Understanding the reestablishment of micro-ecosystem on the soil microbial community after Merapi Volcano eruption through 16S metagenomic analysis

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Abstract. An eruption of Merapi Volcano affected the established soil ecosystem. Succession process will recover the disrupted soil ecosystem. Soil microbial community is known as the first agent for the succession. They play essential roles to construct the micro-habitat by contributing the ecosystem recovery in nutrient cycling and subsequently initiating the plants-microbes interaction. Since the soil microbial community and its functional profiles become a response to the reestablishment process, their structure community is essential to be understood. This study was addressed to describe the soil microbial community in particular of the presence of plant communities after the eruption and to find out the functional profiles through its community. Using the 16S metagenomic culture-independent analysis, we examined the soil samples of Merapi Volcano after an eruption in three types of soil samples including soil without plants (SC), soil covered with shrubs (LP), and soil covered with high plants (HP). As a result, we obtained that the establishment following the presence of plant community type delineating the pattern diversity value increasingly from SC to LP then HC with Proteobacteria as a prominent Phylum. The presence of the plant community suggested that plants-microbes interaction constructed the establishment of microbial community structure where the microbial community in SC led the nitrogen metabolism than other soil samples due to the annotated nifH gene abundance. Therefore, we assumed that the microbial community in soil without plants is the early recovery stage and the plant community for establishing the micro-habitat after the volcano eruption.

1. Introduction
Soil microbial communities have been recognized as an important part of the living organism to serve nutrients for other organisms through their role in the nutrient cycling process. However, the ecosystem stress and disturbance by the land transformation or natural disaster disturbs their established structure and subsequently may change their proper functions. Therefore, the characterization of the soil microbial community has been used to evaluate the sustainability before and after of the ecosystem disturbance [1]. Ecologically, microorganism plays like a succession engineer to re-establish the treated ecosystem with their cosmopolitan character, stress tolerance ability, massive genetic pool, and metabolic versatility [2,3].

In nature, the recovery process is a relatively slow process which usually in line with the ecology restoration [4]. Banning et al. [1] reported that the dynamic of soil microbial community reconciles to the ecosystem development pattern such as edaphic variables and vegetation. As an example, the change of soil microbial community in the fire-adapted woodland is reflected in the difference of soil nutrient content and vegetation composition [5]. The relationship between plants and microbes is occurred to initiate the recovery ecosystem by improving the pyroclastic soil quality after the eruption [5]. The effect of disturbance on soil microbial community and their function happens transiently. This may link with a further dependent relationship of the plant and microbes for the succession process.
Therefore, soil microbial composition and their functional profiles are devoted to viewing the community pattern of the vegetation development during the recovery process. Soil microbial serve the important function in soil biogeochemical processes that valuable for the plant community such as nitrogen fixation, phosphate solubilization, and plant growth hormone synthesis.

Soil microbes tend to respond to the environmental stress faster than the vegetation above ground. Harris [8] reveals that it is hard to define the microbial community facilitates the establishment of plant community in succession stages. It is necessary to investigate the insight of ecosystem work under succession process since the diversity of plant community above the soil alter the soil microbial community structure [9,10]. The different plants species are able to shape the rhizosphere microbial community which indicate that different plant species host specific microbial community and functions [11,12]. By measuring the size, composition, and activity of the soil microbial community are fruitful for explaining the ecosystem status [8]. The reestablishment process of the ecosystem can be recognized by the role of soil microbial community to construct the plant vegetation [4]. In addition, Cong [13] suggested future changes in the environment, which have consequences for forest succession, will alter the microbial community structure and functional potential.

Currently, the advanced metagenomic is able to show the larger number of sequences related to the soil microbial community in taxonomic position and functions pathways [14]. Therefore, in this study, we unravelled the soil microbial community and their major functions by the 16S metagenomic methods of the soil samples from Merapi Volcano in line with the partial vegetation development after the eruption.

2. Material and Method

2.1. Soil sampling

Approximately 10 gram of soil samples collected in Merapi Volcano. The soil types were considered by the presence of the plant community where triple spots of the soil without plants (SC), beneath the shrubs (LP), and under the high plants (HP) were collected in zip-lock plastic and subsequently kept with ice gel to maintain the community dynamic before preserved at -80°C for further analysis. We did not define exactly the plant species where the soil samples collected but the shrubs considered to the plants community with 0-1 cm of stem diameter and approximately 100 cm height while the higher plants described with 10-30 cm of stem diameter and more than 100 cm of plants community height. In addition, soil samples collections were simulated to the soil representing to the soil impacted volcano eruption but no plant communities until succeed by the plant communities. Three spots of samples collection were addressed to SC at Hargobinangun (E: 110° 26’ 25”; W: 07° 35’ 7”), LP at Purwobinangun (E: 110° 26’ 15”; W: 07° 35’ 6”), HP at Purwobinangun (110° 26’ 11”; W: 07° 35’ 8”) in Yogyakarta, Indonesia.

2.2. Preparation for Soil DNA extraction, amplification, and indexing library

The extraction of the soil DNA was performed by following the ZymoBIOMICS DNA Miniprep kit protocol (Cat. R2002, Zymo Research Corporation, USA). The obtained DNA then was amplified by the 16S ribosomal DNA following the Illumina 16S Metagenomic Sequencing Library Preparation protocol (15044223-B; Illumina, USA) using V3 forward primer (5-CGTCAGCMGCCGCGTAAG-3) and V4 reverse primer (5- GACTACHVGGGTATCTAATCC-3) [15] by using MyTaq HS DNA polymerase (BIO-25049, Bioline, UK). Indexing and sequencing of the PCR amplicons were outsourced to the sequencing company in the Republic of Korea (https://dna.macrogen.com/).

2.3. Taxonomic and functional profile analysis

The obtained fastq data was analyzed with QIIME2 2019.4 [16]. The raw sequences data were demultiplexed using q2-demux plugin and followed by denoising using DADA2 [17]. All amplicon sequence variants (ASVs) were filtered with total abundance ≥ 100 ASVs and at least show up in 2 samples using q2-feature-table plugin. The filtered ASVs were aligned with MAFFT [18] and used to
construct phylogeny with Fasttree 2 [19] using q2-phylogeny plugin. Alpha diversity including Shannon diversity index, Pielou’s Evenness, observed OTUs and Faith’s Phylogenetic Diversity [20] and Beta diversity including unweighted UniFrac distance [21], Bray-Curtis dissimilarity and Principle Coordinate Analysis were calculated using q2-diversity plugin after the samples were rarefied to 19743 sequences per sample to retain more samples. Taxonomy were incorporate to ASVs using q2-feature-classifier classify-sklearn naive Bayes taxonomy [22] against the SILVA 132 reference sequences at 99% similarity level. Analysis of enzymatic activities and metabolism especially on nitrogen metabolism was performed using functional taxonomic prediction of PICRUSt2 [23]. Statistical analysis, venn diagram and visualization were conducted using agricolae package [24], VennDiagram package [25] and ggplot package, respectively in R.

3. Results and Discussion

The 352012 total denoised sequences of 16S metagenomic results revealed that the soil bacteria community consisted 1052 OTUs classified to 15 phyla, 42 class, 118 order, 191 families, 305 genera and 388 species. Proteobacteria is the predominant phyla among all soil samples (40.84±1.44%) followed by Actinobacteria (26.60±0.93%), Chloroflexi (14.45±1.38%), Acidobacteria (8.87±0.59%), Verrucomicrobia (2.78±0.22%), and Rokubacteria (2.69±0.34%) as the six biggest phyla (Figure 1) while other phyla only have proportion around 3.76 % in community. According to the Figure 1, HSD Tukey test showed that the abundance of Proteobacteria in HP was higher than LP and SC whether Chloroflexi in SC was higher than other soil samples. Meanwhile, Actinobacteria, Acidobacteria, Rokubacteria, and Verrucomicrobia were not significantly different which means no change during reestablishment.

In term of soil bacteria diversity, SC samples have the lowest diversity value than HP and LP samples (p-value = 0.034) (Figure 2A). The diversity value is determined by the number of OTUs that presents on each soil sample (Figure 2B). HP has higher number of OTUs (599) than LP (400) and SC (283) which means that HP has higher diversity with average Shannon index 8.57±0.10 followed by LP and SC about 8.17±0.15 and 6.96±0.57, respectively. Interestingly, both HP and LP shown significant different of diversity value based on the HSD Tukey test compared to SC samples. On the
other hand, the number of OTUs of HP samples shared greater to the LP rather than SC. Moreover, HP has greatest unique OTUs number followed SC and LP. Among all soil samples, there are 16 OTUs as core OTUs that present in all soil samples including the family of Bacillaceae, Bradyrhizobiaceae, Hyphomicrobiaceae, Micromonosporaceae, Planctomycetaceae, Sphingomonadaceae, Streptomycetaceae, Xanthobacteraceae, uncultured Acidimicrobiales and uncultured Planctomycetes.

Figure 2. Alpha diversity (A) and shared OTU of Merapi soil bacteria (B).

Shared OTUs number and diversity index value correlated the soil bacteria community relationship among samples which HP and LP were closely clustered to the distinctive SC samples (Figure 3). The Principal Component Analysis (PCA) depicted the relationships among communities were separated into three clusters which clearly revealed following the presence of the plant communities on that soil samples with 70% proportion explained. Both HP and LP have a close relationship (19%) compared to the distance of SC cluster (51%).

Figure 3. Principal Component Analysis of Merapi soil bacteria

We also generated the functional profiles of soil bacteria by using genome database annotation in KEGG (Figure 4). We specified the profile of the nitrogen metabolism which was evaluated to show dynamic of functional genes establishment directing to the presence of plant communities. The result showed that the genes *nasA* and *nirA* involved in assimilatory nitrate reduction followed by genes *nirK*, *nirS*, *narG* and *nosZ* which related to denitrification and nitrification gene *amoA* were strongly found in HP whereas nitrogen fixation gene *nifH* and genes *narG* and *nrfA* associated with dissimilatory nitrate reduction predominant in SC. Both HP and SC nitrogen metabolism enzymes
were actively on their bacteria community. The major contributor of nasA and nirA gene in HP are mainly Rhizobiales from Proteobacteria which more abundant in HP than LP and SC, whereas Chloroflexi and Actinobacteria contributed more nasA gene in SC. Rhodospirillales, Rhizobiales and Desulfurellales are the main contributor of nifH gene in SC. Related gene of denitrification pathway were contributed mainly from Actinobacteria and Proteobacteria. The nosZ gene that encode the nitrous-oxide reductase to release nitrogen that higher in SC was provided more by Chloroflexi. The dissimilatory nitrate reduction pathway involved gene, especially nrfA gene, were present in Myxococcales from Deltaproteobacteria class.

Figure 4. Prediction based abundance of key gene involved in nitrogen cycle from Merapi soil bacteria

Microbes have a strong relationship with plants which directly or indirectly determine the dynamic of their community structure and functionality [7,10,26,27], because every plant species is associated with their soil microbial community structure [12,28,29]. This is correlated to the SC samples which out of plant community have the lowest diversity instead of HP and LP so that in PCA analysis SC clustered separately. The presence of the plant community strongly revealed the soil microbial diversity of the HP and LP where the establishing pattern of the constructed soil microbial community structure has seemed on the diversity index value which increased from the soil without plants (SC), with shrubs (LP) to the high plants (HP). The soil community structure of HP was determined as an established soil community structure which may present previously before the volcano eruption. In addition, shared OTUs of HP and LP were driven by the presence of the plant community which shared highest rather than SC. It is originally determined by the type of plant community which influence the soil microbial community structure driving the ecosystem processes [30–33]. Under the reestablishment process, Proteobacteria have a strong portion in the structure of all samples while the low diversity value in SC samples was induced by the aridity of soil. Soil without vegetation would increase its aridity and reducing the soil microbial diversity [34]. Therefore, the Chloroflexi abundance rose significantly in SC samples also might be correlated with the aridity of soil. In addition, soil ecology, especially on microbial community might be altered after the eruption that exposed by the eruption material such as sulphur dioxide (SO₂) [32,35]. The SO₂ stimulates the pioneer microbial community to alter the chemical property inhabiting the new environment. The soil microbial community was changed by the eruption material content which can provide the growth nutrient since the organic materials were decreased [35]. Not only from the eruption effect but also seemed in the conversion of the forest for agricultural purposes decreasing the organic content so that
microbial community might adapt simultaneously for recovery [36]. Previously, soil microbial community might be richer before the change of the soil texture and pH affected by the eruption distributes the change of community structure [37].

In terms of linking the community structure and function, the predominant phyla, Proteobacteria, largely consists of nitrogen metabolising bacteria for nitrogen cycling under the succession processes. Nitrogen is one of the important parts in nutrient cycling for the plant as a growth-limiting factor, which affects the leaf area, leaf chlorophyll content and photosynthetic rate lead to biomass production [38,39]. Soil microbiome was involved in soil biogeochemical process including converting the organic nitrogen into inorganic nitrogen and vice versa [7]. The available nitrogen in the soil regulates the nitrogen cycle mediated bacteria. Nitrogen-fixing bacteria would lead the succession when the concentration of nitrogen in the soil is limited, followed by vegetation growth at adequate nitrogen concentration [40]. This correlated with the high abundances of the nifH gene in SC than other samples showed the soil microbial community in SC tend to obtain the nitrogen through fixation pathway. The soil microbial community in HP and LP retrieve the nitrogen through assimilatory nitrate reduction, denitrification, and nitrification pathway. These pathways were more preferred in soil covered with vegetation because of the availability of organic matter from the plant species that enrich the copiotrophic bacteria i.e. Actinobacteria and Proteobacteria [41,42]. The succession process showed by distinct changes of microbial diversity from SC into HP which implied the functional capacity of the community providing supporting evidence that different vegetation characteristics could stimulate varying soil microorganisms due to the development of various microhabitats supporting various species population.

The gene composition and profile of bacterial community structure related to the N-cycle in volcanic soil of Merapi appear to be quite complex, and differ from other ecosystems [43]. Different behaviour was observed in paddy soil. In general there are five key N-cycling gene involved in N fixation (nifH), ammonia oxidation (amoA) by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), and nitrite reduction (nirS and nirK) along profiles (0–100 cm depth) of different paddy soils across China from north to south. The microbes and functional genes involved in the N-cycling significantly decreased with soil depth; however, AOA were enriched in deeper soil layers (20–40 cm). Microbial community structure and abundance was affected by soil depth and geographic region. Less microbial and gene abundance was on the deeper horizons relative to top-soils. The gene involved in N-cycling of Archea group was more abundant than that of bacteria group in the paddy soil profile, and nirS and nirK abundances were dominant in topsoil and deeper soil, respectively. High abundances and low vertical changes of N-cycling genes suggest more dynamic N-transformations. They also observed that soil properties and climate parameters had a significant relationship with N-cycling gene abundances. The abundance of different N-cycling genes was affected by different environmental parameters, which should be studied further to explore their roles in N cycling for effective ecosystem restoration. Our analyses revealed that different group of microorganisms contributed on the N-cycling which implies that the importance of microbial community structure analyses for restoration studies. In order to get more views on the effect of soil profiles, further studies should also verify the effect of soil layer on microbial community structures and its physiological role.

4. Conclusion
The diversity of microbial community and their function simulated the reestablishment process of disturbing ecosystem from the volcano eruption. Enhanced the diversity index of soil microbial community was altered by the presence of plant community where SC has a lowest value followed LP and HP. Analogically, the reestablishment of micro-habitat is start from the soil without plants to shrubs and then high plants community. SC was an initiating stage of micro-habitat recovery which led the nitrogen metabolism function to serve the plant nutrient under the succession process.
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