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SOIL BIOLOGY

Characterization of Bacterial and Fungal Communities in Soils under Different Farming Systems. The Cacao Plantation in Sulawesi Island—Indonesia[1]

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Abstract—The cacao plantations in Sulawesi Island, Indonesia are responsible for a great part of the local economy; however, their soils still need to be deeply explored. Our study focused on evaluation of the microbial communities in cacao soils according to their location and applied management system. Four soil samples were taken from six cacao farms under two kinds of systems (conventional and organic). 16S and ITS rDNA amplicon sequencing analyses of soils were also performed to identify bacteria and fungi, respectively, whereby their relative abundance and diversity were determined. In general view, the bacterial and fungal communities were affected by management system at the local and general levels. Bacterial analyses indicated that the number of operational taxonomic units and bacterial diversity were higher under the organic system in Kulawi, Palolo, and Poso farms. The composition and biodiversity of fungi were clearly different between organic and conventional systems and between different places (coastal and inland). The effect of agricultural management was observed in each location individually and in general.

Keywords: agricultural management, amplicon sequencing, location, microbial diversity

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INTRODUCTION

Indonesia is the third largest cacao producer in the world, and the economy of cacao production has an immense impact on small holders and commercial farmers [13]. Understanding the components of soil microbiota, and the functional associations between microbes and associated plant roots, plays a vital role in improving cacao production. The functional and composition dynamics of soil microbial communities under different soil conditions could be used to help monitor agricultural practices and to propose better soil management strategies [17]. Accurate taxonomic characterization of the soil microbial genetic profile is indispensable for determining microbial diversity and its role in soil fertility [8].

Agricultural systems with reduced inputs, including synthetic fertilizers and pesticides, would greatly contribute on sustainable production improvement. Their effects on microbial diversity and composition would induce a better understanding of the systems. Some researchers have reported the increasing of soil microbiota richness by organic farming after 10 years in this system [21]. Moreover, the soil nutrients, soil enzyme activities, and bacterial richness and diversity could be improved by organic management, with some major bacterial lineages that contribute to nutrient (C, N, S and P) cycling were enriched [34].

Studies on soil microbial diversity have been performed for different agronomic purposes, ranging from the investigation of plant microbial associations to the prediction of future environmental changes and flora successions [14, 19, 26, 35]. Sometimes the soil organic carbon could be more sensitive variations caused by land uses than the microbial diversity indexes [24], and the long-term effect of soil disturbances might cause lower response in the soil microbiota profiles [3]. Analyses of soil fungal and bacterial diversity could provide insight into soil development processes and microbial dynamics for better soil management, which in turn improves cacao production. Even though Indonesia is a great contributor to the

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[1] Supplementary materials are available for this at doi: 10.1134/S1064229319100144 and are accessible for authorized users.

global cacao supply, the soil microbial metagenomics are not widely exploited, and have rarely been applied in the assessment of cacao soil management practices. Specially using the next generation sequencing (NGS). Such method is a rapid, high-throughput technology for use in such endeavors [12] and has further enhanced the speed of microbial fingerprint analysis due to their automation, relative simplicity in sample preparation and sequence assembly [5].

Therefore, the study of soil microbial diversity could generate important data for better understanding of soil health status, thereby sustaining and increasing the production of cacao to meet the present demands. Considering the main cacao producing regions of Indonesia, the current study may provide important information about the fungal and bacterial composition of soil in conventional and organic farming.

MATERIALS AND METHODS

Study areas and soil sampling. Soil samples were collected from six cacao farms located in Palolo, Kulawi, Sarjo, Sidondo, Parigi, and Poso on Sulawesi Island, Indonesia, where two kinds of systems were visited at each site (conventional and organic). In fact, the organic system was Environmental Friendly System, characterized by the less utilization of chemical fertilizers and pesticides, without any Certification Agency in that area. The soil managements including the fertilization, pest and diseases control in both systems were previously described [1]. The liquid and solid fertilizer applied in organic system composed of animal manures and crop residues from local farmers, but there was no specific formula standardized for that. The soil groups were Haplic Acrisols (Ochric) (Kulawi and Parigi), Gleyic Acrisols (Palolo) and Dystric Fluvisols (Sidondo, Poso and Sarjo), according to FAO GeoNetwork database [9]. Four samples in single cores were taken from each site, composing 48 experimental units. Sampling was performed in January of 2015 and at each sampling site, cores of 10 cm diameter and 10 cm depth were sampled at the third plant inside in the middle of the canopy. Each sample individually was sieved at 2 mm, homogenized and a portion was taken from the original and kept in a freezer (–18°C) for subsequent DNA analysis. Another part was air-dried and submitted to soil chemical analysis at Bogor University—Indonesia, whose results are shown in Suppl. Table 1.

Amplicon sequencing analysis of soils. DNA extraction, amplification of both 16S rRNA and internal transcribed spacer (ITS) genes were conducted for bacteria and fungi respectively in each sample. DNA from the total microbial community was isolated from approximately 0.25 g of soil per sample using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) targeting the isolated microbial genomic DNA from all soil types according to the manufacturer's instructions. The following steps were performed from the PCR to the 16S and ITS rDNA amplicon sequencing analysis using the Illumina MiSeq 250bp at Genome Quebec, Montreal, Canada. The bacterial community (16S) was amplified with 534f and 783r oligonucleotides [8]. Whereas the ITS1and ITS2 [20] were applied to amplify the soil fungal community with their respective adapters, following by library construction (Fluidigm Co., USA). The validation and quantification, followed by pooling and sample loading were carried out using MiSeq System equipment (Illumina Co., USA).

Analysis of Illumina-derived data was performed by re-assembling the pair-ended files with Galaxy cloud (https://usegalaxy.org), and bioinformatics analysis of the bacterial data was performed using CloVR pipeline (http://clovr.org) [29] and Qiime software (qiime.org) [15]. Operational Taxonomic Units (OTU) clusters were defined by a 97% identity threshold and the datasets were submitted to rarefaction analysis before estimate the diversity. The quality filtering was applied at a Phred score ≥ Q20. The taxonomy base analysis for the 16S genes was accessed by Ribosomal Database Project (RDP) classifier supplemented with BLAST, and for the ITS by the Unite Community (https://unite.ut.ee/). Relative abundance and diversity index were used to evaluate how the microbial communities in Cacao soils are influenced by the location and management system. Differences for the relative abundance and diversity indexes were estimated by analysis of variance (ANOVA) after check the normality distribution with the Shapiro-Wilk test. The genetic features (abundance of OTU, species and genus) were selected by Hierarchical Feature Engineering (HFE) [23]. After feature selection their effects were checked by the Random Forest and the Principal Component Analysis (PCA), where it was evaluated if the group of treatments such as location, system, place are distinguishable features or not. Such analysis was supplemented by the Venn diagram in order to observe the effects of systems and place. The correlations with the selected soil properties were performed with mean of relative abundance and diversity indexes, but only those that showed and significant value were plotted in the tables.

RESULTS

Composition and biodiversity of bacteria. In this study, we assessed and compared the composition of soil bacterial communities present in six locations divided in two systems of Sulawesi Island. Indonesia by Illumina MiSeq-based analysis of 16S rRNA gene sequences. The sampled areas covered inland (Kulawi, Palolo, and Sidondo) and coastal (Parigi, Poso, and Sarjo) areas of this island.

The number of sequences per sample ranged from 25851 to 61366, with an average of 41824. We classified 560544 (82.85%) quality sequences, with values considered acceptable according to other studies [6, 16].

The number of OTUs, standardized with 12940 sequences, of bacterial communities derived from the sites is described in Fig. 1, which revealed

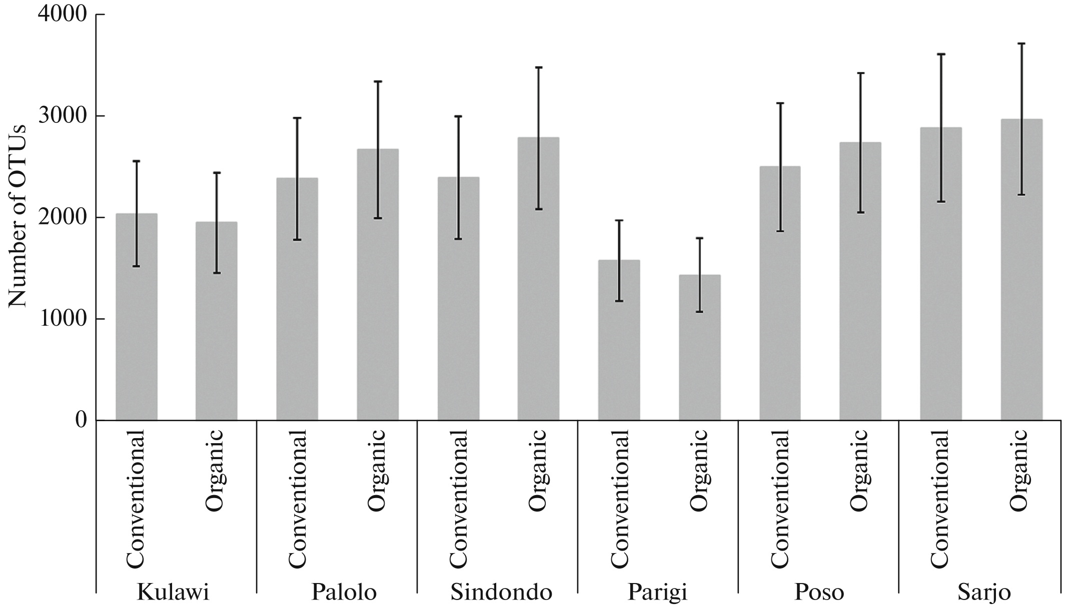
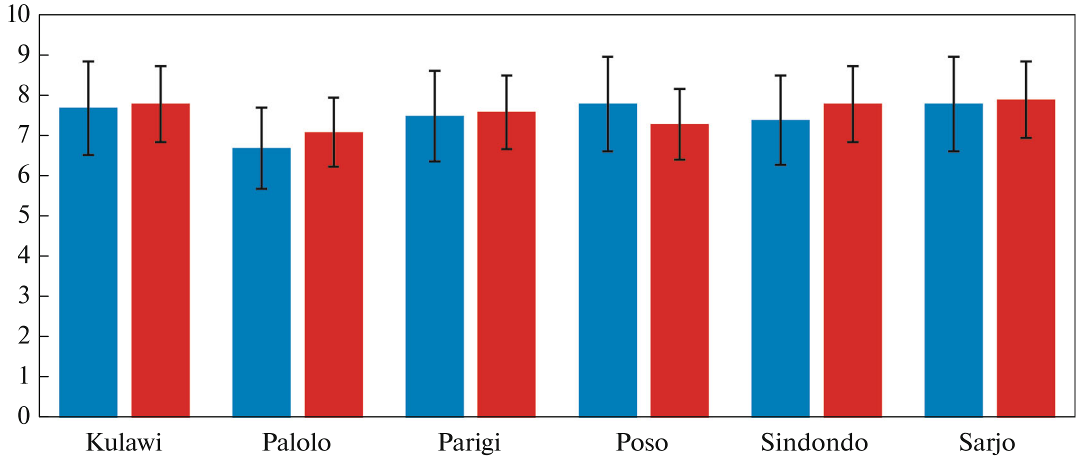


Fig. 1. Number of operational taxonomic units (OTUs) within the 16S rRNA gene sequences of soils cultivated under conventional and organic systems in six areas of Sulawesi Island, Indonesia. Bars represent standard error (SE).

(a)

Shannon-Wiener index



(b)

Chao index

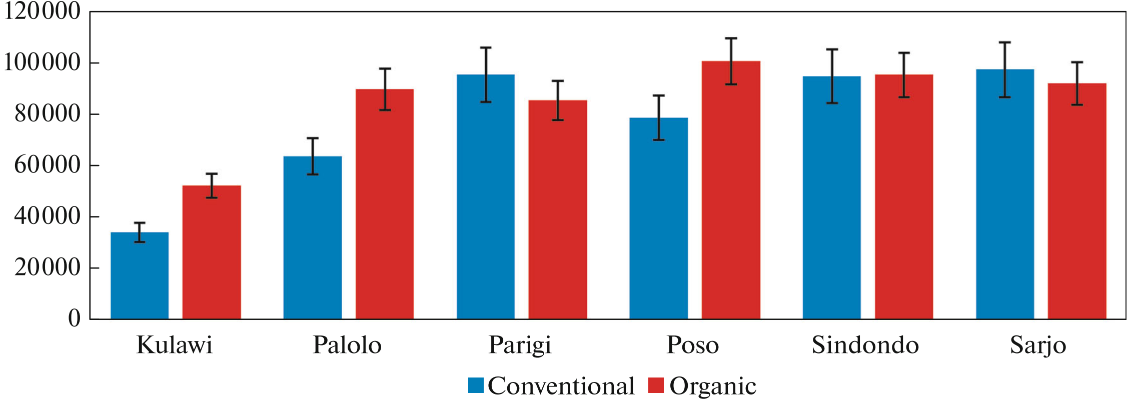


Fig. 2. Bacterial biodiversity index of soils cultivated under organic and conventional systems in Sulawesi Island, Indonesia. Bars represent standard error (SE).

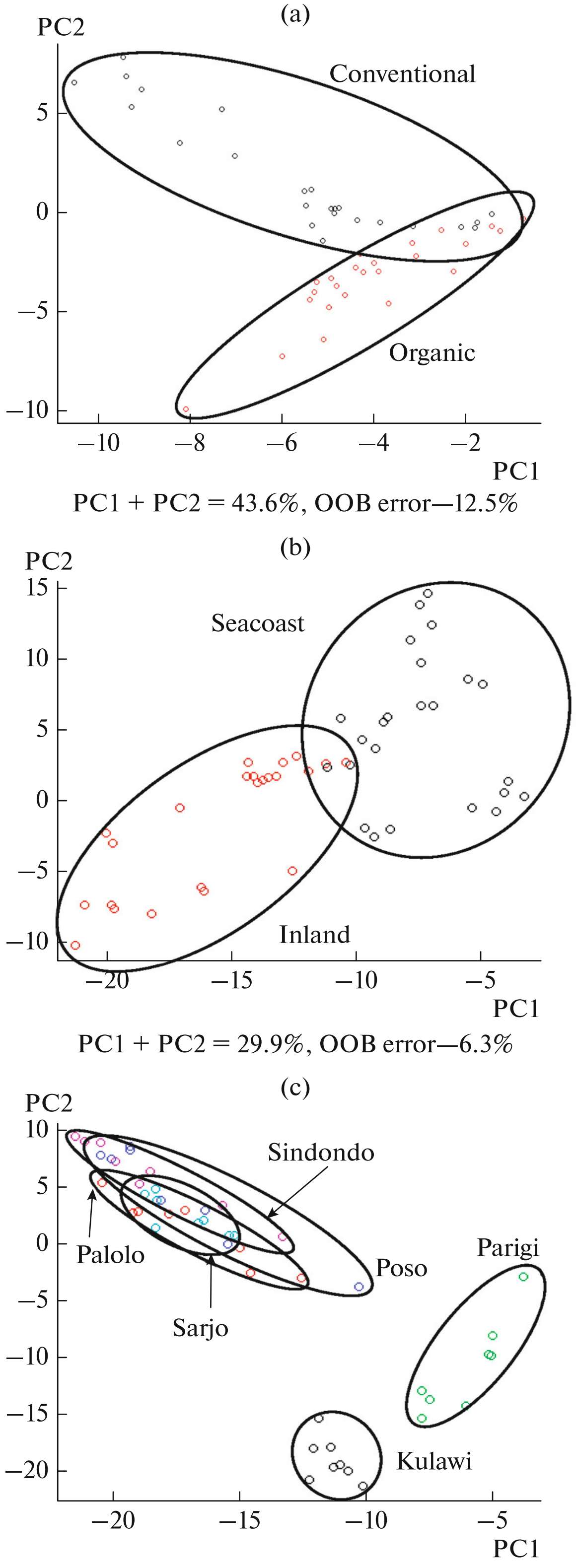
higher values under the organic system in five locations, with the exception of the Sidondo and Kulawi areas.

The Shannon index of diversity ranged from 6.7 to 7.7 in conventional systems and from 7.1 to 7.8 in organic systems. In general, the differences between the systems were not significant (Fig. 2a). The Chao index were higher in organic systems in Kulawi and Palolo and no differences were found between systems in other locations (Fig. 2b). Higher bacterial diversity was observed in samples from areas with lower chemical fertilizer use (Fig. 2 and Table S1). Nevertheless, in different systems within the same location, large variation in diversity was observed.

PCA graphs clearly indicated that the overall data had a significant separation between Organic and Conventional areas (Fig. 3a), as well as for Inland and Seacoast (Fig. 3b) after the selection of features. In terms of location only Kulawi and Parigi could be separated from other areas (Fig. 3c). However, the Random Forest data demonstrated that for all of treatments there was distinguishable taxonomic features. Furthermore, the selected variables changed depending the group label. The distribution of OTUs among the systems and places, represented by the Venn diagram revealed that 80.3% of OTUs belong to both treatments, with these divisions, where around 10% (between 8 and 12%) were individually located in each system (organic, conventional) and/or place (coast, inland) (Fig. S1).

Most of theclassifiable sequences were affiliated with 21 classes across the entire data set. The dominant phyla within the 16S genes across all samples were *Acidobacteria* (0.15%), *Actinobacteria* (1.14%), *Bacteroidetes* (0.53%), *Chloroflexi* (5.56%), *Crenarchaeota* (13.54%), *Eurarchaeota* (0.43%), *Firmicutes* (0.56%), *Alphaproteobacteria* (25.72%), and *Verrucomicrobia* (2.63%), in all sequences that were classified below the domain level (Fig. 4). In addition, the dominant classa, *Aplhaproteobacteria*, was present in all samples and the relative abundances of the five dominant classes, represented approximately 55% of all classified sequences. Thus, despite the different surveying efforts and sampling sites used in these studies, a variety of soils distributed among locations and treated within different systems were found to contain the same dominant bacterial groups. The *Alphaproteobacteria* were more abundant in conventional plots in Sidondo and Poso.

About the relative abundance of each of the 15 most represented classes in the Cacao farms. For almost all of them, a significant ($P < 0.05$) difference in distribution between the locations and systems was apparent and their distribution are shown in Table S2. Most sequences associated with *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Alphaproteobacteria*, and *Verrucomicrobia* were derived from organic systems, whereas those related to *Crenarchaeota*, *Euyar-*



PC1 + PC2 = 43.6%, OOB error—12.5%

PC1 + PC2 = 29.9%, OOB error—6.3%

PC1 + PC2 = 30.7%, OOB error—0%

Fig. 3. Principal component analyses of selected OTUs of 16S rRNA genes after features selection and their respective out-of-bag (OOB) errors rate according to system (a), Place (b) and Location (c) in Sulawesi Island—Indonesia.

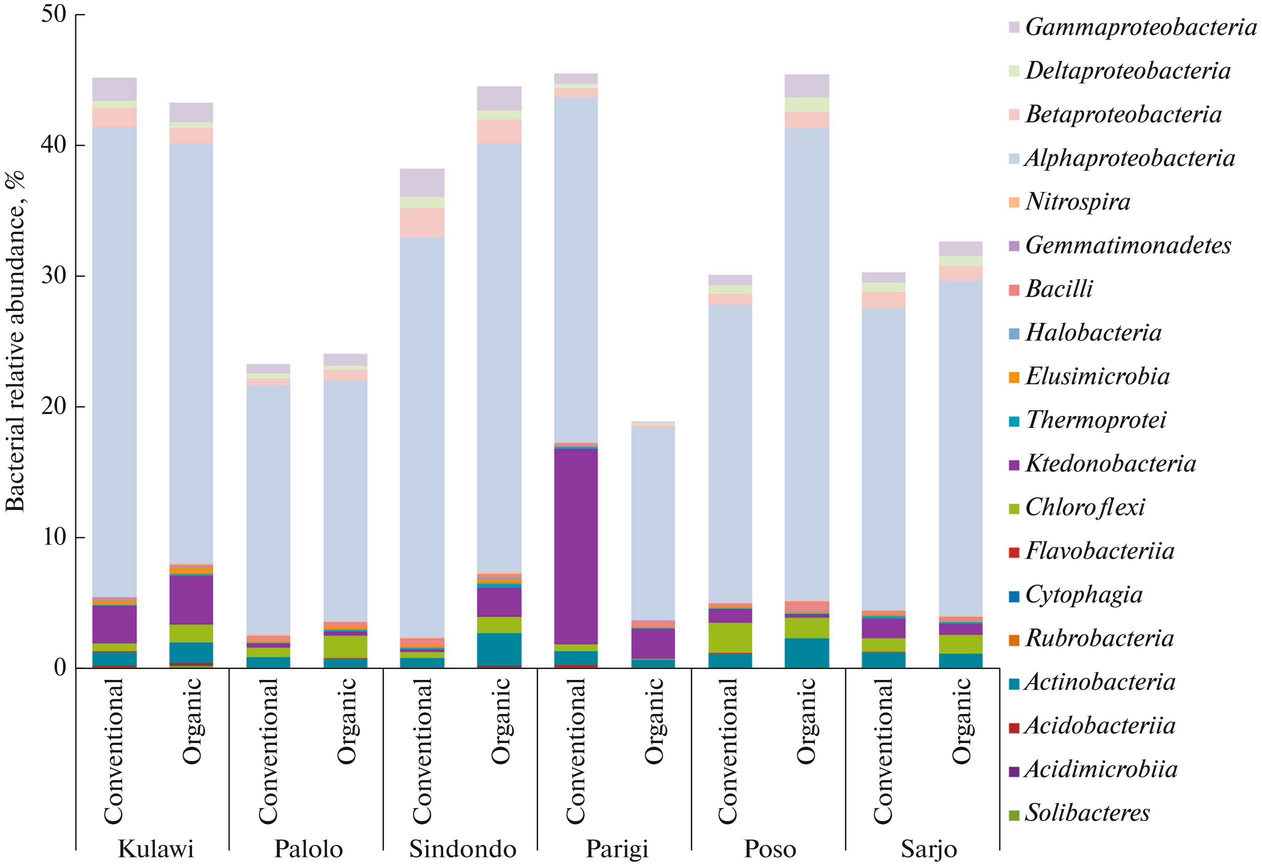


Fig. 4. Distribution of bacteria classes in Cacao Soils cultivated under conventional and organic systems in six areas of Sulawesi Island—Indonesia.

chaeota, *Firmicutes* originated from conventional systems.

A relationship between the bacterial community and soil properties could be detected. The Chao index was positively correlated with Na and negatively with Al. the highest values were obtained by K and *Ktedonobacteria*, *Acidobacteria* and *Gemmatimodanetes* (Table S4).

Composition and biodiversity of fungi. In total, 3230449 ITS rDNA sequences were obtained from all soil samples, and 2357935 (72%) of these could be assigned to amplicon sequencing analysis of fungi. The following results are displayed by class level due to the high amount of data. The OTU graph (Fig. 5) was generated using 10000–55000 sequences per sample, and rarefaction analysis of fungal communities derived from the sites revealed a higher number of OTUs in organic systems, whereas this pattern changed with higher numbers of sequences per sample. Concerning the location, Kulawi Palolo, and Sarjo had the highest number of OTUs, and Parigi had the lowest. The greatest fungal biodiversity was observed in the organic systems within each location; however, in general, no significance was observed between systems among all soil samples when using the Shannon index (Fig. 6a), with the exception of Kulawi. Conversely, the Chao index indicated the presence of greater diversity in organic systems in Sidondo (Fig. 6b). The PCA revealed significant difference between Kulawi and Poso, Sarjo and Parigi, also Palolo area could be distinguished to other areas too. In addition, within the ITS genes, with a clear separation between systems and places were detected. For the location only the Kulawi area was distinguishable compared to other for axes PC1 and PC2. The Venn diagram indicated that 62 and 54% of OTUs belong to both systems and places respectively. Additionally, the organic and conventional systems contain around 20% of the OTUs. Regarding the effect of place, the coast had 11% and the inland areas 35% of OTUs (Fig. S1).

A comparison of ITS rDNA profiles at the class level revealed that the composition of fungal communities differed among locations and systems. The Cacao soils mostly contained the classes *Agaricomycetes*, *Ascomycota*, *Dothideomycetes*, *Eurotiomycetes*, and *Sordariomycetes*. The relative abundance of Sordariomycetes and *Agaricomycetes* in soil samples from areas using the organic system was higher than that in areas using the conventional system. However, the opposite pattern was found for *Dothideomycetes* in Sidondo and Poso (Fig. 8).

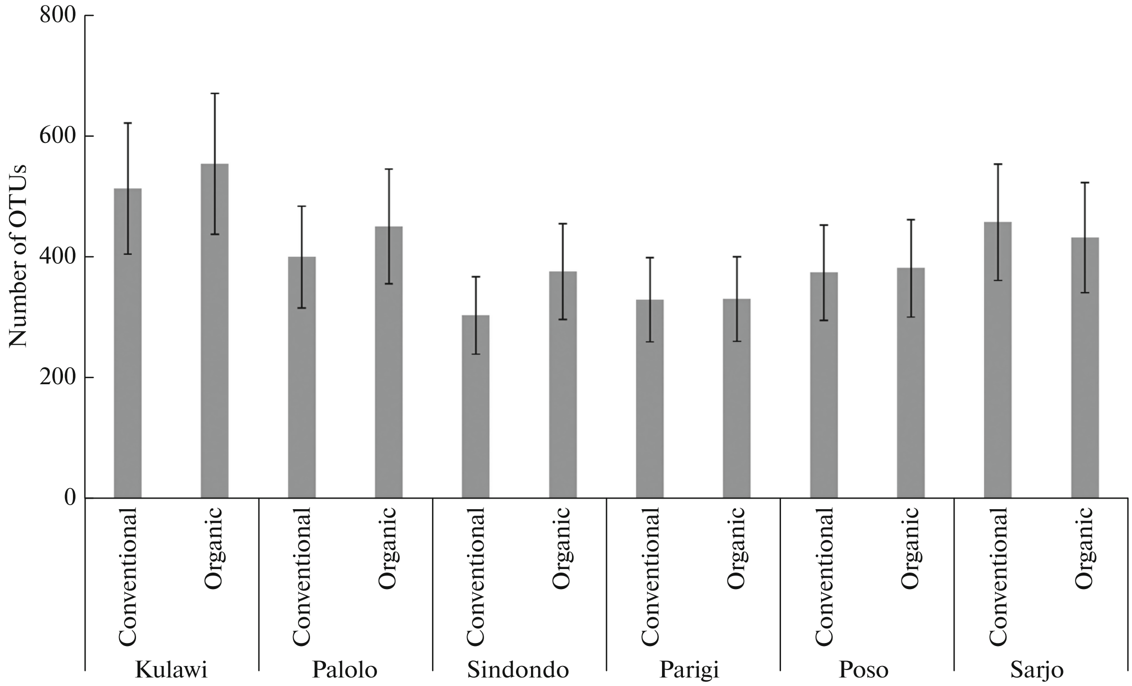
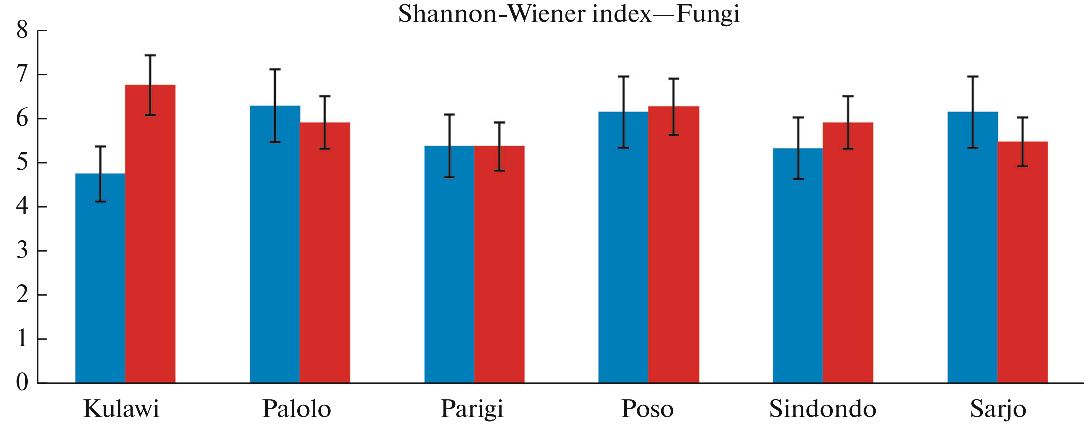
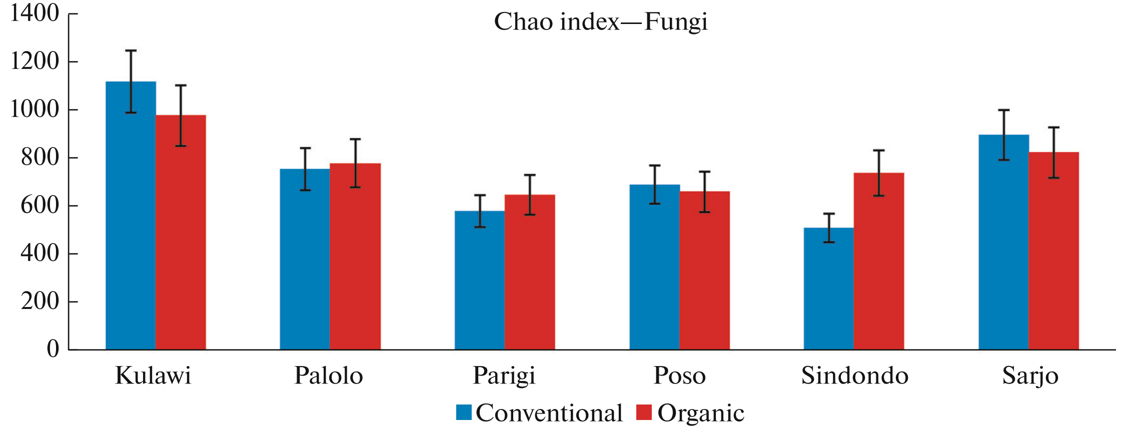


Fig. 5. Number of operational taxonomic units (OTUs) within the ITS rRNA sequences of soils cultivated under conventional and organic systems in six areas of Sulawesi Island—Indonesia.


(a)

Shannon-Wiener index—Fungi


(b)

Chao index—Fungi

Fig. 6. Fungal biodiversity index of soils cultivated under organic and conventional systems in Sulawesi Island—Indonesia. Bars represent standard error (SE).

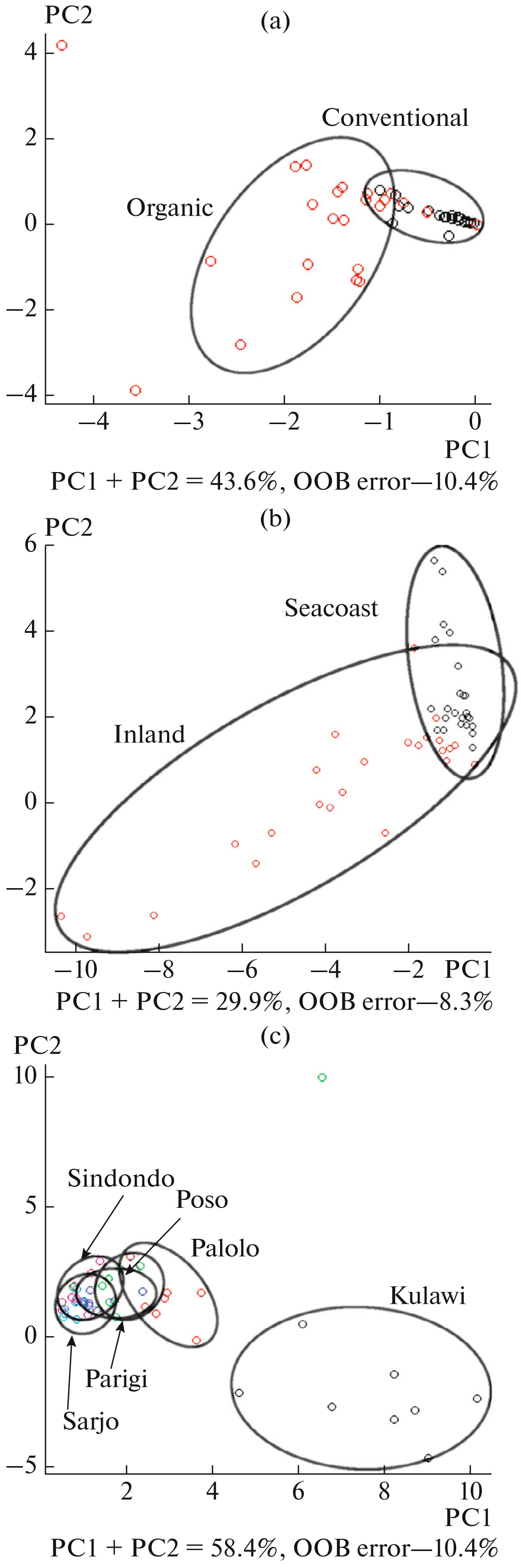


Fig. 7. Principal component analyses of selected OTUs of ITS rRNA genes after features selection and their respective out-of-bag (OOB) errors rate, according to system (a), Place (b) and Location (c) in Sulawesi Island, Indonesia.

Accordingly, the relative abundance of other fungal classes such as *Entorrhizomycetes* and *Agaricomycetes* was lower in conventional areas than in the organic areas (Table S3). This indicates that the abdication of chemical fertilizers and pesticides could increase the populations of some fungal species in the same location. In general, the composition of fungi was significantly affected by location and system ($P < 0.05$). Significant correlations were found for fungal classes and soil characteristics. The *Ustilagomycetes* and *Glomeromycetes* index were positive correlated with Mg and K. The *Zygomycetes* and *Dothideomycetes* had relationship with H too (Table S5).

DISCUSSION

It is essential to understand the soil conditions in order to use sustainable methodologies for cocoa agriculture, ensuring the quality of soil, balanced microorganism communities. The metagenomic analysis provided a profile of the microorganism communities in soil, including both bacteria and fungus, allowing to establish one criterion to evaluate the local management system.

Focusing on the bacterial data, we found that the distribution and abundance of predominant taxa in soils from Palolo and Poso were *Betaproteobacteria* and *Alphaproteobacteria*. Despite these differences, all regions had a predominance of the taxon *Alphaproteobacteria*, which is able to survive in old soils with low levels of nutrients. It was also noted that the treatment effect was only significant in Poso, where soil has been preserved and used less in agriculture.

For both microbial groups, it was possible to distinguish between organic and conventional systems as well as for seacoast and inland areas after a selection of OTUs. This could occur may be due to the age of the implanted system, therefore later on such difference might be even more detectable with the original OTU. Furthermore, the Venn diagram besides confirming such data, could show that the bacteria are more distributed around the areas and systems, whereas the fungi were more specific, sometimes reaching more than 30% (inland). In fact, the soil microbial status seems to be very sensitive to any disturbance as compared to other chemical and physical properties [32, 33]. Moreover, the effect or organic management strongly changes the microbial profile as compared to conventional systems [7, 18], and the bacterial dynamics are usually affected by long-term management, especially comparable with organic systems [2]. So the evaluation of microbial community could easily notice any effect of any alteration on soil. However, other indicator, such as the Greenhouse gas could be useful to estimate the soil health under both conventional and organic systems [22].

Significant effects by system have been found in the Kulawi region, which is in contrast to Parigi, located

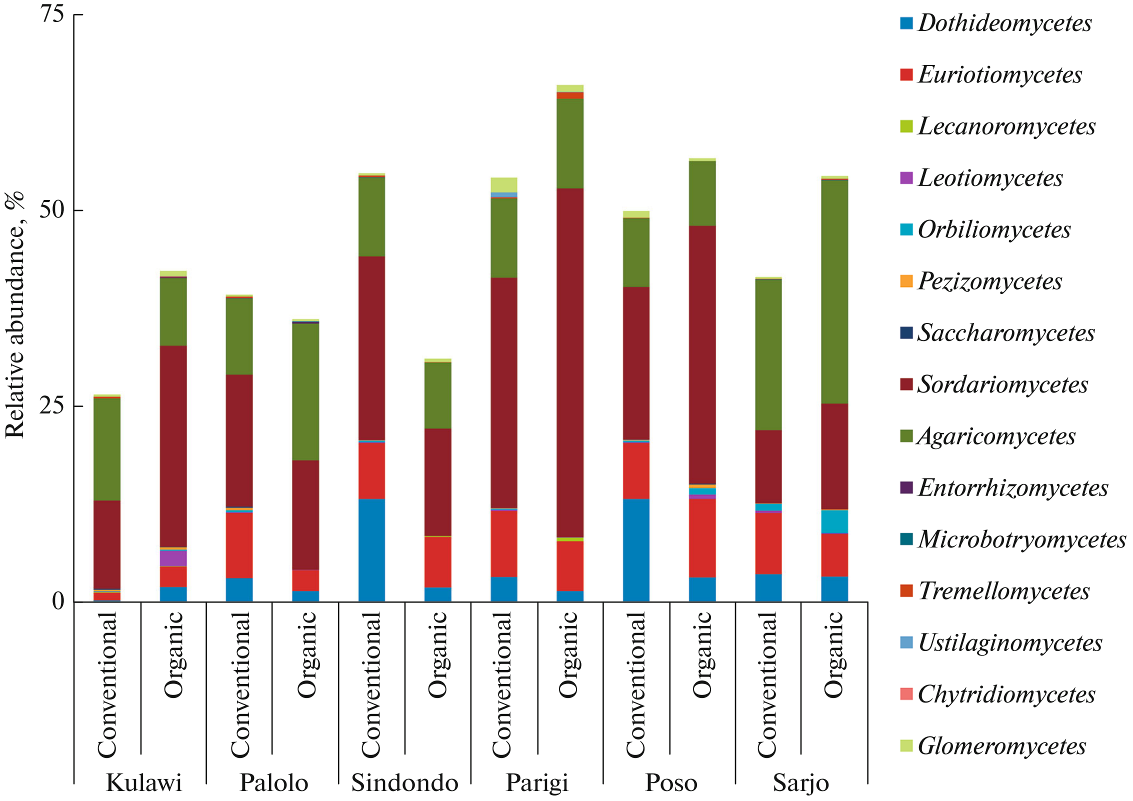


Fig. 8. Distribution of fungal classes in Cacao soils cultivated under conventional and organic systems in six areas of Sulawesi Island, Indonesia.

in inland and seacoast regions, respectively. General effects were also found compared the inland and seacoast areas. This may be related to the specific properties of these soils. The dynamics of bacterial communities in dunes could be influenced by their location, where those distant from the shores were clearly different to the ones nearby [11]. Also, the microbial community composition, as determined by phospholipid fatty acid analysis, differed among vegetation areas with special regarding to fungal "fingerprints" in inland plots compared to seacoast [30]. Despite this, high diversity was confirmed by the Shannon-Wiener index for Palolo, especially under organic treatments, and Sidondo. Interestingly, in Poso, conventional treatment had a better effect than organic treatment. As fungi comprise a large part of soil biodiversity and help to ensure soil health due to their unique biological and ecological features [4], we also analyzed the DNA of mycobiomes, fungal communities.

These data revealed that Parigi soils possess a higher frequency of *Basidiomycota*, *Agaricomycetes*, and *Sordariomycetes* only when under organic treatment; however, no significant differences were identified for OTUs of fungus in both treatments. This suggests that fungi communities are more sensitive to location than bacteria, where this last group is more spread. Interestingly, a local effect, as demonstrated by the PCA, was found especially in Kulawi, Sarjo, and Palolo, which is in contrast to the effects in Parigi and Poso. However, the organic management was found to have a significant higher diversity and relative abundance for some microbial phylla in Kulawi, Parigi, and Sidodo. In addition, it was found with a higher contrast found between Poso and Kulawi, as well as Sarjo and Parigi; this may be explained by differences in soil characteristics, as suggested by the interesting diversity found in coast and inland soils. Consistent with previous studies in which the pH of soil was shown to drive differences in microbial communities [25, 27, 28], these data suggest that seacoast soils may be favorable for specific types of fungus.

In the current research, the sampling time was unique, so seasonal changes might also occur in these areas. In agrochernozems soils the mineral fertilizers had less effect on bacterial community than the seasonality [31]. The soil properties in Kulawi were more acidic, and Parigi soils are more neutral, followed by abundance in N, organic C and low availability of P in Palolo. Interestingly, these regions contrast in terms of fungal abundance, but not bacterial communities. Thus, in all of these cases the effects of soil type on microbial community should be considered too, besides their chemical properties only.

CONCLUSIONS

This study provides knowledge about soil microorganism communities in Cacao plantation of Sulawesi Island – Indonesia, with special regarding to the differences between the organic and conventional management, and areas at seacoast and inland. These differences in terms of microbial community were clearly detected at local and general level after selection of microbial groups. The composition of bacterial and fungal communities can be directly or indirectly affected by soil chemical properties, and agricultural management systems, with consequent impacts on soil function.

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