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Metagenomic Sequencing Analysis and Microbial Identification on Various Landcover

[1] Rossyda Priyadarshini *, [1] Elly Syafriani [1] Medina Uli Alba Somala, [2] Amir Hamzah

[1] Department of Agrotechnology, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Surabaya, Indonesia
[2] Department of Agrotechnology, Faculty of Agriculture, University of Tribhuwana Tungggadewi, Malang, Indonesia

Abstract

Soil degradation will affect the availability of soil nutrients. Microbes role was important in supplying soil nutrient. How is microbes supply the nutrients can be learned through the metagenome technique. Metagenome is one of the molecular techniques that can identify microbial communities from soil samples that are used quickly and precisely. Through the metagenome technique, not only information about the identity of microbial species can be obtained, but also the enzymes produced, which will affect the soil nutrients availability. This study aims to detect and identify the impact of land cover types on microbial types. Soil samples were taken under agricultural land (LP) and agroforestry (AF). Each type of land cover was taken at 4 (four) different locations. The method used in this research is a molecular-based species identification method through metagenome analysis. This study showed that there are variations in microbial species in various types of land cover. This information is important for sustainable agricultural management in the future. Our study showed that on all of the land cover types, Proteobacteria were the most abundant phylum in all of the land cover patterns. Among them, s_Sphingomonas_melonis; f_Sphingomonadaceae; g_Sphingomonas; c_Alphaproteobacteria; p_Proteobacteria; and o_Sphingomonadales were the most abundant in the LP groups, while p_Acidobacteria and c_Deltaproteobacteria were the most abundant in AF groups. This composition of soil metagenomic was associated with the soil physic-chemical properties, especially pH and soil organic matter.

Keywords: metagenomic technique, soil bacteria, 16S rRNA gene amplicon, land cover types

Introduction

The soil microbial community was an important actor in providing the ecosystem services, that are essential for human life, carbon and nutrient cycles, and crop productivity (Doran and Zeiss, 2000; Schimel and Schaeffer, 2012; Nie *et al.*, 2015). The function of the soil ecosystem is truly dependent on the activity and complexity of the microbial community; where the activity is influenced by physical, chemical, and biological aspects of the soil ecosystem (Fierer *et al.*, 2012).

Land cover patterns can change the microbial community in the soil as well as the function of the soil ecosystem including the C cycle (de Vries *et al.*, 2013; Schimel and Schaeffer, 2012; Mackelprang *et*

* Corresponding author
Email address: ossy_rsd@gmail.com

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al., 2011). Land cover pattern will affect diversity and composition of soil bacterial due to changes soil pH, total C, and C/N ratio (Wijayanti, 2015).

Understanding the role of microbes in providing carbon and nutrient cycles is very complex and difficult to learn, because of its wide diversity, both in terms of taxonomy and function (Bardgett and McAlister, 1999; Singh *et al.,* 2006). Information about the potential and function of the gene will describe the microbial role in providing ecosystem functions while providing an overview of land management pattern effect on microbial population (Manoharan *et al.,* 2017; Yang *et al.,* 2014).

Assessment of the contribution of soil microbial diversity on soil function is interesting to study due to high abundance of microbes in soil (Hughes *et al.,* 2001). Nowadays, it has been developed the DNA sequencing methods that will enhance the understanding of relationship between soil's characteristics, land cover types, and the soil microbiome diversity.

Many studies showed that soil properties changes due to land cover change significantly affects the soil microbiome. Many soil properties, just like soil C and nutrient content, soil organic matter, texture, pH, and salinity, deeply impact the soil microbiome (David, 2012). These soil characteristics were affected by the types of vegetation and land-use practices (Xu *et al.,* 2014). The soil microbial communities' structures are affected by changing land cover, environment factors, soil properties, diversity and vegetation structures, and land-use patterns (Wang *et al.,* 2013).

The microbial communities affect the soil function, and drive either the microbial structure and their activities, that will influence the C storage; C:N ratio, soil pH, nitrogen mineralization and phosphorus conversion. Coversely, land management activities (vegetation types, fertilizer input, pesticides,tillage) also controlled the soil microbial function. Those aspects were differ depend on the drivers scale. Therefore, assessment the impact of land management practices on soil microbial pattern (structure, composition, genetic diversity) and soil chemical properties will be usefull in planning, managing and designing land cover types.

This studies aim to learn the critical factor that is affect the changes of functional genetic diversity and microbial taxonomic composition in different land cover patterns and also assess the relationship between soil characteristics related to functional patterns and microbial taxonomies.

Research Method

Site Description and Sampling

Soil samples were taken on farmland located at 5°20'-6°18'north latitude (NL) and 7°-8°15' east longitude (EL) at Batu district, East Java between June- September 2019.

Two different land cover patterns, agroforestry (AF) and agricultural land (LP) were observed. Each land cover was taken from 4(four) different locations. Soil samples were taken at each land cover, 4 samples for each location. Then, soil samples were analyzed their soil biological and chemical characteristics.

Soil Physico-Chemical Characteristics Analysis

Soil sample characteristics were determined at the beginning of this experiment to collect data as a baseline for soil properties characterization. This parameter was evaluated, i.e: pH, soil organic matter (SOM), available P, available K, and total N.

Soil chemical and physical properties were determined at the Laboratory of Soil Health at Agriculture Faculty, University of Pembangunan Nasional Veteran Jawa Timur, East Java, Indonesia. Soil pH was measured under 1:2 soil/water suspension. Total C were extracted and determined by the Kjeldahl method; while the available phosphorus determined by Bray 1. Soil Potassium was extracted using NH4OAc pH 7.

Metagenomic Sequencing

Metagenomics methods used to study the abundance and diversity of microbial communities in various environment. Metagenomics methods usually needs the 16S ribosomal RNA (rRNA) genes, the common aplicon sequencing methods in identifying and characterizing microbial communities, especially bacteria and fungi in a soil sample.

Metagenomic sequences were conducted in two steps: (a) DNA extraction (b) PCR (amplification, quantification, mixing, and purification), (c) Library data preparation, (c) Sequencing and assembly, (d) Functional annotation, (d) Statistical analysis. The fourth step, functional annotation was a vital step caused only 1-2% bacteria can be cultured in laboratory. For advance observation, it was used NGS based on 16S rRNA. The workflow is as follows:

DNA Extraction

DNA was extracted using Power Soil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA) following the manufacturer's instructions. The quality of DNA from each extraction was determined by 132 electrophoresis using 0.8% agarose gel and also by DNA quantification using a nanospectrophotometer. For sequencing, DNA extraction from each habitat (6 samples) was collected into one sample. All samples are sorted and compared; and the amount of DNA was assessed using fluorescence using the Quan-iT Pico-Green dsDNA kit. After that a metagenomic library was prepared using 454139 GS Junior Titanium Rapid DNA according to the manufacturer's instructions. PCR emulsions (emPCR) were carried out according to the Manual Amplification Method using the Lib-L kit.

Amplification of 16S rRNA Gene

16S rRNA genes is common technique to marker the taxonomic of all bacteria. This technique can examine the genetic diversity of bacteria. 16 rRNA sequencing consist of nine hypervariable regions (V1-V9) ranging 30-100 base pairs long and conserved regions. These hypervariable regions has different conservation degrees; wherever the more conservation degress correlate with higher-rank of taxonomy, wherever less conserved regions indicate to lower ranks, such as genus and species. Moreever, the content of conserved sequences on 16S genes was high, so it was possible to use the universal primers. It also used widely in characterizing the diversity of microbial communities. The 16 rRNA used as targets for PCR primers.

The universal primer for bacteria was used to amplify the 16S rRNA bacteria. After this amplification, the related primer were continued by the second step of PCR. Sequencing of 16 rRNA by PCR amplicons was conducted using Ilumina sequencing technology. Illumina offers short reads on 2x250 or 2x300 bp, but it also preferred on longer reads. This method was started by homogenizing the soil samples sieved through 2 mm to release from rocks and plant material. Total DNA was extracted using PowerMax Soil DNA Isolation Kit. Then DNA was checked by 1% of agarose gel electrophoresis, and the quality was measured using spectrophotometer at A260/280 nm ratio. After that, each sample were barcoded for DNA sequencing. Illumina sequencing procedure using Illlumina adaptor A, Illumina adaptor B and barcode; it was added on the 50-eds and 30-ends of primers respectively. The QIIME software was use to process the raw sequencing data, and the OTUs of bacteria were determined to a similarity of 97%.

Data Analysis Procedures

The raw data was tested their validity by combining and filtering the data. The clean data, then is used in clustering the OTUs and get the annotation of species of each OTUs. The unique OTUs was visualize using Venn diagrams. While the relative abundance of bacterial OTUs between samples was represented by PCA (Principal Component Analysis) based on genus profile.

From these OTUs data, the distribution, abundance, and also evenness and relative species was analyzed using alpha diversity and beta diversity. Besides, in order to explain the community structures differences between samples or among groups, it was conducted PCA analysis to explain the phylogenetic trees construction through downstream statistical analysis. PCA will illustrate general pattern of microbial. Whereas the significant differences of community composition and structure between groups were analyzed using T-test, LEfSe, Anosim, and MRPP. LEfSe (Line Discriminat Analysis (LDA) Effect Size) analysis was conducted to statistically analyze the microbial population.

Result and Discussion

Soil Physico-Chemical Characteristics

The initial physico-chemical characteristics of soil samples were analyzed at each types of land cover (Table 1.). Our study showed that the pH value, soil organic matter, phosphorus availability, and potassium content of each site were varied. There were significant differences of all soil chemical characteristics between land cover. Overall, all of the sites were categorized as slightly acid – acid.

Table 1. Soil properties of land cover types

No.	Land cover	pH	Soil Organic Matter (SOM)	Available P	Available K	N Total (%)
1.	Agricultural Land (LP)	6.18±0.052	3.01±0.274	66.39±2.652	0.72±0.303	0.43±0.01
2.	Agroforestry (AF)	5.43±0.043	1.02±0.214	48.43±7.064	1.50±0.442	0.13±0.02

Soil properties (C, P, K, pH) among land cover pattern were vary. Soil pH, soil C, available P, and total nitrogen were measured on each type of land cover (Table 1.). Overall, the nutrient status of LP was better than AF due to the management process, such as fertilization.

Taxonomic Composition of Bulk Soil

Proteobacteria was important taxa in soil, they provide soil function related to the biogeochemical cycle. For all of soil samples it was found that Proteobacteria was the most abundant bacteria, comprising of around 60% all of the phyla in AF, and more than 75% in LP. The high abundance of Proteobacteria indicated that these phyla have an important role in these communities of microbial. The Proteobacteria that is found in the two samples were Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria. Among them, the most abundant bacteria s the in the two types of landcover waAlphaproteobacteria . Whereas the second abundant phylum in AF was Acidobacteria, and less abundant of this taxa was in LP land cover. However, Firmicutes and Actinobacteria were the most abundant in LP.

The differentiation in the microbes abundance and their role in soil function were studied on agroforestry (AF) and agricultural (LP) landcover. In this study, it was found that Proteobacteria and

Firmicutes were the dominant bacterial phyla on all of the land cover pattern (Figure 3.) The high abundance of Proteobacteria in agricultural samples was also reported by Dai *et al.* (2018), who stated that eventhough management practices (such as fertilization) will decrease bacterial diversity it will also favors the population of Proteobacteria and Actinobacteria in agroecosystems. The high of Proteobacteria abundance in agriculture could be responsible for improving on soil function which will promote the plant growth. Most genera of Proteobacteria phylum are categorized as PGPR (plant growth-promoting rhizobacteria), that will enhance the nutrient availability and plant health (Lugtenberg and Kamilova, 2009).

Agricultural practices were reported that in this area there were bacterial diversity loss, caused by the natural habitats conversion into intensive managed systems (Mahamane and Mahamane, 2005; Underwood *et al.*, 2009; Ding *et al,* 2013; Dudley and Alexander, 2017). Many research was also conclude the same things that loss of soil microbial diversity was associated with agricultural management. Some agricultural practices, crop rotation, fertilizer, and also pesticide have potency in sustaining of soil biodiversity Altieri (1999) and also enhancing ecosystem function (Dube *et al.,* 2019).

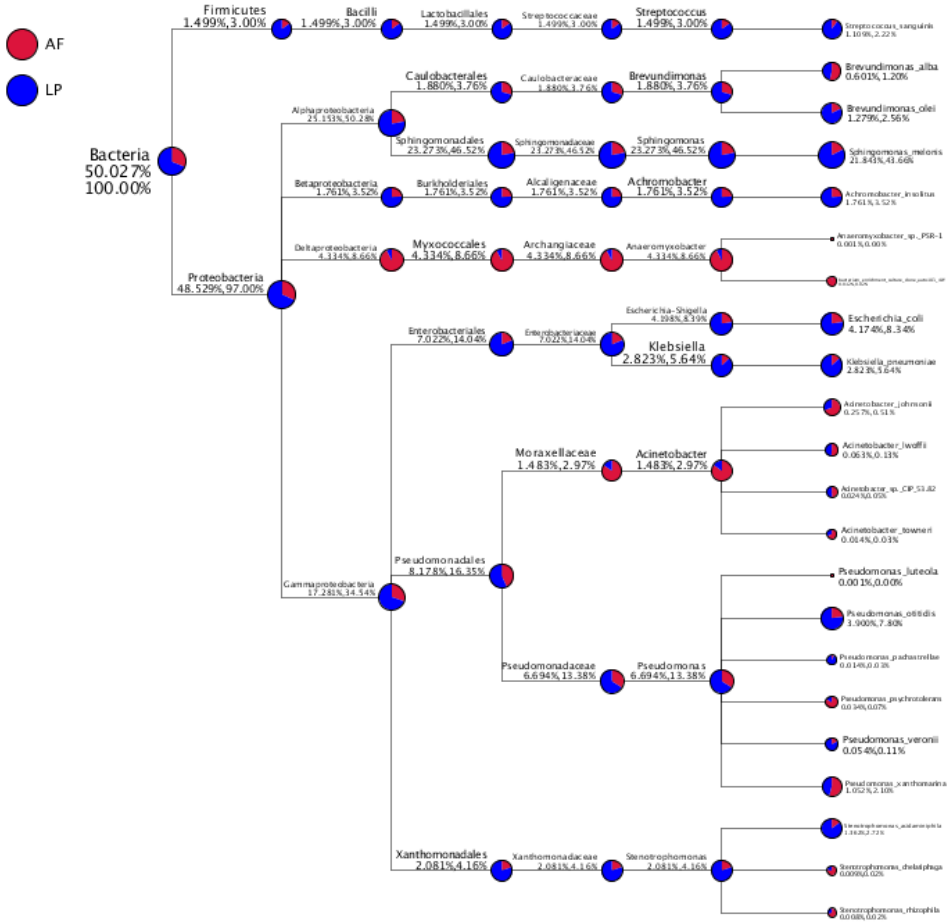


Figure 1. Taxonomy tree in a single sample. Different colors represent different taxonomic ranks. The relative abundance of species showed by the size of circle stands. The percentage of whole taxon were represent as the first number below the taxonomic name; while the percentage of selected taxon showed on the second number.

Planting many kinds of crops will facilitate the nutrient suplly, eiher in the form of root exudates and plant residue. This things resulted in selection and emerging many types of microbes that will restore the soil biological activity (Szoboszlay *et al.*, 2017, Tieman *et al.*, 2017). Our results indicate that Proteobacteria is the highest abundance in all of the land cover patterns.

Gemmatimonadetes, Latescibacteria, and Nitrospirae were phyla with a relative abundance smaller than 0.1% on the total microbes in all samples. It means that both of landcover types were categorized as "healthy". Nitrospirae was one of the microorganisms that is usefull as soil quality indicator. Nitrospirae was categorized as Gram-negative nitrite-oxidizing organism with 0.9-2.2 x 0.2 - 0.4 micrometer in size. Some of the Nitrospirae species perform important functions in the nitrogen cycle. This species have a vital role in nitrogen cyle in the second step of nitrification , i.e. by performing nitrite oxidation.

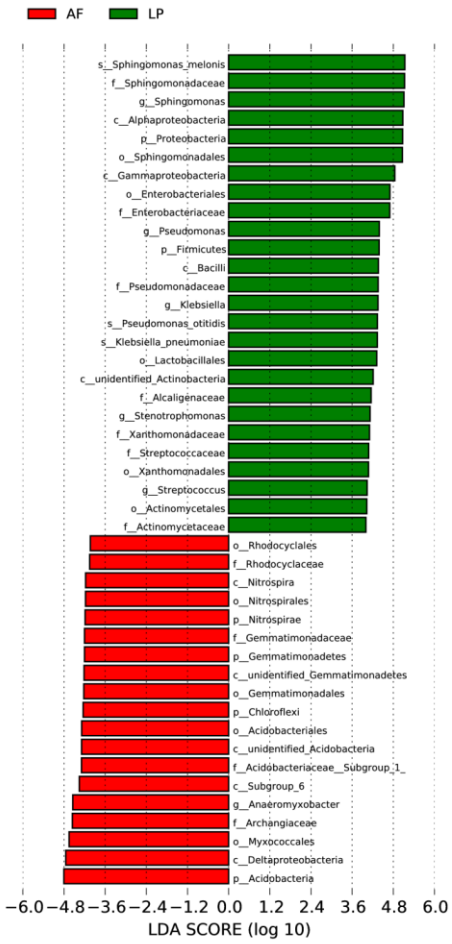


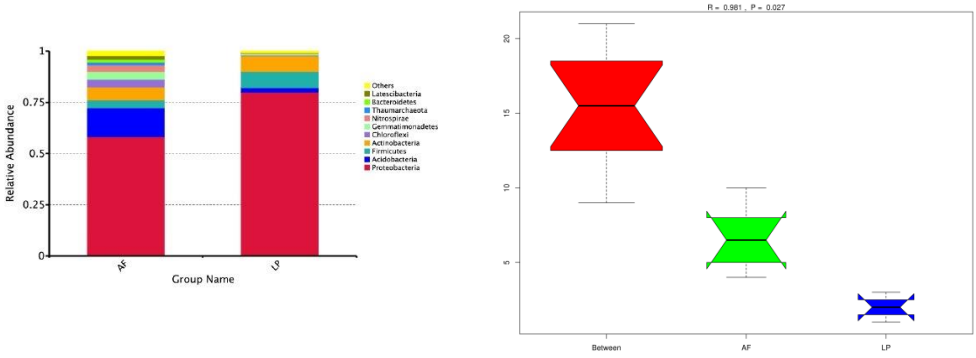
Figure 2. The LEfSe method to Characterize the Significant Bacteria in Soil on Various Land Cover. The vertical coordinate visualizes the taxonomic unit, while the horizontal bar visualizes the Linear Discriminant Analysis (LDA) score (log 10). The bar represents the abundance of taxonomical unit.

Microbial organisms need soil C and N-total for their growth. This nutrient were strong influence by management practices, especially long-term fertilization. The microbial taxa that is dependent on their soil characteristics were : genus *Nitrososphaera* (phylum *Thaumarchaeota*), *Nitrospira* of *Nitrospirae*, *Hydrogenophaga*, and *Thiobacillus and Sulfuricurvum*(phylum *Proteobacteria*), and several subgroups of *Acidobacteria* (Gu *et al.*, 2017; Greenblum *et al.*, 2012).

OTUs analysis between samples on bacterial communities was used the Venn diagram. This Venn diagram showed that 1452 OTUs shared between LP and AF; 59 OTUs belong to LP; and no one on AF. There was a total of 2020 genera from the AF and LP samples, and among the total genera, 1452 genera exist in both samples, AF and LP. The LDA represents the differences between the AF and LP groups at the genus level (Figure 2.). s_Sphingomonas_melonis; f_Sphingomonadaceae; g_Sphingomonas; c_Alphaproteobacteria; p_Proteobacteria;and o_Sphingomonadales were the most abundant in the LP groups, while p_Acidobacteria and c_Deltaproteobacteria were the most abundant in AF groups. This dominance was observed in all group members therefore these genera were contributed to the differences between the LP and AF groups.

Sphingomodales were commonly found on plant roots (Haichar et al., 2008). Our study revealed that the increasing of Sphingomodales abundance which is known as metabolizing nutrients microbial will cause an increase of Proteobacteria abundance. Our study revealed that the increasing of Sphingomodales abundance which is known as metabolizing nutrients microbial will cause an increase of Proteobacteria abundance. Sphingomodales was a potential gene in root–accociated diazotroph that contain the nitrogenase gene. However, it is important to remember that the phylum Alphaproteobacteria that is known as OTUs biomarker, were also important for plant growth, due to their content of PGPR (plant growth-promoting growth) rhizobacteria (Pini *et al.*, 2011). Commonly, the Alpha and gamma-bacteria in the rhizosphere were higher than in the bulk soil (Pascual *et al.*, 2018).

The taxonomic analysis showed that bacteria belong to Acidobacteria, Actinobacteria, Firmicutes, Proteobacteria, and Thaumarchaecota. Soil microbes profile showed that at the phylum level, AF landcover have the microbial abundance higher than in LP. The diversity of microbial species was also greater at AF than LP. This difference was also shown on the anosim analysis.



(a) (b)

Figure 3. The (a) Microbial Community Relative Abundance and (b) Anosim Analysis on AF and LP Landcover

The anosim analysis tested the differences between two land cover, which is represented by the R-value and confidence degree. The positive R-value and the confidence degree represented by the P-value showed significant differences between landcover types. The R-value (0.981) and P-value (0.027) mean that soil microbial on AF was significantly different from the LP land cover.

The Influence of Soil Properties on Microbial Community Structure

This research showed that the dominant bacterial taxa found in this field sites mainly belong to phylum Proteobacteria (primarily Alphabacteria, Betabacteria, and Gammabacteria), Actinobacteria, Firmicutes, and Acidobacteria. It seems that this taxa were also dominantly found in diverse mountains along elevation gradients (Praeg *et al.,* 2019). Acidobacteria was categorized as oligotrophic microbes (Fierer *et al*., 2012; Cederlund *et al.,* 2014), it means that they are related with the recalcitrant carbon and low pH (Lldo *et al.,* 2017), and therefore sensitive to pH changes. The soil pH value was belong to acid-slightly acid ; thus it was affect the Acidobacteria, either their abundance, community structure and also diversity (Chu *et al.,* 2010). This strengthens our research which is observed as a large abundance of Acidobacteriaon AF that has low pH (4.77), compared with the LP (pH = 5.46). Gemmatimonadales were also affected by pH as well.

Actinobacteria, as the third most abundant phylum in our study, was known as to survive in any condition, therefore the abundance between both landcover was similar (AF = 0,15%; LP=0,20%) was almost similar. Actinobacteria was important in the decomposition of all of the recalcitrant matter (Rehakov *et al.,* 2015).

The bacteria found abundant in bulk soil was Alphaproteobacteria and Gammaproteobacteria, which is also found by byYao et al., (2017) and Praeg, Pauli, and Illmer (2019). They are affect by soil carbon (Yao *et al.,* 2017) and also pH status of the bulk soil(Kim *et al.,* 2014). The OTU's number of AF land cover higher than LP land cover, while on the species rank, the LP land cover higher than AF land cover. This is probably due to the soil carbon content on LP land cover (Table 1.) was higher than AF land cover. This is probably because there was a fertilization addition on LP land cover. Carbon was needed for microbe metabolism as an energy source so that the growth and activities of soil microbe will be increased.

Environmental characteristics, such as elevation, altitude, and soil characteristics influenced the community prokaryotic pattern. The diversity of prokaryotic bacteria was positively attributed to pH and C/N ratios. The key factor determine the microbial diversity pattern was pH value (Singh *et al.,* 2012; Shen *et al.,* 2013; Bartram *et al.,* 2014; Zhang *et al.,* 2015). Generally, diversity and abundance of bacterial taxa positively increasing liniearly with the pH increasing; and inversely decreasing with the organic carbon content (Shen *et al.,* 2015). Community structure diversity were strongly related with the vegetation and nutrient status, and soil pH (Yao *et al*., 2017), where the pH value have the strongest influence.

Conclusion

In our study, the alpha diversity of soil microbial between land covers significantly different. It seems that it influenced by the soil pH, because this parameter affects the ecological parameter, thus influence the nutrient supply and microorganisms' growth.

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References

Altieri, M.A. (1999). The ecological role of biodiversity in agroecosystems. *Agric. Ecosyst. Environ.74*, 19–31.

Bardgett, R.D., and McAlister, E. (1999). The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. *Biology and Fertility of Soils*, *29*, 282–290.

Bartram, A. K., Jiang, X., Lynch, M. D. J., Masella, A. P., Nicol, G. W., and Dushoff, J., et al. (2014). Exploring links between pH and bacterial community composition in soils from the craibstone experimental farm. *FEMS Microbiol. Ecol.*, *87*, 403–415. doi: 10.1111/1574-6941. 12231.

Cederlund, H., Wessén, E., Enwall, K., Jones, C. M., Juhanson, J., and Pell, M., et al. (2014). Soil carbon quality and nitrogen fertilization structure bacterial communities with predictable responses of major bacterial phyla. Agric. *Ecosyst. Environ. Appl. Soil Ecol.*, *84*, 62–68. doi: 10.1016/j.apsoil.2014. 06.003.

Chu, H., Fierer, N., Lauber, C. L., Caporaso, J. G., Knight, R., and Grogan, P. (2010). Soil bacterial diversity in the arctic is not fundamentally different from that found in other biomes. *Environ. Microbiol.*, *12*, 2998–3006. doi: 10.1111/j.1462-2920.2010.02277.x.

Dai, Z., Su, W., Chen, H., Barberan, A., Zhao, H., Yu, M., Yu, L., Brookes, P.C., Schadt, C.W. and Chang, S.X. et al. (2018). Long-term nitrogen fertilization decreases bacterial diversity and favours the growth of Actinobacteria and Proteobacteria in agro-ecosystems across the globe. *Glob. Chang. Biol*., *24*, 3452–3461.

David, M. M., Power, M. E., Comín, F. A. and Yockteng, R. (2012). Structural and functional loss in restored wetland ecosystems. *Plos Boilogy*, *10*, e1001247, https://doi.org/10.1371/journal.pbio.1001247.

de Vries, F.T., Thebault, E., Liiri, M., Birkhofer, K., Tsiafouli, M.A., Bjornlund, L., Jorgensen, H.B., Brady, M.V., Christensen, S., de Ruiter, P.C., d'Hertefeldt, T., Frouz, J., Hedlund, K., Hemerik, L., Hol, W.H.G., Hotes, S., Mortimer, S.R., Setala, H., Sgardelis, S.P., Uteseny, K., van der Putten, W.H., Wolters, V., and Bardgett, R.D. (2013). Soil food web properties explain ecosystem services across European land cover systems. *Proceedings ofthe National Academy of Sciences of the United States of America, 110*, 14296-14301.

Ding, G.C., Piceno, Y.M., Heuer, H., Weinert, N., Dohrmann, A.B., Carrillo, A., Andersen, G.L., Castellanos, T., Tebbe, C., and Smalla, K. (2013). Changes of soil bacterial diversity as a consequence of agricultural land cover in a semi-arid ecosystem. *PLoS ONE, 8*; e59497.

Doran, J.W.,and Zeiss, M.R. (2000). Soil health and sustainability: managing the biotic component of soil quality. *Applied Soil Ecology*, *15*, 3-11.

Dube J. P., Valverde A., Steyn J. M., Cowan D. A., and van der Waals J. E. (2019). *Differences in Bacterial Diversity, Composition and Function due to Long-Term Agriculture in Soils in the Eastern Free State of South Africa*. Diversity 11;61.

Dudley, N. and Alexander, S. (2017). Agriculture and biodiversity: A review. *Biodiversity, 18*;45–49.

Fierer, N., Leff, J.W., Adams, B.J., Nielsen, U.N., Bates, S.T., Lauber, C.L., Owens, S., Gilbert, J.A., Wall, D.H., and Caporaso, J.G. (2012). Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 21390-21395.

Haichar, F. E. Z., Marol, C., Berge, O., Rangel-Castro, J. I., Prosser, J. I., and Balesdent, J., et al. (2008). Plant host habitat and root exudates shape soil bacterial community structure. *ISME J.*, *2*, 1221–1230. doi: 10.1038/ismej.2008.80

Hughes J. B., Hellmann J. J., Ricketts T. H., and Bohannan B.J.M. (2001). Counting the uncountable: statistical approaches to estimating microbial diversity. *Appl. Environ. Microbiol.*, *67*, 4399–4406. 10.1128/AEM.67.10.4399-4406.2001

Greenblum, S., Turnbaugh, P. J., and Borenstein, E. (2012). Metagenomic systems biology of the human gut microbiome reveals topological shifts associated with obesity and inflammatory bowel disease. *Proc. Natl. Acad. Sci. U.S.A.*, *10*,594–599.

Gu Y., Wang Y., Lu S., Xiang Q., and Yu X., et al. (2017). Long-term fertilization structures bacterial and archaeal communities along soil depth gradient in a paddy soil. *Front. Microbiol., 8*, 1516.

Haichar, F. E. Z., Marol, C., Berge, O., Rangel-Castro, J. I., Prosser, J. I., and Balesdent, J., et al. (2008). Plant host habitat and root exudates shape soil bacterial community structure. *ISME J., 2*, 1221–1230. doi: 10.1038/ismej.2008.80

Hughes, J. B., Hellmann, J. J., Ricketts, T. H., and Bohannan, B. J. M. (2001). Counting the uncountable: statistical approaches to estimating microbial diversity. *Appl. Environ. Microbiol.*, *67*, 4399–4406. doi: 10.1128/AEM.67.10. 4399-4406.2001

Kämpfer, P., Denner, E. B., Meyer, S., Moore, E. R., and Busse, H. J. (1997). Classification of "Pseudomonas azotocolligans" Anderson 1955, 132, in the genus Sphingomonas as Sphingomonas trueperi sp. nov. *Int. J. Syst. Bacteriol., 47*, 577–583. doi: 10.1099/00207713-47-2-577.

Kim, H. M., Jung, J. Y., Yergeau, E., Hwang, C. Y., Hinzman, L., and Nam, S., et al. (2014). Bacterial community structure and soil properties of a subarctic tundra soil in Council, Alaska. *FEMS Microbiol. Ecol., 89*, 465–475. doi: 10.1111/1574-6941.12362.

Lladó, S., López-Mondéjar, R., and Baldrian, P. (2017). Forest soil bacteria: diversity, involvement in ecosystem processes, and response to global change. *Microbiol. Mol. Biol. Rev., 81*, e00063–e00016. doi: 10.1128/MMBR.00063-16.

Lugtenberg, B. and Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol., 63*, 541–556.

Mackelprang, R., Waldrop, M.P., De Angelis, K.M., David, M.M., Chavarria, K.L., Blazewicz, S.J., Rubin, E.M., and Jansson, J.K. (2011). Metagenomic analysis of a permafrost microbial community reveals a rapid response to thaw. *Nature, 480*, 368-U120.

Mahamane, L. and Mahamane, S. (2005)**.** Biodiversity of ligneous species in semi-arid to arid ones of southwestern Niger according to anthropogenic and natural factors. *Agric. Ecosyst. Environ., 105*, 267–271.

Manoharan, L., Ahren, D., Urich. T. and Hedlund, K. (2017). Effects of land cover management on soil microbes to degrade organic matter through captured metagenomics and metatranscriptomics. *Geophysical Research Abstracts, 19*, EGU2017-18162

Nie, M., Bell, C., Wallenstein, M.D., and Pendall, E. (2015). Increased plant productivity and decreased microbial respiratory C loss by plant growth-promoting rhizobacteria under elevated CO2. *Scientific Reports 5*.

Pascual, J., Blanco, S., Ramos, J. L., and van Dillewijn, P. (2018). Responses of bulk and rhizosphere soil microbial communities to thermoclimatic changes in a Mediterranean ecosystem. *Soil Biol. Biochem.,118*, 130–144. doi: 10.1016/j.soilbio.2017.12.0,13.

Pini, F., Galardini, M., Bazzicalupo, M., and Mengoni, A. (2011). Plantbacteria association and symbiosis: are there common genomic traits in alphaproteobacteria? *Genes, 2*, 1017–1032. doi: 10.3390/genes2041017.

Praeg N, Pauli H and Illmer P. (2019). Microbial Diversity in Bulk and Rhizosphere Soil of Ranunculus glacialis Along a High-Alpine Altitudinal Gradient. *Frontiers in Microbiology, 10* (14). 1-9. doi: 10.3389/fmicb.2019.01429.

Rehákov,á, K., Chronáková, A., Krištufek, V., Kuchtová, B., Capková, K., and Scharfen, J., et al. (2015). Bacterial community of cushion plant Thylacospermum ceaspitosum on elevational gradient in the Himalayan cold desert. Front. *Microbiol., 6*, 304. doi: 10.3389/fmicb.2015.00304

Schimel, J.P., and Schaeffer, S.M. (2012). Microbial control over carbon cycling in soil. *Frontiers in Microbiology, 33*(1), 1-7.

Shen, C., Xiong, J., Zhang, H., Feng, Y., Lin, X., and Li, X., et al. (2013). Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. *Soil Biol. Biochem., 57*, 204–211. doi: 10.1016/j.soilbio.2012.07.013.

Shen, C., Ni, Y., Liang, W., Wang, J., and Chu, H. (2015). Distinct soil bacterial communities along a small-scale elevational gradient in alpine tundra. *Front. Microbiol., 6*, 582. doi: 10.3389/fmicb.2015.00582.

Singh, B.K., Munro, S., Reid, E., Ord, B., Potts, J.M., Paterson, E., and Millard, P. (2006). Investigating microbial community structure in soils by physiological, biochemical and molecular fingerprinting methods. *European Journal of Soil Science, 57*, 72–82.

Szoboszlay, M., Dohrmann, A., Poeplau, C., Don, A., and Tebbe, C. (2017). Impact of land cover change and soil organic carbon quality on microbial diversity in soils across Europe. *FEMS Microbiol. Ecol., 93*,146.

Tiemann, L., Grandy, A., Atkinson, E., Marin-Spiotta, E., and McDaniel, M. (2015). Crop rotational diversity enhances belowground communities and functions in an agroecosystem. *Ecol. Lett., 18*, 761–771.

Underwood, E.C., Viers, J.H., Klausmeyer, K.R., Cox, R.L., and Shaw, M.R. (2009). Threats and biodiversity in the Mediterranean biome. *Divers. Distrib*., *15*,188–197.

Wang, Q., He, T., Wang, S., and Liu, L. (2013). Carbon input manipulation affects soil respiration and microbial community composition in a subtropical coniferous forest. *Agric. For. Meteorol*., *178*, 152–160.

Wijayanti, M. (2015). Bacterial Diversity and Lipase Producing Bacteria in Forest and Oil Palm Plantation in Jambi. *Dissertation*. IPB: Bogor.

Xu, H. J. et al. (2014). Biochar impacts soil microbial community composition and nitrogen cycling in an acidic soil planted with rape. *Environmental Science & Technology*, *48*, 9391–9399, https://doi.org/10.1021/es5021058.

Yang, Y.F., Gao, Y., Wang, S.P., Xu, D.P., Yu, H., Wu, L.W., Lin, Q.Y., Hu, Y.G., Li, X.Z., He, Z.L., Deng, Y., and Zhou, J.Z. (2014). The microbial gene diversity along an elevation gradient of the Tibetan grassland. *Isme Journal*, *8*, 430-440.

Yao, F., Yang, S., Wang, Z., Wang, X., Ye, J., and Wang, X., et al. (2017). Microbial taxa distribution is associated with ecological trophic cascades along an elevation gradient. *Front. Microbiol., 8*, 2071. doi: 10.3389/fmicb.2017. 02071.

Zhang, Y., Cong, J., Lu, H., Li, G., Xue, Y. and Deng, Y., et al. (2015). Soil bacterial diversity patterns and drivers along an elevational gradient on Shennongjia Mountain, China. *Microb. Biotechnol., 8*, 739–746. doi: 10.1111/1751-7915.12288.