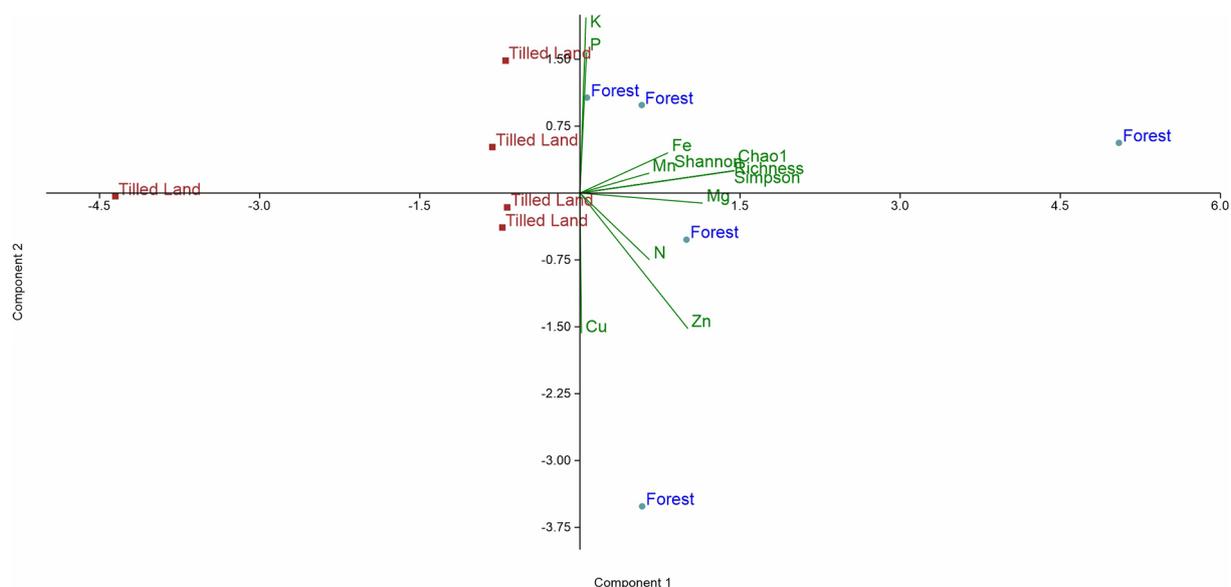
Figure 5. Interaction among soil micro-and macronutrients and bacterial diversity indices in forest and tilled soil

Maintaining soil bacterial diversity is vital for preserving microbial community diversity and ecosystem services; due to anthropogenic activities altering the microbial community, it might be difficult to restore it to its original condition (Sui et al., 2019). Further study is needed to evaluate the combined effects of agricultural cultivation activities such as soil, tillage, mulching and fertilizer application on microbial diversity and structure to evaluate the business as usual effects on soil sustainability and find the best management for soil sustainability in UB forest.

CONCLUSIONS

Forest and tilled soil have different characteristics, both in abiotic and biotic components. Forest soil has greater soil bacterial diversity due to optimal micro-and macronutrient availability than tilled soil. Thus, forest soil serves a better environment for bacterial growth and development than tilled land. Therefore, tilled land management had diminished soil nutrients, specifically Mg, Fe and Zn, and soil bacteria diversity and population, affecting soil fertility to support plant growth and crop production.

Acknowledgements

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