Land-use change influences microbial and carbon dynamics in soils of the tropics

by

Emily J. Díaz Vallejo

A dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

(Geography)

at the

UNIVERSITY OF WISCONSIN-MADISON

2023

Date of final oral examination: 07/03/2023

The dissertation is approved by the following members of the Final Mentor and Examination Committee:

Erika Marín-Spiotta, Professor, Geography
Thea Whitman, Associate Professor, Soil Science
Kris Sankaran, Assistant Professor, Statistics
Joseph Mason, Professor, Geography

Dedication

I wholeheartedly dedicate this work to my parents, Hilda Vallejo and Juan Díaz, whose unwavering support have been instrumental in enabling me to realize my dreams. I firmly believe that the triumph of this endeavor is not solely mine, but rather a shared victory and a testament to the profound impact they have had on shaping who I am today.

Acknowledgments

I would like to express my sincere gratitude to the individuals who have provided invaluable support and assistance throughout the completion of this project. Their contributions have been instrumental in its success. First and foremost, I extend my deepest appreciation to my adviser, Erika Marín-Spiotta, whose support and guidance have been pivotal to my career development. I am truly grateful for her continuous input and mentorship. I am also thankful to Elliot Vaughan and Jamie Woolet for their commitment to fieldwork, lab work, and statistical support. Their expertise and dedication have significantly enhanced the quality of my project. I extend my heartfelt thanks to Chelsie Rodriguez and Milan Stolpman, whose involvement in generating new ideas and processing samples has been invaluable. Their contributions have brought fresh perspectives and enriched the overall research. I extend my appreciation to Joe Mason, Jack Williams, Ken Keefover-Ring, and Thea Whitman for generously providing access to lab materials and space essential for conducting my research, and continuous mentorship and guidance. Their support has been indispensable in facilitating the smooth execution of my work. I would like to express my gratitude to Kris Sankaran for providing exceptional statistical assistance. Your expertise and guidance in analyzing and interpreting data have been invaluable to the success of this project. I would like to acknowledge Para la Naturaleza and the owners of individual private properties for granting me permission to access the research sites. Their cooperation and willingness to accommodate my needs have been instrumental in the successful completion of this study. I am sincerely grateful to the members of the Biogeolab, whose steady support has created a nurturing and collaborative research environment. Their assistance and encouragement have played a significant role in shaping my project. Finally, I would like to express my profound gratitude to all the funding sources that have supported my research economically. I extend my thanks to the Pruitt Minority Fellowship, O.N. Allen Soil and Environmental Microbiology Small Grant, UW-Madison Geography Department, and the NSF CAREER BCS-1349952.

Abstract

This dissertation explores the influence of land use changes on soil dynamics and functionality in tropical regions. Specifically, it addresses three main research questions. Firstly, it examines the global impact of land use changes on microbial biomass, abundance, and traits in tropical regions while identifying knowledge gaps and areas for further research in tropical microbial ecology. Secondly, it investigates how tropical secondary forest succession affects microbial function in diverse soil environments and explores the connection between changes in microbial function and soil carbon variability across different successional stages. Lastly, it aims to define a benchmark tool for soil carbon assessment in a variety of tropical climates, land use practices, and soil types, with potential applications for farmers, land managers, soil researchers, policymakers, and individuals interested in soil health assessment.

The research findings underscore the significant effects of land use conversions on soil microbial communities and their associated ecosystem functions. To better understand the global-scale response of tropical regions to environmental changes, a comprehensive understanding of the diverse climates, vegetation, management practices, and soil conditions in these regions is essential. Additionally, this study examines the dynamics of microbial communities during forest succession and highlights the urgent need for further research to comprehend microbial functionalities in disturbed tropical forest soils. Furthermore, it emphasizes the importance of establishing benchmarks for assessing soil carbon in tropical soils, taking into account the impacts of land use change, and developing guidelines or protocols accordingly.

This dissertation identifies knowledge gaps and suggests future research directions, including the exploration of a broader range of soil types, investigations into dry systems, examination of specific soil characteristics, and the incorporation of advanced molecular techniques. The ultimate goal of this work is to contribute to the understanding of soil dynamics, provide insights for sustainable soil management, and offer strategies for climate change mitigation in tropical regions.

Table of Content

Introduction

Land use change in tropical regions has become a critical issue due to the increasing demand for agricultural and wood products, grazing for cattle, and residential and urban development (Aide et al. 2013), resulting in significant impacts on soil carbon (Powers and Marín-Spiotta 2017). The carbon inputs to soils from plant detritus in the tropics are roughly balanced by losses to the atmosphere through the respiration of soil microorganisms that feed on that material (Mitchard 2018). However, even a 1% imbalance in soil carbon effluxes over influxes would be equivalent to approximately 10% of global anthropogenic carbon emissions (Nottingham et al. 2020), emphasizing the substantial effect that tropical land cover changes can have on global emissions. Current estimates suggest that without efforts to alter the current trajectory of deforestation and land use management practices, the tropics will become a net source of carbon to the atmosphere in the coming decades (Mitchard 2018). However, these estimates are based on limited and highly variable data, and there is a significant knowledge gap regarding the response of soil carbon to land use change in tropical soils.

Studies evaluating the respond of SOC to the effects of deforestation for agricultural, and livestock uses results in global losses of SOC, with the exception of certain pastures, but it has revealed high variability in the response of soil carbon to land use change (Guo and Gifford 2002, Don et al. 2011, Powers et al. 2011). This variability is further complicated by large geographic biases in available data, indicating a lack of comprehensive sampling across the diverse climates and soils found in the tropical region (Powers et al. 2011), suggesting the need to address factors influencing soil organic carbon in the tropics. Microbial communities in soil play vital roles in ecosystem processes, including litter decomposition and organic matter transformations (Friesen et al. 2011, Wieder et al. 2013). Monitoring changes in microbial

communities and their functions can provide valuable insights into the processes influencing carbon dynamics. Also, climate and physicochemical soil properties have been widely recognized as significant drivers of dynamics at both global and regional scales (Luo et al. 2017, Wiesmeier et al. 2019). Therefore, to improve our understanding of soil carbon dynamics in the tropics, it is crucial to evaluate factors that affect soil carbon across a wide range of climates, soils, and environmental conditions.

The field of soil health offers indices and benchmarks that can be instrumental in understanding soil carbon changes. Soil health refers to the continued capacity of soil to function as a vital living ecosystem, supporting plants, animals, and humans (Lehmann and Kleber 2015). By establishing benchmarks, we can assess the effects of various factors and inform future assessment and management strategies. This knowledge will provide farmers, land managers, and policymakers with a baseline for implementing measures to maintain or enhance soil health. Moreover, it will enable climate mitigation strategists to develop effective plans for the future. Therefore, my Ph.D. research aimed to investigate how land-use change affects microbial communities and soil carbon across different climates, land uses, and soil properties.

In the first chapter, I investigated how land-use change influences microbial communities and soil carbon across a wide range of climate conditions, land uses, and soil properties. I conducted a global meta-analysis using published data from comparative studies in tropical regions. By analyzing 83 paired studies that reported data on microbial biomass, abundance, composition, and enzyme activity under representative land-use change transitions in the tropics, I calculated response ratios for different land conversion types, such as from reference forests to agriculture, pastures, plantations, and secondary forests. I found that microbial biomass decreased with forest conversion to agriculture and plantations. Also, microbial abundance and

enzyme activity showed variable results depending on the specific type of forest conversion, while microbial diversity and richness did not exhibit any difference. Notably, the published studies reviewed in the meta-analysis were not representative of the full range of biophysical conditions observed in the tropics, with an overrepresentation of sites in moist regions in the American tropics.

In the second chapter, I conducted a case study utilizing a chronosequence of sites representing a catena to investigate further how microbial function changes, in this case, during forest succession as a result of pasture abandonment on different soil orders. For this, I collected soil samples across a series of chronosequences in Puerto Rico on different soil orders, measured soil organic carbon, and analyzed DNA targeting ITS2 and 16S genes to quantify fungal and bacterial communities. I found that forest succession significantly affected bacterial diversity and fungal community composition across different successional stages. Also, I found variations in the relative abundance of certain bacterial phyla following pasture abandonment. While fungal phyla exhibited minimal changes across succession, distinct trends emerged when considering functional characteristics. Interestingly, I also found that the variation of bacterial phyla, as well as the variation of fungal functional groups across succession, were modulated by soil characteristics. Furthermore, I found that microbial functional characteristics partially explained variations in soil carbon concerning forest age and soil order, underscoring the complex relationship within these disturbed tropical forest soils.

In the third chapter, I conducted a regional scale analysis to understand how land use change affects soil carbon across a diversity of tropical soil environments. For this, I developed an assessment of soil carbon benchmark models using available data for Puerto Rico across various climates, land uses, and soil types, with a specific focus on agroecosystems. By

collecting data from the United States Natural Resources Conservation Services (USDA NRCS) Kellogg database and relevant publications, I obtained a dataset of 586 pedons representing nine out of ten soil orders in Puerto Rico, encompassing five land uses. I evaluated factors influencing soil organic carbon within the 0-30 cm depth and examined two benchmark styles for soil health. My findings revealed significant variations in SOC across different land uses, with different factors controlling SOC levels in pastures, agricultural lands, and forests. When evaluating a Soil Health Gap benchmark model on my sites, the gaps in soil carbon between forests and agricultural or pasture lands varied depending on soil order and climate conditions. This suggests that certain managed lands may contain more carbon than forest systems, with soil environment and climate modulating this relationship. I also evaluated the Scores Benchmark model using the distribution of soil carbon across multiple environmental conditions and concluded that this benchmark would be more suitable for tropical soils than the Soil Health Gap Benchmark. However, these Benchmark styles could have advantages and disadvantages depending on the area's scale and Soil health goal.

Research Questions:

Chapter 1: How do land use changes affect microbial biomass, abundance, and traits in tropical regions globally? What are the knowledge gaps in the literature and areas for further research in tropical microbial ecology?

Chapter 2: How does tropical secondary forest succession influence microbial function in different soil environments? How do changes in microbial function influence soil carbon variability across successional stages?

Chapter 3: Can we define a benchmark tool for soil carbon in a diversity of tropical climates, land use, and soils can be used as a tool for farmers, land managers, soil researchers, policymakers, and anyone interested in soil health assessment?

References

Aide, T. M., M. L. Clark, H. R. Grau, D. López-Carr, M. A. Levy, D. Redo, M. Bonilla-Moheno, G. Riner, M. J. Andrade-Núñez, and M. Muñiz. 2013. Deforestation and Reforestation of Latin America and the Caribbean (2001–2010). Biotropica 45:262–271.

Don, A., J. Schumarcher, and A. Freibauer. 2011. Impact of tropical land-use change on soil organic carbon stocks – a meta-analysis. Global Change Biology 17:1658–1670.

Friesen, M. L., S. S. Porter, S. C. Stark, E. J. von Wettberg, J. L. Sachs, and E. Martinez-Romero. 2011. Microbially mediated plant functional traits. Annual Review of Ecology, Evolution and Systematics 42:23–46.

Guo, L. B., and R. M. Gifford. 2002. Soil carbon stocks and land use change: a meta-analysis. Global Change Biology 8:345–360.

Lehmann, J., and M. Kleber. 2015. The contentious nature of soil organic matter. Nature 528:60–68.

Luo, Z., W. Feng, Y. Luo, J. Baldock, and E. Wang. 2017. Soil organic carbon dynamics jointly controlled by climate, carbon inputs, soil properties and soil carbon fractions. Global Change Biology 23:4430–4439.

Mitchard, E. T. 2018. The tropical forest carbon cycle and climate change. Nature 559:527–534.

Nottingham, A. T., P. Meir, E. Velasquez, and B. L. Turner. 2020. Soil carbon loss by experimental warming in a tropical forest. Nature 584:234–237.

Powers, J. S., M. D. Corre, T. E. Twine, and E. Veldkamp. 2011. Geographic bias of field observations of soil carbon stocks with tropical land-use changes precludes spatial extrapolation. Proceedings of the National Academy of Sciences 108:6318–6322.

Powers, J. S., and E. Marín-Spiotta. 2017. Ecosystem Processes and Biogeochemical Cycles in Secondary Tropical Forest Succession. Annual Review of Ecology, Evolution, and Systematics 48:497–519.

Wieder, W. R., G. B. Bonan, and S. D. Allison. 2013. Global soil carbon projections are improved by modelling microbial processes. Nature Climate Change 3:909–912.

Wiesmeier, M., L. Urbanski, E. Hobley, B. Lang, M. von Lützow, E. Marín-Spiotta, B. van Wesemael, E. Rabot, M. Ließ, N. Garcia-Franco, U. Wollschläger, H.-J. Vogel, and I. Kögel-Knabner. 2019. Soil organic carbon storage as a key function of soils - A review of drivers and indicators at various scales. Geoderma 333:149–162.

Chapter 1: A meta-analysis of tropical land-use change effects on the soil microbiome: Emerging patterns and knowledge gaps

Introduction

Tropical regions are currently experiencing some of the fastest rates of land-use change globally (Aide et al., 2012). From 2010-2015, tropical forests were lost at a rate of 5.5 M ha/yr, greater than the loss of boreal forests (0.08M ha/yr), or in contrast with an increase in temperate and subtropical (Keenan et al., 2018, Veldkamp et al., 2020). Habitat loss is the primary driver of plant and animal diversity declines worldwide (Millennium Ecosystem Assessment, 2005), yet the effects of changes in land use and land cover (hereafter, land-use change) on the abundance and diversity of soil microorganisms and their function has not been well documented. Soil microorganisms are key players in multiple ecosystem processes, such as plant fitness (Friesen et al., 2011), litter decomposition, nutrient availability, and soil organic matter (SOM) transformations (Turner et al., 2013; Wieder et al., 2013). Losses of soil biodiversity and food web complexity can affect carbon (C) and nutrient cycling (de Graaff et al., 2015), yet whether land-use change results in microbial species richness declines or shifts in community assemblages and activity is still unknown.

Land-use change, such as deforestation or forest clearing for agriculture and livestock grazing, can influence the soil microbiome through shifts in plant communities and management practices, which can alter the amount, timing, and spatial distribution of substrates for microbial growth (Cai et al., 2018; Krashevska et al., 2018). For example, perennial and annual cropping systems differ in rhizosphere inputs, which influence fungal communities and total microbial biomass (Liang et al., 2012; Zhang et al., 2019; Zhang et al., 2018). Grazing and fertilization can affect soil resources and alter the abundance of microbial groups involved in nitrogen (N)

cycling (Meyer et al., 2013). Management practices, such as the use of mechanization for clearing and tillage, can transform soil physical and chemical properties and the spatial relationship among organisms, which can alter heterotrophic food webs and biogeochemical cycling (Mathew et al., 2012).

Modifications in pathways and rates of biogeochemical processes coupled to changes in overall microbial biomass or the abundance of key microbial groups (Bai et al., 2019; Docherty & Gutknecht, 2012; Potthast et al., 2012) may result from the preferential use of different substrates by distinct microorganisms (Paterson et al., 2008; Strickland et al., 2009; Zhong et al., 2020). For example, the forest soil microbiome may be better adapted to degrading lignin-rich litter than the microbiome of grassland soils (Cleveland et al., 2003). Bacteria are expected to assimilate more decomposed substrates, whereas fungi may preferentially degrade more recent plant C inputs (Frey, 2019; Poll et al., 2006).

Enzyme activities can be used as a proxy for microbial function and are sensitive to changes in plant cover and management (Sinsabaugh et al., 2002; Zhao et al., 2018). Enzymes involved in plant litter decomposition catalyse the breakdown of compounds, such as cellulose, hemicellulose, and lignin, and control the release of plant- and microbe-available nutrients from organic forms (Horwath & Paul, 2015). These activities can be sensitive to changes in plant cover and management (Sinsabaugh et al. 2002). For example, some studies have found that agricultural practices can reduce levels of enzyme activity compared to forest soils due to soil disturbance by tillage (Acosta-Martínez et al., 2007). Other studies have found that rates of enzyme activity can increase with pasture establishment after deforestation (Tischer et al., 2015). However it is important to consider how plant diversity effects on decomposition and nutrient cycling create biogeochemical heterogeneity, as well as microbial functional redundancy, to

understand how the soil microbiome is affected by forest clearing and conversion to agriculture or livestock grazing, especially in species-rich tropical regions (Chaer et al., 2009; Smith et al., 2015; Townsend et al., 2008).

Here we conduct a meta-analysis on the response of soil microbial biomass, composition, and functional activity under different land-cover types representative of major land-use changes transitions in tropical regions. We focused on soil bacteria and fungi. We identify current knowledge gaps in the literature, including geographic biases in available data. Our understanding of the diversity of tropical soil microorganisms lags behind temperate regions. Identifying how tropical land-use change alters interactions among soil microbial community composition and function can provide insight into feedbacks between tropical ecosystems and global change factors, such as climate change (Graham et al., 2016; Powell et al., 2015). We hypothesized that forest conversion to agriculture, pasture, and tree plantation would decrease soil microbial biomass, abundance, and function, with consequences for biogeochemical cycling. We expected that secondary forests regrowing from human land use would recover microbial communities and function. Because microbial communities are dependent on soil resource availability, we expected that changes in microbial communities would mirror expected changes in soil pH, carbon, nitrogen, and phosphorus with land-use change.

Methods

We conducted a literature review using Web of Knowledge and Google Scholar databases to find studies within the tropical latitudes (between 23°26′16″N and 23°26′16″S) using the terms “tropical”, “land use”, “land cover”, “soil microbes”, “microbial biomass”, “fungi”, and “mycorrhizal fungi” (published up until February 2019, the last date we updated our search). This search resulted in 110 primary sources. Eighty-three of those reported data for paired

comparisons between two or more land-use types. We used PlotDigitizers software to estimate values when data were only reported in figures (Huwaldt & Steinhorst, 2015).

We selected studies that were conducted using a paired plot approach to compare reference forests to other land use or vegetation cover types: (a) forests to agriculture (b) forests to pastures, (c) forests to plantations, and (d) forests to secondary forests (Table S1). The studies needed to have a reference forest to be able to compare changes. Studies that only included regrowing forest and secondary forest without reference forests were not selected for this meta-analysis. We averaged data reported from plot replicates within a site (location). However, most studies reported mean values for different land covers.

Most of the studies grouped the data by soil depth, date collected or by season. Due to the limited number of studies reporting each possible combination of categories, we created consolidated variables. We limited our analysis to data for the topsoil layer (0-20 cm depth) to include the largest number of studies. If reported depths fit into the category of 20 cm depth (i.e., 0-5, 5-10, 0-10,10-20 0-15 cm), we averaged the data for the different layers up to 20 cm. If a study reported two or more sampling collection dates or seasons (e.g. wet and dry), we calculated average values. We acknowledge that sampling collection date and season are important factors that could influence microbial communities, yet few studies reported seasonal measurements of enough microbial variables of interest for our meta-analysis.

Land use and vegetation covers were grouped under five categories: “forest”, “agriculture”, “pasture”, “plantation forest” and “secondary forest.” We primarily used site identification provided by the authors. Forests were those presented as reference points by study authors and included unmanaged forests largely undisturbed by human activities. Agriculture included crops and systems with some level of resource inputs. Pastures included managed and

unmanaged grasslands and forage systems that were actively being used to support cattle or other livestock or were recently abandoned but still under grass cover. Plantations were defined as managed systems of woody trees or palms. Secondary forests were identified as successional forests establishing after clearing or after cessation of other land-use activities and could include sites recovering from logging, pasture, agriculture, and other non-forest uses.

We included a large number of microbial and environmental variables in our meta-analysis (Table 1). Studies reported many different microbial measurements depending on their research objective. For microbial variables, we grouped the data based on the type of measurements. For example, we analyzed studies using PLFA and DNA sequencing to measure microbial abundance separately, recognizing that different approaches have different assumptions that can lead to different responses.

Data analysis

We conducted all analyses using R 3.3.2. The effect of land-use change transitions on microbial variables was calculated as the response ratio (RR) using the equation (Gurevitch, 1993; Q. Zhang et al., 2017):

$$RR = \ln(\text{Land-use Change}) - \ln(\text{Forest})$$

where “Land-use Change” and “Forest” are the mean of the replacement land cover and the reference forest system, respectively. Many studies did not report the standard deviation and the number of samples that were used in the analyses. Therefore, in order to include as many studies as possible, we conducted an unweighted meta-analysis as used in Guo & Gifford (2002) and Johnson & Curtis (2001). To determine if there was a significant difference between the reference forest sites and other land uses, we calculated the 95% confidence interval (CI). If the

CI did not overlap with zero, then the response ratio was considered significant. In addition to separate analyses by land-use change transition, we use the general category "forest conversion" to report an average response of soil variables to replacement of reference forest cover by agriculture, pasture, and plantation forests combined.

We performed a one-way ANOVA Type III for an unbalanced design to analyze differences between response ratios across rainfall classes for each land-use change comparison only for microbial biomass carbon. We did not perform the aforementioned analysis with other variables because the number of samples were too low to fit all the land-use change and rainfall classes. To test if microbial biomass mirrored the magnitude of changes in soil properties, we used simple linear regression models between the response ratio of microbial biomass (carbon (C) and nitrogen (N)) and the response ratio of soil properties, specifically pH, organic C, total N and total phosphorus (P) across all land-use changes.

To identify potential geographic biases in available data on tropical soil microbiome response to land-use change (sensu Powers et al., 2011), we assessed how well the literature represented variation in mean annual precipitation (MAP), mean annual temperature (MAT), ecoregions, and soil type observed across the tropics. We calculated the percentage of land in the tropics represented by each climatic category, ecoregion, or soil type using ArcGIS (ArcGIS 10; ESRI 2010). We performed a Chi squared analysis to assess how well the studies represented MAP and MAT (Powers et al., 2011). When missing from the original publication, we used the WorldClim version 2 Global Climate Data set (Hijmans et al., 2005) for compiling climate data, and the Food and Agriculture Organization (FAO) Harmonized World Soil Database v 1.2 (Fischer et al., 2008) to identify soil types. We used The Nature Conservancy's terrestrial ecoregions map, which is based on the World Wildlife Fund ecoregions classification (Olson et

al., 2001). Ecoregions represent potential vegetation types based on climatic variables and were classified as moist broadleaf forest, dry broadleaf forest, coniferous forest, and grassland, savanna, and shrubland. Mean annual temperature was subdivided into three categories (18-21°C, 22-25°C, and 26-30°C) which accounted for the Koppen climate classification system criterion (Köppen, 1900; Kottek & Hantel, 2005). Mean annual precipitation was divided into rainfall classes associated with different tropical forest life zones: dry (<1000 mm), moist (1000-2500 mm) and wet (>2500 mm) representing 17%, 59% and 23% of the sites, respectively. When analysing for biases, we pooled studies from all land-use transitions as the number of studies was too small to compare each category individually. There were too few studies to perform the Chi squared on soil type and ecoregion type, given the large number of categories for each variable, but expected representation of each category was compared qualitatively to observed representation in the literature.

Results

We recorded 10,314 observations of 87 variables from 83 studies (Supplementary database). Thirty-six studies included multiple pairs of sites of the same land-use change comparison. Field sites were located in 25 countries, with the greatest proportion in Brazil (33%), India (7%), and Costa Rica (6%) and the lowest proportion in Argentina, Benin, Cameroon, Colombia, Dominican Republic, Ethiopia, Hawaii, Nigeria, Sumatra, and Tahiti, Taiwan (1%).

Microbial biomass response to land-use change

On average, forest conversion resulted in losses of soil microbial biomass carbon and nitrogen (Figure 1, Table S2). For microbial biomass C, these results were primarily observed in conversion of forest to agriculture and plantations. Conversion to pasture did not affect microbial

biomass C. Only conversion of forest to plantation showed a significant decline in microbial biomass N. Microbial biomass P was not affected by forest conversion. Secondary forests did not show any difference in microbial biomass C, N or P compared to forest values.

Microbial biomass carbon was the most represented variable among the three rainfall classes: dry (23% of studies), moist (52%) and wet (25%) life zones. Wet secondary forests had a greater microbial biomass carbon response ratio than moist secondary forests ($F=3.35$, $P=0.04$, Figure 2, Table S3). There was no effect of rainfall class on microbial biomass carbon during conversion of forest to plantations. In forest conversion to pastures ($F=6.17$, $P<0.01$) and in agricultural lands ($F=18.27$, $P<0.01$), microbial biomass carbon response ratio was greater in dry systems compared to moist systems.

Microbial abundance response to land-use change

On average, forest conversion resulted in a decrease or no change in microbial abundance measured by DNA sequencing and phospholipid fatty acid analysis (PLFA; Figure 3, Table S2). Four studies performed sequencing analysis (one study conducted pyrosequencing and the rest conducted Illumina sequencing). Forest replacement by plantations decreased total bacterial PLFAs and Gram – bacterial PLFAs. Conversion of forests to agriculture resulted in a decrease in the abundance of proteobacteria DNA sequences, total bacterial PLFAs, and Gram – bacterial PLFAs. In contrast, the conversion of forests to pastures increased the abundance of actinobacteria DNA sequences and total fungal PLFA. Secondary forests had greater abundance of Firmicutes and lower actinobacteria DNA sequences than forests.

Enzyme activity response to land-use change

Extracellular enzyme activity generally decreased with forest conversion (Figure 4, Table S2). Plantations showed lower activities for all enzymes reported. Conversion to pastures

increased α-glucosidase and urease activity. Deforestation for agriculture decreased activities of alkaline phosphatase, α-glucosidase and arylsulfatase. Secondary forests had greater levels of α-glucosidase activity and lower levels of ß-glucosidase and arylsulfatase than reference forests. Activities of acid phosphatase, alkaline phosphatase and urease did not differ between forests and secondary forests.

Bacterial diversity response to land-use change

Forest conversion resulted in no change in soil bacterial diversity as measured by Shannon and Simpson indices (Figure 5, Table S2). However, OTU Chao1 richness decreased with forest conversion to agricultural lands (Table S2).

Fungal response to land-use change

Fungal variables showed variable responses to land-use change (Figure 6, Table S2). Conversion from forest to plantation decreased arbuscular mycorrhizal fungi richness (AMF). Fungal spore density from all species in the soil and AMF richness decreased with conversion to agriculture. AMF colonization of roots increased in pastures. Secondary forests had greater arbuscular root colonization and less AMF richness than reference forests.

Microbial biomass responses to changes in soil properties

We tested for relationships between average response ratios for microbial biomass and the response ratios for soil properties across all four land-use change transitions. The response ratios for microbial biomass N (Figure 7e) and microbial biomass P (Figure S2) were positively correlated with the response ratio for soil pH. Microbial biomass C response ratios did not show any relationship with soil pH (Figure 7a). Microbial biomass C and microbial biomass N response ratios increased with organic C, total soil N, and total P response ratios.

How representative are microbial studies of tropical environments?

The available literature reporting microbial biomass and compositional variables for paired land uses does not reflect the diversity of biophysical variables represented in tropical regions. The range of MAP observed in the study sites (from 500 mm to 4,400 mm) was not representative of rainfall variation in the tropics (Chi square $P<0.001$; Figure 8a). Sites in the wet (>2500 mm) and moist (1000-2500 mm) life zones were overrepresented at the expense of drier sites. The range of MAT was also not representative of the temperature variation in the tropics (Chi Square $P=0.035$; Figure 8b). There were not enough studies to perform a Chi square analysis on the representativeness of the study sample set by ecoregion or soil order, although visual interpretation of the data suggest some strong biases in the literature. Grasslands, savannas, shrublands, coniferous forest, dry broadleaf forest and ecoregions were underrepresented while moist broadleaf forests were overrepresented compared to expected by their observed distribution across tropical landscapes (Figure S3a). Moist broadleaf forests composed 71% of field observations. The diversity of tropical soils is underrepresented in the literature. The FAO global soil map identifies 27 soil units in the tropics, yet only 16 of these were represented in the literature reviewed (Figure S3b). Twenty-one percent of the study sites were on Ferrasols and Acrisols. Cambisols were the third most studied soil order (12% of field observations).

Discussion

Our findings show that land-use cover changes in tropical regions affect soil microbial communities and functions. Notably, forest conversion to intensely managed systems decreases microbial biomass, abundance, and enzyme activities. Forest conversion to pastures showed increases, decreases, or no changes to microbial communities and functions, suggesting some

microbial variables may depend on the response of soil properties after deforestation. After abandonment of pasture, agricultural or plantation forest management, secondary forest succession can recover microbial communities and function. Moist ecosystems were overrepresented in the literature, whereas dry ecosystems were greatly underrepresented. Therefore, our understanding of how microbial communities and function are affected by land-use change is biased towards moist and wet regions.

Greater changes in microbial biomass and composition with replacement of forest by more intensively managed land

Measured declines in soil microbial biomass carbon, nitrogen and phosphorus with conversion of forests to agriculture and plantations are consistent with expectations for changes in soil properties in more intensely managed systems. The overall decrease of microbial biomass C and N can be associated with the decrease of soil C and N stocks common in agricultural systems (Bossio et al., 2005). Losses of soil C and N are attributed to the removal of plant residues and nutrients through biomass harvest and to accelerated decomposition of organic matter through soil disturbance during tillage and other soil preparation techniques (Montecchia et al., 2011). Management practices that improve organic matter inputs to soils can remedy observed decreases in microbial biomass in agricultural soils. On sandy soils in the tropical dry forest of eastern Amazonia, the addition of manure fertilizer coupled with irrigation increased microbial biomass C relative to the adjacent forest during the dry season (Medeiros et al., 2015). Banger et al., (2008) found that manure additions increased microbial biomass C on tropical agricultural fields beyond that observed with inorganic fertilizers. Similarly, conversion of conventional agriculture to organic farming in Brazil increased soil microbial biomass C and soil C (Santos et al., 2012). Observed decreases in soil microbial biomass after establishment of

plantations has been attributed to reductions in soil nutrients due to woody biomass harvest and residue management (Liu et al., 2012). Allen et al., (2015) found that reduction in microbial biomass with establishment of plantations in Indonesia was related to soil nutrient availability in highly weathered Acrisols.

Surprisingly, conversion of forests to pastures did not alter soil microbial biomass C, N, or P pools. However, this result could be related to carbon dynamics in soils. Forage grasses common in many tropical ecosystems are characterized by large root C inputs (Fischer et al., 1994) and many studies report no change or even gains in soil C pools with pasture establishment (Cleveland et al., 2003; Marín-Spiotta et al., 2008; Neill et al., 1997; Trumbore et al., 1995). Consistent with our findings for more intensively managed sites, soil C stocks and microbial biomass have been found to decline with increasing pasture age and grazing intensity in the eastern Amazon (Melo et al., 2012) and in Hawai'i (Elmore & Asner, 2006).

Changes in microbial composition but no change in microbial diversity with forest conversion

Forest conversion altered the abundance of different microbial groups depending on the land-use conversion. Conversion to agriculture and tree plantation decreased the abundance of bacterial PLFA biomass, consistent with observed declines in total soil microbial biomass pools. Fungal PLFA biomass, spore abundance and root colonization were greater in pasture soils compared to forests, which could be due to increased fine-root derived C inputs under pasture (Picone, 2000). Distinct microbial communities between tropical forests and pastures have been attributed to differences in root biomass, soil pH, moisture, and nutrient availability (Borneman & Triplett, 1997; Picone, 2000).

Replacement of forest with alternative land uses did not alter microbial community diversity as measured by traditional richness indices, although AMF spore richness decreased

with conversion to pastures and in secondary forests. A recent meta-analysis of tropical rain forest soils found an increase in bacterial alpha diversity and changes in composition with forest conversion to pastures and plantation (Petersen et al., 2019). However, most of these results were not strictly attributed to conversion from forest to other land uses, but to variability in soil properties such as pH and available C for microorganisms.

Reductions in enzyme activity with conversion to intensively-managed systems

Extracellular enzymes catalyse the decomposition of C-rich macromolecules into smaller, soluble compounds that plants and microorganisms can use for energy and nutrient acquisition. As such, they are often measured as indicators of microbial function, activity, and soil health. Overall, our meta-analysis found that extracellular enzyme activity decreased or showed no change with deforestation. Agricultural soils and plantation soils tended to have reduced enzyme activities, whereas pastures and secondary forests showed reduction, increases, or no net change (Acosta-Martínez et al., 2007; Sotomayor-Ramírez et al., 2009). Decreases in microbial functional capacity resulting from land-cover change and long-term agricultural practices can affect microbial influences on nutrient and carbon dynamics.

Variability in enzyme activity response with land-use change can be due to a variety of methodological and ecological factors. Enzyme assay conditions are not standardized across laboratories, which may affect findings (Burns, 1982; German, Marcelo et al., 2012; German et al., 2011). At the same time, extracellular enzymatic activity is regulated by environmental factors such as temperature, moisture, soil pH and substrate availability (Burns & Dick, 2002; Tabatabai, 1994; Tate III, 2002). Soil texture, structure and mineralogy also affect enzymatic activity (Burns, 1982; Quiquampoix et al., 2002; Tate III, 2002). Variability in environmental properties within and across land uses in a study can mask potential effects of changes in

microbial biomass or composition with land management. For example, differences in soil types among replicate sites masked potential effects of land use in Western Kenya (Bossio et al., 2005). Large variability among soil sample replicates and strong seasonal differences in enzyme activity along a forest successional chronosequence on former pastures in Puerto Rico may have also obscured differences between land use and cover types (Smith et al., 2015).

Recovery of forest microbiome during secondary succession

The meta-analysis revealed few differences between secondary forests and reference forest sites. In summary, secondary forests and reference forests had similar amounts of microbial biomass C, N and P, total bacterial and fungal PLFA biomarkers, abundance of Acidobacteria and Proteobacteria, activity rates of acid phosphatse, alkaline phosphatase, and urease, and AMF spore density. This suggests that forest succession can reverse some of the observed declines in microbial biomass properties during deforestation. However, it could also suggest that the variability among studies is too high and values can be either positive or negative resulting in a non-significant response ratio.

Reforestation provides the opportunity to study successional trajectories of soil microbial communities. Mycorrhizal fungi can persist through at least 30-40 years of pasture use (Fischer et al., 1994) and potentially facilitate plant colonization during reforestation. In Costa Rica, studies have found greater microbial biomass carbon, fungal abundance and diversity in secondary forests compared to pastures used for cattle (Hafich et al., 2012; McGee et al., 2019). This result was attributed to increased levels of soil N, organic C, and phosphate with forest succession. As secondary forests aged in a successional chronosequence in Puerto Rico, the amount of fungal PLFA biomarkers decreased, whereas Gram positive bacteria and anaerobic

Gram-negative bacteria became more abundant, resembling the community of primary forest soils (Smith et al., 2015).

Many tropical studies show that succession and restoration can return properties of the soil microbial community to pre-disturbance levels with the recovery of aboveground biomass (Araújo et al., 2013; Knief et al., 2005), yet recovery times can vary. Variability in the length of time for microbial recovery with reforestation is likely affected by environmental properties, such as soil type and climatic controls on primary production and plant inputs, as well as the severity of disturbance associated with land-use change (Carpenter et al., 2001). Recovery of soil microbial biomass and activity comparable to undisturbed old forest in the Dominican Republic occurred within 5-7 years after agricultural abandonment (Templer et al.,2005). In highland wet forests in Western Amazon, bacterial community composition shifted to that of the primary forests within 5-30 years of forest regeneration (Jesus et al., 2009). In north-eastern Brazil, soil microbial biomass and enzyme activity in areas that had been logged recovered in less than 10 years, but stoichiometry of microbial biomass carbon-to-nitrogen ratios differed, reflecting potential legacy effects of N losses from land use (Araújo et al., 2013). Overall, the soil microbiome during tropical reforestation shows relatively rapid recovery to levels of biomass and community composition of undisturbed forests, although time to recovery can vary greatly with past land-use intensity and soil type.

Microbial response to forest conversion is positively related to changes in soil properties

Soil physical and chemical properties play a strong role in regulating microbial communities by influencing gradients of environmental conditions, such as pH, oxygen, temperature and moisture, that affect microbial physiology (Morris & Blackwood, 2007; Russo et al., 2012). The response of certain microbial variables, including microbial biomass C, N and

P were positively correlated to response ratios of soil pH, and soil organic carbon, total nitrogen and total phosphorus concentrations. Experimental manipulations in a lowland tropical forest showed significant shifts in microbial community structure in response to plant litter and throughfall inputs, attributed to changes in the amount and bioavailability of C and N substrates reflecting top-down control in soils (Nemergut et al., 2010). In contrast, along a soil fertility gradient and nutrient addition experiment in Hawaii, the effect of invasive tree species on soil microbes was mediated by soil N and P levels, highlighting the important bottom-up control of soil resources on microbial composition (Kao-Kniffin & Balser, 2008). High levels of N availability result in greater microbial biomass and activity in soils (Potthast et al., 2012) and low P availability constrains processes such as decomposition and microbial use of available carbon in highly weathered tropical soils (Cleveland et al., 2002).

A recent synthesis concluded that tropical deforestation can have long-term effects on soil properties, such as pH, base saturation, and bulk density, in addition to changes in organic matter (Veldkamp et al., 2020). Considering a soil's physical, chemical and biological component is important for helping us understand land-use change effects on the soil microbiome. Measurements at the appropriate spatial and temporal scale that consider heterogeneity in factors influencing soil microbial communities and their function are necessary to reveal directional responses to temporal seasonality and environmental changes. Other challenges in quantifying and predicting the response of soil microbes to land-use change are paucity in environmental data, including soil properties, or even lack of soil descriptions in many microbial studies.

Gaps in our knowledge of microbial response to tropical land-use change

Our current understanding of the response of the tropical soil microbiome to some of the most common land-use conversions come from a limited number of sites that are not

representative of the diversity of environmental conditions found in the tropics. Similar geographic biases have been reported for the literature on soil C response to land-use change in the global tropics (Marín-Spiotta & Sharma, 2013; Powers et al., 2011). Identifying these gaps in our knowledge is crucial, given the strong influence of environmental variables on soil microbial processes.

Our finding that drier regions (<1000 mm/y MAP) were underrepresented in the literature is important because soil microbial community composition and function are known to be especially sensitive to changes in soil moisture (Barbhuiya et al., 2004; Cleveland et al., 2003; Costa et al., 2013; Liu et al., 2012; Saynes et al., 2005; Singh et al., 2010). Moisture fluctuations play an important role in microbial communities and on their contribution to biogeochemical cycling of C, N, and other important plant nutrients (Bouskill et al., 2016; Liptzin & Silver, 2015; Yuste et al., 2017). Particularly, transitions from dry to wet seasons increase respiration and denitrification rates resulting in greater CO2 and N2O fluxes from soil to the atmosphere (Calvo-Rodriguez et al., 2020; Waring & Powers 2016). Yet, uncertainties in our ability to predict microbial response to land-use change is magnified by projected increased variability in rainfall and frequency or severity of droughts in the tropics (Mora et al., 2013; Uriarte et al., 2016). Changes in the length and temporal distribution of wet and dry seasons may affect microbial function and community structure through changes in soil water content, affecting a soil's capacity to buffer microbes from extreme temperatures (Schipper et al., 2007; Tucker & Reed, 2016; Wood et al., 2013).

Bias in the types of soils studied also require caution when extrapolating results across the tropics, because soil types can influence substrate availability, enzyme activity, and soil microbial biomass (Cleveland et al., 2003). Expanding soil microbial research on sites that

represent large land areas of the tropics yet are poorly studied will enhance our understanding of biogeochemical consequences of potential changes in microbial communities. Currently, most of the available data on soil microbial response to land-use change comes from the American tropics, especially Brazil, whereas other areas, such as the African tropics, are underrepresented. This geographic bias, identified also for research on soil C response to land-use change (Powers et al., 2011), has many causes and implications (e.g., Wilson et al., 2016) but is especially problematic for informing regional projections and land management policies.

Conclusions

Tropical deforestation to intensively managed systems alters soil microbial community structure and function with consequences for biogeochemical cycling and nutrient pools. Our meta-analysis revealed the following trends in the literature: (1) Conversion of forests to agriculture and tree plantations typically yielded reductions in microbial biomass and enzyme activity and shifts in community composition, (2) Differences between pastures and forests were more variable, with some studies reporting gains, losses or no net change in microbial biomass with pasture establishment, reflecting reported heterogeneity in the response of soil C pools in the literature, and (3) Secondary forests recovered microbial biomass and diversity but microbial composition and structure remained different from that of reference forests.

The diversity of ecoregions, rainfall classes, and soil orders found across the global tropics was not well represented in the literature on soil microbial response to land-use change. Microbial land-use change studies have been conducted in only 60% of the FAO soil orders expected in the tropics. Given the importance of soil physical and chemical properties on microbial processes, these limitations affect our understanding of the mechanisms controlling C cycling and nutrient availability in tropical soils. Moist and wet sites are overrepresented

compared to drier sites, which hinders predictions about the response of tropical soil microbes to future climate change, especially given projected variability in rainfall for many regions of the tropics. Despite these geographic uncertainties, emergent trends indicate differential response of soil microbial communities and related ecosystem functions to land-use conversions.

Figures

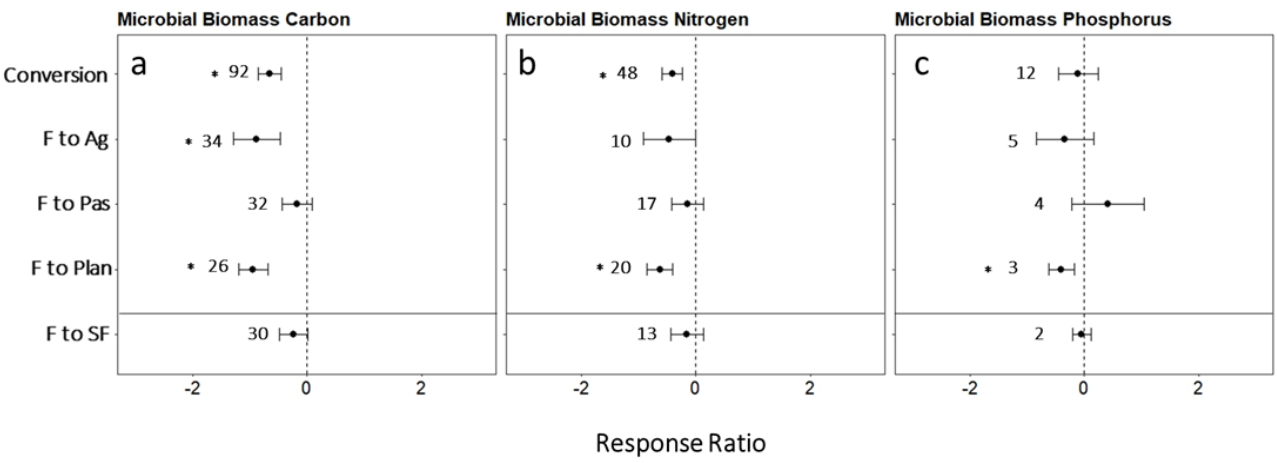

Figure 1. Microbial biomass response ratios. Values represent average response ratios with 95% confidence intervals. The numbers with each ratio represent the number of paired sites. The conversion value is the mean of conversion from forest to agriculture, pasture, and plantation. F = forests, Ag = agriculture, Pas = pastures, Plan = plantations and SF = secondary forests. Asterisk (*) shows significant response.

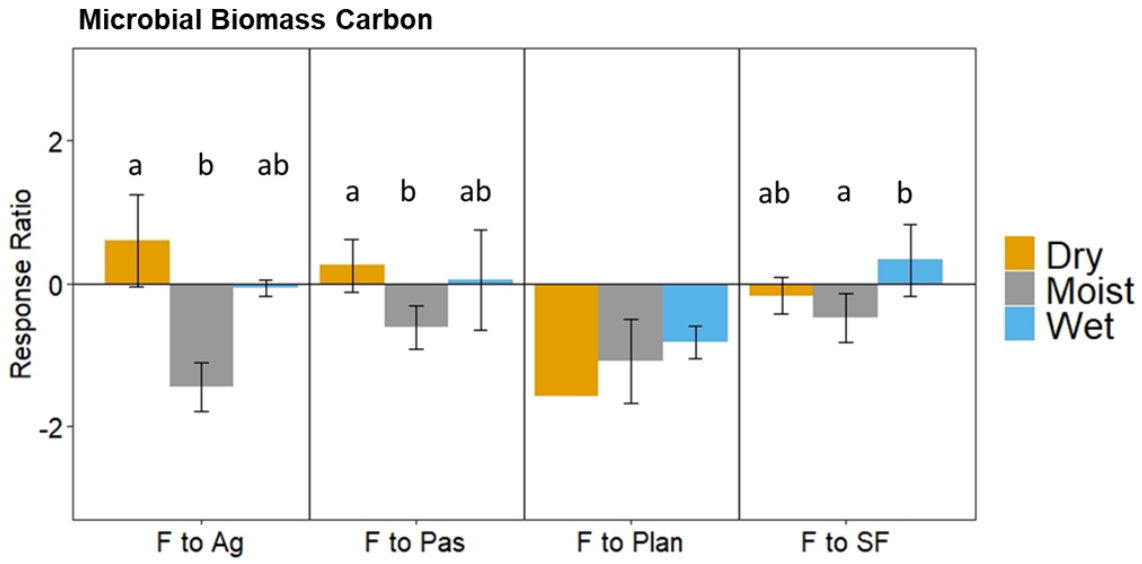

Figure 2. Microbial biomass carbon response ratios across rainfall classes. Bars represent average response ratio and lines represent standard errors. F = forests, Ag = agriculture, Pas = pastures, Plan = plantations and SF = secondary forests. Small case letters show differences among rainfall classes within each land-use change ($p < 0.05$).

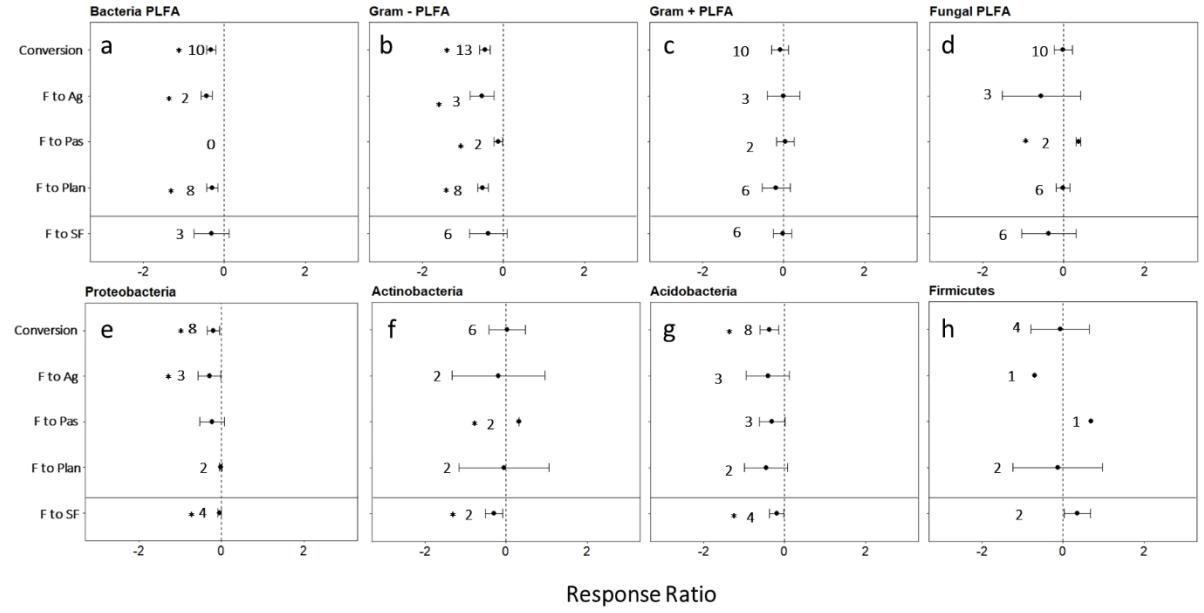

Figure 3. Microbial abundance response ratios. Values represent average response ratios with 95% confidence intervals. The numbers with each ratio represent the number of paired sites. The conversion value is the mean of conversion from forest to agriculture, p pasture, and plantation. F = forests, Ag = agriculture, Pas = pastures, Plan = plantations, SF = secondary forests and PLFA = phospholipid-derived fatty acids. Asterisk (*) shows significant response.

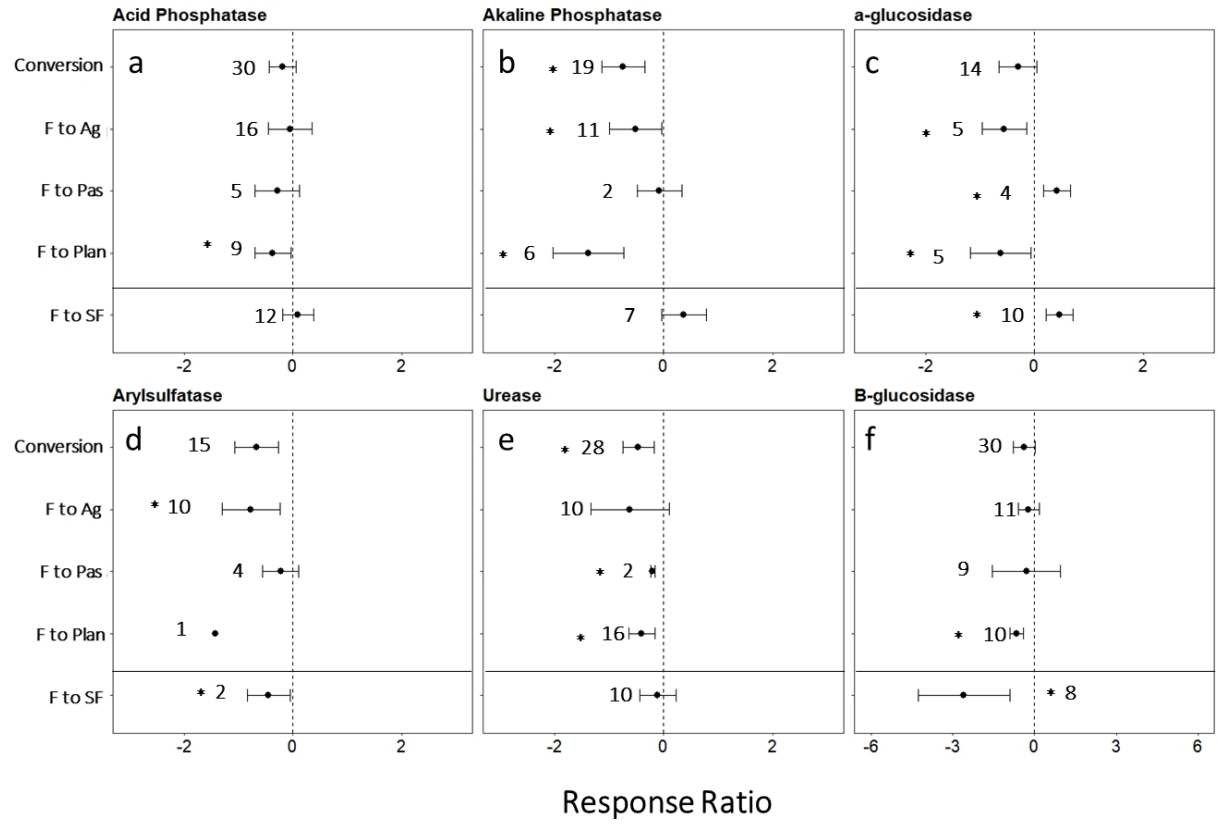

Figure 4. Enzyme activity response ratios. Values represent average response ratios with 95% confidence intervals. The numbers with each ratio represent the number of paired sites. The conversion value is the mean of conversion from forest to agriculture, pasture, and plantation. F = forests, Ag = agriculture, Pas = pastures, Plan = plantations and SF = secondary forests. Asterisk (*) shows significant response.

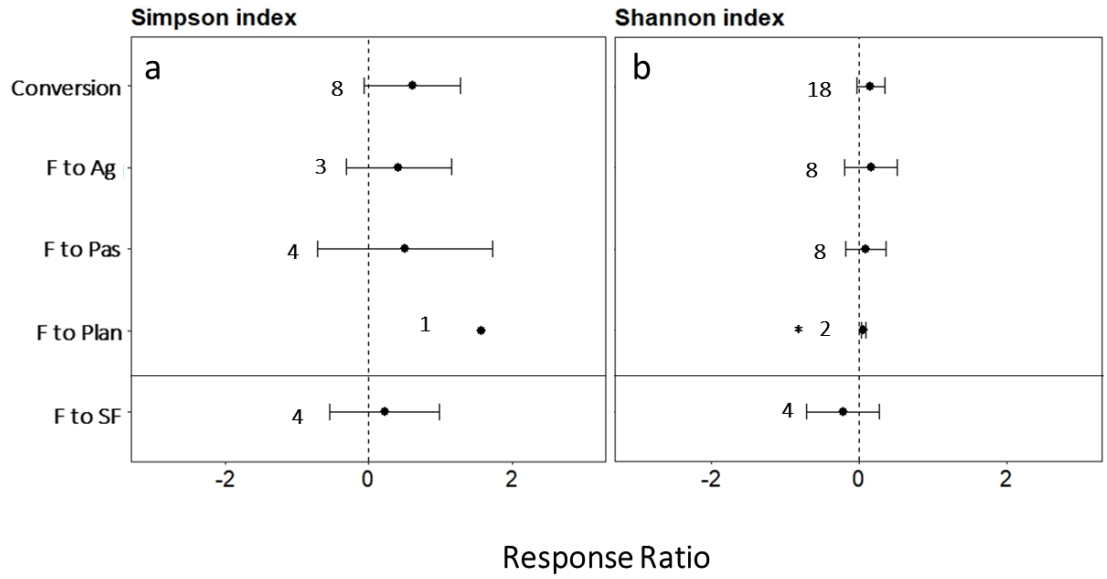

Figure 5. Bacteria diversity and richness response ratios. Values represent average response ratios with 95% confidence intervals. The numbers with each ratio represent the number of paired sites. The conversion value is the mean of conversion from forest to agriculture, pasture, and plantation. F = forests, Ag = agriculture, Pas = pastures, Plan = plantations and SF = secondary forests. Asterisk (*) shows significant response.

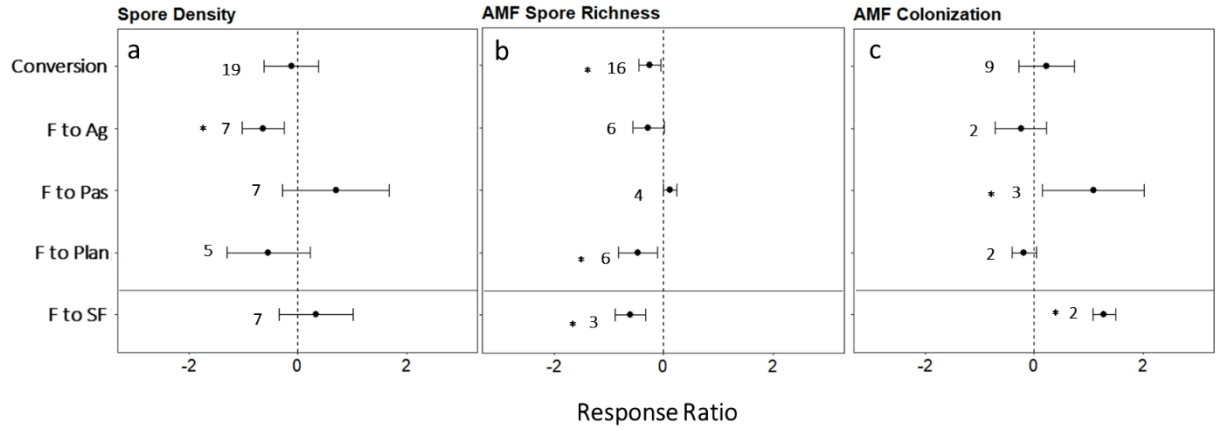

Figure 6. Fungal measurements response ratios. Values represent average response ratios with 95% confidence intervals. The numbers with each ratio represent the number of paired sites. The conversion value is the mean of conversion from forest to agriculture, pasture, and plantation. F = forests, Ag = agriculture, Pas = pastures, Plan = plantations, SF = secondary forests and AMF = arbuscular mycorrhizal fungi. Asterisk (*) shows statistically significant response.

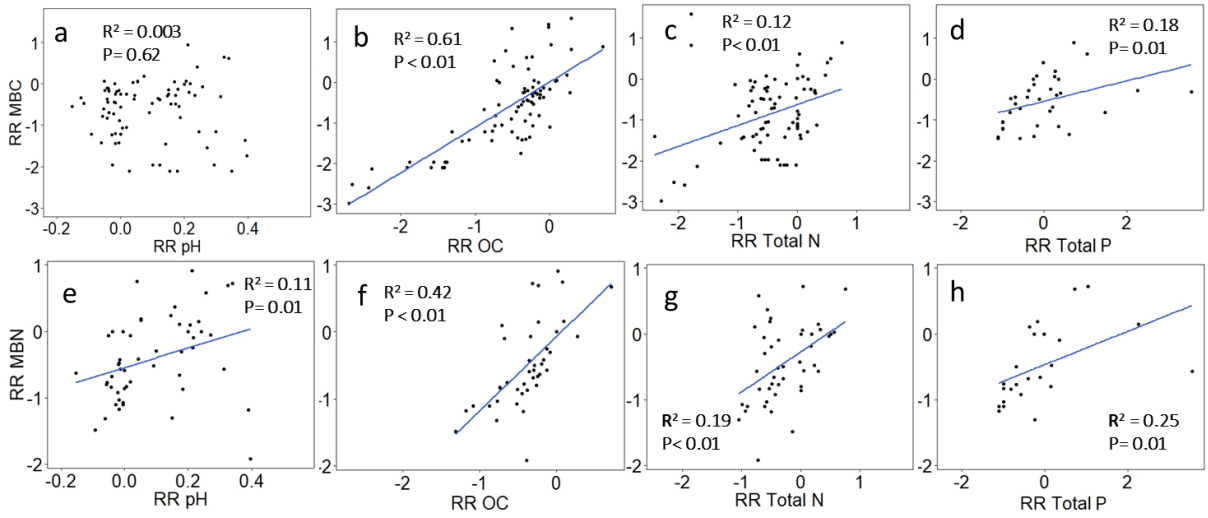

Figure 7. Relationship between response ratio of microbial biomass and soil properties including forest conversion to agriculture, pastures, plantations, and secondary forests. RR = response ratio, MBC = microbial biomass carbon, MBN = microbial biomass nitrogen and N = soil nitrogen, OC = organic carbon, P = phosphorus.

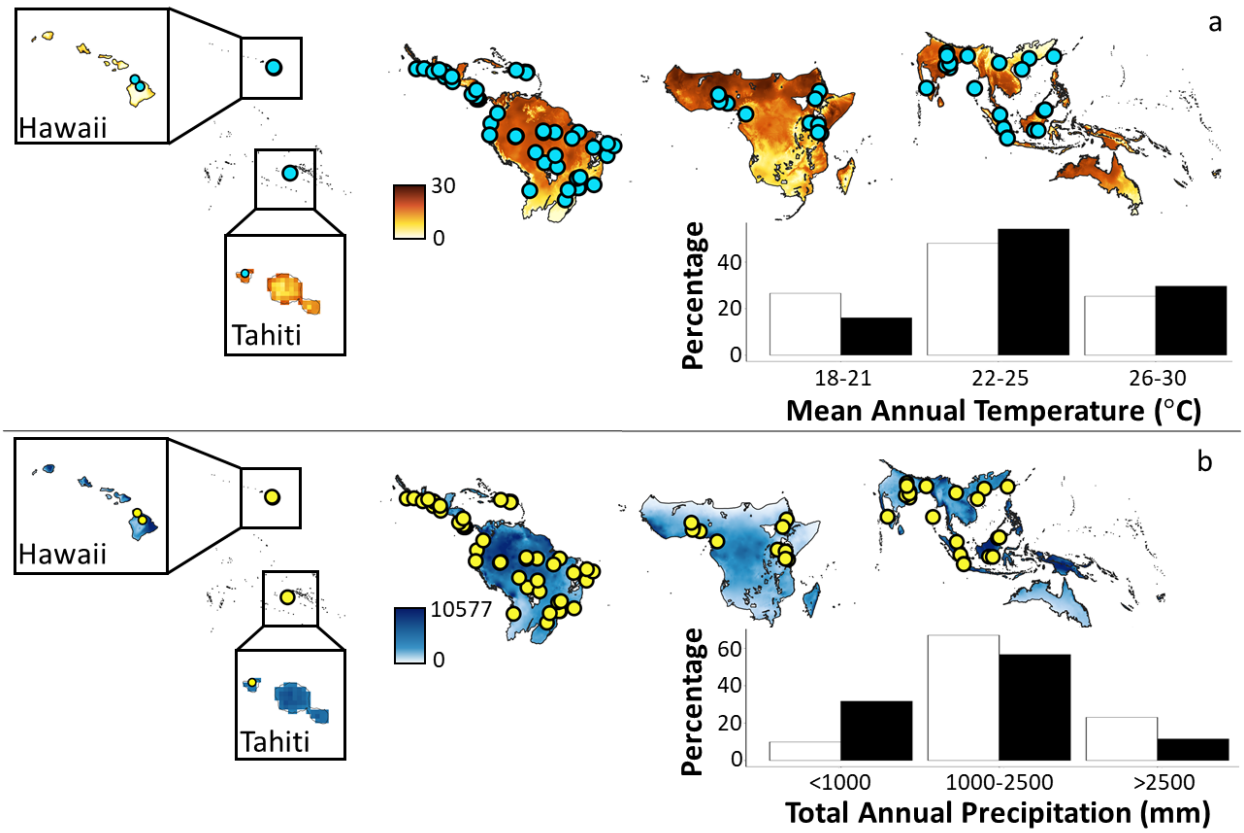

Figure 8. The location of study sites on maps of distribution of (a) mean annual temperature and (b) mean annual precipitation across the tropics. The bar graphs represent the percent distribution of study sites in white and the percent distribution of land cover in study sites in white and the percent distribution of land cover in black.

Table 1. Environmental and microbial variables reported in the meta-analysis

Environmental variables		Microbial Biomass	Colonies	OTU Abundace	PLFA	Enzyme	Bacteria Diversity	Fungal variables	Fungal Diversity
Site information		Carbon	Fungal	Archea	Total	a-Galactosidase	Similarity	Spore Density	Spores Shannon Diversiry
	Land change	Nitrogen	Bacteria	Bacteria	Bacteria	B-Glucosaminidase	Simpson	AMF Spore Abundance	Spores Simpson
	Mean annual temperature (MAT)	Phosphorus		Fungi	Gram Positive	B-Glucosidase	Shannon Diversity	AMF Spore Abundance	AMF Spore Richness
	Mean annual precipitation (MAP)			Actinomycetes	Gram Negative	a-Glucosidase	Whitaker Global	AMF Total Spore Species	AMF Diversity Jacknife
	Temperature class			Proteobacteria	Fungal	Xylanase	Pielou Evenness	Extractable Glomalin	AMF Shannon Diverisy
	Rainfall class			Actinobacteria		B-Xylosidase	Fisher Diversity	Total Glomalin	AMF Shannon Eveness
Soil properties				Acidobacteria		Acetylglucosaminidase	OTU Jackniefe	AMF Root Colonization	AMF Pielou Evenness
	Organic carbon			Firmicutes		Phosphatase	OTU Richness	ECM Root Colonization	AMF Simpson
	Total carbon			ECM		Phophomonoesterase			OTU Richness
	Total nitrogen					Urease			OTU True B Diversiry
	Available phosphorus					Acid Phosphatase			
	Total phosphorus					Alkaline Phosphatase			
	pH					Cellobiohydrolase			
						Chitinase			
						Phenol Oxidase			
						Peroxidase			
						Laccase			
						Cellulase			
						Arylsulfatase			

Supplementary

Table S1. List of references and location for comparison of paired study sites under four main land change transitions in the tropics.

Reference	Location	Land change transition
Acosta-Martinez et al. 2007	Puerto Rico	Forest vs Pasture
Ahmed et al. 2019	Ethiopia	Forest vs Pasture
Burke et al. 2003	Hawaii, Ecuador, Brazil	Forest vs Pasture
Carney et al. 2004	Costa Rica	Forest vs Pasture
Cenciani et al. 2009	Brazil	Forest vs Pasture
Cleveland et al. 2003	Costa Rica	Forest vs Pasture
deCarvaljo et al. 2016	Brazil	Forest vs Pasture
Fischer et al. 1994	Costa Rica	Forest vs Pasture
Fracetto et al. 2013	Brazil	Forest vs Pasture
Frazão et al. 2010	Brazil	Forest vs Pasture
Gavito et al. 2008	Mexico	Forest vs Pasture
Goss-Souza et al.2017	Brazil	Forest vs Pasture
Groffman et al. 2001	Costa Rica	Forest vs Pasture
Hamaoui et al. 2016	Brazil	Forest vs Pasture
Jewda et al. 2009	Kenya	Forest vs Pasture
Jewda et al. 2012	Kenya	Forest vs Pasture
Johnson and Wedin 1997	Costa Rica	Forest vs Pasture
Lammel et al. 2015	Brazil	Forest vs Pasture
Lammel et al. 2017	Brazil	Forest vs Pasture
Leal et al. 2013	Brazil	Forest vs Pasture
Leal et al. 2009	Brazil	Forest vs Pasture
Luizao et al. 1992	Brazil	Forest vs Pasture
Luizao et al. 1999	Brazil	Forest vs Pasture
Medeiros et al. 2015	Brazil	Forest vs Pasture
Melo et al. 2012	Brazil	Forest vs Pasture
Mendes et al. 2015a	Brazil	Forest vs Pasture
Mendes et al. 2015b	Brazil	Forest vs Pasture
Mgana and Kuyakov 2014	Tanzania	Forest vs Pasture
Mirza et al. 2014	Brazil	Forest vs Pasture
Mueller et al. 2016	Brazil	Forest vs Pasture
Navarrete et al. 2011	Brazil	Forest vs Pasture
Ndaw et al. 2009	Brazil	Forest vs Pasture
Ormeño-Orillo et al. 2012	Mexico	Forest vs Pasture
Pabst et al. 2013	Tanzania	Forest vs Pasture
Potthast et al. 2012	Ecuador	Forest vs Pasture
Prasad et al. 1994	India	Forest vs Pasture

To see the full list and bibliography, go to Díaz-Vallejo, E.J., Seeley, M., Smith, A.P. and Marín-Spiotta, E. (2021), A meta-analysis of tropical land-use change effects on the soil microbiome: Emerging patterns and knowledge gaps. Biotropica, 53: 738-752. https://doi-org.ezproxy.library.wisc.edu/10.1111/btp.12931

Table S2. Mean of all response ratio of soil microorganisms variables for forest to agriculture pasture, plantation, and secondary forest transition. The overall group represent the mean of forest conversion to agriculture, pasture, and plantation. (F = Forest, A = Agriculture, Pas = Pasture, Plan =Plantation, S=Secondary Forest, SD= Standard Deviation, N = Number of samples, AMF = Arbuscular mycorrhizal fungi and ECM = Ectomycorrhizal fungi). Asterisk (*) shows significant response.

Category	Variable	F_A	SD	N	F_Pas	SD	N	F_Plan	SD	N	F_S	SD	N	Conversion	SD	N
Microbial Biomass																
	Carbon	-0.881 *	1.221	34	-0.177	0.758	32	-0.939 *	0.6518	26	-0.241	0.6953	30	-0.6529 *	1.2205	92
	Nitrogen	-0.461	0.743	10	-0.147	0.598	17	-0.626 *	0.2148	20	-0.152	0.5168	13	0.0411 *	0.6236	48
	Phosphorus	-0.336	0.574	5	0.4063	0.645	4	-0.401 *	0.1989	3	-0.044	0.1181	2	0.1049	0.6196	12
Colonies																
	Fungal										-0.511		1			
	Bacteria										-0.145		1			
OTU Abundace																
	Archea	0.1331	0.285	3	-0.441	0.672	2							-0.0967	0.5023	5
	Bacteria	-1.496 *	1.106	4	-0.605 *	0.524	3							-1.1139 *	0.9642	7
	Fungi	-4.125 *	1.02	3	-0.448	0.347	2							-2.6516 *	2.1462	5
	Actinomycetes	0.0812		1	-0.037	0.312	2							0.0022	0.2306	3
	Proteobacteria	-0.295 *	0.239	3	-0.224	0.265	3	-0.015	0.0211	2	-0.045 *	0.044	4	-0.1984 *	0.2241	8
	Actinobacteria	-0.191	0.824	2	0.324 *	0.002	2	-0.054	0.7989	2	-0.294 *	0.1578	2	0.0263	0.5658	6
	Acidobacteria	-0.409	0.469	3	-0.306	0.284	3	-0.454	0.3844	2	-0.196 *	0.1775	4	-0.38161 *	0.335	8
	Firmicutes	-0.693		1	0.6931		1	-0.133	0.7924	2	0.3466 *	0.2323	2	-0.0664	0.7317	4
PLFA																
	Total	-0.767 *	0.679	9	0.0722	0.208	2	-0.164	0.4481	6	-0.357	0.4682	3	-0.4552 *	0.6458	17
	Bacteria	-0.435 *	0.098	2				-0.295 *	0.2025	8	-0.309	0.3829	3	-0.3229 *	0.1909	10
	Gram Positive	-0.002	0.356	3	0.0409	0.152	2	-0.185	0.4014	5	-0.026	0.2745	6	-0.0841	0.337	10
	Gram Negative	-0.531 *	0.258	3	-0.132 *	0.076	2	-0.512 *	0.1894	8	-0.377	0.5777	6	-0.4577 *	0.2313	13
	Fungal	-0.558	0.696	2	0.3638 *	0.049	3	-0.018	0.2467	8	-0.369	0.5903	3	-0.0129	0.4025	13
Enzyme																
	a-Galactosidase	-0.346 *	0	3	0.2229 *	0	3							-0.0614	0.3114	6
	B-Glucosaminidase	-0.107 *	0	3	0.2664 *	0	3							0.07961	0.2045	6
	B-Glucosidase	-0.213	0.645	11	-0.3	1.918	9	-0.653 *	0.3893	10	-2.591 *	2.4325	8	-0.3856	1.1152	30
	a-Glucosidase	-0.553 *	0.468	5	0.411 *	0.254	4	-0.626 *	0.6356	5	0.4573 *	0.4001	10	-0.3034	0.6537	14
	Xylanase				0.2452	0.48	3	0.0561	0.2346	2				0.1695	0.3735	5
	B-Xylosidase	-0.385 *	0.094	2												
	Acetylglucosaminidase				-0.242	0.393	4				-2.533 *	2.278	5	-0.2419	0.393	4
	Phosphatase	0.7492 *	0.141	2												
	Phophomonoesterase				-0.591 *	0.126	3	-0.927 *	0.3133	9				-0.8429 *	0.3119	12
	Urease	-0.616	1.154	10	-0.203 *	0.029	2	-0.402 *	0.4907	16	-0.107	0.5327	10	-0.4644 *	0.7703	28
	Acid Phosphatase	-0.049	0.818	16	-0.29	0.458	5	-0.371 *	0.5019	9	0.0909	0.507	12	-0.1855	0.6833	30
	Alkaline Phosphatase	-0.512 *	0.806	11	-0.078	0.296	2	-1.38 *	0.8033	6	0.3686	0.5501	7	-0.7406 *	0.8725	19
	Cellobiohydrolase				0.8274 *	0.293	4	2.1284		1	0.9276 *	0.3641	5	1.0875 *	0.6349	5
	Chitinase	-0.687		1				0.5196		1				-0.0838	0.8533	3
	Phenol Oxidase	0.2604 *	0.007	2												
	Peroxidase	1.2436 *	0.337	2												
	Laccase	-0.588		1												
	Cellulase	-0.214		1				0.7818 *	0.4273	2				0.4497	0.6496	3
	Arylsulfatase	-0.768 *	0.869	10	-0.225	0.339	4	-1.429	NA	1	-0.448 *	0.2867	2	-0.6676 *	0.784	15
Bacteria Diversity																
	Similarity				0.0556		1									
	Simpson	0.4173	0.651	3	0.5056	1.245	4	1.5699		1	0.223	0.7794	4	0.6055	0.9693	8
	Shannon Diversity	0.164	0.51	8	0.0962	0.387	8	0.0639 *	0.0216	2	-0.216	0.4968	4	0.16067	0.40502	18
	Whitaker Global	0.0371	0.126	3	-0.047	0.119	3							-0.0051	0.1187	6
	Pielou Evenness	-0.111		1	-0.392	1.117	3				-0.06		1	-0.3215	0.9226	4
	Fisher Diversity				0.977		1	0.4292		1				0.7031	0.3873	2
	OTU Jackniefe	-0.513		1	-0.024		1				-1.471		1	-0.2686	0.3454	2
	OUT Chao1	-0.283 *	0.167	2	-0.087	0.493	3				-0.626	0.5448	2	-0.16571	0.37408	5
	OTU ACE	1.635	2.217	2	0.14	0.629	3				-0.442	0.8053	2	-0.57001	1.53983	5

Table S2. Continue

Category	Variable	F_A		SD	N	F_Pas		SD	N	F_Plan		SD	N	F_S		SD	N	Conversion		SD	N
Fungal Spores																					
	Spore Density	-0.637	*	0.525	7	0.6989		1.32	7	-0.54		0.8688	5	0.3349		0.91	7	-0.1191		1.1198	19
	Shannon Diversiry									-0.056			1	-0.1651			1				
	Simpson									0.2231			1	0.6286			1				
	AMF Spore Abundance	1.1629		1.72	4	1.1314		1.7934	5	-0.075		0.3006	2	2.995	*	1.9766	2	0.9234	*	1.5577	11
	AMF Spore Richness	-0.275		0.351	6	0.1192		0.1225	4	-0.467	*	0.4437	6	-0.6004	*	0.2449	3	-0.2485	*	0.4062	16
	AMF Diversity Jacknife	0.1805	*	0.026	2	0.0756		0.2157	2	0.0278		0.1624	2					0.0946		0.14	6
	AMF Shannon Diverisy	0.3742			1	0.0619		0.0018	2					0.251			1	0.1659		0.1803	3
	AMF Shannon Eveness	0.3805			1	-0.077			1					0.1054			1	0.1517		0.3234	2
	AMF Pielou Evenness					0.026	*	0.0119	2												
	AMF Simpson					0.1453		0.1656	2												
	AMF Total Spore Species	-0.25		0.487	8	-0.07		0.2485	6	-0.412	*	0.2764	6	-0.4092	*	0.5125	7	-0.2447	*	0.3772	20
Fungal OTU																					
	ECM Abundance									-0.767		2.5153	2	0.5394		2.7514	2				
	OTU Richness					-0.487			1	-0.078	*	0.0292	2	0.149		0.2479	3	-0.2142		0.2373	3
	True B Diversiry									-0.014		0.0116	2	-0.0027		0.0115	2				
Root Colonization																					
	AMF Root Colonization	-0.24		0.34	2	1.0903	*	0.8243	3	0.0039		0.1033	2	1.2836	*	0.1453	2	0.2279		0.7892	9
	ECM Root Colonization													-0.2016			1				
Proteins																					
	Extractable Glomalin	-0.484			1									-0.3542			1				
	Total Glomalin	-0.618			1									-0.1297	*	0.0742	2				

Table S3. Results of all ANOVAs for microbial biomass carbon across rainfall class.

	F value	p value
Forest to Secondary Forest	3.56	0.04
Forest to Plantation	0.94	0.41
Forest to Pasture	6.18	0.01
Forest to Agriculture	18.27	0.00

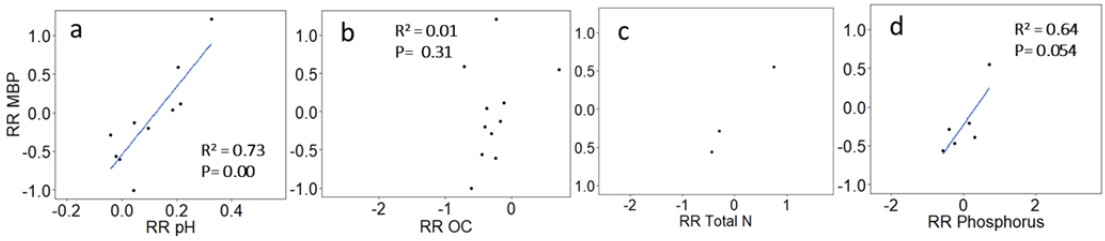

Figure S1. Relation between response ratio of microbial biomass phosphorus with soil properties. RR = response ratio, MBP = microbial biomass phosphorus, N = nitrogen, OC = organic carbon.

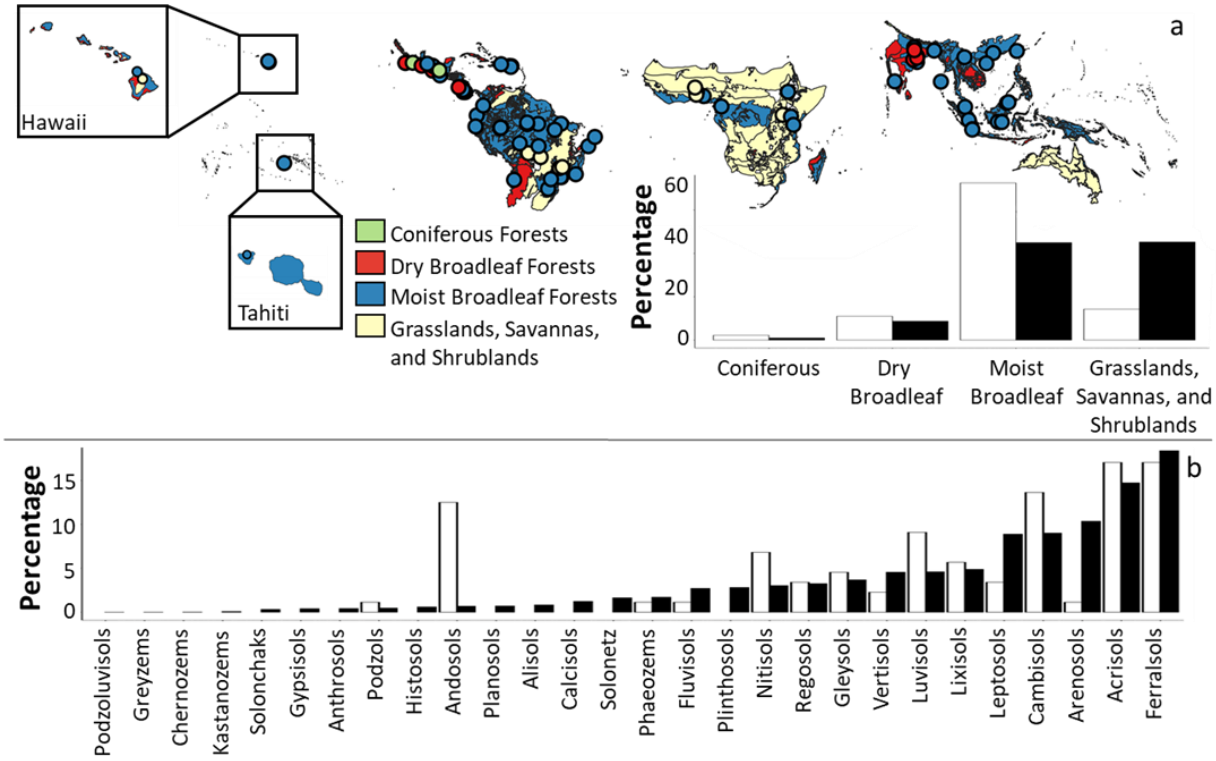

Figure S2. Study sites across ecoregions and soil orders in the tropics. (a) Map of ecoregions across the tropics. Points on the map represent study locations. (b) The bar graph compares soil order representation in the tropics. In the bar graphs, the percent distribution of study sites is in white and the percent distribution of land cover is in black.

References

Acosta-Martínez, V., Cruz, L., Sotomayor-Ramírez, D., & Pérez-Alegría, L. (2007). Enzyme activities as affected by soil properties and land use in a tropical watershed. *Applied Soil Ecology, 35*(1), 35-45. doi:10.1016/j.apsoil.2006.05.012

Aide, T. M., Clark, M. L., Grau, H. R., Lopez-Carr, D., Levy, M. A., Redo, D., . . . Muniz, M. (2012). Deforestation and Reforestation of Latin America and the Caribbean (2001–2010). *Biotropica, 0*(0), 1-10.

Allen, K., Corre, M. D., Tjoa, A., & Veldkamp, E. (2015). Soil Nitrogen-Cycling Responses to Conversion of Lowland Forests to Oil Palm and Rubber Plantations in Sumatra, Indonesia. *PLoS One, 10*(7), e0133325. doi:10.1371/journal.pone.0133325

Araújo, A. S. F., Cesarz, S., Leite, L. F. C., Borges, C. D., Tsai, S. M., & Eisenhauer, N. (2013). Soil microbial properties and temporal stability in degraded and restored lands of Northeast Brazil. *Soil Biology and Biochemistry, 66*, 175-181. doi:10.1016/j.soilbio.2013.07.013

Bai, Z., Wu, X., Lin, J.-J., Xie, H.-T., Yuan, H.-S., & Liang, C. (2019). Litter-, soil- and C:N-stoichiometry-associated shifts in fungal communities along a subtropical forest succession. *Catena, 178*, 350-358. doi:10.1016/j.catena.2019.03.037

Banger, K., Kukal, S. S., Toor, G., Sudhir, K., & Hanumanthraju, T. H. (2008). Impact of long-term additions of chemical fertilizers and farm yard manure on carbon and nitrogen

sequestration under rice-cowpea cropping system in semi-arid tropics. *Plant and Soil, 318*(1-2), 27-35. doi:10.1007/s11104-008-9813-z

Barbhuiya, A. R., Arunachalam, A., Pandey, H. N., Arunachalam, K., Khan, M. L., & Nath, P. C. (2004). Dynamics of soil microbial biomass C, N and P in disturbed and undisturbed stands of a tropical wet-evergreen forest. *European Journal of Soil Biology, 40*(3-4), 113-121. doi:10.1016/j.ejsobi.2005.02.003

Borneman, J., & Triplett, E. W. (1997). Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. *Applied and Environmental Microbiology, 63*(7), 2647-2653.

Bossio, D. A., Girvan, M. S., Verchot, L., Bullimore, J., Borelli, T., Albrecht, A., . . . Osborn, A. M. (2005). Soil microbial community response to land use change in an agricultural landscape of western Kenya. *Microbial Ecology, 49*(1), 50-62. doi:10.1007/s00248-003-0209-6

Bouskill, N. J., Wood, T. E., Baran, R., Hao, Z., Ye, Z., Bowen, B. P., . . . Gilbert, B. (2016). Belowground response to drought in a tropical forest soil. II. Change in microbial function impacts carbon composition. *Frontiers in Microbiology, 7*, 323.

Brinkmann, N., Schneider, D., Sahner, J., Ballauff, J., Edy, N., Barus, H., . . . Polle, A. (2019). Intensive tropical land use massively shifts soil fungal communities. *Sci Rep, 9*(1), 3403. doi:10.1038/s41598-019-39829-4

Burns, R. G. (1982). Enzyme activity in soil: location and a possible role in microbial ecology. *Soil Biology and Biochemistry, 14*(5), 423-427.

Burns, R. G., & Dick, R. P. (2002). *Enzymes in the environment: activity, ecology, and applications*: CRC Press.

Cai, X., Lin, Z., Penttinen, P., Li, Y., Li, Y., Luo, Y., . . . Fu, W. (2018). Effects of conversion from a natural evergreen broadleaf forest to a Moso bamboo plantation on the soil nutrient pools, microbial biomass and enzyme activities in a subtropical area. *Forest Ecology and Management, 422*, 161-171. doi:10.1016/j.foreco.2018.04.022

Calvo-Rodriguez, S., Kiese, R., & Sánchez-Azofeifa, G. A. (2020). Seasonality and budgets of soil greenhouse gas emissions from a tropical dry forest successional gradient in Costa Rica. *Journal of Geophysical Research: Biogeosciences*, 125(9), e2020JG005647.

Carpenter, F. L., Mayorga, S. P., Quintero, E. G., & Schroeder, M. (2001). Land-use and erosion of a Costa Rican Ultisol affect soil chemistry, mycorrhizal fungi and early regeneration. *Forest Ecology and Management, 144*(1-3), 1-17. doi:Doi 10.1016/S0378-1127(00)00361-3

Chaer, G., Fernandes, M., Myrold, D., & Bottomley, P. (2009). Comparative resistance and resilience of soil microbial communities and enzyme activities in adjacent native forest and agricultural soils. *Microbial Ecology, 58*(2), 414-424. doi:10.1007/s00248-009-9508-x

Cleveland, C. C., Townsend, A. R., Schmidt, S. K., & Constance, B. C. (2003). Soil microbial dynamics and biogeochemistry in tropical forests and pastures, southwestern Costa Rica. *Ecological Applications, 13*(2), 314-326. doi:Doi 10.1890/1051-0761(2003)013[0314:Smdabi]2.0.Co;2

Costa, D., Freitas, H., & Sousa, J. P. (2013). Influence of seasons and land-use practices on soil microbial activity and metabolic diversity in the "Montado ecosystem". *European journal of soil biology, 59*, 22-30.

de Graaff, M. A., Adkins, J., Kardol, P., & Throop, H. L. (2015). A meta-analysis of soil biodiversity impacts on the carbon cycle. *Soil, 1*(1), 257-271. doi:10.5194/soil-1-257-2015

Docherty, K. M., & Gutknecht, J. L. (2012). The role of environmental microorganisms in ecosystem responses to global change: current state of research and future outlooks. *Biogeochemistry, 109*(1), 1-6.

Eaton, W. D., Giles, E., & Barry, D. (2010). Microbial community indicators of soil development in tropical secondary forests (Costa Rica). *Ecological Restoration, 28*(3), 236-238.

Elmore, A. J., & Asner, G. P. (2006). Effects of grazing intensity on soil carbon stocks following deforestation of a Hawaiian dry tropical forest. *Global Change Biology, 12*(9), 1761-1772.

ESRI, A. "ArcGIS 10.1." Environmental Systems Research Institute, Redlands, CA, USA (2012).

Fischer, C. R., Janos, D. P., Perry, D. A., Linderman, R. G., & Sollins, P. (1994). Mycorrhiza inoculum potentials in tropical secondary success. *Biotropica, 26*, 369-377.

Fischer, G., Nachtergaele, F., Prieler, S., Van Velthuizen, H., Verelst, L., & Wiberg, D. (2008). Global agro-ecological zones assessment for agriculture (GAEZ 2008). *IIASA, Laxenburg, Austria and FAO, Rome, Italy, 10*.

Frey, S. D. (2019). Mycorrhizal Fungi as Mediators of Soil Organic Matter Dynamics. *Annual Review of Ecology, Evolution, and Systematics, 50*(1), 237-259. doi:10.1146/annurev-ecolsys-110617-062331

Friesen, M. L., Porter, S. S., Stark, S. C., von Wettberg, E. J., Sachs, J. L., & Martinez-Romero, E. (2011). Microbially mediated plant functional traits. *Annual Review of Ecology, Evolution, and Systematics, 42*(1), 23-46. doi:10.1146/annurev-ecolsys-102710-145039

German, D. P., Marcelo, K. R., Stone, M. M., & Allison, S. D. (2012). The M ichaelis–M enten kinetics of soil extracellular enzymes in response to temperature: a cross-latitudinal study. *Global Change Biology, 18*(4), 1468-1479.

German, D. P., Weintraub, M. N., Grandy, A. S., Lauber, C. L., Rinkes, Z. L., & Allison, S. D. (2011). Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biology and Biochemistry, 43*(7), 1387-1397.

Graham, E. B., Knelman, J. E., Schindlbacher, A., Siciliano, S., Breulmann, M., Yannarell, A., . . . Nemergut, D. R. (2016). Microbes as Engines of Ecosystem Function: When Does Community Structure Enhance Predictions of Ecosystem Processes? *Front Microbiol, 7*, 214. doi:10.3389/fmicb.2016.00214

Guo, L. B., & Gifford, R. (2002). Soil carbon stocks and land use change: a meta analysis. *Global Change Biology, 8*(4), 345-360.

Gurevitch, J. (1993). Meta-analysis: combining the results of independent experiments. *Design and analysis of ecological experiments*.

Hafich, K., Perkins, E. J., Hauge, J. B., Barry, D., & Eaton, W. D. (2012). Implications of land management on soil microbial communities and nutrient cycle dynamics in the lowland tropical forest of northern Costa Rica. *Tropical Ecology, 53*(2), 215-224.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology: A Journal of the Royal Meteorological Society, 25*(15), 1965-1978.

Horwath, W., & Paul, E. (2015). Carbon cycling: the dynamics and formation of organic matter. *Soil microbiology, ecology and biochemistry, 4*, 339-382.

Huwaldt, J. A., & Steinhorst, S. (2015). Plot Digitizer, version 2.6. 8. In: Software.

Jesus, E. d. C., Marsh, T. L., Tiedje, J. M., & Moreira, F. M. d. S. (2009). Changes in land use alter the structure of bacterial communities in Western Amazon soils. *The ISME Journal, 3*(9), 1004-1011. doi:10.1038/ismej.2009.47

Johnson, D. W., & Curtis, P. S. (2001). Effects of forest management on soil C and N storage: meta analysis. *Forest Ecology and Management, 140*(2-3), 227-238.

Kao-Kniffin, J., & Balser, T. C. (2008). Soil fertility and the impact of exotic invasion on microbial communities in Hawaiian forests. *Microbial Ecology, 56*(1), 55-63.

Keenan, R. J., Reams, G. A., Achard, F., de Freitas, J. V., Grainger, A., & Lindquist, E. (2015). Dynamics of global forest area: Results from the FAO Global Forest Resources Assessment 2015. *Forest Ecology and Management*, 352, 9-20.

Knief, C., Vanitchung, S., Harvey, N. W., Conrad, R., Dunfield, P. F., & Chidthaisong, A. (2005). Diversity of methanotrophic bacteria in tropical upland soils under different land uses. *Applied and Environmental Microbiology, 71*, 3826-3831.

Köppen, W. (1900). Attempted climate classification in relation to plant distributions. *Geogr Z, 6*, 657-679.

Kottek, M., & Hantel, M. (2005). 17 Global climate maps (Part 12/12). In *Observed Global Climate* (pp. 124-144): Springer.

Krashevska, V., Malysheva, E., Klarner, B., Mazei, Y., Maraun, M., Widyastuti, R., & Scheu, S. (2018). Micro-decomposer communities and decomposition processes in tropical lowlands as affected by land use and litter type. *Oecologia, 187*(1), 255-266. doi:10.1007/s00442-018-4103-9

Liang, C., Jesus, E. d. C., Duncan, D. S., Jackson, R. D., Tiedje, J. M., & Balser, T. C. (2012). Soil microbial communities under model biofuel cropping systems in southern Wisconsin, USA: Impact of crop species and soil properties. *Applied Soil Ecology, 54*, 24-31. doi:http://doi.org/10.1016/j.apsoil.2011.11.015

Liptzin, D., & Silver, W. L. (2015). Spatial patterns in oxygen and redox sensitive biogeochemistry in tropical forest soils. *Ecosphere, 6*(11), 1-14.

Liu, L., Gundersen, P., Zhang, T., & Mo, J. (2012). Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. *Soil Biology and Biochemistry, 44*(1), 31-38. doi:10.1016/j.soilbio.2011.08.017

Marín-Spiotta, E., Swanston, C. W., Torn, M. S., Silver, W. L., & Burton, S. D. (2008). Chemical and mineral control of soil carbon turnover in abandoned tropical pastures. *Geoderma, 143*(1-2), 49-62.

Marín-Spiotta, E., & Sharma, S. (2013). Carbon storage in successional and plantation forest soils: a tropical analysis. *Global Ecology and Biogeography, 22*(1), 105-117.

Mathew, R. P., Feng, Y., Githinji, L., Ankumah, R., & Balkcom, K. S. (2012). Impact of no-tillage and conventional tillage systems on soil microbial communities. *Applied and Environmental Soil Science, 2012*, 1-10. doi:10.1155/2012/548620

Medeiros, E. V. d., Notaro, K. d. A., Barros, J. A. d., Moraes, W. d. S., Silva, A. O., & Moreira, K. A. (2015). Absolute and specific enzymatic activities of sandy entisol from tropical dry forest, monoculture and intercropping areas. *Soil and Tillage Research, 145*, 208-215. doi:10.1016/j.still.2014.09.013

Melo, V. S., Desjardins, T., Silva Jr, M. L., Santos, E. R., Sarrazin, M., & Santos, M. M. L. S. (2012). Consequences of forest conversion to pasture and fallow on soil microbial biomass and activity in the eastern Amazon. *Soil Use and Management, 28*(4), 530-535. doi:10.1111/sum.12003

Meyer, A., Focks, A., Radl, V., Keil, D., Welzl, G., Schoning, I., . . . Schloter, M. (2013). Different land use intensities in grassland ecosystems drive ecology of microbial communities involved in nitrogen turnover in soil. *Plos One, 8*(9). doi:UNSP e73536 DOI 10.1371/journal.pone.0073536

Millennium Ecosystem Assessment. (2005). *Ecosystems and Human Well-Being: Biodiversity Synthesis*. Retrieved from Washington, D.C.:

Montecchia, M. S., Correa, O. S., Soria, M. A., Frey, S. D., García, A. F., & Garland, J. L. (2011). Multivariate approach to characterizing soil microbial communities in pristine and agricultural sites in Northwest Argentina. *Applied Soil Ecology, 47*(3), 176-183. doi:10.1016/j.apsoil.2010.12.008

Mora, C., Frazier, A. G., Longman, R. J., Dacks, R. S., Walton, M. M., Tong, E. J., . . . Anderson, J. M. (2013). The projected timing of climate departure from recent variability. *Nature, 502*(7470), 183-187.

Morris, S. J., & Blackwood, C. B. (2007). The ecology of soil organisms. In *Soil microbiology, ecology and biochemistry* (pp. 195-229): Elsevier.

Neill, C., Melillo, J. M., Steudler, P. A., Cerri, C. C., de Moraes, J. F., Piccolo, M. C., & Brito, M. (1997). Soil carbon and nitrogen stocks following forest clearing for pasture in the southwestern Brazilian Amazon. *Ecological Applications, 7*(4), 1216-1225.

Nemergut, D. R., Cleveland, C. C., Wieder, W. R., Washenberger, C. L., & Townsend, A. R. (2010). Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest. *Soil Biology and Biochemistry, 42*(12), 2153-2160.

Olson, D. M., Dinerstein, E., Wikramanayake, E. D., Burgess, N. D., Powell, G. V., Underwood, E. C., . . . Morrison, J. C. (2001). Terrestrial Ecoregions of the World: A New Map of Life on EarthA new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. *BioScience, 51*(11), 933-938.

Paterson, E., Osler, G., Dawson, L. A., Gebbing, T., Sim, A., & Ord, B. (2008). Labile and recalcitrant plant fractions are utilised by distinct microbial communities in soil: Independent of the presence of roots and mycorrhizal fungi. *Soil Biology and Biochemistry, 40*(5), 1103-1113. doi:10.1016/j.soilbio.2007.12.003

Petersen, I. A. B., Meyer, K. M., & Bohannan, B. J. (2019). Meta-analysis reveals consistent bacterial responses to land use change across the tropics. *Frontiers in Ecology and Evolution, 7*, 391.

Picone, C. (2000). Diversity and Abundance of Arbuscular-Mycorrhizal Fungus Spores in Tropical Forest and Pasture1. *Biotropica, 32*(4). doi:10.1646/0006-3606(2000)032[0734:Daaoam]2.0.Co;2

Poll, C., Ingwersenm, J., M., S., Gerzabek, M. H., & Kandeler, E. (2006). Mechanisms of solute transport affect small-scale abundance and function of soil microorganisms in the detritusphere. *European Journal of Soil Science 57*, 583-595.

Potthast, K., Hamer, U., & Makeschin, F. (2012). Land-use change in a tropical mountain rainforest region of southern Ecuador affects soil microorganisms

and nutrient cycling. *Biogeochemistry, 111*, 151-167. doi:10.1007/s10533-011-9626-7

Powell, J. R., Welsh, A., & Hallin, S. (2015). Microbial functional diversity enhances predictive models linking environmental parameters to ecosystem properties. *Ecology, 96*, 1985-1993.

Powers, J. S., Corre, M. D., Twine, T. E., & Veldkamp, E. (2011). Geographic bias of field observations of soil carbon stocks with tropical land-use changes precludes spatial extrapolation. *Proceedings of the National Academy of Sciences, 108*(15), 6318-6322.

Quiquampoix, H., Servagent-Noinville, S., & Baron, M.-H. (2002). Enzyme adsorption on soil mineral surfaces and consequences for the catalytic activity. *Enzymes in the environment. Marcel Dekker, New York*, 285-306.

Russo, S. E., Legge, R., Weber, K. A., Brodie, E. L., Goldfarb, K. C., Benson, A. K., & Tan, S. (2012). Bacterial community structure of contrasting soils underlying Bornean rain forests: Inferences from microarray and next-generation sequencing methods. *Soil Biology and Biochemistry, 55*, 48-59.

Santos, V. B., Araujo, A. S. F., Leite, L. F. C., Nunes, L. A. P. L., & Melo, W. J. (2012). Soil microbial biomass and organic matter fractios during transition from covnentional to organic farming systems. *Geoderma, 170*, 227-231. doi:0.1016/j.geoderma.2011.11.007

Saynes, V., Hidalgo, C., Etchevers, J. D., & Campo, J. E. (2005). Soil C and N dynamics in primary and secondary seasonally dry tropical forests in Mexico. *Applied Soil Ecology, 29*(3), 282-289. doi:10.1016/j.apsoil.2004.11.007

Schipper, L., Baisden, W., Parfitt, R., Ross, C., Claydon, J., & Arnold, G. (2007). Large losses of soil C and N from soil profiles under pasture in New Zealand during the past 20 years. *Global Change Biology, 13*(6), 1138-1144.

Singh, B. K., Bardgett, R. D., Smith, P., & Reay, D. S. (2010). Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nat Rev Micro, 8*(11), 779-790.

Sinsabaugh, R. L., Carreiro, M. M., & Alvarez, S. (2002). Enzyme and microvial dynamics of litter decomposition. In R. G. Burns & R. P. Dick (Eds.), *Enzymes in the Environment: Activity, Ecology, and Applications* (pp. 249-265): CRC Press.

Smith, A. P., Marín-Spiotta, E., & Balser, T. (2015). Successional and seasonal variations in soil and litter microbial community structure and function during tropical post-agricultural forest regeneration: A multi-year study. *Global Change Biology*(21), 3532–3547. doi:10.1111/gcb.12947

Sotomayor-Ramírez, D., Espinoza, Y., & Acosta-Martínez, V. (2009). Land use effects on microbial biomass C, β-glucosidase and β-glucosaminidase activities, and availability, storage, and age of organic C in soil. *Biology and Fertility of Soils, 45*(5), 487-497.

Strickland, M. S., Lauber, C., Fierer, N., & Bradford, M. A. (2009). Testing the functional significance of microbial community composition. *Ecology, 90*(2), 441-451. doi:10.1890/08-0296.1

Tabatabai, M. (1994). Soil enzymes. *Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties, 5*, 775-833.

Tate III, R. L. (2002). Microbiology and enzymology of carbon and nitrogen cycling. *Enzymes in the environmental Marcel Dekker. Inc Nueva York*, 227-248.

Templer, P. H., Groffman, P. M., Flecker, A. S., & Power, A. G. (2005). Land use change and soil nutrient transformations in the Los Haitises region of the Dominican Republic. *Soil Biology and Biochemistry, 37*(2), 215-225. doi:10.1016/j.soilbio.2004.07.031

Tischer, A., Blagodatskaya, E., & Hamer, U. (2015). Microbial community structure and resource availability drive the catalytic efficiency of soil enzymes under land-use change conditions. *Soil Biology and Biochemistry, 89*, 226-237. doi:10.1016/j.soilbio.2015.07.011

Townsend A, Asner G, Cleveland C (2008) The biogeochemical heterogeneity of tropical forests. Trends in Ecology & Evolution, 23, 424–431.

Trumbore, S. E., Davidson, E. A., Barbosa de Camargo, P., Nepstad, D. C., & Martinelli, L. A. (1995). Belowground cycling of carbon in forests and pastures of Eastern Amazonia. *Global Biogeochemical Cycles, 9*(4), 515-528.

Tucker, C. L., & Reed, S. C. (2016). Low soil moisture during hot periods drives apparent negative temperature sensitivity of soil respiration in a dryland ecosystem: a multi-model comparison. *Biogeochemistry, 128*(1-2), 155-169.

Turner, B. L., Lambers, H., Condron, L. M., Cramer, M. D., Leake, J. R., Richardson, A. E., & Smith, S. E. (2013). Soil microbial biomass and the fate of phosphorus during long-term ecosystem development. *Plant and Soil, 367*(1-2), 225-234. doi:10.1007/s11104-012-1493-z

Uriarte, M., Schwartz, N., Powers, J. S., Marín-Spiotta, E., Liao, W., & Werden, L. K. (2016). Impacts of climate variability on tree demography in second growth tropical forests: the importance of regional context for predicting successional trajectories. *Biotropica, 48*(6), 780-797.

Veldkamp, E., Schmidt, M., Powers, J. S., and Corre, M.D. (2020). Deforestation and reforestation impacts on soils in the tropics. *Nature Reviews Earth & Environment,* 1-16.

Waring, B. G., & Powers, J. S. (2016). Unraveling the mechanisms underlying pulse dynamics of soil respiration in tropical dry forests. *Environmental Research Letters*, 11(10), 105005.

Wieder, W. R., Bonan, G. B., & Allison, S. D. (2013). Global soil carbon projections are improved by modelling microbial processes. *Nature Climate Change*. doi:doi:10.1038/nclimate1951

Wilson, K. A., Auerbach, N. A., Sam, K., Magini, A. G., Moss, A. S. L., Langhans, S. D., . . . Meijaard, E. (2016). Conservation research is not happening where it is most needed. *PLoS Biology, 14*(3).

Wood, T. E., Detto, M., & Silver, W. L. (2013). Sensitivity of soil respiration to variability in soil moisture and temperature in a humid tropical forest. *PLoS One, 8*(12).

Yuste, J. C., Hereş, A.-M., Ojeda, G., Paz, A., Pizano, C., García-Angulo, D., & Lasso, E. (2017). Soil heterotrophic CO2 emissions from tropical high-elevation ecosystems (Páramos) and their sensitivity to temperature and moisture fluctuations. *Soil Biology and Biochemistry, 110*, 8-11.

Zhang, H., Liu, T., Wang, Y., & Tang, M. (2019). Exogenous arbuscular mycorrhizal fungi increase soil organic carbon and change microbial community in poplar rhizosphere. *Plant, Soil and Environment, 65*(No. 3), 152-158. doi:10.17221/2/2019-pse

Zhang, K., Cheng, X., Shu, X., Liu, Y., & Zhang, Q. (2018). Linking soil bacterial and fungal communities to vegetation succession following agricultural abandonment. *Plant and Soil, 431*(1-2), 19-36. doi:10.1007/s11104-018-3743-1

Zhang, Q., Yang, J., Koide, R. T., Li, T., Yang, H., & Chu, J. (2017). A meta-analysis of soil microbial biomass levels from established tree plantations over various land uses, climates and plant communities. *Catena, 150*, 256-260. doi:10.1016/j.catena.2016.11.028

Zhao, F. Z., Ren, C. J., Han, X. H., Yang, G. H., Wang, J., & Doughty, R. (2018). Changes of soil microbial and enzyme activities are linked to soil C, N and P stoichiometry in afforested ecosystems. *Forest Ecology and Management, 427*, 289-295. doi:10.1016/j.foreco.2018.06.011

Zhong, Z., Zhang, X., Wang, X., Fu, S., Wu, S., Lu, X., . . . Yang, G. (2020). Soil bacteria and fungi respond differently to plant diversity and plant family composition during the secondary succession of abandoned farmland on the Loess Plateau, China. *Plant and Soil, 448*(1-2), 183-200. doi:10.1007/s11104-019-04415-0

Chapter 2: Soil bacterial and fungal traits across secondary forest succession in a soil weathering gradient.

Introduction

Recent advances in microbial ecology have highlighted the importance of understanding the resilience of microbial communities and their effect on ecosystem processes following disturbances (Antwis et al. 2017). Human-induced changes in ecosystems, such as deforestation and the subsequent expansion of secondary forest areas, have significant global consequences on microbial communities, including changes in microbial biomass, abundance, enzyme activity, diversity, and richness (Petersen et al. 2019, Díaz-Vallejo et al. 2021). Alterations in microbial communities due to land use change can affect biogeochemical processes, such as soil organic carbon and nutrient dynamics. However, the functional implications of microbial changes, particularly in the context of secondary forest succession, remain poorly understood (Bissett et al. 2013), especially in tropical regions.

Multiple studies have demonstrated that microbial communities undergo shifts during secondary succession, and soil properties influence these shifts. Increased litter inputs during forest succession have been found to positively impact fungal beta diversity, while changes in soil properties primarily affect bacterial composition and diversity (Wang et al. 2002, Cline and Zak 2015). The regrowth of tropical forests leads to enhanced microbial biomass, abundance, and enzyme activities comparable to those in reference or primary forests (Díaz-Vallejo et al. 2021). Moreover, microbial biomass and community composition can recover relatively quickly during secondary succession, although the recovery times may vary (Templer et al. 2005, da C Jesus et al. 2009, Araújo et al. 2013). On local scales, forest succession has shown relationships with increased microbial abundance, diversity, and soil nitrogen levels, organic carbon, and

phosphate (Hafich et al. 2012, McGee et al. 2019). Globally, studies have shown positive correlations between land use-induced changes in microbial biomass and fluctuations in soil pH, carbon, nitrogen, and phosphorus levels (Díaz-Vallejo et al., 2021), indicating that is soil properties increases, microbial biomass will increase too. Therefore, changes in soil properties due to land use change and forest recovery can modulate microbial communities.

Understanding the microbial response to secondary forest succession in the tropics requires considering soil nutrient change effects on microbial processes along with the inherent heterogeneity of tropical soils (Townsend et al. 2008). Previous studies have highlighted the wide range of variability in the impact of secondary forest succession on soil nutrient trends in tropical regions (Powers and Marín-Spiotta 2017), which can potentially influence microbial responses spatially. Tropical soil properties exhibit variations both vertically, in terms of soil depth, and horizontally across space, following the concept of a soil catena, where matter fluxes are spatially linked across the landscape (Sommer and Schlichting 1997). This natural phenomenon plays a crucial role in influencing nutrient distribution (Van Langenhove et al. 2021), thereby affecting microbial activity (Semenov et al. 2013). Within a soil catena, variations in soil nutrients can modulate microbial processes, such as organic matter decomposition (Kaspari and Yanoviak 2008, Powers and Salute 2011). Furthermore, the interplay of sulfur and potassium levels can also impact microbial respiration rates (Luizão et al. 2007). Additionally, soil pH can serve as a spatial regulator of the availability and influence of macro and micronutrients on soil microbial processes (Pansu et al. 2010). Thus, any changes in soil properties spatially along a soil catena play a crucial role in shaping microbial communities and their functional traits.

Previous research has highlighted the high variability of microbial responses to afforestation in the tropics (Díaz-Vallejo et al. 2021). One of the key challenges in predicting microbial responses to secondary forest succession in the tropics is the aforementioned soil heterogeneity. Existing studies on tropical ecosystems focusing on microbial communities and soil carbon changes related to land use and secondary forest succession have primarily focused on highly weathered soils (Powers et al. 2011, Marín-Spiotta and Sharma 2013, Díaz-Vallejo et al. 2021), which introduces biases in our understanding. Given the tremendous diversity of tropical soils, studying secondary forest succession across a tropical catena encompassing different soil orders could provide valuable insights into the responses of microbial functional traits and their potential implications for biogeochemical trends. Therefore, investigating secondary forest succession across various soil orders in tropical regions is essential for comprehensively understanding the responses of microbial functional traits and their potential implications for biogeochemical trends.

The functional traits approach holds significant advantages when evaluating the functional implications of microbial changes across tropical forest secondary succession in varying soil environments. Originally established in the study of plant and animal ecology, this approach has garnered increasing interest in microbial ecology by shifting the focus from taxonomy and community composition to functional characterization (Green et al. 2008). Adopting the functional traits approach provides a comprehensive framework for understanding how microbial communities respond to environmental changes and the subsequent implications for soil biogeochemistry (Green et al. 2008, Fierer 2017, Blagodatskaya et al. 2021, Romillac and Santorufo 2021). The functional traits approach allows researchers to examine the life history strategies of microorganisms, which represent a set of traits that are associated with

physiological and evolutionary trade-offs favored under different environmental conditions (Malik et al. 2020). By characterizing microbial functional traits, researchers can gain insights into how disturbances might impact microbial communities and their potential effects on critical soil processes, such as soil organic carbon dynamics (Fierer 2017).

Functional characterizations of bacteria can provide valuable insights into their ecological relevance (Louca et al. 2016). For instance, chemolithotrophs bacteria derive energy from the oxidation of inorganic compounds (Kulakowski et al. 2018). Ureolytic bacteria, break down urea into ammonia and carbon dioxide. Similarly, methanotrophic bacteria utilize methane as a carbon and energy source, while phototrophic bacteria use carbon dioxide and light (Kulakowski et al. 2018). All these functional characterizations can potentially impact soil carbon dynamics through processes such as fixation, transformation, or release into the atmosphere. Fungi can also be characterized based on their trophic strategies as saprotrophs, symbiotrophs, and pathotrophs (Jastrow et al. 2007, Štursová et al. 2012, Treseder and Lennon 2015). These functional groups can have a differential influence on soil carbon dynamics. Saprotrophic fungi contribute to rapid soil carbon turnover through the degradation of complex organic compounds, while mycorrhizal fungi, particularly ectomycorrhizal fungi, promote carbon persistence by competing with saprotrophs (Averill and Hawkes 2016, Shah et al. 2016). However, little is known about changes in microbial functional characterization and their potential role in influencing soil processes across succession, particularly in the tropics (see McGee et al. 2019).

Therefore, the aims of this study were to evaluate 1) how microbial communities change with tropical forest succession in different soil orders along a weathering catena and 2) to evaluate if microbial functional traits can explain soil carbon variation. We hypothesized that soil microbial community composition, diversity, and function will change across forest regrowth on

former pastures and that soil orders will modulate variability, but the direction of change will vary for specific functional groups. We also hypothesized that microbial traits will explain a portion of the variability of soil carbon across succession due to the importance of those traits for carbon cycle processes in soil. Understanding the resilience and functional traits of microbial communities during forest succession in tropical regions, particularly in relation to soil properties and different soil orders, is crucial for comprehensively assessing their impact on ecosystem processes and soil carbon dynamics, thereby providing valuable insights for managing and conserving tropical soils and their associated biogeochemical cycles.

Methods

Site description

The field sites are located in the Municipios of Ciales, Florida, and Arecibo in the northern-central karst region of Puerto Rico (18°21'02" N, 66°35'13" W). The area's climate is classified as subtropical moist forest in the Holdridge life zone (Ewel and Whitmore 1973), with annual rainfall averaging 2,000 mm and the mean annual temperature ranging from 17-29 °C (Daly et al. 2003). The bedrock of the study site is primarily limestone from the upper Oligocene and the Tertiary (USGS). Soils in this area represent a gradient of weathering from less weathered at the top of steep karst hills to more highly weathered soil in the valleys. A predictable catena is found along the topography, starting from the top: Inceptisols to Mollisols, to Alfisols, to Ultisols (Vaughan et al. 2019). Because climate and parent material are the same in this region, topography is the primary soil-forming factor differing in this catena (Vaughan et al. 2019). Hillslopes in this area have a gentle relief with moist soil conditions (Ewel and Whitmore 1973). For the study, the sites selected were located in the Mollisols and Alfisols areas of the catena. Mollisols represent the Soller series, described by clayey, mixed, active, isohyperthermic,

shallow Typic Haprendolls (NRCS Official Series Description). Alfisols represent the Tanama series, described by clayey, mixed, active, isohyperthermic Lithic Hapludalfs (NRCS Official Series Description).

The clearing of forest areas for agricultural purposes started with European settlement during the 1500s and continued until the 1940s (Herman et al. 2008). In early 1820, pastures were 55% of the island (Wadsworth 1950). By the 1900s, 78% of the land was under agricultural or livestock use (Wadsworth 1950). Agriculture was focused on sugarcane and pineapple in lowlands and shade coffee in high elevations, declining 95 and 88 %, respectively, from the 1950s to the 1970s (Kennaway and Helmer 2007). Agriculture land abandonment and forest recovery started in the second half of the 20th century as a result of an economic shift toward industry (Franco et al. 1997). Forest cover increased from 18 percent in the 1950s to 57 percent in 2004 (Brandeis et al. 2009). Pasture lands decreased from 55% to less than 20% during the forest cover increase (Grau et al. 2003). The current land cover in our study sites is primarily a mix of secondary forests and pastures.

The study sampling design consists of sites within Mollisols and Alfisols with different forest successional stages categorized as pastures and secondary forests with ages from 15 to 60 years. Successional ages were classified using historical aerial images and measuring time after the last visible clearing and confirmed, when possible, with local landowners, neighbors, and land managers. To facilitate analyses, forest age was reclassified as forest age classes: 15-29 years (n = 7), 30-39 years (n = 3), 40-49 years (n = 4), > 50 years (n = 2), and active pastures (0 years, n= 8). Within each soil order, representative sites were selected with representative successional ages (Alfisols n = 14 and Mollisols n = 11).

At each site, we collected paired soil cores (5 cm diameter x 10 cm depth) at three replicate semi-randomized locations for six cores per site. After collection, the soil was refrigerated, and a sub-sample was stored in a freezer at -20 °C. Samples were shipped from Puerto Rico to the University of Wisconsin- Madison in a cooler with ice bags and stored again in a -80 °C freezer after arrival.

Soil carbon measurements

Soil subsamples for elemental analysis were air-dried and ground to a fine powder using a SpexMill 8000D. Samples were tested for inorganic carbon using 10% HCl following (Nelson and Sommers 1983). Samples that tested positive were acid fumigated for 24 hours following (Harris et al. 2001). All samples were analyzed in a Flash 2000 Elemental Analyzer for total carbon and nitrogen (non-fumigated samples) and organic carbon (fumigated samples). Aspartic acid was used as a standard, and aspartic acid and soil reference material were used to check every ten samples.

DNA extractions

Soils were extracted for DNA sequencing analysis using 0.25 g of frozen soil subsamples. We performed DNA extractions for each sample with a blank every 24 extractions. Following the manufacturer's protocol, we used the DNEasy PoweLyzer PowerSoil DNA extraction kit (QIAGEN, Germantown, MD). DNA extracted was amplified in triplicates with PCR, targeting the ITS2 gene region with 5.8S-Fun ITS4-Fun primers (Taylor et al. 2016) and the v4 region of the 16S gene using 515f and 806r primers (Walters et al., 2015) with barcodes and Illumina sequencing adapters added (Kozich et al. 2013). PCR amplicons were pooled per sample, purified, and normalized using a SequalPrep Normalization Plate (96, ThermoFisher Scientific,

Waltham, MA) and cleaned up with a Wizard gel cleanup Kit. All pooled samples were submitted to the UW-Madison Biotechnology Center for 2x300 PE Illumina MiSeq sequencing.

DNA processing

The results from the ITS2 forward and reverse sequences were merged, quality filtered, and primer clipped using the ITSx function from the QIIME2 2020.2 software package (Boylen et al. 2019). The dada2 denoise-paired algorithm was implemented to determine amplicon sequence variant level OTUs (Callahan et al. 2016). We tested trimming and truncating parameters to determine optimal sequence rotation and quality control values. We used the UNITE 2022.10.16 database (UNITE-Community, 2017) as the ITS2 reference database at 97% ID to assign taxonomy. We trained the classifier using QIIME2 feature-classifier classify sklearn (a naïve Bayes classifier). The classifier was trained for the region-specific to the primers 5.8S-Fun ITS4-Fun.

We quality-filtered and trimmed the 16S sequence reads and dereplicated them using dada2 (Callahan et al. 2016). We assigned taxonomies to the 16S reads with the aligned 515f-806r region of the 97% ID OTUs from the Silva 138 database using a mothur classif.seqsknn method (version 138; Quast et al. 2013). There reads can be use to classify taxonomy to bacteria and archaea, but for this work we focus only on bacteria results.

Functional traits

To assign potential functional traits to the organisms, we used ecological traits, which are functional assignments based on the life strategy, phenotypic, and quantitative genomic traits of a taxon using its nomenclature for assignation (can target different taxonomic ranks; Djemiel et al. 2022). For 16S data, we used the FAPROTAX database (Louca et al. 2016) to assign metabolic functions, ecological traits, or large functional groups relevant to prokaryotes. We used

FUNGuild using all confident levels to maximize sequence classifications (Nguyen et al. 2016) for ITS data, which assigns ecological traits to fungi based on their taxonomy.

Bioinformatics and statistical approach

All bioinformatics were completed primarily using R packages phyloseq (McMurdie and Holmes 2013), ggplot2, and dplyr (Wickham et al. 2015, Wickham 2016). We did not average by the site to represent the variability of our study area, resulting in a total of 75 samples. To compare community composition across samples, we used Bray-Curtis dissimilarities on Hellinger-transformed relative abundances (Legendre and Gallagher 2001). The community composition was presented using NMDS ordinations. To test if forest successional ages or soil order influenced community composition, we performed a PERMANOVA using the adonis function in the vegan R package (Oksanen et al. 2013). Diversity indices (Shannon's Diversity and Shannon's Evenness) were estimated using the vegan package in R (Oksanen et al. 2013). To test for differences across successional ages and between soil orders for microbial diversity, phylum relative abundance, and functional characterizations, we performed ANOVAs with interactions and Tukey HSD for multiple comparison corrections. We also used linear regression with cubic smoothing spline to visualize trends across succession and the effect of soil order on the trends, comparing models with and without the soil order effect using an ANOVA. To test how functional groups could potentially influence soil carbon, we performed linear regression models with the abundance of the functional group relative abundance and the interaction of successional ages + functional group relative abundance and the interaction of soil orders. Data that did not fit assumptions for ANOVA or linear regressions were transformed. We considered there to be a significant effect when the p-value <0.05, but we also considered there to be a

marginal effect explaining potential trends if the p-value was <0.10 due to our highly variable system and the limited number of field samples.

Results

Bacterial diversity

Bacterial Shannon's Evenness index differed after pasture abandonment (p-value = 0.004; Table 1; Figure 1a), with a significant interaction between forest age class and soil order (p-value = 0.012). Tukey's HSD showed higher evenness in pastures compared to forests of 15-29 years (p-value = 0.088), 30-39 years (p-value = 0.09), and >50 years (p-value = 0.022). Bacterial evenness decreased with forest age, but in Mollisols it increased at forests of 40 years or more. We found no significant difference in Richness or Shannon Diversity index for bacteria across succession or between soil orders. However, there was a marginal difference (p-value = 0.084) for Shannon diversity and forest age. This might indicate that Shannon diversity decreases from pastures to early-stage forests but increases again at older stages.

The bacterial NMDS (K = 3, Stress 0.143; Figure 2a) did not show any clear groupings between the two soil orders or any pattern with forest age. The PERMANOVA for bacterial communities showed a significant effect on soil order (p-value = 0.001), forest age class (p-value = 0.001), and their interaction (p-value = 0.001; Table 2). However, the betadisperse analysis showed that the forest age class (p-value = 0.001) and soil order (p-value = 0.03; Table 3) groupings were heterogeneous, meaning that the dispersion of the samples may influence the significance of the forest age class and soil order grouping in the PERMANOVA.

Fungal diversity

Fungal richness, Shannon diversity index, or Shannon's Evenness index did not differ with forest age class or soil order (Table 1).

The fungal NMDS (k=3, Stress = 0.149; Fig 2b) showed a gradual change in community composition across forest age class and different grouping between soil orders. The PERMANOVA for the fungal communities showed a significant effect between soil orders (p-value = 0.001), forest age class (p-value = 0.001), and their interaction (p-value = 0.001; Table 2). The betadisperse analysis showed a homogeneous dispersion in the fungal data supporting the PERMANOVA results of soil order grouping (p-value = 0.561) but not for forest age class (p-value = 0.001; Table 3).

Bacteria relative abundance

The most abundant bacterial phyla in our samples were Actinobactiota (15.5%), Acidobacteriota (13.19%), and Proteobacteria (12%; see the others in Table 4), which are also known to be highly abundant in soils (Janssen 2006, Lauber at al., 2009). To test how the most abundant bacterial phylum's relative abundance differed across forest age and soil order, we used a two-way ANOVA (Table 5, Figure 3) followed by a Tukey HSD (Table 6). We found that Actinobacteria relative abundance significantly differed by soil order (p-value = 0.007), with an interaction between forest age and soil order (p-value = 0.036). Mollisols had a greater abundance of Actinobacteria than Alfisols (p-value = 0.009); this difference was highlighted in pastures. Acidobacteriota relative abundance differed among forest age classes (p-value = <0.001) and the interaction between forest age and soil order (p-value < 0.001). The Tukey HSD showed that Acidobacteriota relative abundance was lower in pastures of Mollisols compared to forest of 15-29 years (p-value = 0.012), 40-49 years (p-value = 0.001), and > 50 years (p-value = 0.020). The relative abundance of Proteobacteria differed by forest age class (p-value = 0.04). Tukey HSD only showed a difference between forests of 15-29 years and 30-39 years (p-value

0.097) and between forests of 40-49 years and 30-39 years (p-value = 0.090), suggesting a decrease from pasture to early stages, an increase in mid-stages, and a decrease later.

We also report the results of Methylomirobilota (5%) and Verrucomicrobiota (4.4%) due to their importance as indicators of land use change. Methylomirobilota relative abundance also significantly differed among forest age classes (p-value < 0.001). The Tukey HSD showed that pastures have lower Methylomirobilota relative abundances than forests of 15-29 years (p-value < 0.001), 40-49 years (p-value < 0.001), and >50 years (p-value < 0.001). Verrucomicrobiota relative abundance showed a significant difference in forest age classes (p-value < 0.001), soil orders (p-value =0.003), and the interaction between forest age and soil orders (p-value = 0.009). The Tukey HSD showed that pastures in Alfisols have higher Verrucomicrobiota relative abundances than forests of 15-29 years (p-value = 0.007), 40-49 years (p-value = 0.017), and >50 years (p-value = 0.067), and, in general, Alfisols have greater relative abundances than Mollisols (p-value = 0.022).

To visualize the trends of changes in the relative abundacnes of bacterial phyla across forest ages, we used natural spline lines with 3 degrees of freedom (Figure 4). Most of our models represented a variability from R^2 <0.01 to 0.41. Acidobacteriota (R^2 = 0.41), and Methylomirabilota (R^2 = 0.31), increased non-linearly from pastures to older-stage forests. Actinobacteriota (R^2 = 0.18) and Verrucomicrobiota (R^2 = 0.33) decreased non-linearly from pasture to older-stage forests. Proteobacteria (R^2 = 0.03) did not show any clear trend across successional stage. Soil order had a significant effect on all the aforementioned phyla except Proteobacteria and Methylomirabilota (Table 7). These findings indicate that the successional patterns in bacterial phyla are influenced by soil order.

Fungi relative abundance

The most abundant fungal phyla at our sites were Ascomycota (30.7%) and Basidomycota (16.1%). We were not able to assign taxonomy at the phylum level to 15% of our fungal community (Table 4). To test how fungal phyla's relative abundances differ across forest age and soil order, we used a two-way ANOVA (Table 8, Figure 7) followed by a Tukey HSD (Table 9). Mortierellomycota relative abundance differed among forest age classes (p-value <0.001) and by soil orders (p-value = 0.035). The Tukey HSD revealed that forests of 40–49 years had more Mortierellomycota than pastures (p-value <0.001), and forests of 15-29 years (p-value = 0.016), and 30–39 years (p-value = 0.017). We also found that Glomeromycota was higher in pastures compared to forests (p-value = 0.056). None of the other fungal phyla differed among forest age classes and soil orders.

To see the trends in fungal phyla across forest ages, we used natural spline lines with 3 degrees of freedom (Figure 6). Our models represented a variability from <0.01 to 0.21% (measured by R^2). Mortierellomycota was the phylum with the best-represented variability (R^2 = 0.21), indicating a non-linear increase across forest succession. Ascomycota (R^2 = 0.08) and Glomeromycota (R^2 = 0.06) showed a non-linear decrease across succession, while Basidomycota (R^2 = 0.03) showed a non-linear increase. The variation in these phyla was very low, represented by forest age and soil order. None of the fungal relative abundances varied by soil order (Table 10).

Bacteria functional traits

A total of 12% of the bacterial OTUs were classified were classified using the FAPROTAX database. From those, we chose to use functional classifications directly relevant to the soil carbon cycle (see Table 11 for relative abundances). We used a two-way ANOVA to test

how different bacterial functional groups differed across forest age classes and soil orders (Table 12, Figure 7) and Tukey HSD (Table 13). Chitinolytic bacteria relative abundance differed across forest age classes (p-value <0.001), soil orders (p-value < 0.001), and their interaction (p-value < 0.001). The Tukey HSD test showed lower Chitinolytic bacteria relative abundance in pastures compared to forests of15 to 29 years (p-value < 0.001) and 30-39 years (p-value = 0.079), but in Alfisols, it increased at forests of 30-39 years and decrease again with forest age. Methylotrophic bacteria differed across forest ages (p-value = 0.003), with greater relative abundance in pastures compared to forests of 15-29 years (p-value = 0.011), 40-49 years (p-value = 0.015) and >50 years (p-value = 0.071). Cellulolytic bacteria relative abundance differed across forest ages (p-value = 0.043) and soil orders (p-value = 0.009). Their relative abundance was greater in Alfisols than Mollisols (p-value = 0.015) and in pastures than forests of 15-29 years (p-value = 0.065). Aromatic compound degradation bacteria relative abundance decreased from pastures to older forests of 40-49 years (p-value = 0.005) and > 50 years (p-value = 0.099). Bacteria involved in aromatic hydrocarbon degradation were more abundant in Alfisols than in Mollisols (p-value 0.068). Chemotrophic bacteria relative abundance showed a complex pattern with succession, decreasing from pastures to forests of 15-29 years (p-value = 0.004), then increasing in forests of 30-39 years (p-value < 0.001), and decreasing again in the older forests, 40-49 years (p-value < 0.001) and >50 years (p-value = 0.001).

To see trends across succession in these functional classifications, we used spline lines with 3 degrees of freedom (Figure 8). The variability of these models ranged from 0.06 to 0.41 (measured by R^2). Chemotrophic ($R^2 = 0.15$) and Chitinolytic ($R^2 = 0.10$) bacteria increased in the early stages of forest succession and then decreased in Mollisols, whereas Cellulolytic (R2 = 0.15) bacteria showed the opposite trend. In Alfisols, Cellulolytic and Chemotrophic bacteria

decreased after pasture abandonment, increased during early forest succession, and declined in older forests, while Chitinolytic bacteria showed the opposite trend. These results suggest that successional trends in bacterial functional classification vary with soil order (Table 14).

Fungi functional traits

A total of 48% of the fungal OTUs were classified using the FUNGuild database. For trophic-level classification, there were 34.1% of Saprotrophs, 12.2% of Pathotrophs, and 15.5% of Symbiotrophs (Table 11). We used a two-way ANOVA to test how different fungal functional groups differed across forest age classes and soil orders (Table 15, Figure 9) and Tukey HSD (Table 16). Saprotrophic fungi relative abundance was lower in pastures than in forests of 40-49 years (p-value = 0.026) and was greater in Alfisols compared to Mollisols (p-value = 0.058). Symbiotrophs' relative abundance differed across forest ages (p-value = 0.003) and soil order (p-value = 0.011). The forests of 40-49 years had a greater relative abundance of Symbiotrophs than pastures (p-value = 0.009), forests of 15-29 years (p-value = 0.021), and 30-39 years (p-value = 0.010), suggesting an increase during succession. Alfisols had more symbiotrophs than Mollisols (p-value = 0.036). Pathotrophs did not differ between forest age classes or soil orders.

We used spline line models to visualize trends in these trophic categories across forest age varied from <0.01 to 0.14 (measured by R^2; Figure 10, Table 17). Saprotrophs increased with forest age, while Pathotrophs showed a decrease with succession, followed by an increase in older forests. However, the models for Saprotrophs and Pathotrophs only explained a small percentage of the variability of the data. The relative abundance of Symbiotrophs increased with forest age on Alfisols only.

For guild classification, 1.06% were Arbuscular mycorrhizal fungi, 2.01% were Ectomycorrhizal fungi, 1.24% were Plant pathogens, and 5.28% were Wood Saprotrophs across

all samples (Table 11). Arbuscular mycorrhizal fungi's relative abundance was greater in pastures than in forests (p-value = 0.056; Figure 11). Ectomycorrhizal relative abundance was greater in forests of >50 years than in pastures (p-value = 0.036) and forests of 15–29 years (p-value = 0.034), suggesting an increase across succession. Plant pathogens' relative abundance was greater in Alfisols than in Mollisols (p-value = 0.014). We found no differences in the relative abundances of arbuscular mycorrhizal fungi and wood saprotrophs across successional stages or soil orders.

The spline line models represented <0.01 to 0.13 of the variability of the data (measured by R^2). Arbuscular mycorrhizal fungi decreased with forest age (R^2 = 0.1; Figure 12, Table 17) with no difference across soil orders. Wood saprotrophs showed no change with reforestation in Mollisols, whereas in Alfisols, their abundance increased early on, followed by a decrease later with succession (R^2 = 0.13).

Bacteria's relationship to soil carbon

Actinobacteria relative abundances were negatively related to SOC (β = -0.128, p-value = 0.041, R^2 = 0.36), and the effect was stronger in forests >50 yrs. (β = -0.199, p-value 0.051, R^2 = X; Table 18). Verrucomicrobiota relative abundances were negatively related to soil carbon (β = -0.619, p-value 0.027, R^2 = 0.48) with a significant effect on soil order (p-value = 0.007). Firmicutes relative abundances were only positively related to soil carbon in forests > 50 years (β = 1.584, p-value 0.041), representing a variability of 39%. In contrast, Acidobacteria was positively related to SOC (β = 0.128526, p-value = 0.078, R^2 = 0.41), and the effect was more significant in forests >50 years (β = 0.303, p-value < 0.001). Proteobacteria was positively related to SOC (β = 0.181, p-value = 0.032, R^2 = 0.54), and the effect was more substantial in forests of 30-39 years (β = 0.066, p-value = 0.005). Methylomirabilota was only positively

related to soil carbon for forests of 30-39 years (β = 0.619, p-value 0.045) and negatively to forests of > 50 years (β = -0.733, p-value 0.034), and this model explained 38% of the variability. Of the bacteria related to soil carbon, none show a significant effect on different soil types.

Bacterial functionality was related to soil carbon. Chitinolytic bacteria were negatively related to soil carbon (β = -5.931e-15, p-value < 0.001, R^2 = 0.42), and this relationship was modulated across forest age classes. Fermentation bacteria was negatively related to soil carbon (β = - 3.101e-14, p-value < 0.001, R^2 = 0.38), and this relationship was modulated across forest age classes.

Fungi's relationship to soil carbon

Ascomycota relative abundances were positively related to soil carbon (β = 0.035, p-value = 0.001, R^2 = 0.50; Table 19) with a stronger effect in forests of > 50 years (β = 0.046, p-value = 0.007) and showed an inverse relationship in forest of 30-39 years (β = -0.0638, p-value = 0.002). Motirellomycota relative abundances were positively related to soil carbon in forests of 30-39 years (B = -3.04, p-value 0.007), and negatively related soil carbon forests of > 50 years (β = -0.347449, p-value = 0.008) this model represented 37% of the variability. Basidomycota relative abundances were only negatively related to forest soil carbon of 30-39 years (β = -0.050, p-value = 0.003, R^2= 0.41). Gloreomycota relative abundances were negatively related to soil carbon (β = -3.037869, p-value = 0.058, R^2 = 0.17), with a strong effect on soil order (p-value <0.001).

Pathotrophs were negatively correlated to soil carbon in Mollisols while positively correlated to carbon in Alfisols (β = -1.485e-01, p-value 0.003). Ectomycorrhizal fungi were negatively related to soil carbon in the forest of 15-29 years (B = -0.427, p-value 0.035) and positively related in the forest of 30-29 (β = 0.844, p-value 0.006) and 40-49 years (β = 0.604, p-

value = 0.039) model represents a variability of 40%. Plant pathogens were positively related to soil carbon in forests of 30-39 years (β = 0.462644, p-value = 0.049) with the model representing 32% of the variability. Wood saprotrophs were positively related to soil carbon (β = 0.0342, p-value 0.001, R^2 = 0.43), with soil order having a strong influence (p-value = 0.010).

Discussion

This study aimed to investigate the effects of tropical forest succession on microbial communities in different soil orders along a weathering catena, as well as to assess whether microbial functional traits explained the variation in soil carbon across succession and soil orders. When examining bacterial diversity, we found that forest succession had a significant impact on alpha diversity but not beta diversity. On the other hand, fungal beta diversity was influenced by forest succession, while alpha diversity remained relatively stable. Our results suggest that there is a difference in microbial response to forest succession. Interestingly, we observed distinct variations in the relative abundance of certain bacteria following pasture abandonment, with some bacteria showing differences solely between forests and pastures, while others exhibited changing trends across different forest ages. While fungal phyla displayed minimal changes across succession, analyzing their functionality revealed important trends. This suggests a potential shift in the functional roles of fungi in response to environmental changes. Notably, our findings highlight the crucial role of soils in shaping the microbial response to forest succession, linking that part of the variability found in microbial communities across succession is the result of diverse soil environments. Additionally, we found that microbial functional characteristics partially explained variations in soil carbon related to forest age and soil order, underscoring the complex relationship within these disturbed ecosystems. Although our study provides valuable insights, further research is needed to enhance the classification of

microbial functionalities and roles in disturbed forest soil, particularly in tropical regions. In the subsequent sections, we dip into the implications of our findings, offering a more detailed exploration and discussion of their significance.

Forest succession influences bacterial and fungal beta and alpha diversity differently.

Forest succession plays a crucial role in influencing microbial diversity, particularly in relation to bacterial and fungal communities. When assessing alpha diversity, which measures the richness and evenness of species within a specific habitat, distinct responses were observed between bacteria and fungi. For bacteria, alpha diversity was affected by changes in vegetation, indicating alterations in the number and relative abundance of bacterial taxa within specific habitats. Notably, pastures exhibited greater bacterial evenness compared to any forest successional age class. Additionally, a trend of decreasing Shannon diversity was observed from pastures to early-stage forests, followed by an increase in older stages. These results differ from findings in tropical and subtropical regions with similar karst soil as in our study, where bacterial diversity typically increases across secondary succession (Ren et al. 2017, Wang et al. 2022). Enhanced bacterial diversity across secondary succession can be attributed to increased carbon and nitrogen levels (Wang et al. 2022), and higher plant diversity following afforestation has also been associated with increased bacteria diversity (Ren et al. 2017). However, a recent meta-analysis of tropical rainforest soils found an increase in bacterial alpha diversity and changes in composition with forest conversion to pastures and plantations (Petersen et al. 2019). Yet, most of these results were not strictly attributed to carbon for bacteria. In our study sites, the reduction in bacterial diversity in secondary forests may be attributed to early successional stage plants exploiting nutrients that are limiting availability for microorganisms (Powers and Marín-Spiotta 2017), leading to changes in bacterial alpha diversity. When looking at fungal alpha diversity, we

found that it remained relatively stable despite vegetation cover changes, indicating a consistent number and relative abundance of fungal taxa.

In contrast to alpha diversity, beta diversity focuses on variations in species composition between different habitats or areas. For bacteria, beta diversity shows significant response to land use change but it exhibit significant variation too, indicating that the composition of bacterial species remained relatively consistently variable across different habitats or areas. The high variability of bacterial communities during forest succession can be attributed to bacteria's ability to adjust gene expression for various metabolic pathways based on resource availability in the environment (McGee et al. 2019). Unless there are significant disturbances in the soil, such as topsoil removal, bacterial communities will remain highly variable. Conversely, fungal beta diversity was influenced by forest succession, indicating differences in fungal species composition between pastures and forests, as well as across different successional stages. Differences in fungal community composition between pastures and forests may be attributed to changing patterns in nutrient acquisition during transitions in vegetation communities (Mueller et al. 2016, Cho et al. 2017). Moreover, variations in the chemical composition of organic matter returned through plant litter across succession (Marín-Spiotta et al. 2008) could contribute to differences in fungal communities (Bai et al. 2019). These findings highlight the intricate dynamics of microbial communities during forest succession and underscore the crucial role of vegetation composition and nutrient inputs through litter deposition in shaping patterns of bacterial and fungal diversity.

Bacterial relative abundance varied after pasture abandonment.

The relative abundance of different bacteria phyla exhibited significant variations during forest succession after pasture abandonment. Actinobacteria, Acidobacteria, and Proteobacteria

were the most abundant, which is consistent with previous reports of soils in the tropics (Schneider et al. 2015, Zhang et al. 2017). Some bacterial groups differed more between pastures and forests than with forest age. Acidobacteria, for example, increased after pasture abandonment but had similar abundances across different forest ages. Similar findings were reported in the Brazilian Amazon (Navarrete et al. 2015). Acidobacteria is known to be abundant in the phyllosphere of tropical trees (Kim et al. 2013), suggesting litter could influence the increase of abundance in forest soils. The lack of changes in Acidobacteria across succession can be the result of the length of recovery of tropical forests. Forest structure can recover in 20 years after pasture abandonment (Marín-Spiotta et al. 2007), and biomass and stem density have also shown fast recovery in wet tropical forests (Poorter et al. 2019). This suggests that the fast recovery of forests could also be influencing a fast recovery of Acidobacteria which may contribute to no changes across later successional stages. Another phylum, Verrucomicrobia, showed higher relative abundance in pastures compared to forests. This finding aligns with results from the Brazilian Amazon, where an increase of 11.6% in Verrucomicrobia's relative abundance was observed in pastures, and the community composition within this phylum significantly differed from the forest community (Ranjan et al. 2015). In line with previous literature, our findings align with the proposal that Acidobacteria and Verrucomicrobia can serve as potential indicators of soil land use effects, specifically in the context of forest-to-pasture conversion (Pajares et al., 2016).

Differences in relative abundance across forest ages were observed in several phyla, including Proteobacteria, Myxococcota, Latescibateriota, and the functional classification of Chemotrophic bacteria. Proteobacteria decreased from pastures to early stages of succession, followed by an increase in mid-stages and a subsequent decline in older forests. This trend

suggests that the increasing soil organic matter as a result of leaf litter during afforestation may promote the growth of Proteobacteria (Zhang et al. 2020), which we supported by Protobacteria positively related to SOC in our study. Notably, the higher abundance of Proteobacteria and Chemotrophs in pastures and mid-stages suggests variability in nitrogen availability across succession, as Proteobacteria and some bacteria Chemotrophs are known to play a role in the nitrogen cycle and thrive in nitrogen-rich soil environments (Pfister et al. 2010). Our results support our hypothesis that bacteria abundance and functionality will vary due to pasture abandonment and forest succession, although the implications for ecosystem function require further investigation.

Saprotrophic and symbiotrophic fungi increase across forest succession.

Saprotrophic and Symbiotrophic fungi exhibit varying abundance patterns throughout forest succession, with notable findings related to specific fungal phyla. Our analysis revealed that the most prevalent fungal phyla were Ascomycota and Basidiomycota, which aligns with previous studies in tropical soil environments (Ren et al. 2017, McGee et al. 2019). Surprisingly, we observed no differences in the relative abundance of Ascomycota and Basidiomycota across different stages of forest succession. Still, our spline model analysis showed a non-linear decrease in the relative abundances of Ascomycota across the succession stages, while Basidiomycota exhibited a non-linear increase. However, due to the high variability observed in their relative abundances, our models could only explain less than 8% of the variation, indicating the influence of other factors in driving their dynamics. Ascomycota and Basidiomycota may be more strongly influenced by resource availability rather than vegetation change across succession.

Glomeromycota, a phylum primarily associated with arbuscular mycorrhizal fungi, showed a decrease after pasture abandonment. This observation implies that pastures may have a greater relative abundance of arbuscular mycorrhizal fungi compared to forest systems. Similar observations were found in Brazilian Amazon were Glomeromycota spores were more abundant in pastures and early secondary forests (Leal et al., 2009). Supporting this statement, when evaluating the functional classification of arbuscular mycorrhizal fungi, we also noticed a marginal trend of greater relative abundance of arbuscular mycorrhizal fungi in pastures compared to forests. Notably, while Glomeromycota exhibited a decrease after pasture abandonment, there was an increase in the relative abundance of Symbiotrophic fungi. However, these results could be supported by the increase in the relative abundance of ectomycorrhizal fungi in older forest stages, which could indicate a switch from different mycorrhizal fungi symbiotic associations as result of vegetation change.

Interestingly, Mortierellomycota, a phylum consisting of decomposers (Benny et al 2016), also was more abundant in older forest stages. This finding aligns with expectations, as late-successional forests receive greater carbon inputs through above-ground litter deposition, especially coarse woody debris, favoring the carbon-decomposing fungi (Cornwell et al. 2008). The positive relationship of saprotrophic fungi and ectomycorrhizal fungi at later forest stages was surprising due to the expectation that organic matter accumulation will increase with forest age, influencing a higher relative abundance of decomposers in soils and a lower relative abundance of mycorrhizal fungi (Averill and Hawkes 2016, Schilling et al. 2016). A possible explanation for this could be that ectomycorrhizal fungi can function as saprotrophic fungi decomposing organic matter in soil due to potentially greater litter inputs, which has been supported by their evolutionary history (Lindahl and Tunlid 2015, Averill 2016).

Soils have a strong effect in modulating microbial response to forest succession.

Soils play a crucial role in shaping the microbial response to forest succession, and our study highlights the importance of considering soil heterogeneity when evaluating the impact of land use on microbial communities in tropical regions. Our findings demonstrate that soil type influences the relative abundances of Actinobacteria and Myxococcota across forest succession. Generally, Mollisols exhibit greater relative abundances of Actinobacteria and Myxococcota than Alfisols. However, Mollisols show a decrease in relative abundance from pastures to early stages of forest succession, followed by an increase in older stages, while no apparent differences were observed in Alfisols. The effect of our alkaline, high organic matter Mollisols on these phyla has previously been seen in other studies, especially for Actinobacteria. Previous research has found that Actinobacteria are more abundant in alkaline soils rich in organic matter (Goodfellow and Williams 1983), and their relative abundance may be linked to the decomposition of organic matter (Pfister et al. 2010, Becerra-Lucio et al. 2021). This would suggest that in our catena system, the weathering of Mollisols into Alfisols, reducing organic matter and pH, may be modulating bacteria relative abundances.

Symbiotrophic fungi relative abundance appeared to increase in older stages of succession in Alfisols, while in Mollisols, it seemed to decrease in forests older than 50 years. Many studies have reported that symbiotic relative abundance negatively correlates with saprotrophic relative abundance (specifically ectomycorrhizal fungi) due to competition for nitrogen in soils (Cairney 2012, Averill et al. 2014). However, if soil nutrients are limited at late stages, ectomycorrhizal fungi would be more abundant at later stages (Cox et al. 2010). The availability of organic matter in Mollisols may promote a decrease in symbionts, while a more

weathered soil in which Alfisols have less organic matter could imply fewer resources for microorganisms compared to Mollisols leading to a higher relative abundance of symbionts.

Our findings emphasize that microbial communities respond not only to vegetation changes but also to the availability of resources and the chemical characteristics of soil environments. Future research should incorporate the diversity of soils found in tropical regions to gain a comprehensive understanding of how land use changes affect not only biogeochemical cycles but also the organisms inhabiting the soil, which can have potential implications for ecosystem recovery and function across forest succession.

Microbial functional characteristics can explain some of the variations of soil carbon.

Our research highlights the significant role of microbial functional characteristics in explaining variations in soil carbon content. Despite the absence of a clear trend in SOC concentrations across succession, our findings demonstrate that microbial functional characteristics in combination with soil orders and successional stages could explain 30-50% of the variability in carbon content. This underscores the importance of understanding microbial communities' response to environmental change and how it relates to soil carbon cycle (Schimel and Schaeffer 2012). Notably, the relationship between different microbial groups varied depending on the successional stage and soil type.

Among the bacteria functional groups, Fermentation and chitinolytic bacteria were found to be significantly negatively related to soil carbon, with this relationship being modulated across forest age classes. In contrast, bacteria phyla emerged as strong predictors of soil carbon across succession and soil orders. Actinobacteria, which exhibited a negative correlation with SOC, particularly in older forests, are frequently associated with decomposition processes in the mineral horizons of soils, contributing to the breakdown of polysaccharides and phenolic

compounds during plant biomass decay (Šturšová et al. 2012, Větrovský et al. 2014). On the other hand, Acidobacteria showed a positive correlation with SOC, with a more pronounced effect observed in older forests. Proteobacteria also positively correlated with SOC, especially in forests aged 30-39 years. Certain groups of bacteria within the Proteobacteria phylum have been identified as expressing ligninolytic and cellulolytic capabilities involved in organic material decomposition (Brown and Chang 2014).

Furthermore, our research examined the functional characterization of fungi and their influence on soil carbon. We found that pathogenic fungi exhibited a negative correlation with soil carbon in Mollisols while showing a positive correlation in Alfisols. We hypothesize that the negative relationship observed in Mollisols can be attributed to the accumulation of pathogens in the rhizosphere, which directly depletes carbon and nutrients from plant tissues and reduces root uptake capacity. This, in turn, leads to negative feedback on plant growth (Bever et al. 1997), resulting in reduced inputs of carbon to the soil. Conversely, we hypothesize that in Alfisols, pathogens can act as saprotrophs, decomposing simple organic compounds (Deacon 1997) and promoting the transformation of carbon-stable compounds in the soil (Treseder and Lennon 2015, Kyaschenko et al. 2017). Despite these findings, our understanding of pathogenic fungi and their role in biogeochemical cycles remains significantly limited. However, it is recognized that the presence of pathogenic fungi can affect the input and decomposition of plant organic matter by other microorganisms, thereby influencing carbon cycling in the soil (Zanne et al. 2020). Therefore, considering the role of pathogens is important for comprehending soil carbon dynamics.

Additionally, we found a positive relationship between wood saprotrophs and soil carbon. Saprotrophic fungi, including wood saprotrophs, play a critical role in the decomposition of

organic matter in the soil. They contribute to the breakdown of lignin and cellulose compounds, leading to the transformation of carbon into more stable forms within the soil which may they contribute to increases of carbon or could be responding to more available carbon in the soil (Treseder and Lennon 2015, Kyaschenko et al. 2017). Ectomycorrhizal fungi also displayed a positive correlation with SOC at later stages. This indicates their role in increasing soil carbon in late successional stages forests by limiting decomposition processes carried out by other organisms (e.g., bacteria), thereby preventing carbon loss through CO_2 (Cairney 2012, Averill and Hawkes 2016, Shah et al. 2016, Baskaran et al. 2017). Fungal phyla relative abundance, particularly Ascomycota and Basidiomycota, can also contribute to our understanding of soil carbon Ascomycota showed a positive association with soil carbon, with the strength of this association varying depending on the succession stage and soil type. . In contrast, Basidiomycota exhibited a negative relationship with forest soil carbon, specifically in the age range of 30-39 years. Both Ascomycota and Basidiomycota can rapidly metabolize organic substrates derived from root exudates, and their relative abundances are influenced by litter decomposition (Hannula et al. 2012, Bastida et al. 2013). Although no direct relationship between Ascomycota and Basidiomycota and succession was observed, their associations with soil carbon align with expectations based on their ecological roles.

Challenges in using functional characterization to understand the resilience of microbial communities and their impact on ecosystem processes.

The use of functional traits has been important in providing a framework for earth system modelers to understand soil biogeochemistry responses to environmental changes (Wieder et al. 2013). However, the use of functional traits to understand the resilience of microbial communities and their impact on ecosystem processes presents several challenges. Although

functional traits provide valuable insights, our study revealed that the complete representation of bacterial and fungal communities was limited, with only 12% and 48% of reads classified, respectively. This suggests that a substantial portion of the microbial diversity that could be influencing ecosystem function during pasture abandonment and forest succession remains unclassified. However, this is not unique to our study. Although these databases present a great opportunity to study microbial function response to environmental changes, they have limitations (Djemiel et al. 2022). The FAPROTAX database, primarily populated with bacterial and Archaeal communities, poses limitations as it assigns ecological traits based on species names (Louca et al. 2016). Thus, taxa lacking species-level classification cannot be adequately assessed at higher taxonomic levels. Also, FAPROTAX was developed from information on ocean microbes (Louca et al. 2016). The representation of the database for soil accounts for less than 25% (Djemiel et al. 2022), which can be the main explanation as to why our samples were poorly assigned.

FunGuild database classification poses challenges due to the importance of the resolution of taxonomic levels classification from UNITE database. Assigning traits to taxa of interest requires taxonomic names at the genus or species level, which may not always be available. In our case, a significant percentage of sequences (15%) could not be classified by the UNITE database, limiting our ability to assign ecological traits as FunGuild uses taxonomic classification where 66% are at the genus level and 34% at the species level (Nguyen et al. 2016). Also, FunGuild provides confidence rankings (“highly probable,” “probable,” and “possible”) representing the likelihood that a taxon belongs to a given guild. However, we did not select the particular confidence resolution to maximize sample classification in our data. The

lack of comprehensive classification and varying levels of confidence in trait assignment within FunGuild can introduce additional uncertainty to a study's findings and implications.

While these methodologies provide a basis for hypothesis development and general assessments of microbial communities and their ecological roles, further research is required to expand the databases and improve the representation of the diversity of microorganisms found in soils. This need is particularly pronounced in tropical ecosystems, which are understudied and subject to potential biases compared to other soil types (Díaz-Vallejo et al. 2021). Addressing these challenges will contribute to a more accurate understanding of microbial communities and their functional contributions in diverse ecosystems.

Conclusion

In conclusion, our study provides insights into the impact of tropical forest succession on microbial communities in different soil orders within a catena. We found that forest succession influenced bacterial alpha-diversity and fungal beta-diversity. The relative abundance of certain bacteria varied following pasture abandonment, while major phyla of fungi showed minimal changes. Saprotrophic and Symbiotrophic fungi increased after pasture abandonment and forest succession. Moreover, soil type played a crucial role in modulating the microbial response to forest succession. Additionally, we found that microbial functional characteristics partially explained the variations in soil carbon with respect to forest age and soil order. These findings highlight the complex dynamics of microbial communities during forest succession and emphasize the need for further research to enhance our understanding of microbial functionalities in disturbed tropical forest soils.

Figures

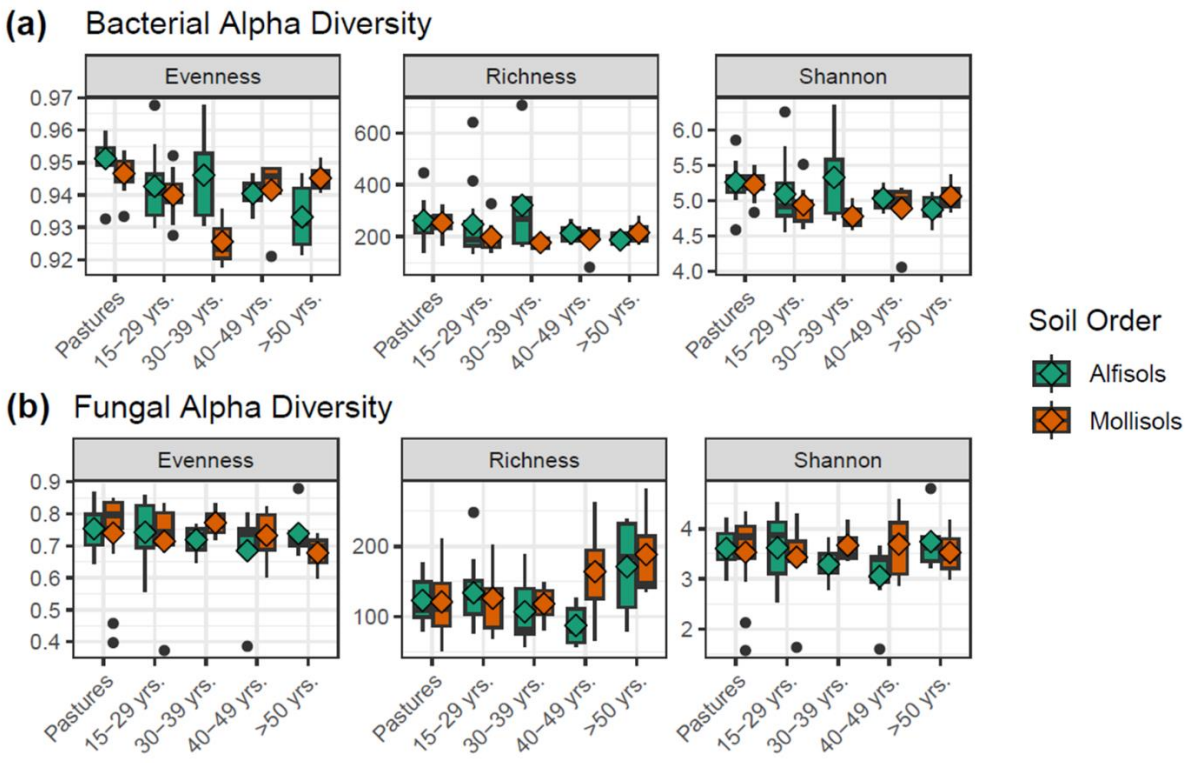

Figure 1. Bacterial and Fungal diversity indexes among forest age classes and soil orders. yrs. = years

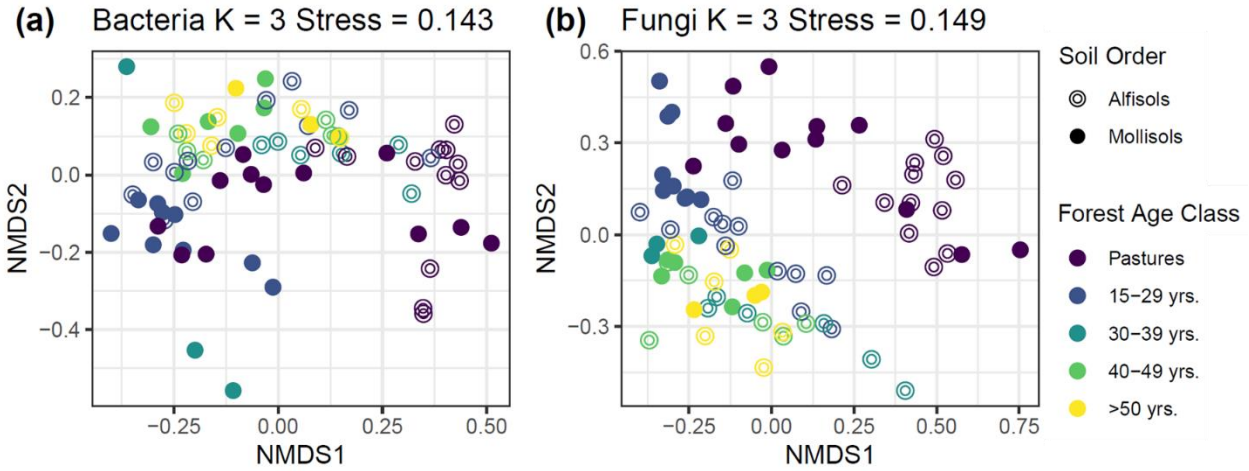

Figure 2. Non-metric scaling analysis for Bacterial and Fungal beta diversity across forest age classes and soil orders. yrs. = years

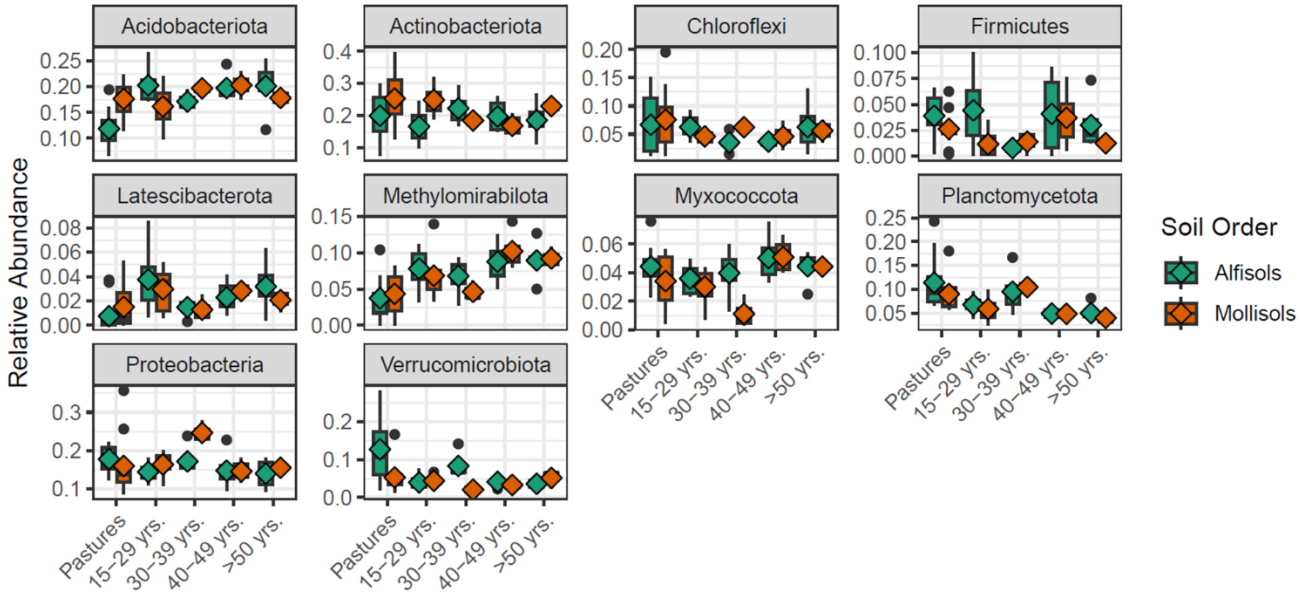

Figure 3. Relative abundances of Bacterial phyla across forest age classes and soil orders. yrs. = years

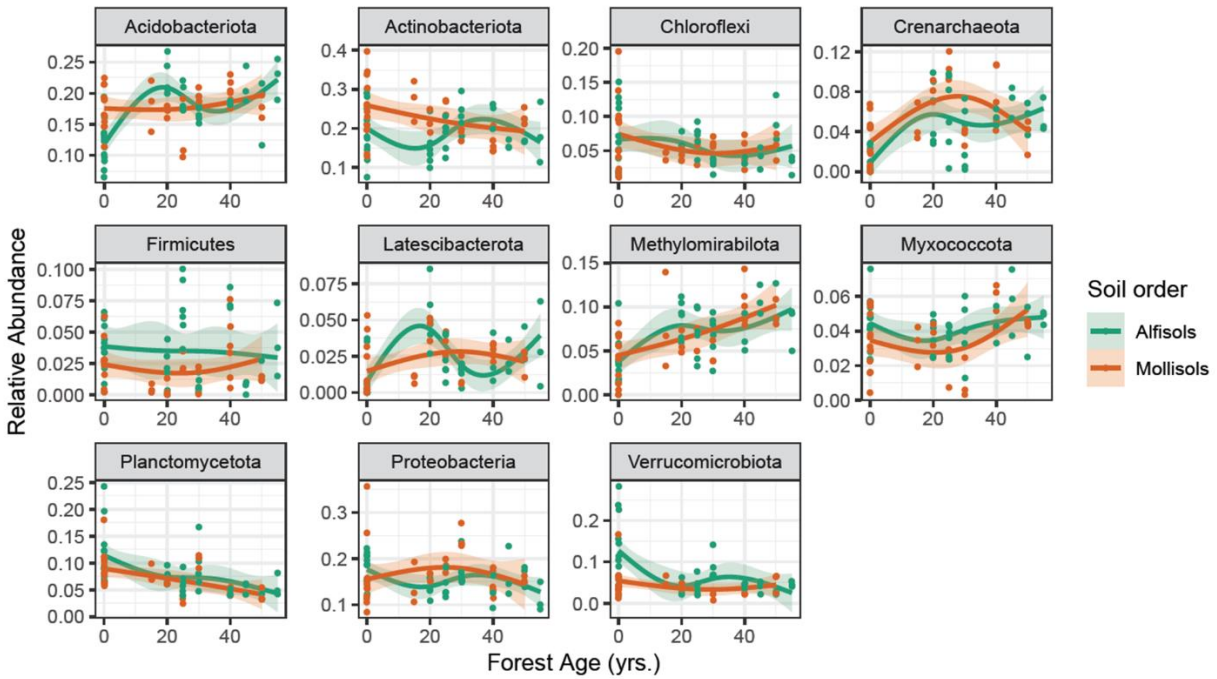

Figure 4. Spline lines trends with 3 degrees of freedom for bacterial relative abundances phyla across forest ages and between soil orders. yrs. = years

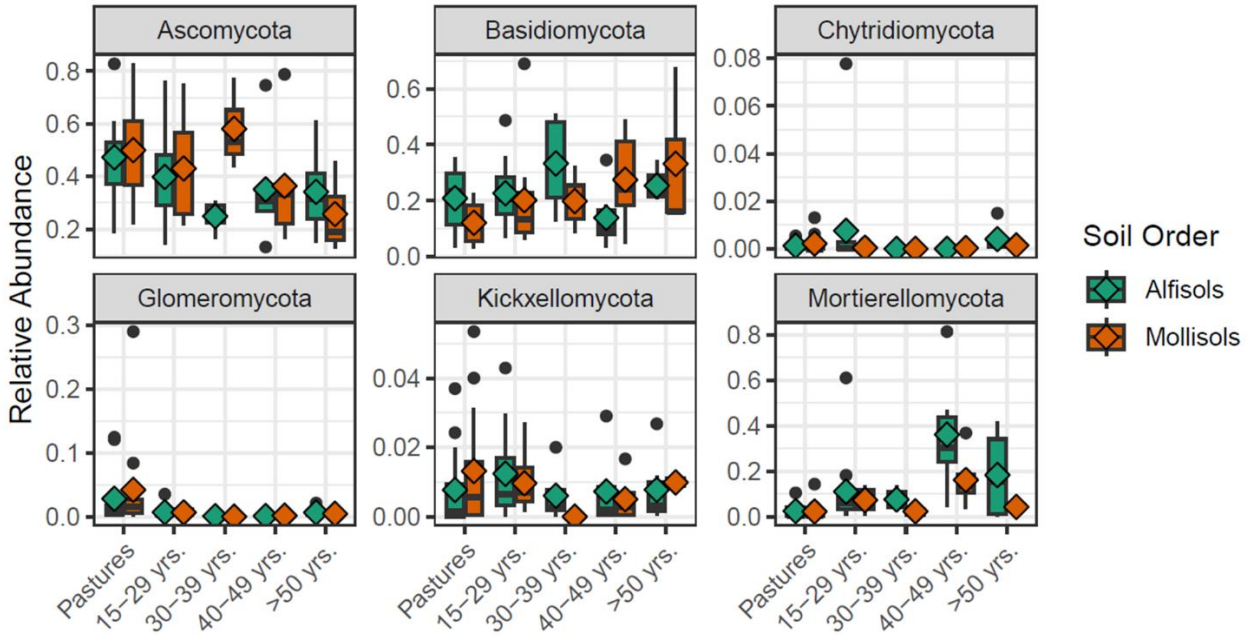

Figure 5. Fungal phyla relative abundances across forest age classes and soil orders. yrs. = years

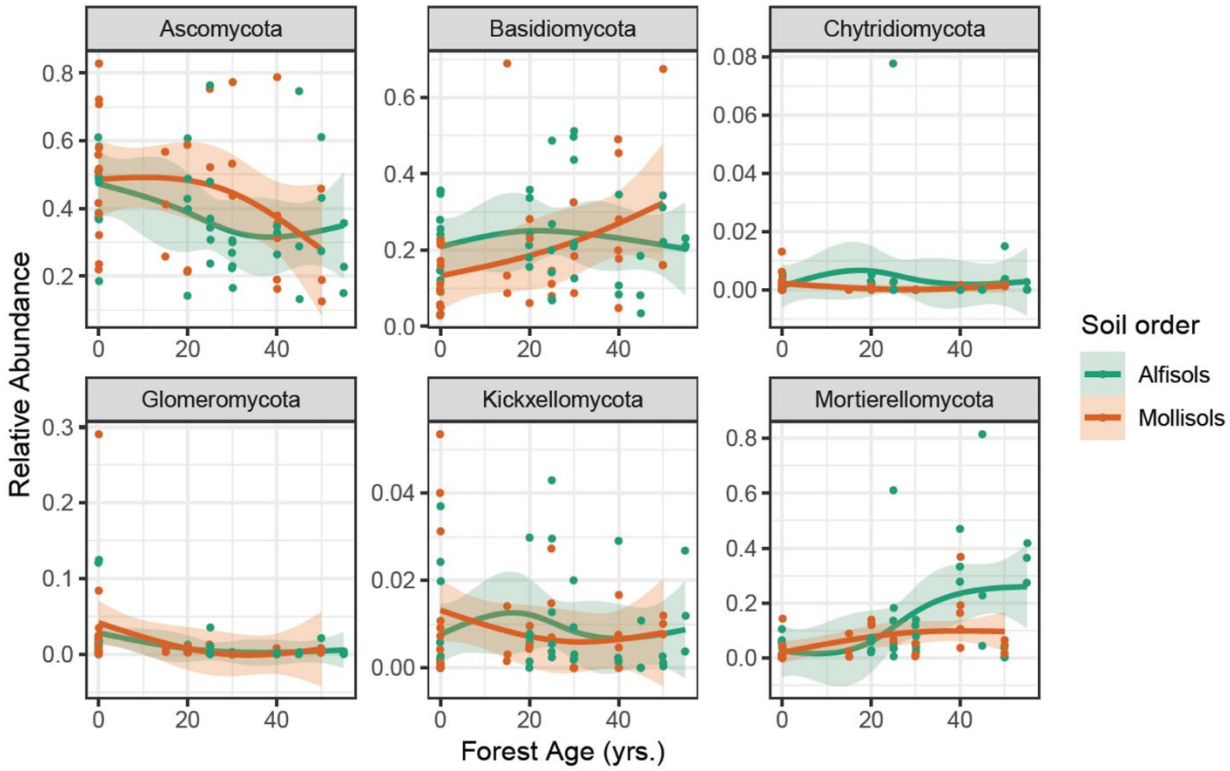

Figure 6. Spline lines trends with 3 degrees of freedom for fungal phyla relative abundances phyla across forest ages and between soil orders. yrs. = years

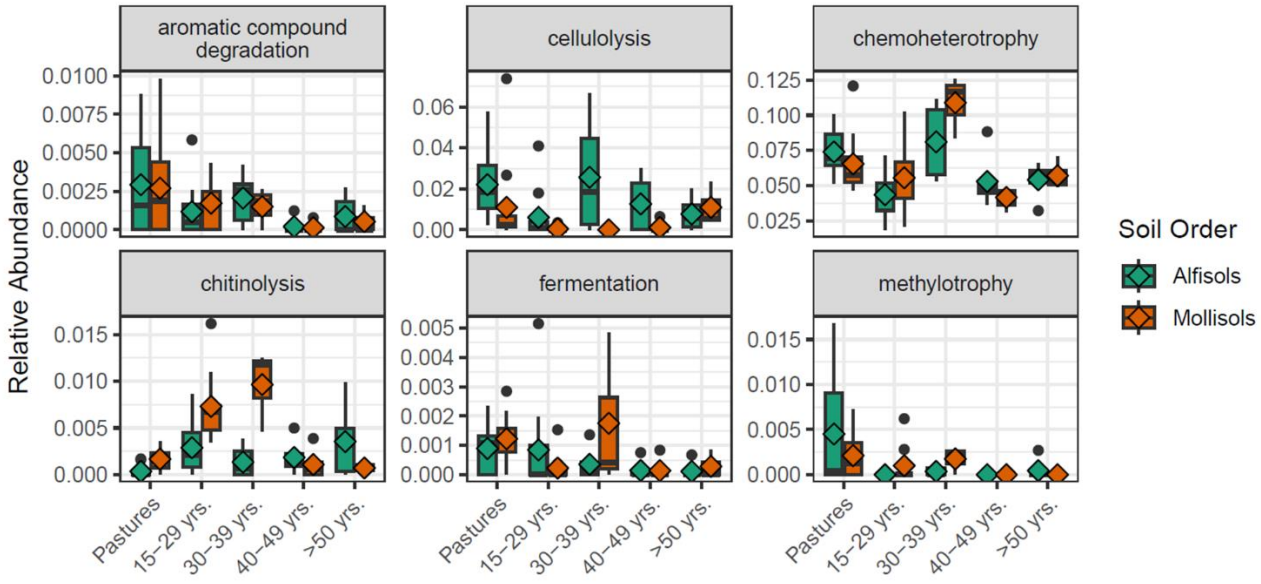

Figure 7. Bacterial functional groups relative abundance across forest age classes and soil orders. yrs. = years

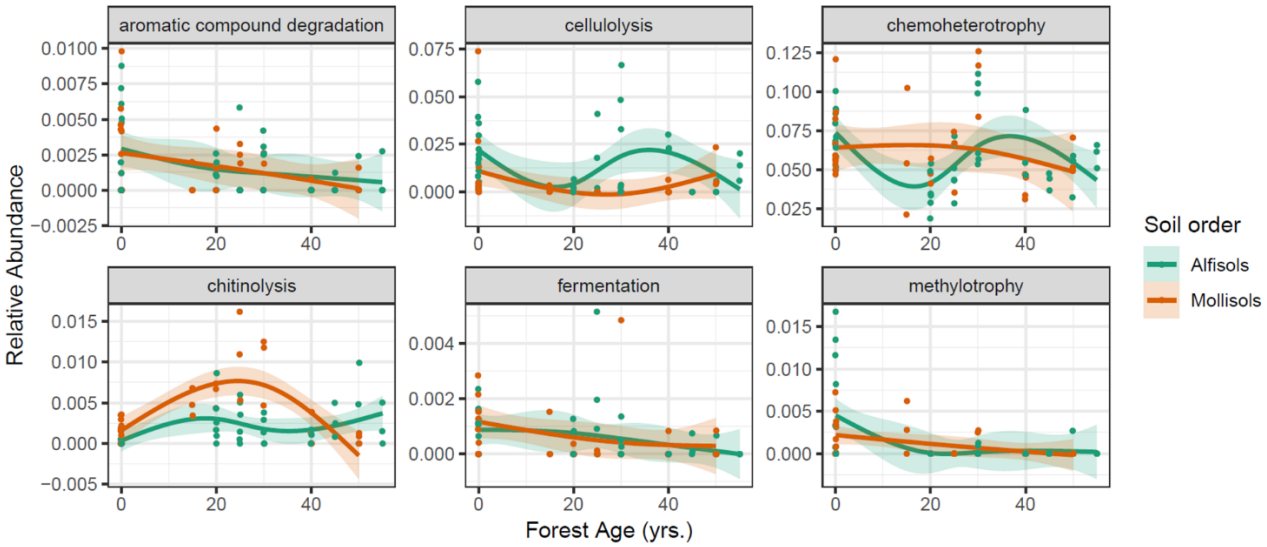

Figure 8. Spline line trends with 3 degrees of freedom for bacterial functional groups relative abundances phyla across forest ages and between soil orders. yrs. = years

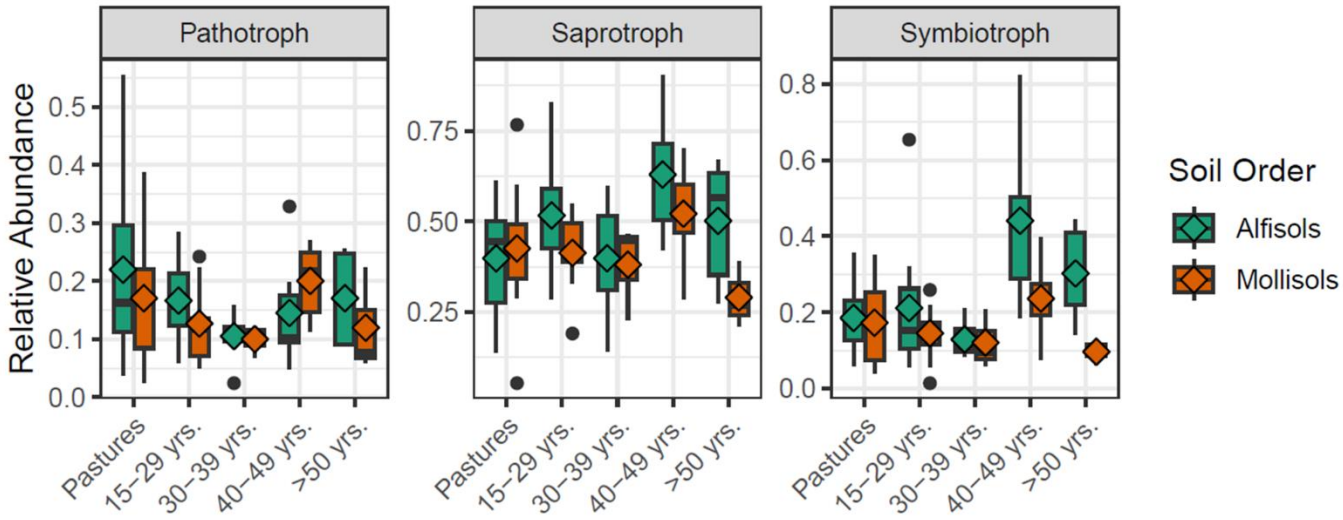

Figure 9. Fungal trophic functional groups relative abundance across forest age classes and soil orders. yrs. = years

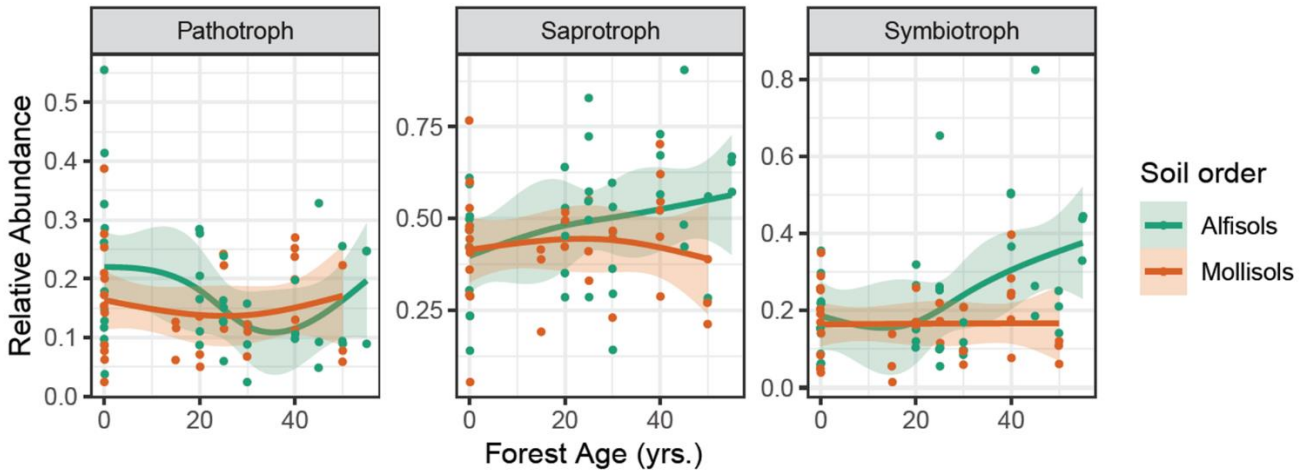

Figure 10. Spline line trends with 3 degrees of freedom for fungal trophic functional groups relative abundances across forest ages and between soil orders. yrs. = years

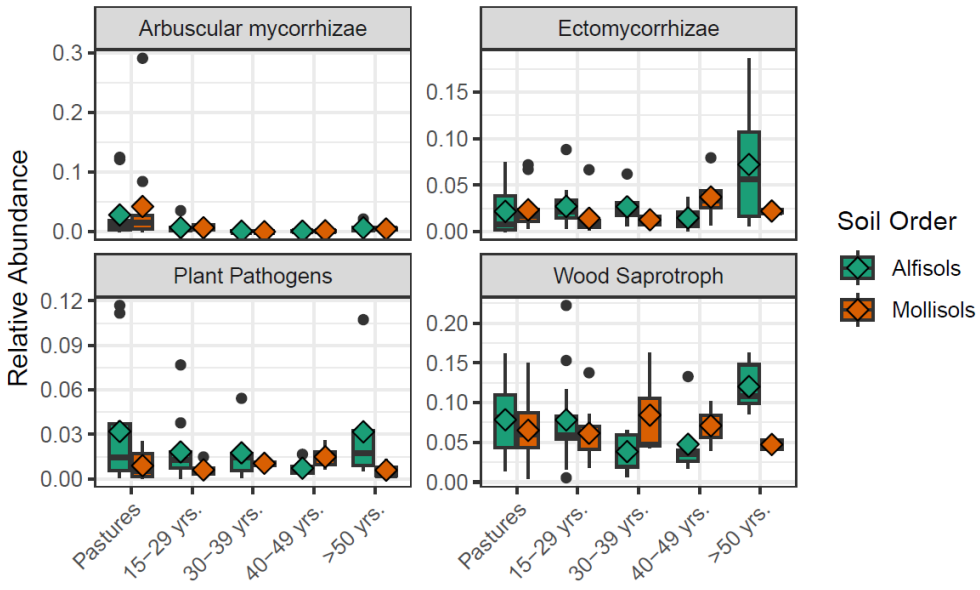

Figure 2. Fungal guild functional groups relative abundance across forest age classes and soil orders. yrs. = years

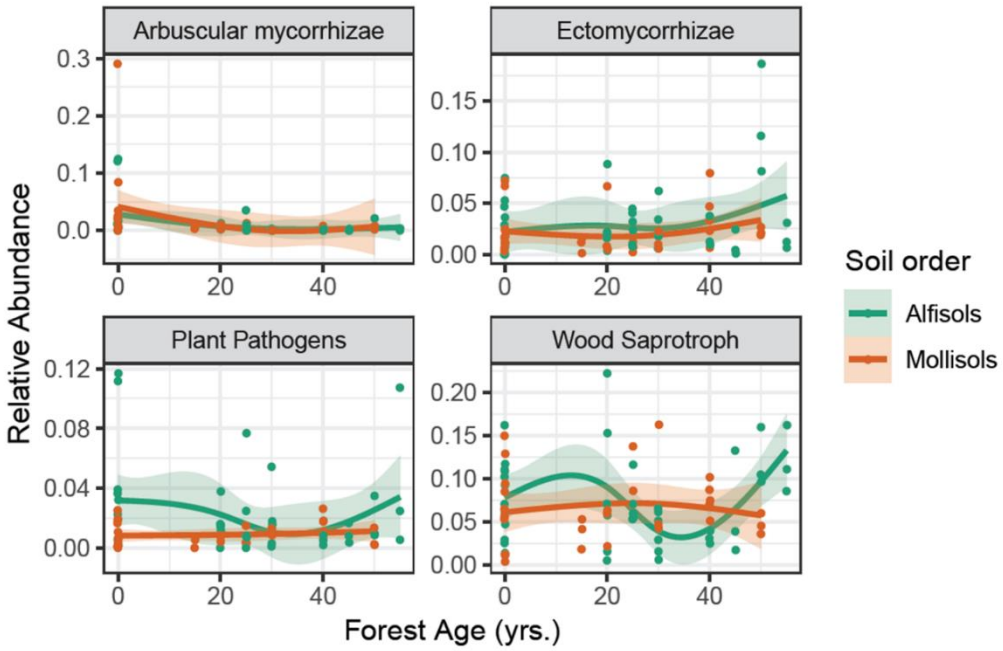

Figure 3. Spline line trends with 3 degrees of freedom for fungal guild functional groups relative abundances across forest ages and between soil orders. yrs. = years

Tables

Table 1. Analysis of Variance Results for testing differences of Bacterial and Fungal diversity indexes among forest age classes, soil orders, and their interaction.

			df	Sum of Squares	Mean of Squares	F value	p-value
Bacteria							
	Richness						
		Forest Age Class	4	0.875	0.219	1.974	0.109
		Soil Order	1	0.205	0.205	1.847	0.179
		Forest Age Class * Soil Order	4	0.439	0.110	0.990	0.419
		Residuals	65	7.205	0.111		
	Shannon Diversity Index						
		Forest Age Class	4	1.100	0.275	2.154	0.084
		Soil Order	1	0.253	0.253	1.978	0.164
		Forest Age Class * Soil Order	4	0.607	0.152	1.189	0.324
		Residuals	65	8.299	0.128		
	Shannon Evenness						
		Forest Age Class	4	0.001	0.000	4.215	0.004
		Soil Order	1	0.000	0.000	2.091	0.153
		Forest Age Class * Soil Order	4	0.001	0.000	3.470	0.012
		Residuals	65	0.005	0.000		
Fungi							
	Richness						
		Forest Age Class	4	1.182	0.295	2.086	0.093
		Soil Order	1	0.155	0.155	1.097	0.299
		Forest Age Class * Soil Order	4	1.003	0.251	1.770	0.146
		Residuals	65	9.207	0.142		
	Shannon Diversity Index						
		Forest Age Class	4	0.631	0.158	0.364	0.833
		Soil Order	1	0.038	0.038	0.088	0.767
		Forest Age Class * Soil Order	4	1.797	0.449	1.039	0.394
		Residuals	65	28.124	0.433		
	Shannon Evenness						
		Forest Age Class	4	0.014	0.003	0.281	0.889
		Soil Order	1	0.001	0.001	0.046	0.831
		Forest Age Class * Soil Order	4	0.025	0.006	0.507	0.731
		Residuals	65	0.788	0.012		

Table 2. PERMANOVA results for Bacterial and Fungal Communities differences among soil order, forest age classes, and the interaction between them

		df	Sum Of Squares	R2	F value	p-value
Bacteria						
	Soil Order	1	0.715	0.021	1.656	0.001
	Forest Age Class	4	2.679	0.080	1.551	0.001
	Soil Order * Forest Age Class	4	2.170	0.065	1.256	0.001
	Residual	65	28.067	0.835		
	Total	74	33.631	1		
Fungi						
	Soil Order	1	0.946	0.029	2.511	0.001
	Forest Age Class	4	4.133	0.127	2.743	0.001
	Soil Order * Forest Age Class	4	2.877	0.089	1.909	0.001
	Residual	65	24.486	0.755		
	Total	74	32.443	1		

Table 3. Betadisper results from Bacterial and Fungal Communities differences among soil order and forest age classes.

		df	Sum of Squares	Mean Squares	F value	# Permutations	p-value
Bacteria							
	Forest Age Class	4	0.028	0.007	9.368	999.000	0.001
	Residuals	70	0.052	0.001			
	Soil Order	1	0.003	0.003	5.057	999	0.032
	Residuals	73	0.045	0.001			
Fungi							
	Forest Age Class	4	0.044	0.011	11.115	999	0.001
	Residuals	70	0.069	0.001			
	Soil Order	1	0.000	0.000	0.380	999	0.561
	Residuals	73	0.063	0.001			

Table 4 Baterial and Fungal relative abundances across all samples

	Phylum	Relative abundance (%)
Bacteria		
	Actinobacteriota	15.566
	Acidobacteriota	13.187
	Proteobacteria	12.037
	Planctomycetota	5.682
	Methylomirabilota	5.019
	Verrucomicrobiota	4.409
	Chloroflexi	4.311
	Myxococcota	2.924
	Firmicutes	2.189
	Latescibacterota	1.676
Fungi		
	Ascomycota	30.668
	Basidiomycota	16.108
	Unknown	15.053
	Mortierellomycota	7.430
	Glomeromycota	1.059
	Rozellomycota	0.975
	Kickxellomycota	0.672
	Mucoromycota	0.378
	Chytridiomycota	0.168
	Entorrhizomycota	0.014
	Blastocladiomycota	0.004
	Basidiobolomycota	0.001

Table 5. Analysis of Variance Results for testing differences of Bacterial phyla relative abundances among forest age classes, soil orders, and their interaction.

	df	Sum of Squares	Mean of Squares	F value	p-value
Actinobacteriota					
Forest Age	4	0.031	0.008	1.437	0.232
Soil Order	1	0.041	0.041	7.551	0.008
Forest Age	4	0.059	0.015	2.726	0.037
Residuals	65	0.354	0.005		
Acidobacteriota					
Forest Age	4	0.045	0.011	7.308	0.000
Soil Order	1	0.002	0.002	1.103	0.298
Forest Age	4	0.043	0.011	6.985	0.000
Residuals	65	0.100	0.002		
Planctomycetota					
Forest Age	4	0.030	0.007	2.612	0.043
Soil Order	1	0.003	0.003	0.985	0.325
Forest Age	4	0.021	0.005	1.818	0.136
Residuals	65	0.184	0.003		
Methylomirabilota					
Forest Age	4	0.037	0.009	11.607	0.000
Soil Order	1	0.000	0.000	0.005	0.945
Forest Age	4	0.003	0.001	0.827	0.513
Residuals	65	0.052	0.001		
Verrucomicrobiota					
Forest Age	4	0.049	0.012	5.573	0.001
Soil Order	1	0.021	0.021	9.435	0.003
Forest Age	4	0.033	0.008	3.673	0.009
Residuals	65	0.144	0.002		
Chloroflexi					
Forest Age	4	0.012	0.003	2.028	0.101
Soil Order	1	0.000	0.000	0.061	0.805
Forest Age	4	0.004	0.001	0.719	0.582
Residuals	65	0.093	0.001		
Myxococcota					
Forest Age	4	0.003	0.001	4.371	0.003
Soil Order	1	0.001	0.001	6.390	0.014
Forest Age	4	0.001	0.000	1.654	0.171
Residuals	65	0.013	0.000		
Firmicutes					
Forest Age	4	0.005	0.001	2.350	0.063
Soil Order	1	0.005	0.005	8.130	0.006
Forest Age	4	0.003	0.001	1.363	0.257
Residuals	65	0.037	0.001		
Latescibacterota					
Forest Age	4	0.007	0.002	6.483	0.000
Soil Order	1	0.000	0.000	0.013	0.910
Forest Age	4	0.001	0.000	0.962	0.434
Residuals	65	0.018	0.000		

Table 6. Tukey HSD results for SOC pair comparisons for Bacterial phyla relative abundance differences among forest age classes, soil orders, and their interaction.

	Difference	Lowe CI	Upper CI	p-value
Actinobacteriota				
15-29 yrs.-Pastures	-0.034	-0.101	0.033	0.624
30-39 yrs.-Pastures	-0.025	-0.113	0.063	0.932
40-49 yrs.-Pastures	-0.059	-0.138	0.021	0.251
>50 yrs.-Pastures	-0.037	-0.125	0.051	0.769
30-39 yrs.-15-29 yrs.	0.009	-0.081	0.099	0.999
40-49 yrs.-15-29 yrs.	-0.025	-0.106	0.057	0.915
>50 yrs.-15-29 yrs.	-0.003	-0.093	0.087	1.000
40-49 yrs.-30-39 yrs.	-0.034	-0.133	0.066	0.878
>50 yrs.-30-39 yrs.	-0.012	-0.118	0.094	0.998
>50 yrs.-40-49 yrs.	0.022	-0.078	0.121	0.973
Mollisols-Alfisols	0.048	0.012	0.084	0.010
Acidobacteriota				
15-29 yrs.-Pastures	0.045	0.007	0.083	0.012
30-39 yrs.-Pastures	0.038	-0.012	0.087	0.221
40-49 yrs.-Pastures	0.063	0.018	0.108	0.002
>50 yrs.-Pastures	0.056	0.006	0.105	0.020
30-39 yrs.-15-29 yrs.	-0.007	-0.058	0.043	0.994
40-49 yrs.-15-29 yrs.	0.018	-0.028	0.064	0.807
>50 yrs.-15-29 yrs.	0.011	-0.040	0.061	0.977
40-49 yrs.-30-39 yrs.	0.025	-0.031	0.081	0.711
>50 yrs.-30-39 yrs.	0.018	-0.042	0.078	0.917
>50 yrs.-40-49 yrs.	-0.007	-0.063	0.049	0.996
Mollisols-Alfisols	0.007	0.017	0.030	0.584
Planctomycetota				
15-29 yrs.-Pastures	-0.021	-0.067	0.024	0.680
30-39 yrs.-Pastures	0.033	-0.026	0.093	0.526
40-49 yrs.-Pastures	-0.028	-0.082	0.026	0.591
>50 yrs.-Pastures	-0.030	-0.090	0.029	0.619
30-39 yrs.-15-29 yrs.	0.055	-0.006	0.115	0.097
40-49 yrs.-15-29 yrs.	-0.007	-0.062	0.049	0.997
>50 yrs.-15-29 yrs.	-0.009	-0.069	0.052	0.994
40-49 yrs.-30-39 yrs.	-0.061	-0.129	0.006	0.090
>50 yrs.-30-39 yrs.	-0.063	-0.135	0.008	0.109
>50 yrs.-40-49 yrs.	-0.002	-0.069	0.065	1.000
Mollisols-Alfisols	0.011	-0.015	0.037	0.399

Methylomirabilota				
15-29 yrs.-Pastures	0.036	0.012	0.059	0.001
30-39 yrs.-Pastures	0.021	-0.009	0.052	0.304
40-49 yrs.-Pastures	0.058	0.031	0.086	0.000
>50 yrs.-Pastures	0.053	0.023	0.084	0.000
30-39 yrs.-15-29 yrs.	-0.014	-0.045	0.017	0.704
40-49 yrs.-15-29 yrs.	0.023	-0.005	0.051	0.170
>50 yrs.-15-29 yrs.	0.018	-0.014	0.049	0.512
40-49 yrs.-30-39 yrs.	0.037	0.003	0.072	0.029
>50 yrs.-30-39 yrs.	0.032	-0.005	0.069	0.122
>50 yrs.-40-49 yrs.	-0.005	-0.040	0.029	0.993
Mollisols-Alfisols	-0.003	-0.019	0.014	0.762
Verrucomicrobiota				
15-29 yrs.-Pastures	-0.055	-0.100	-0.011	0.007
30-39 yrs.-Pastures	-0.033	-0.091	0.025	0.517
40-49 yrs.-Pastures	-0.060	-0.113	-0.007	0.017
>50 yrs.-Pastures	-0.056	-0.114	0.003	0.067
30-39 yrs.-15-29 yrs.	0.023	-0.037	0.082	0.822
40-49 yrs.-15-29 yrs.	-0.005	-0.058	0.049	0.999
>50 yrs.-15-29 yrs.	0.000	-0.060	0.059	1.000
40-49 yrs.-30-39 yrs.	-0.027	-0.093	0.038	0.772
>50 yrs.-30-39 yrs.	-0.023	-0.093	0.047	0.891
>50 yrs.-40-49 yrs.	0.004	-0.061	0.070	1.000
Mollisols-Alfisols	-0.030	-0.056	-0.004	0.023
Chloroflexi				
15-29 yrs.-Pastures	-0.018	-0.049	0.013	0.482
30-39 yrs.-Pastures	-0.030	-0.071	0.011	0.250
40-49 yrs.-Pastures	-0.033	-0.070	0.004	0.100
>50 yrs.-Pastures	-0.012	-0.053	0.028	0.912
30-39 yrs.-15-29 yrs.	-0.012	-0.053	0.030	0.931
40-49 yrs.-15-29 yrs.	-0.015	-0.053	0.023	0.803
>50 yrs.-15-29 yrs.	0.006	-0.036	0.047	0.995
40-49 yrs.-30-39 yrs.	-0.003	-0.049	0.043	1.000
>50 yrs.-30-39 yrs.	0.017	-0.032	0.067	0.855
>50 yrs.-40-49 yrs.	0.021	-0.025	0.067	0.719
Mollisols-Alfisols	0.003	-0.015	0.021	0.713

Myxococcota				
15-29 yrs.-Pastures	-0.006	-0.019	0.006	0.649
30-39 yrs.-Pastures	-0.009	-0.026	0.007	0.509
40-49 yrs.-Pastures	0.012	-0.003	0.027	0.174
>50 yrs.-Pastures	0.005	-0.011	0.022	0.892
30-39 yrs.-15-29 yrs.	-0.003	-0.020	0.013	0.984
40-49 yrs.-15-29 yrs.	0.018	0.003	0.033	0.012
>50 yrs.-15-29 yrs.	0.011	-0.005	0.028	0.316
40-49 yrs.-30-39 yrs.	0.021	0.003	0.039	0.017
>50 yrs.-30-39 yrs.	0.015	-0.005	0.034	0.244
>50 yrs.-40-49 yrs.	-0.007	-0.025	0.012	0.856
Mollisols-Alfisols	-0.007	-0.015	0.000	0.044
Firmicutes				
15-29 yrs.-Pastures	-0.002	-0.023	0.019	0.998
30-39 yrs.-Pastures	-0.023	-0.051	0.004	0.141
40-49 yrs.-Pastures	0.007	-0.018	0.032	0.941
>50 yrs.-Pastures	-0.009	-0.037	0.019	0.906
30-39 yrs.-15-29 yrs.	-0.021	-0.050	0.007	0.234
40-49 yrs.-15-29 yrs.	0.009	-0.017	0.035	0.863
>50 yrs.-15-29 yrs.	-0.007	-0.035	0.022	0.968
40-49 yrs.-30-39 yrs.	0.030	-0.001	0.062	0.065
>50 yrs.-30-39 yrs.	0.015	-0.019	0.048	0.736
>50 yrs.-40-49 yrs.	-0.016	-0.047	0.016	0.640
Mollisols-Alfisols	-0.138	-0.026	-0.002	0.022
Latescibacterota				
15-29 yrs.-Pastures	0.023	0.010	0.037	0.000
30-39 yrs.-Pastures	0.003	-0.015	0.021	0.992
40-49 yrs.-Pastures	0.014	-0.002	0.031	0.110
>50 yrs.-Pastures	0.017	-0.001	0.035	0.072
30-39 yrs.-15-29 yrs.	-0.021	-0.039	-0.002	0.021
40-49 yrs.-15-29 yrs.	-0.009	-0.026	0.008	0.565
>50 yrs.-15-29 yrs.	-0.006	-0.025	0.012	0.876
40-49 yrs.-30-39 yrs.	0.012	-0.009	0.032	0.508
>50 yrs.-30-39 yrs.	0.014	-0.008	0.036	0.361
>50 yrs.-40-49 yrs.	0.003	-0.018	0.023	0.996
Mollisols-Alfisols	-0.001	-0.010	0.008	0.784

Table 7. Linear regression with cubic smoothing spline results from Bacterial phyla relative abundance across forest age. The effect of soil order is evaluated by conducting an ANOVA on regression with and without soil order.

	Res.Df	RSS	df	Sum of Sq	F value	p-value	R2
Actinobacteriota							
Forest Age	71	0.27442					0.28
Forest Age * Soil Order	67	0.214	4	0.060418	4.729	0.002019	0.18
Acidobacteriota							
Forest Age	71	0.099728					0.2
Forest Age * Soil Order	67	0.070411	4	0.029317	6.9741	9.35E-05	0.41
Proteobacteria							
Forest Age	71	0.14144					0.03
Forest Age * Soil Order	67	0.13245	4	0.008992	1.1371	0.3467	0.03
Planctomycetota							
Forest Age	71	0.07967					0.24
Forest Age * Soil Order	67	0.07519	4	0.00448	0.9981	0.4149	0.24
Methylomirabilota							
Forest Age	71	0.051625					0.33
Forest Age * Soil Order	67	0.049951	4	0.001674	0.5614	0.6915	0.31
Verrucomicrobiota							
Forest Age	71	0.15244					0.15
Forest Age * Soil Order	67	0.11343	4	0.039016	5.7615	0.000479	0.33
Chloroflexi							
Forest Age	71	0.084191					0.03
Forest Age * Soil Order	67	0.082414	4	0.001776	0.361	0.8355	< 0.01
Myxococcota							
Forest Age	71	0.015644					0.07
Forest Age * Soil Order	67	0.014292	4	0.001352	1.5849	0.1885	0.1
Firmicutes							
Forest Age	71	0.044817					< 0.01
Forest Age * Soil Order	67	0.040314	4	0.004503	1.871	0.1257	0.04
Latescibacterota							
Forest Age	71	0.018822					0.21
Forest Age * Soil Order	67	0.016464	4	0.002358	2.399	0.05866	0.27

Table 8. Analysis of Variance Results for testing differences of fungal phyla relative abundances among forest age classes, soil orders, and their interaction.

		df	Sum of Squares	Mean of Squares	F value	p-value
Ascomycota						
	Forest Age Class	4	0.957	0.239	1.590	0.187
	Soil Order	1	0.215	0.215	1.429	0.236
	Forest Age Class * Soil Order	4	0.732	0.183	1.217	0.312
	Residuals	65	9.780	0.151		
Basidiomycota						
	Forest Age Class	4	0.314	0.078	1.683	0.165
	Soil Order	1	0.001	0.001	0.019	0.890
	Forest Age Class * Soil Order	4	0.338	0.085	1.816	0.136
	Residuals	65	3.027	0.047		
Chytridiomycota						
	Forest Age Class	4	0.000	0.000	0.611	0.656
	Soil Order	1	0.000	0.000	0.792	0.377
	Forest Age Class * Soil Order	4	0.000	0.000	0.597	0.666
	Residuals	65	0.006	0.000		
Glomeromycota						
	Forest Age Class	4	0.019	0.005	2.430	0.056
	Soil Order	1	0.001	0.001	0.277	0.601
	Forest Age Class * Soil Order	4	0.001	0.000	0.162	0.957
	Residuals	65	0.129	0.002		
Kickxellomycota						
	Forest Age Class	4	0.000	0.000	0.800	0.530
	Soil Order	1	0.000	0.000	0.006	0.937
	Forest Age Class * Soil Order	4	0.000	0.000	0.531	0.713
	Residuals	65	0.010	0.000		
Mortierellomycota						
	Forest Age Class	4	1.030	0.257	5.721	0.001
	Soil Order	1	0.209	0.209	4.636	0.035
	Forest Age Class * Soil Order	4	0.306	0.077	1.703	0.160
	Residuals	65	2.924	0.045		

Table 9. Tukey HSD results for SOC pair comparisons for Fungal phyla relative abundance differences among forest age classes, soil orders, and their interaction.

	Difference	Lowe CI	Upper CI	p-value
Ascomycota				
15-29 yrs.-Pastures	-0.153	-0.480	0.175	0.688
30-39 yrs.-Pastures	-0.232	-0.660	0.196	0.555
40-49 yrs.-Pastures	-0.220	-0.607	0.168	0.510
>50 yrs.-Pastures	-0.332	-0.761	0.096	0.202
30-39 yrs.-15-29 yrs.	-0.079	-0.516	0.357	0.986
40-49 yrs.-15-29 yrs.	-0.067	-0.464	0.330	0.990
>50 yrs.-15-29 yrs.	-0.180	-0.616	0.257	0.778
40-49 yrs.-30-39 yrs.	0.012	-0.471	0.496	1.000
>50 yrs.-30-39 yrs.	-0.100	-0.617	0.416	0.982
>50 yrs.-40-49 yrs.	-0.113	-0.596	0.371	0.966
Mollisols-Alfisols	0.130	-0.053	0.313	0.162
Basidiomycota				
15-29 yrs.-Pastures	0.080	-0.103	0.264	0.735
30-39 yrs.-Pastures	0.176	-0.064	0.416	0.253
40-49 yrs.-Pastures	0.063	-0.154	0.280	0.925
>50 yrs.-Pastures	0.172	-0.068	0.412	0.273
30-39 yrs.-15-29 yrs.	0.095	-0.149	0.340	0.810
40-49 yrs.-15-29 yrs.	-0.017	-0.239	0.205	0.999
>50 yrs.-15-29 yrs.	0.092	-0.153	0.336	0.832
40-49 yrs.-30-39 yrs.	-0.113	-0.383	0.158	0.771
>50 yrs.-30-39 yrs.	-0.004	-0.293	0.286	1.000
>50 yrs.-40-49 yrs.	0.109	-0.162	0.380	0.793
Mollisols-Alfisols	-0.024	-0.128	0.080	0.652
Chytridiomycota				
15-29 yrs.-Pastures	0.003	-0.005	0.011	0.850
30-39 yrs.-Pastures	-0.002	-0.012	0.009	0.990
40-49 yrs.-Pastures	-0.002	-0.011	0.008	0.990
>50 yrs.-Pastures	0.001	-0.009	0.012	0.995
30-39 yrs.-15-29 yrs.	-0.005	-0.015	0.006	0.744
40-49 yrs.-15-29 yrs.	-0.004	-0.014	0.005	0.701
>50 yrs.-15-29 yrs.	-0.001	-0.012	0.009	0.995
40-49 yrs.-30-39 yrs.	0.000	-0.012	0.012	1.000
>50 yrs.-30-39 yrs.	0.003	-0.010	0.016	0.955
>50 yrs.-40-49 yrs.	0.003	-0.009	0.015	0.953
Mollisols-Alfisols	-0.002	-0.007	0.002	0.351

Glomeromycota				
15-29 yrs.-Pastures	-0.031	-0.068	0.005	0.118
30-39 yrs.-Pastures	-0.038	-0.085	0.010	0.180
40-49 yrs.-Pastures	-0.037	-0.080	0.006	0.122
>50 yrs.-Pastures	-0.032	-0.080	0.015	0.318
30-39 yrs.-15-29 yrs.	-0.006	-0.055	0.042	0.996
40-49 yrs.-15-29 yrs.	-0.006	-0.049	0.038	0.997
>50 yrs.-15-29 yrs.	-0.001	-0.049	0.047	1.000
40-49 yrs.-30-39 yrs.	0.001	-0.053	0.054	1.000
>50 yrs.-30-39 yrs.	0.005	-0.052	0.062	0.999
>50 yrs.-40-49 yrs.	0.005	-0.049	0.058	0.999
Mollisols-Alfisols	0.008	-0.013	0.029	0.448
Kickxellomycota				
15-29 yrs.-Pastures	0.001	-0.009	0.011	1.000
30-39 yrs.-Pastures	-0.007	-0.020	0.007	0.643
40-49 yrs.-Pastures	-0.004	-0.016	0.008	0.843
>50 yrs.-Pastures	-0.002	-0.015	0.011	0.992
30-39 yrs.-15-29 yrs.	-0.007	-0.021	0.006	0.563
40-49 yrs.-15-29 yrs.	-0.005	-0.017	0.007	0.769
>50 yrs.-15-29 yrs.	-0.003	-0.016	0.011	0.978
40-49 yrs.-30-39 yrs.	0.002	-0.013	0.017	0.994
>50 yrs.-30-39 yrs.	0.004	-0.012	0.021	0.935
>50 yrs.-40-49 yrs.	0.002	-0.013	0.017	0.992
Mollisols-Alfisols	0.000	-0.005	0.006	0.862
Mortierellomycota				
15-29 yrs.-Pastures	0.089	-0.096	0.275	0.661
30-39 yrs.-Pastures	0.037	-0.206	0.279	0.993
40-49 yrs.-Pastures	0.348	0.128	0.567	0.000
>50 yrs.-Pastures	0.141	-0.101	0.384	0.484
30-39 yrs.-15-29 yrs.	-0.053	-0.300	0.194	0.975
40-49 yrs.-15-29 yrs.	0.258	0.034	0.483	0.016
>50 yrs.-15-29 yrs.	0.052	-0.196	0.299	0.977
40-49 yrs.-30-39 yrs.	0.311	0.037	0.585	0.018
>50 yrs.-30-39 yrs.	0.105	-0.188	0.397	0.854
>50 yrs.-40-49 yrs.	-0.206	-0.480	0.067	0.227
Mollisols-Alfisols	-0.102	-0.214	0.019	0.074

Table 10. Linear regression with cubic smoothing spline results from Fungal phyla relative abundance across forest age. The effect of soil order is evaluated by conducting an ANOVA on regression with and without soil order.

	Res.Df	RSS	df	Sum of Sq	F value	p-value	R2
Ascomycota							
Forest Age	71	2.3098					0.06
Forest Age * Soil Order	67	2.145	4	0.16478	1.2867	0.284	0.08
Basidiomycota							
Forest Age	71	1.4716					0.01
Forest Age * Soil Order	67	1.3612	4	0.11045	1.3592	0.2575	0.03
Chytridiomycota							
Forest Age	71	0.006236					< 0.01
Forest Age * Soil Order	67	0.006037	4	0.000199	0.552	0.6982	< 0.01
Glomeromycota							
Forest Age	71	0.098293					0.1
Forest Age * Soil Order	67	0.097103	4	0.00119	0.2053	0.9346	0.06
Kickxellomycota							
Forest Age	71	0.010254					< 0.01
Forest Age * Soil Order	67	0.009973	4	0.000281	0.4718	0.7562	< 0.01
Mortierellomycota							
Forest Age	71	1.2633					0.18
Forest Age * Soil Order	67	1.1449	4	0.11838	1.7319	0.1533	0.21

Table 11. Bacterial and Fungal functional groups' relative abundances

	Functional Group	Relative abundance (%)
Bacteria		
	Aromatic compound degradation	0.123
	Cellulolysis	0.787
	Chemoheterotrophy	4.57
	Chitinolysis	0.203
	Fermentation	0.0482
	Methylotrophy	0.0987
Fungi		
	Pathotroph	12.2
	Saprotroph	34.1
	Symbiotroph	15.5
	Arbuscular mycorrhizae	1.06
	Ectomycorrhizae	2.01
	Plant Pathogens	1.24
	Wood Saprotroph	5.28

Table 12. Analysis of Variance Results for testing differences of Bacterial functional groups relative abundances among forest age classes, soil orders, and their interaction.

		df	Sum of Squares	Mean of Squares	F value	p-value
Chitinolysis						
	Forest Age Class	4	0.0002	0.0000	8.3260	0.0000
	Soil Order	1	0.0001	0.0001	14.5180	0.0003
	Forest Age Class * Soil Order	4	0.0002	0.0000	7.7240	0.0000
	Residuals	65	0.0004	0.0000		
Fermentation						
	Forest Age Class	4	0.0000	0.0000	2.6220	0.0427
	Soil Order	1	0.0000	0.0000	0.2250	0.6368
	Forest Age Class * Soil Order	4	0.0000	0.0000	1.7620	0.1472
	Residuals	65	0.0001	0.0000		
Methylotrophy						
	Forest Age Class	4	0.0001	0.0000	4.2940	0.0038
	Soil Order	1	0.0000	0.0000	0.3630	0.5490
	Forest Age Class * Soil Order	4	0.0000	0.0000	1.2180	0.3118
	Residuals	65	0.0006	0.0000		
Methanol Oxidation						
	Forest Age Class	4	0.0000	0.0000	0.7620	0.5540
	Soil Order	1	0.0000	0.0000	1.4830	0.2280
	Forest Age Class * Soil Order	4	0.0000	0.0000	0.7500	0.5610
	Residuals	65	0.0000	0.0000		
Cellulolysis						
	Forest Age Class	4	0.0026	0.0006	2.6120	0.0433
	Soil Order	1	0.0017	0.0017	7.1220	0.0096
	Forest Age Class * Soil Order	4	0.0010	0.0002	1.0230	0.4023
	Residuals	65	0.0159	0.0002		
Aromatic Compound Degradation						
	Forest Age Class	4	0.0001	0.0000	3.5890	0.0105
	Soil Order	1	0.0000	0.0000	0.0030	0.9571
	Forest Age Class * Soil Order	4	0.0000	0.0000	0.1450	0.9647
	Residuals	65	0.0003	0.0000		
Aromatic Hydrocarbon Degradation						
	Forest Age Class	4	3.57E-06	8.92E-07	0.794	0.5335
	Soil Order	1	4.48E-06	4.49E-06	3.992	0.0499
	Forest Age Class * Soil Order	4	3.49E-06	8.73E-07	0.777	0.5443
	Residuals	65	7.30E-05	1.12E-06		
Chemoheterotrophy						
	Forest Age Class	4	0.017764	0.004441	11.216	5.57E-07
	Soil Order	1	0.000104	0.000104	0.262	0.6106
	Forest Age Class * Soil Order	4	0.003543	0.000886	2.237	0.0746
	Residuals	65	0.025736	0.000396		

Table 13. Tukey HSD results for SOC pair comparisons for Bacterial functional groups relative abundance differences among forest age classes, soil orders, and their interaction.

	Difference	Lowe CI	Upper CI	p-value
Chitinolysis				
15-29 yrs.-Pastures	0.004	0.001	0.006	0.001
30-39 yrs.-Pastures	0.003	0.000	0.006	0.080
40-49 yrs.-Pastures	0.000	-0.003	0.003	0.993
>50 yrs.-Pastures	0.002	-0.002	0.005	0.672
30-39 yrs.-15-29 yrs.	-0.001	-0.004	0.003	0.982
40-49 yrs.-15-29 yrs.	-0.003	-0.006	0.000	0.029
>50 yrs.-15-29 yrs.	-0.002	-0.006	0.001	0.383
40-49 yrs.-30-39 yrs.	-0.003	-0.006	0.001	0.289
>50 yrs.-30-39 yrs.	-0.002	-0.006	0.003	0.828
>50 yrs.-40-49 yrs.	0.001	-0.003	0.005	0.915
Mollisols-Alfisols	0.186	0.000	0.003	0.017
Fermentation				
15-29 yrs.-Pastures	0.000	-0.001	0.000	0.471
30-39 yrs.-Pastures	0.000	-0.001	0.001	0.971
40-49 yrs.-Pastures	-0.001	-0.002	0.000	0.068
>50 yrs.-Pastures	-0.001	-0.002	0.000	0.143
30-39 yrs.-15-29 yrs.	0.000	-0.001	0.001	0.970
40-49 yrs.-15-29 yrs.	0.000	-0.001	0.001	0.722
>50 yrs.-15-29 yrs.	0.000	-0.001	0.001	0.825
40-49 yrs.-30-39 yrs.	-0.001	-0.002	0.001	0.508
>50 yrs.-30-39 yrs.	-0.001	-0.002	0.001	0.612
>50 yrs.-40-49 yrs.	0.000	-0.001	0.001	1.000
Mollisols-Alfisols	0.000	0.000	0.006	0.574
Methylotrophy				
15-29 yrs.-Pastures	-0.003	-0.005	0.000	0.012
30-39 yrs.-Pastures	-0.003	-0.006	0.001	0.195
40-49 yrs.-Pastures	-0.003	-0.006	0.000	0.016
>50 yrs.-Pastures	-0.003	-0.006	0.000	0.072
30-39 yrs.-15-29 yrs.	0.000	-0.003	0.004	0.997
40-49 yrs.-15-29 yrs.	0.000	-0.003	0.003	0.994
>50 yrs.-15-29 yrs.	0.000	-0.003	0.003	1.000
40-49 yrs.-30-39 yrs.	-0.001	-0.004	0.003	0.967
>50 yrs.-30-39 yrs.	-0.001	-0.004	0.003	0.995
>50 yrs.-40-49 yrs.	0.000	-0.003	0.004	0.999
Mollisols-Alfisols	0.000	-0.002	0.001	0.778

Methanol Oxidation					
	15-29 yrs.-Pastures	0.000	0.000	0.000	0.597
	30-39 yrs.-Pastures	0.000	-0.001	0.000	0.801
	40-49 yrs.-Pastures	0.000	-0.001	0.000	0.736
	>50 yrs.-Pastures	0.000	-0.001	0.000	0.801
	30-39 yrs.-15-29 yrs.	0.000	0.000	0.000	1.000
	40-49 yrs.-15-29 yrs.	0.000	0.000	0.000	1.000
	>50 yrs.-15-29 yrs.	0.000	0.000	0.000	1.000
	40-49 yrs.-30-39 yrs.	0.000	0.000	0.000	1.000
	>50 yrs.-30-39 yrs.	0.000	-0.001	0.001	1.000
	>50 yrs.-40-49 yrs.	0.000	0.000	0.000	1.000
	Mollisols-Alfisols	0.000	0.000	0.000	0.286
Cellulolysis					
	15-29 yrs.-Pastures	-0.013	-0.027	0.001	0.065
	30-39 yrs.-Pastures	0.001	-0.017	0.019	1.000
	40-49 yrs.-Pastures	-0.010	-0.026	0.006	0.432
	>50 yrs.-Pastures	-0.008	-0.026	0.010	0.720
	30-39 yrs.-15-29 yrs.	0.014	-0.004	0.032	0.218
	40-49 yrs.-15-29 yrs.	0.003	-0.013	0.020	0.982
	>50 yrs.-15-29 yrs.	0.005	-0.013	0.023	0.933
	40-49 yrs.-30-39 yrs.	-0.011	-0.031	0.010	0.581
	>50 yrs.-30-39 yrs.	-0.009	-0.030	0.013	0.788
	>50 yrs.-40-49 yrs.	0.002	-0.018	0.022	0.999
	Mollisols-Alfisols	-0.009	-0.017	-0.019	0.015
Aromatic Compound Degradation					
	15-29 yrs.-Pastures	-0.001	-0.003	0.000	0.179
	30-39 yrs.-Pastures	-0.001	-0.003	0.001	0.782
	40-49 yrs.-Pastures	-0.003	-0.005	-0.001	0.006
	>50 yrs.-Pastures	-0.002	-0.004	0.000	0.100
	30-39 yrs.-15-29 yrs.	0.000	-0.002	0.003	0.981
	40-49 yrs.-15-29 yrs.	-0.001	-0.003	0.001	0.484
	>50 yrs.-15-29 yrs.	-0.001	-0.003	0.002	0.933
	40-49 yrs.-30-39 yrs.	-0.002	-0.004	0.001	0.361
	>50 yrs.-30-39 yrs.	-0.001	-0.004	0.002	0.789
	>50 yrs.-40-49 yrs.	0.001	-0.002	0.003	0.970
	Mollisols-Alfisols	0.000	-0.001	0.011	0.933

Aromatic Hydrocarbon Degradation				
15-29 yrs.-Pastures	0.000	-0.001	0.001	0.935
30-39 yrs.-Pastures	0.000	-0.002	0.001	0.785
40-49 yrs.-Pastures	-0.001	-0.002	0.001	0.634
>50 yrs.-Pastures	-0.001	-0.002	0.001	0.716
30-39 yrs.-15-29 yrs.	0.000	-0.001	0.001	0.984
40-49 yrs.-15-29 yrs.	0.000	-0.001	0.001	0.952
>50 yrs.-15-29 yrs.	0.000	-0.001	0.001	0.966
40-49 yrs.-30-39 yrs.	0.000	-0.001	0.001	1.000
>50 yrs.-30-39 yrs.	0.000	-0.001	0.001	1.000
>50 yrs.-40-49 yrs.	0.000	-0.001	0.001	1.000
Mollisols-Alfisols	0.000	-0.001	0.000	0.068
Chemoheterotrophy				
15-29 yrs.-Pastures	-0.022	-0.039	-0.005	0.004
30-39 yrs.-Pastures	0.023	0.000	0.045	0.046
40-49 yrs.-Pastures	-0.024	-0.044	-0.004	0.013
>50 yrs.-Pastures	-0.016	-0.038	0.007	0.296
30-39 yrs.-15-29 yrs.	0.045	0.022	0.068	0.000
40-49 yrs.-15-29 yrs.	-0.002	-0.022	0.019	1.000
>50 yrs.-15-29 yrs.	0.007	-0.016	0.030	0.925
40-49 yrs.-30-39 yrs.	-0.047	-0.072	-0.021	0.000
>50 yrs.-30-39 yrs.	-0.038	-0.065	-0.011	0.002
>50 yrs.-40-49 yrs.	0.008	-0.017	0.034	0.892
Mollisols-Alfisols	0.001	-0.010	0.013	0.821

Table 14. Linear regression with cubic smoothing spline results from Bacterial Functional groups' relative abundance across forest age. The effect of soil order is evaluated by conducting an ANOVA on regression with and without soil order.

	Res.Df	RSS	df	Sum of Sq	F value	p-value	R2
Chitinolysis							
Forest Age	71	0.000675					0.16
Forest Age * Soil Order	67	0.000452	4	0.000223	8.2863	1.72E-05	0.41
Fermentation							
Forest Age	71	6.70E-05					0.07
Forest Age * Soil Order	67	6.56E-05	4	1.34E-06	0.3432	0.8478	0.04
Methylotrophy							
Forest Age	71	0.000586					0.17
Forest Age * Soil Order	67	0.000546	4	4.00E-05	1.2264	0.308	0.18
Methanol Oxidation							
Forest Age	71	1.02E-05					<0.01
Forest Age * Soil Order	67	9.55E-06	4	6.60E-07	1.1573	0.3376	0.01
Cellulolysis							
Forest Age	71	0.018222					0.05
Forest Age * Soil Order	67	0.015348	4	2.87E-03	3.137	0.01998	0.15
Aromatic Compound Degradation							
Forest Age	71	0.000322					0.11
Forest Age * Soil Order	67	0.00032	4	1.75E-06	0.0918	0.9847	0.06
Chemoheterotrophy							
Forest Age	71	0.035434					0.09
Forest Age * Soil Order	67	0.033015	4	0.00242	1.2277	0.3075	0.1

Table 15. Analysis of Variance Results for testing differences of Fungal functional groups relative abundances among forest age classes, soil orders, and their interaction.

			df	Sum of Squares	Mean of Squares	F value	p-value
Throphic							
	Pathotroph						
		Forest Age Class	4	0.114	0.029	1.716	0.157
		Soil Order	1	0.022	0.022	1.335	0.252
		Forest Age Class * Soil Order	4	0.042	0.010	0.628	0.644
		Residuals	65	1.084	0.017		
	Saprotroph						
		Forest Age Class	4	1.434	0.359	2.959	0.026
		Soil Order	1	0.448	0.448	3.700	0.059
		Forest Age Class * Soil Order	4	0.607	0.152	1.252	0.298
		Residuals	65	7.878	0.121		
	Symbiotroph						
		Forest Age Class	4	0.830	0.208	4.463	0.003
		Soil Order	1	0.317	0.317	6.827	0.011
		Forest Age Class * Soil Order	4	0.381	0.095	2.049	0.098
		Residuals	65	3.022	0.047		
Guilds							
	Arbuscular mycorrhizae						
		Forest Age Class	4	0.019	0.005	2.430	0.056
		Soil Order	1	0.001	0.001	0.277	0.601
		Forest Age Class * Soil Order	4	0.001	0.000	0.162	0.957
		Residuals	65	0.129	0.002		
	Ectomycorrhizae						
		Forest Age Class	4	0.010	0.003	2.765	0.035
		Soil Order	1	0.001	0.001	0.951	0.333
		Forest Age Class * Soil Order	4	0.008	0.002	2.185	0.080
		Residuals	65	0.060	0.001		
	Plant Pathogens						
		Forest Age Class	4	0.002	0.000	0.675	0.612
		Soil Order	1	0.004	0.004	6.164	0.016
		Forest Age Class * Soil Order	4	0.003	0.001	1.067	0.380
		Residuals	65	0.038	0.001		
	Wood Saprotroph						
		Forest Age Class	4	0.012	0.003	1.263	0.294
		Soil Order	1	0.002	0.002	0.704	0.405
		Forest Age Class * Soil Order	4	0.021	0.005	2.286	0.070
		Residuals	65	0.151	0.002		

Table 16. Tukey HSD results for SOC pair comparisons for fungal functional groups relative abundance differences among forest age classes, soil orders, and their interaction.

	Difference	Lowe CI	Upper CI	p-value
Pathotroph				
15-29 yrs.-Pastures	-0.06662	-0.17377	0.040538	0.416205
30-39 yrs.-Pastures	-0.12193	-0.2621	0.018242	0.117936
40-49 yrs.-Pastures	-0.03705	-0.16383	0.089743	0.924188
>50 yrs.-Pastures	-0.06053	-0.2007	0.079637	0.746048
30-39 yrs.-15-29 yrs.	-0.05531	-0.19818	0.087563	0.814143
40-49 yrs.-15-29 yrs.	0.029572	-0.1002	0.159344	0.968279
>50 yrs.-15-29 yrs.	0.006085	-0.13679	0.148959	0.999953
40-49 yrs.-30-39 yrs.	0.084883	-0.07325	0.243015	0.563998
>50 yrs.-30-39 yrs.	0.061396	-0.10765	0.230446	0.846731
>50 yrs.-40-49 yrs.	-0.02349	-0.18162	0.134646	0.993594
Mollisols-Alfisols	-0.0255	-0.08617	0.035184	0.451125
Saprotroph				
15-29 yrs.-Pastures	0.117153	-0.18174	0.416047	0.807224
30-39 yrs.-Pastures	-0.04655	-0.43753	0.34443	0.997274
40-49 yrs.-Pastures	0.384192	0.030535	0.737848	0.026531
>50 yrs.-Pastures	0.043654	-0.34733	0.434636	0.997878
30-39 yrs.-15-29 yrs.	-0.1637	-0.56223	0.234821	0.779199
40-49 yrs.-15-29 yrs.	0.267039	-0.09494	0.629018	0.246567
>50 yrs.-15-29 yrs.	-0.0735	-0.47203	0.325026	0.985445
40-49 yrs.-30-39 yrs.	0.430743	-0.01034	0.871831	0.058886
>50 yrs.-30-39 yrs.	0.090205	-0.38134	0.561747	0.983315
>50 yrs.-40-49 yrs.	-0.34054	-0.78163	0.100549	0.206429
Mollisols-Alfisols	-0.14074	-0.31234	0.038595	0.10644
Saprotroph				
15-29 yrs.-Pastures	0.016138	-0.17676	0.20904	0.999317
30-39 yrs.-Pastures	-0.06739	-0.31972	0.184947	0.944308
40-49 yrs.-Pastures	0.276303	0.048059	0.504547	0.00984
>50 yrs.-Pastures	0.078707	-0.17363	0.33104	0.905691
30-39 yrs.-15-29 yrs.	-0.08352	-0.34073	0.173678	0.892398
40-49 yrs.-15-29 yrs.	0.260165	0.02655	0.49378	0.0215
>50 yrs.-15-29 yrs.	0.062569	-0.19463	0.31977	0.959896
40-49 yrs.-30-39 yrs.	0.343689	0.059019	0.628359	0.010109
>50 yrs.-30-39 yrs.	0.146093	-0.15823	0.450418	0.664778
>50 yrs.-40-49 yrs.	-0.1976	-0.48227	0.087074	0.304468
Mollisols-Alfisols	-0.11989	-0.23221	7564128	0.367816

Arbuscular mycorrhizae				
15-29 yrs.-Pastures	-0.03145	-0.06762	0.004724	0.118256
30-39 yrs.-Pastures	-0.03773	-0.08505	0.009588	0.179895
40-49 yrs.-Pastures	-0.03699	-0.07979	0.00581	0.122025
>50 yrs.-Pastures	-0.03239	-0.07971	0.014925	0.318129
30-39 yrs.-15-29 yrs.	-0.00628	-0.05451	0.041951	0.996139
40-49 yrs.-15-29 yrs.	-0.00554	-0.04935	0.038268	0.996551
>50 yrs.-15-29 yrs.	-0.00094	-0.04918	0.047289	0.999998
40-49 yrs.-30-39 yrs.	0.00074	-0.05264	0.054123	1
>50 yrs.-30-39 yrs.	0.005338	-0.05173	0.062407	0.99894
>50 yrs.-40-49 yrs.	0.004598	-0.04879	0.057982	0.999234
Mollisols-Alfisols	0.008013	-0.01293	0.028955	0.448151
Ectomycorrhizae				
15-29 yrs.-Pastures	-0.00096	-0.02724	0.025317	0.999975
30-39 yrs.-Pastures	-0.00062	-0.035	0.03375	0.999999
40-49 yrs.-Pastures	0.003397	-0.0277	0.034489	0.99805
>50 yrs.-Pastures	0.035874	0.0015	0.070248	0.036515
30-39 yrs.-15-29 yrs.	0.000337	-0.0347	0.035375	1
40-49 yrs.-15-29 yrs.	0.004358	-0.02747	0.036182	0.995312
>50 yrs.-15-29 yrs.	0.036835	0.001798	0.071872	0.034508
40-49 yrs.-30-39 yrs.	0.00402	-0.03476	0.042799	0.998412
>50 yrs.-30-39 yrs.	0.036498	-0.00496	0.077954	0.110542
>50 yrs.-40-49 yrs.	0.032477	-0.0063	0.071256	0.14315
Mollisols-Alfisols	-0.00852	-0.02367	0.006627	0.265948
Plant Pathogens				
15-29 yrs.-Pastures	-0.00795	-0.02898	0.013086	0.827197
30-39 yrs.-Pastures	-0.00573	-0.03324	0.021781	0.97714
40-49 yrs.-Pastures	-0.01003	-0.03492	0.014852	0.790691
>50 yrs.-Pastures	0.002764	-0.02475	0.030275	0.998597
30-39 yrs.-15-29 yrs.	0.002215	-0.02583	0.030257	0.999455
40-49 yrs.-15-29 yrs.	-0.00209	-0.02756	0.023383	0.99937
>50 yrs.-15-29 yrs.	0.010709	-0.01733	0.038751	0.821504
40-49 yrs.-30-39 yrs.	-0.0043	-0.03534	0.026734	0.995084
>50 yrs.-30-39 yrs.	0.008494	-0.02469	0.041674	0.951958
>50 yrs.-40-49 yrs.	0.012797	-0.01824	0.043834	0.776835
Mollisols-Alfisols	-0.01399	-0.02514	-0.00284	0.01461
Wood Saprotroph				
15-29 yrs.-Pastures	-0.00041	-0.04215	0.04132	1
30-39 yrs.-Pastures	-0.01942	-0.07401	0.035176	0.856328
40-49 yrs.-Pastures	-0.0139	-0.06328	0.035483	0.933249
>50 yrs.-Pastures	0.026271	-0.02832	0.080864	0.662758
30-39 yrs.-15-29 yrs.	-0.019	-0.07465	0.036644	0.873565
40-49 yrs.-15-29 yrs.	-0.01348	-0.06403	0.03706	0.944508
>50 yrs.-15-29 yrs.	0.026685	-0.02896	0.082332	0.665652
40-49 yrs.-30-39 yrs.	0.005518	-0.05607	0.067108	0.999105
>50 yrs.-30-39 yrs.	0.045687	-0.02015	0.111529	0.304781
>50 yrs.-40-49 yrs.	0.040169	-0.02142	0.101758	0.366985
Mollisols-Alfisols	-0.01022	-0.03349	0.013056	0.384456

Table17. Linear regression with cubic smoothing spline results from Fungal Functional groups' relative abundance across forest age. The effect of soil order is evaluated by conducting an ANOVA on regression with and without soil order.

	Res.Df	RSS	df	Sum of Sq	F value	p-value	R2
Pathotroph							
Forest Age	71	0.66893					0.02
Forest Age * Soil Order	67	0.61281	4	0.05612	1.5339	0.2024	0.05
Saprotroph							
Forest Age	71	1.9478					<0.01
Forest Age * Soil Order	67	1.788	4	0.15976	1.4966	0.2131	0.04
Symbiotroph							
Forest Age	71	1.3301					0.08
Forest Age * Soil Order	67	1.1657	4	0.16437	2.3618	0.06192	0.14
Arbuscular Mycorrhizae							
Forest Age	71	0.098293					0.1
Forest Age * Soil Order	67	0.097103	4	0.00119	0.2053	0.9346	0.06
Ectomycorrhizae							
Forest Age	71	0.063852					0.03
Forest Age * Soil Order	67	0.061964	4	0.001888	0.5102	0.7284	<0.01
Plant Pathogens							
Forest Age	71	0.03983					<0.01
Forest Age * Soil Order	67	0.034651	4	0.005179	2.5035	0.05038	0.07
Wood Saprotroph							
Forest Age	71	0.1452					<0.01
Forest Age * Soil Order	67	0.1207	4	0.024502	3.4003	0.01362	0.13

Table 18. Regression analysis results from testing SOC to Bacterial relative abundance and its interaction with forest age class and soil order.

	Estimate	Estimate	Error	t- value	p-value	F value	R2
Actinobacteriota							
	Intercept	0.081	0.014	5.697	0.000	4.88	0.37
	Relative Abundance	-0.129	0.062	-2.084	0.041		
	15-29 yrs	0.021	0.026	0.824	0.413		
	30-39 yrs.	-0.036	0.030	-1.189	0.239		
	40-49 yrs	0.035	0.026	1.323	0.191		
	>50 yrs.	0.059	0.033	1.821	0.073		
	Soil Order	0.027	0.021	1.312	0.194		
	15-29 yrs * Relative Abundance	-0.057	0.111	-0.518	0.606		
	30-39 yrs. *Relative Abundance	0.061	0.128	0.475	0.636		
	40-49 yrs * Relative Abundance	-0.091	0.118	-0.769	0.445		
	>50 yrs. * Relative Abundance	-0.277	0.139	-1.987	0.051		
	Soil Order * Relative Abundance	-0.005	0.084	-0.057	0.955		
Acidobact							
	Intercept	0.017	0.023	0.726	0.470	5.76	0.41
	Relative Abundance	0.200	0.112	1.788	0.079		
	15-29 yrs	0.017	0.033	0.535	0.594		
	30-39 yrs.	0.107	0.049	2.197	0.032		
	40-49 yrs	0.005	0.043	0.113	0.910		
	>50 yrs.	-0.239	0.062	-3.844	0.000		
	Soil Order	0.011	0.028	0.405	0.687		
	15-29 yrs * Relative Abundance	-0.036	0.153	-0.239	0.812		
	30-39 yrs. *Relative Abundance	-0.650	0.237	-2.746	0.008		
	40-49 yrs * Relative Abundance	0.037	0.197	0.187	0.852		
	>50 yrs. * Relative Abundance	1.156	0.304	3.807	0.000		
	Soil Order * Relative Abundance	0.028	0.139	0.201	0.841		
Proteobacteria							
	Intercept	0.021	0.014	1.437	0.156	8.83	0.54
	Relative Abundance	0.181	0.083	2.189	0.032		
	15-29 yrs	-0.005	0.027	-0.170	0.865		
	30-39 yrs.	0.066	0.026	2.549	0.013		
	40-49 yrs	-0.001	0.025	-0.033	0.974		
	>50 yrs.	-0.041	0.027	-1.527	0.132		
	Soil Order	-0.020	0.020	-0.964	0.339		
	15-29 yrs * Relative Abundance	0.082	0.165	0.501	0.618		
	30-39 yrs. *Relative Abundance	-0.420	0.145	-2.900	0.005		
	40-49 yrs * Relative Abundance	0.075	0.148	0.502	0.617		
	>50 yrs. * Relative Abundance	0.059	0.137	0.430	0.669		
	Soil Order * Relative Abundance	0.195	0.110	1.779	0.080		

Planctomycetota							
	Intercept	0.048	0.014	3.359	0.001	3.82	0.30
	Relative Abundance	0.101	0.242	0.417	0.678		
	15-29 yrs	-0.018	0.025	-0.717	0.476		
	30-39 yrs.	-0.024	0.026	-0.940	0.351		
	40-49 yrs	0.023	0.035	0.658	0.513		
	>50 yrs.	-0.020	0.032	-0.623	0.535		
	Soil Order	0.017	0.014	1.243	0.218		
	15-29 yrs * Relative Abundance	0.505	0.477	1.059	0.294		
	30-39 yrs. *Relative Abundance	0.164	0.423	0.388	0.699		
	40-49 yrs * Relative Abundance	-0.122	0.659	-0.185	0.854		
	>50 yrs. * Relative Abundance	0.134	0.514	0.261	0.795		
	Soil Order * Relative Abundance	0.075	0.161	0.468	0.641		
Methylomirabilota							
	Intercept	0.050	0.012	4.235	0.000	4.89	0.39
	Relative Abundance	0.031	0.141	0.223	0.824		
	15-29 yrs	0.004	0.026	0.138	0.891		
	30-39 yrs.	-0.065	0.025	-2.592	0.012		
	40-49 yrs	0.014	0.023	0.597	0.553		
	>50 yrs.	0.022	0.023	0.966	0.338		
	Soil Order	0.041	0.013	3.222	0.002		
	15-29 yrs * Relative Abundance	-0.017	0.280	-0.062	0.951		
	30-39 yrs. *Relative Abundance	0.688	0.303	2.270	0.027		
	40-49 yrs * Relative Abundance	-0.057	0.241	-0.237	0.814		
	>50 yrs. * Relative Abundance	-0.494	0.301	-1.639	0.106		
	Soil Order * Relative Abundance	-0.290	0.165	-1.760	0.083		
Verrucomicrobiota							
	Intercept	0.074	0.009	8.459	0.000	7.44	0.49
	Relative Abundance	-0.398	0.177	-2.253	0.028		
	15-29 yrs	0.017	0.016	1.040	0.302		
	30-39 yrs.	-0.046	0.016	-2.895	0.005		
	40-49 yrs	0.029	0.020	1.475	0.145		
	>50 yrs.	0.019	0.017	1.112	0.270		
	Soil Order	0.026	0.009	2.779	0.007		
	15-29 yrs * Relative Abundance	-0.202	0.358	-0.563	0.575		
	30-39 yrs. *Relative Abundance	0.387	0.324	1.195	0.236		
	40-49 yrs * Relative Abundance	-0.318	0.477	-0.667	0.507		
	>50 yrs. * Relative Abundance	-0.290	0.362	-0.803	0.425		
	Soil Order * Relative Abundance	-0.151	0.147	-1.030	0.307		

Chloroflexi							
	Intercept	0.035	0.008	4.428	0.000	7.10	0.48
	Relative Abundance	0.428	0.146	2.929	0.005		
	15-29 yrs	0.001	0.014	0.095	0.925		
	30-39 yrs.	0.023	0.016	1.454	0.151		
	40-49 yrs	0.024	0.017	1.411	0.163		
	>50 yrs.	-0.069	0.020	-3.488	0.001		
	Soil Order	0.033	0.010	3.187	0.002		
	15-29 yrs * Relative Abundance	0.122	0.216	0.565	0.574		
	30-39 yrs. *Relative Abundance	-0.971	0.289	-3.355	0.001		
	40-49 yrs * Relative Abundance	-0.210	0.335	-0.628	0.532		
	>50 yrs. * Relative Abundance	1.368	0.392	3.486	0.001		
	Soil Order * Relative Abundance	-0.271	0.146	-1.853	0.069		
Myxococcota							
	Intercept	0.031	0.013	2.456	0.017	7.76	0.50
	Relative Abundance	0.390	0.280	1.397	0.167		
	15-29 yrs	0.010	0.030	0.327	0.745		
	30-39 yrs.	-0.044	0.025	-1.722	0.090		
	40-49 yrs	0.054	0.023	2.336	0.023		
	>50 yrs.	0.040	0.018	2.207	0.031		
	Soil Order	0.050	0.015	3.464	0.001		
	15-29 yrs * Relative Abundance	-0.068	0.640	-0.107	0.915		
	30-39 yrs. *Relative Abundance	0.829	0.568	1.461	0.149		
	40-49 yrs * Relative Abundance	-0.815	0.503	-1.620	0.110		
	>50 yrs. * Relative Abundance	-1.309	0.421	-3.109	0.003		
	Soil Order * Relative Abundance	-0.740	0.338	-2.190	0.032		
Firmicutes							
	Intercept	0.054	0.006	8.618	0.000	5.38	0.3941
	Relative Abundance	0.241	0.242	0.998	0.322		
	15-29 yrs	0.020	0.011	1.796	0.077		
	30-39 yrs.	-0.018	0.012	-1.548	0.127		
	40-49 yrs	0.015	0.010	1.571	0.121		
	>50 yrs.	-0.031	0.012	-2.699	0.009		
	Soil Order	0.028	0.009	3.032	0.004		
	15-29 yrs * Relative Abundance	-0.332	0.307	-1.080	0.284		
	30-39 yrs. *Relative Abundance	-0.819	0.620	-1.322	0.191		
	40-49 yrs * Relative Abundance	-0.197	0.233	-0.845	0.401		
	>50 yrs. * Relative Abundance	1.585	0.761	2.083	0.041		
	Soil Order * Relative Abundance	-0.361	0.274	-1.321	0.191		

Latescibacterota							
	Intercept	0.046	0.007	6.284	0.000	5.17	0.38
	Relative Abundance	0.106	0.315	0.334	0.739		
	15-29 yrs	0.009	0.013	0.692	0.492		
	30-39 yrs.	-0.034	0.014	-2.363	0.021		
	40-49 yrs	-0.014	0.015	-0.902	0.371		
	>50 yrs.	-0.001	0.017	-0.040	0.968		
	Soil Order	0.020	0.009	2.164	0.034		
	15-29 yrs * Relative Abundance	-0.039	0.440	-0.090	0.929		
	30-39 yrs. *Relative Abundance	0.685	0.701	0.976	0.333		
	40-49 yrs * Relative Abundance	0.925	0.526	1.758	0.084		
	>50 yrs. * Relative Abundance	-0.166	0.895	-0.185	0.854		
	Soil Order * Relative Abundance	0.276	0.338	0.815	0.418		
cellulolysis							
	Intercept	0.059	0.005	11.561	< 2e-16	6.15	0.43
	Relative Abundance	-0.527	0.288	-1.828	0.072		
	15-29 yrs	0.013	0.009	1.513	0.135		
	30-39 yrs.	-0.023	0.009	-2.585	0.012		
	40-49 yrs	0.019	0.007	2.564	0.013		
	>50 yrs.	0.005	0.008	0.552	0.583		
	Soil Order	0.024	0.007	3.613	0.001		
	15-29 yrs * Relative Abundance	-0.357	0.669	-0.533	0.596		
	30-39 yrs. *Relative Abundance	0.249	0.619	0.402	0.689		
	40-49 yrs * Relative Abundance	-0.795	0.598	-1.331	0.188		
	>50 yrs. * Relative Abundance	-0.313	0.463	-0.677	0.501		
	Soil Order * Relative Abundance	-0.709	0.407	-1.740	0.087		
chemoheterotrophy							
	Intercept	0.066	0.014	4.794	0.000	5.80	0.42
	Relative Abundance	-0.236	0.217	-1.086	0.282		
	15-29 yrs	-0.004	0.030	-0.138	0.890		
	30-39 yrs.	0.040	0.029	1.405	0.165		
	40-49 yrs	0.020	0.023	0.886	0.379		
	>50 yrs.	-0.027	0.024	-1.105	0.273		
	Soil Order	-0.018	0.015	-1.177	0.244		
	15-29 yrs * Relative Abundance	0.180	0.500	0.359	0.721		
	30-39 yrs. *Relative Abundance	-0.828	0.444	-1.863	0.067		
	40-49 yrs * Relative Abundance	-0.228	0.414	-0.552	0.583		
	>50 yrs. * Relative Abundance	0.057	0.331	0.172	0.864		
	Soil Order * Relative Abundance	0.619	0.224	2.769	0.007		

chitinolysis							
	Intercept	0.044	0.004	10.129	0.000	9.65	0.56
	Relative Abundance	4.413	1.608	2.745	0.008 **		
	15-29 yrs	0.007	0.008	0.980	0.331		
	30-39 yrs.	-0.002	0.008	-0.289	0.774		
	40-49 yrs	0.010	0.008	1.306	0.196		
	>50 yrs.	-0.020	0.009	-2.409	0.019 *		
	Soil Order	0.009	0.007	1.336	0.186		
	15-29 yrs * Relative Abundance	1.385	3.264	0.424	0.673		
	30-39 yrs. *Relative Abundance	-3.093	2.767	-1.118	0.268		
	40-49 yrs * Relative Abundance	-3.101	2.816	-1.101	0.275		
	>50 yrs. * Relative Abundance	1.215	2.268	0.536	0.594		
	Soil Order * Relative Abundance	1.427	1.954	0.730	0.468		
aromatic_compound_degradation							
	Intercept	0.055	0.005	10.234	0.000	3.75	0.29
	Relative Abundance	0.698	4.484	0.156	0.877		
	15-29 yrs	0.009	0.009	0.981	0.330		
	30-39 yrs.	-0.022	0.010	-2.143	0.036		
	40-49 yrs	0.017	0.008	2.077	0.042		
	>50 yrs.	0.001	0.011	0.102	0.919		
	Soil Order	0.022	0.008	2.888	0.005		
	15-29 yrs * Relative Abundance	3.282	8.000	0.410	0.683		
	30-39 yrs. *Relative Abundance	-3.681	7.444	-0.495	0.623		
	40-49 yrs * Relative Abundance	-10.311	12.617	-0.817	0.417		
	>50 yrs. * Relative Abundance	-10.496	10.301	-1.019	0.312		
	Soil Order * Relative Abundance	1.087	2.704	0.402	0.689		
fermentation							
	Intercept	0.048	0.005	10.259	0.000	4.89	0.37
	Relative Abundance	14.604	8.296	1.760	0.083		
	15-29 yrs	0.006	0.008	0.729	0.469		
	30-39 yrs.	-0.014	0.009	-1.585	0.118		
	40-49 yrs	0.020	0.007	2.667	0.010		
	>50 yrs.	-0.013	0.008	-1.550	0.126		
	Soil Order	0.028	0.007	3.968	0.000		
	15-29 yrs * Relative Abundance	14.634	18.208	0.804	0.425		
	30-39 yrs. *Relative Abundance	-14.332	15.711	-0.912	0.365		
	40-49 yrs * Relative Abundance	-25.150	17.616	-1.428	0.158		
	>50 yrs. * Relative Abundance	1.310	13.167	0.100	0.921		
	Soil Order * Relative Abundance	-13.200	7.779	-1.697	0.095 .		
methylotrophy							
	Intercept	0.046	0.004	11.803	< 2e-16	0.52	0.44
	Relative Abundance	16.702	4.369	3.823	0.000		
	15-29 yrs	0.010	0.007	1.524	0.132		
	30-39 yrs.	-0.014	0.008	-1.740	0.087		
	40-49 yrs	0.016	0.006	2.626	0.011		
	>50 yrs.	-0.024	0.008	-2.908	0.005		
	Soil Order	0.023	0.006	3.775	0.000		
	15-29 yrs * Relative Abundance	19.400	7.559	2.566	0.013		
	30-39 yrs. *Relative Abundance	-20.534	8.224	-2.497	0.015		
	40-49 yrs * Relative Abundance	-26.580	8.310	-3.199	0.002		
	>50 yrs. * Relative Abundance	NA	NA	NA	NA		
	Soil Order * Relative Abundance	-1.097	2.560	-0.429	0.670		

Table 19. Regression analysis results from testing SOC to Fungal relative abundance and its interaction with forest age class and soil order.

	Estimate	Estimate	Error	t- value	p-value	F value	R2
Ascomycota							
	Intercept	0.035947	0.006301	5.706	3.34E-07	7.93	0.5074
	Relative Abundance	0.037288	0.010861	3.433	0.001059		
	15-29 yrs	0.000323	0.011167	0.029	0.976993		
	30-39 yrs.	0.010313	0.011485	0.898	0.372608		
	40-49 yrs	0.022064	0.009877	2.234	0.029054		
	>50 yrs.	-0.0273	0.010932	-2.498	0.015129		
	Soil Order	0.033217	0.009442	3.518	0.000813		
	15-29 yrs * Relative Abundance	0.013717	0.020432	0.671	0.504452		
	30-39 yrs. *Relative Abundance	-0.0639	0.020255	-3.155	0.002463		
	40-49 yrs * Relative Abundance	-0.01614	0.015783	-1.023	0.310295		
	>50 yrs. * Relative Abundance	0.046796	0.016956	2.76	0.007562		
	Soil Order * Relative Abundance	-0.0267	0.013341	-2.001	0.04968		
Basidiomycota							
	Intercept	0.061018	0.007474	8.164	1.84E-11	5.694	0.411
	Relative Abundance	-0.03081	0.024013	-1.283	0.204146		
	15-29 yrs	0.017826	0.011205	1.591	0.116632		
	30-39 yrs.	-0.05	0.012395	-4.034	0.000151		
	40-49 yrs	0.011397	0.010018	1.138	0.259596		
	>50 yrs.	-0.00183	0.012816	-0.143	0.886645		
	Soil Order	0.023724	0.009631	2.463	0.016508		
	15-29 yrs * Relative Abundance	-0.04538	0.035799	-1.268	0.209557		
	30-39 yrs. *Relative Abundance	0.101669	0.033928	2.997	0.003902		
	40-49 yrs * Relative Abundance	0.012944	0.029437	0.44	0.661632		
	>50 yrs. * Relative Abundance	0.009648	0.035962	0.268	0.789356		
	Soil Order * Relative Abundance	0.010462	0.033329	0.314	0.754626		
Glomeromycota							
	Intercept	0.054569	0.00489	11.16	< 2e-16	3.98	0.307
	Relative Abundance	-3.03787	1.786165	-1.701	0.093916		
	15-29 yrs	0.00929	0.009112	1.02	0.311811		
	30-39 yrs.	-0.02644	0.009216	-2.869	0.005598		
	40-49 yrs	0.015535	0.008276	1.877	0.06515		
	>50 yrs.	-0.00402	0.008834	-0.455	0.650495		
	Soil Order	0.023439	0.006441	3.639	0.000554		
	15-29 yrs * Relative Abundance	-0.47607	1.397289	-0.341	0.734457		
	30-39 yrs. *Relative Abundance	7.553919	4.493007	1.681	0.097664		
	40-49 yrs * Relative Abundance	0.927845	2.20803	0.42	0.675759		
	>50 yrs. * Relative Abundance	-8.1362	6.070124	-1.34	0.18494		
	Soil Order * Relative Abundance	-0.05002	0.164411	-0.304	0.76195		

Kickxellomycota							
	Intercept	0.056284	0.00502	11.213	< 2e-16	4.674	0.3532
	Relative Abundance	-0.7376	0.419488	-1.758	0.08355		
	15-29 yrs	0.020453	0.009419	2.171	0.03367		
	30-39 yrs.	-0.02348	0.009459	-2.482	0.01574		
	40-49 yrs	0.016841	0.008584	1.962	0.05419		
	>50 yrs.	0.002585	0.009038	0.286	0.77583		
	Soil Order	0.021552	0.007451	2.892	0.00524		
	15-29 yrs * Relative Abundance	-1.25444	0.782845	-1.602	0.11407		
	30-39 yrs. *Relative Abundance	1.054322	0.951952	1.108	0.27227		
	40-49 yrs * Relative Abundance	-0.18558	0.702723	-0.264	0.79257		
	>50 yrs. * Relative Abundance	-2.05204	1.037895	-1.977	0.05241		
	Soil Order * Relative Abundance	-0.0144	0.553857	-0.026	0.97935		
Mortierellomycota							
	Intercept	0.05696	0.005398	10.552	1.47E-15	5.015	0.3738
	Relative Abundance	-0.06545	0.046485	-1.408	0.164042		
	15-29 yrs	0.011428	0.008547	1.337	0.185983		
	30-39 yrs.	-0.037	0.010089	-3.667	0.000506		
	40-49 yrs	0.018322	0.008147	2.249	0.028015		
	>50 yrs.	0.016836	0.011421	1.474	0.145421		
	Soil Order	0.023403	0.00708	3.306	0.001568		
	15-29 yrs * Relative Abundance	-0.09267	0.08879	-1.044	0.300635		
	30-39 yrs. *Relative Abundance	0.326227	0.118486	2.753	0.0077		
	40-49 yrs * Relative Abundance	-0.04851	0.048153	-1.007	0.317645		
	>50 yrs. * Relative Abundance	-0.34745	0.128173	-2.711	0.008639		
	Soil Order * Relative Abundance	-0.02319	0.054396	-0.426	0.671297		
Pathotroph							
	Intercept	3.71E-02	8.08E-03	4.593	2.14E-05	5.212	3.85E-01
	Relative Abundance	7.59E-02	4.94E-02	1.535	0.12983		
	15-29 yrs	-3.03E-05	1.29E-02	-0.002	0.99813		
	30-39 yrs.	-2.54E-02	1.65E-02	-1.533	0.13018		
	40-49 yrs	7.26E-03	1.34E-02	0.543	0.58922		
	>50 yrs.	7.79E-03	1.91E-02	0.409	0.68414		
	Soil Order	5.06E-02	1.05E-02	4.844	8.63E-06		
	15-29 yrs * Relative Abundance	5.51E-02	6.19E-02	0.889	0.37729		
	30-39 yrs. *Relative Abundance	2.96E-02	1.19E-01	0.25	0.80345		
	40-49 yrs * Relative Abundance	3.35E-02	6.55E-02	0.511	0.61131		
	>50 yrs. * Relative Abundance	-1.00E-01	1.50E-01	-0.668	0.50627		
	Soil Order * Relative Abundance	-1.49E-01	4.91E-02	-3.023	0.00362		

Saprotroph							
	Intercept	0.044486	0.009024	4.93	6.29E-06	3.986	0.3074
	Relative Abundance	0.00839	0.012151	0.69	0.49242		
	15-29 yrs	0.000977	0.015267	0.064	0.94917		
	30-39 yrs.	-0.02611	0.017567	-1.486	0.14223		
	40-49 yrs	0.014022	0.014452	0.97	0.33564		
	>50 yrs.	0.00403	0.018459	0.218	0.82789		
	Soil Order	0.041762	0.014026	2.977	0.00412		
	15-29 yrs * Relative Abundance	0.010664	0.022113	0.482	0.63131		
	30-39 yrs. *Relative Abundance	0.009627	0.027386	0.352	0.72635		
	40-49 yrs * Relative Abundance	0.002232	0.017393	0.128	0.89831		
	>50 yrs. * Relative Abundance	-0.0156	0.028946	-0.539	0.59192		
	Soil Order * Relative Abundance	-0.02713	0.021348	-1.271	0.20837		
Symbiotroph							
	Intercept	0.043991	0.007822	5.624	4.57E-07	3.878	0.2996
	Relative Abundance	0.026526	0.03407	0.779	0.43913		
	15-29 yrs	0.009407	0.012871	0.731	0.46756		
	30-39 yrs.	-0.02512	0.015698	-1.6	0.11454		
	40-49 yrs	0.017386	0.0107	1.625	0.10918		
	>50 yrs.	-0.00384	0.017181	-0.224	0.82371		
	Soil Order	0.03207	0.011125	2.883	0.00539		
	15-29 yrs * Relative Abundance	-0.01335	0.043861	-0.304	0.7619		
	30-39 yrs. *Relative Abundance	0.016704	0.082717	0.202	0.84062		
	40-49 yrs * Relative Abundance	-0.0038	0.028807	-0.132	0.8954		
	>50 yrs. * Relative Abundance	0.001359	0.103578	0.013	0.98957		
	Soil Order * Relative Abundance	-0.02929	0.047344	-0.619	0.53839		
Arbuscular mycorrhizae							
	Intercept	0.054569	0.00489	11.16	< 2e-16	3.98	0.307
	Relative Abundance	-3.03787	1.786165	-1.701	0.093916		
	15-29 yrs	0.00929	0.009112	1.02	0.311811		
	30-39 yrs.	-0.02644	0.009216	-2.869	0.005598		
	40-49 yrs	0.015535	0.008276	1.877	0.06515		
	>50 yrs.	-0.00402	0.008834	-0.455	0.650495		
	Soil Order	0.023439	0.006441	3.639	0.000554		
	15-29 yrs * Relative Abundance	-0.47607	1.397289	-0.341	0.734457		
	30-39 yrs. *Relative Abundance	7.553919	4.493007	1.681	0.097664		
	40-49 yrs * Relative Abundance	0.927845	2.20803	0.42	0.675759		
	>50 yrs. * Relative Abundance	-8.1362	6.070124	-1.34	0.18494		
	Soil Order * Relative Abundance	-0.05002	0.164411	-0.304	0.76195		

Ectomycorrhizae						
Intercept	0.055672	0.005609	9.925	1.68E-14	5.543	0.4031
Relative Abundance	-0.23251	0.162414	-1.432	0.1572		
15-29 yrs	0.017619	0.009134	1.929	0.05824		
30-39 yrs.	-0.04363	0.010388	-4.2	8.54E-05		
40-49 yrs	-0.00126	0.009213	-0.137	0.89159		
>50 yrs.	-0.004	0.011419	-0.35	0.72732		
Soil Order	0.025574	0.008353	3.062	0.00323		
15-29 yrs * Relative Abundance	-0.42704	0.198317	-2.153	0.03513		
30-39 yrs. *Relative Abundance	0.844524	0.298414	2.83	0.00624		
40-49 yrs * Relative Abundance	0.604037	0.28669	2.107	0.03911		
>50 yrs. * Relative Abundance	-0.17797	0.398718	-0.446	0.65688		
Soil Order * Relative Abundance	0.060658	0.288395	0.21	0.83409		
Plant Pathogens						
Intercept	0.055156	0.005426	10.164	6.60E-15	4.206	0.3228
Relative Abundance	-0.44044	0.275967	-1.596	0.11549		
15-29 yrs	0.017003	0.009196	1.849	0.06914		
30-39 yrs.	-0.03326	0.009966	-3.337	0.00142		
40-49 yrs	0.004196	0.010565	0.397	0.69256		
>50 yrs.	-0.00999	0.011615	-0.86	0.39294		
Soil Order	0.021509	0.00888	2.422	0.01831		
15-29 yrs * Relative Abundance	-0.66331	0.429565	-1.544	0.12756		
30-39 yrs. *Relative Abundance	0.926068	0.462644	2.002	0.04963		
40-49 yrs * Relative Abundance	1.204296	0.781817	1.54	0.12848		
>50 yrs. * Relative Abundance	0.526747	0.71414	0.738	0.4635		
Soil Order * Relative Abundance	0.647176	0.718837	0.9	0.37138		
Wood Sapotrophs						
Intercept	0.034212	0.007166	4.774	1.12E-05	6.052	0.4289
Relative Abundance	0.26201	0.080399	3.259	0.00181		
15-29 yrs	0.005724	0.013853	0.413	0.68086		
30-39 yrs.	-0.01579	0.013929	-1.134	0.26114		
40-49 yrs	0.012515	0.012008	1.042	0.30131		
>50 yrs.	-0.02256	0.011883	-1.899	0.0622		
Soil Order	0.028902	0.010924	2.646	0.01028		
15-29 yrs * Relative Abundance	0.013249	0.13952	0.095	0.92465		
30-39 yrs. *Relative Abundance	-0.2041	0.153989	-1.325	0.18982		
40-49 yrs * Relative Abundance	-0.01367	0.148234	-0.092	0.9268		
>50 yrs. * Relative Abundance	0.301264	0.162529	1.854	0.06848		
Soil Order * Relative Abundance	-0.08412	0.135003	-0.623	0.5355		

References

Antwis, R. E., S. M. Griffiths, X. A. Harrison, P. Aranega-Bou, A. Arce, A. S. Bettridge, F. L. Brailsford, A. de Menezes, A. Devaynes, K. M. Forbes, E. L. Fry, I. Goodhead, E. Haskell, C. Heys, C. James, S. R. Johnston, G. R. Lewis, Z. Lewis, M. C. Macey, A. McCarthy, J. E. McDonald, N. L. Mejia-Florez, D. O'Brien, C. Orland, M. Pautasso, W.

D. K. Reid, H. A. Robinson, K. Wilson, and W. J. Sutherland. 2017. Fifty important research questions in microbial ecology. FEMS Microbiology Ecology 93.

Araújo, A. S. F., S. Cesarz, L. F. C. Leite, C. D. Borges, S. M. Tsai, and N. Eisenhauer. 2013. Soil microbial properties and temporal stability in degraded and restored lands of Northeast Brazil. Soil Biology and Biochemistry 66:175–181.

Averill, C. 2016. Slowed decomposition in ectomycorrhizal ecosystems is independent of plant chemistry. Special issue: Food web interactions in the root zone: influences on community and ecosystem dynamics 102:52–54.

Averill, C., and C. V. Hawkes. 2016. Ectomycorrhizal fungi slow soil carbon cycling. Ecology Letters 19:937–947.

Averill, C., B. L. Turner, and A. C. Finzi. 2014. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. Nature 505:543–545.

Bai, Z., X. Wu, J.-J. Lin, H.-T. Xie, H.-S. Yuan, and C. Liang. 2019. Litter-, soil- and C:N-stoichiometry-associated shifts in fungal communities along a subtropical forest succession. CATENA 178:350–358.

Baskaran, P., R. Hyvönen, S. L. Berglund, K. E. Clemmensen, G. I. Ågren, B. D. Lindahl, and S. Manzoni. 2017. Modelling the influence of ectomycorrhizal decomposition on plant nutrition and soil carbon sequestration in boreal forest ecosystems. New Phytologist 213:1452–1465.

Bastida, F., T. Hernández, J. Albaladejo, and C. García. 2013. Phylogenetic and functional changes in the microbial community of long-term restored soils under semiarid climate. Soil Biology and Biochemistry 65:12–21.

Becerra-Lucio, A. A., N. Y. Labrín-Sotomayor, P. A. Becerra-Lucio, F. I. Trujillo-Elisea, A. T. Chávez-Bárcenas, S. Machkour-M'Rabet, and Y. J. Peña-Ramírez. 2021. Diversity and Interactomics of Bacterial Communities Associated with Dominant Trees During Tropical Forest Recovery. Current Microbiology 78:3417–3429.

Bengtsson-Palme, J., M. Ryberg, M. Hartmann, S. Branco, Z. Wang, A. Godhe, P. De Wit, M. Sánchez-García, I. Ebersberger, F. de Sousa, A. Amend, A. Jumpponen, M. Unterseher, E. Kristiansson, K. Abarenkov, Y. J. K. Bertrand, K. Sanli, K. M. Eriksson, U. Vik, V. Veldre, and R. H. Nilsson. 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods in Ecology and Evolution 4:914–919.

Benny, G.L., Smith, M.E., Kirk, P.M., Tretter, E.D., White, M.M. 2016. Challenges and Future Perspectives in the Systematics of Kickxellomycotina, Mortierellomycotina, Mucoromycotina, and Zoopagomycotina . In: Li, DW. (eds) Biology of Microfungi. Fungal Biology. Springer, Cham.

Bever, J. D., K. M. Westover, and J. Antonovics. 1997. Incorporating the Soil Community into Plant Population Dynamics: The Utility of the Feedback Approach. Journal of Ecology 85:561–573.

Bissett, A., M. V. Brown, S. D. Siciliano, and P. H. Thrall. 2013. Microbial community responses to anthropogenically induced environmental change: towards a systems approach. Ecology Letters 16:128–139.

Blagodatskaya, E., M. Tarkka, C. Knief, R. Koller, S. Peth, V. Schmidt, S. Spielvogel, D. Uteau, M. Weber, and B. S. Razavi. 2021. Bridging Microbial Functional Traits With Localized Process Rates at Soil Interfaces. Frontiers in Microbiology 12.

Bolyen, Evan, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. 2019 Nature biotechnology 37.8. 852-857.

Brandeis, T. J., E. H. Helmer, H. Marcano-Vega, and A. E. Lugo. 2009. Climate shapes the novel plant communities that form after deforestation in Puerto Rico and the U.S. Virgin Islands. Forest Ecology and Management 258:1704–1718.

Brown, M. E., and M. C. Chang. 2014. Exploring bacterial lignin degradation. Biocatalysis and biotransformation⬢ Bioinorganic chemistry 19:1–7.

da C Jesus, E., T. L. Marsh, J. M. Tiedje, and F. M. de S Moreira. 2009. Changes in land use alter the structure of bacterial communities in Western Amazon soils. The ISME Journal 3:1004–1011.

Cairney, J. W. G. 2012. Extramatrical mycelia of ectomycorrhizal fungi as moderators of carbon dynamics in forest soil. Soil Biology and Biochemistry 47:198–208.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods 13:581–583.

Cho, H., M. Kim, B. Tripathi, and J. Adams. 2017. Changes in Soil Fungal Community Structure with Increasing Disturbance Frequency. Microbial Ecology 74:62–77.

Cline, L. C., and D. R. Zak. 2015. Soil microbial communities are shaped by plant-driven changes in resource availability during secondary succession. Ecology 96:3374–3385.

Cornwell, W. K., J. H. C. Cornelissen, K. Amatangelo, E. Dorrepaal, V. T. Eviner, O. Godoy, S. E. Hobbie, B. Hoorens, H. Kurokawa, N. Pérez-Harguindeguy, H. M. Quested, L. S. Santiago, D. A. Wardle, I. J. Wright, R. Aerts, S. D. Allison, P. Van Bodegom, V. Brovkin, A. Chatain, T. V. Callaghan, S. Díaz, E. Garnier, D. E. Gurvich, E. Kazakou, J. A. Klein, J. Read, P. B. Reich, N. A. Soudzilovskaia, M. V. Vaieretti, and M. Westoby. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters 11:1065–1071.

Cox, F., N. Barsoum, E. A. Lilleskov, and M. I. Bidartondo. 2010. Nitrogen availability is a primary determinant of conifer mycorrhizas across complex environmental gradients. Ecology Letters 13:1103–1113.

Daly, C., E. H. Helmer, and M. Quiñones. 2003. Mapping the climate of Puerto Rico, Vieques and Culebra. International Journal of Climatology 23:1359–1381.

Deacon, J. W. 1997. Modern mycology. Blackwell Science Oxford.

Díaz-Vallejo, E. J., M. Seeley, A. P. Smith, and E. Marín-Spiotta. 2021. A meta-analysis of tropical land-use change effects on the soil microbiome: Emerging patterns and knowledge gaps. Biotropica 53:738–752.

Djemiel, C., P.-A. Maron, S. Terrat, S. Dequiedt, A. Cottin, and L. Ranjard. 2022. Inferring microbiota functions from taxonomic genes: a review. GigaScience 11.

Ewel, J. J., and J. L. Whitmore. 1973. The ecological life zones of Puerto Rico and the US Virgin Islands. USDA Forest Service, Institute of Tropical Forestry, Research Paper ITF-018 18.

Fierer, N. 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nature Reviews Microbiology 15:579–590.

Franco, P. A., P. L. Weaver, and S. Eggen-McIntosh. 1997. Forest resources of Puerto Rico, 1990. Resour. Bull. SRS-22. Asheville, NC: US Department of Agriculture, Forest Service, Southern Research Station. 4.5 p. Resour. Bull. SRS-22. Asheville, NC: US Department of Agriculture, Forest Service, Southern Research Station. 4.5 p.

Goodfellow, M., and S. T. Williams. 1983. Ecology of actinomycetes. Annual review of microbiology 37:189–216.

Grau, H. R., T. M. Aide, J. K. Zimmerman, J. R. Thomlinson, E. Helmer, and X. Zou. 2003. The Ecological Consequences of Socioeconomic and Land-Use Changes in Postagriculture Puerto Rico. BioScience 53:1159–1168.

Green, J. L., B. J. M. Bohannan, and R. J. Whitaker. 2008. Microbial Biogeography: From Taxonomy to Traits. Science 320:1039–1043.

Hafich, K., E. J. Perkins, J. B. Hauge, D. Barry, and W. D. Eaton. 2012. Implications of land management on soil microbial communities and nutrient cycle dynamics in the lowland tropical forest of northern Costa Rica. Tropical Ecology 53:215–224.

Hannula, S. E., H. T. S. Boschker, W. de Boer, and J. A. van Veen. 2012. 13C pulse-labeling assessment of the community structure of active fungi in the rhizosphere of a genetically starch-modified potato (Solanum tuberosum) cultivar and its parental isoline. New Phytologist 194:784–799.

Harris, D., W. R. Horwáth, and C. Van Kessel. 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Science Society of America Journal 65:1853–1856.

Janssen, P.H., 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied and environmental microbiology, 72:1719-1728.

Herman, J., D. Moorhead, and B. Berg. 2008. The relationship between rates of lignin and cellulose decay in aboveground forest litter. Soil Biology and Biochemistry 40:2620–2626.

Jastrow, J. D., J. E. Amonette, and V. L. Bailey. 2007. Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. Climatic Change 80:5–23.

Kaspari, M., and S. P. Yanoviak. 2008. Biogeography of litter depth in tropical forests: evaluating the phosphorus growth rate hypothesis. Functional Ecology 22:919–923.

Kennaway, T., and E. H. Helmer. 2007. The forest types and ages cleared for land development in Puerto Rico. GIScience & Remote Sensing 44:356–382.

Kim, M., E. Heo, H. Kang, and J. Adams. 2013. Changes in Soil Bacterial Community Structure with Increasing Disturbance Frequency. Microbial Ecology 66:171–181.

Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander, and P. D. Schloss. 2013. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. Applied and Environmental Microbiology 79:5112–5120.

Kulakowski, R., A. Lausen, E. Low-Decarie, and B. Lausen. 2018. Classification Methods for 16S rRNA Based Functional Annotation. Archives of Data Science, Series A (Online First) 4:A17,-23.

Kyaschenko, J., K. E. Clemmensen, E. Karltun, and B. D. Lindahl. 2017. Below-ground organic matter accumulation along a boreal forest fertility gradient relates to guild interaction within fungal communities. Ecology Letters 20:1546–1555.

Lauber, C.L., Hamady, M., Knight, R. and Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and environmental microbiology, 75:5111-5120.

Leal, P.L., Stürmer, S.L. and Siqueira, J.O., 2009. Occurrence and diversity of arbuscular mycorrhizal fungi in trap cultures from soils under different land use systems in the Amazon, Brazil. Brazilian Journal of Microbiology, 40:111-121.

Legendre, P., and E. D. Gallagher. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280.

Lindahl, B. D., and A. Tunlid. 2015. Ectomycorrhizal fungi – potential organic matter decomposers, yet not saprotrophs. New Phytologist 205:1443–1447.

Louca, S., L. W. Parfrey, and M. Doebeli. 2016. Decoupling function and taxonomy in the global ocean microbiome. Science 353:1272–1277.

Luizão, F. J., R. C. C. Luizão, and J. Proctor. 2007. Soil acidity and nutrient deficiency in central Amazonian heath forest soils. Plant Ecology 192:209–224.

Malik, A. A., J. B. H. Martiny, E. L. Brodie, A. C. Martiny, K. K. Treseder, and S. D. Allison. 2020. Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. The ISME Journal 14:1–9.

Marín-Spiotta, E., and S. Sharma. 2013. Carbon storage in successional and plantation forest soils: a tropical analysis: Carbon in reforested and plantation soils. Global Ecology and Biogeography 22:105–117.

Marín-Spiotta, E., W. L. Silver, and R. Ostertag. 2007.Long-Term patterns in tropical reforestation: Plant community and aboveground biomass accumulation. Ecological Applications 17:828–839.

Marín-Spiotta, E., C. W. Swanston, M. S. Torn, W. L. Silver, and S. D. Burton. 2008. Chemical and mineral control of soil carbon turnover in abandoned tropical pastures. Geoderma 143:49–62.

McGee, K. M., W. D. Eaton, S. Shokralla, and M. Hajibabaei. 2019. Determinants of Soil Bacterial and Fungal Community Composition Toward Carbon-Use Efficiency Across Primary and Secondary Forests in a Costa Rican Conservation Area. Microbial Ecology 77:148–167.

McMurdie, P. J., and S. Holmes. 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLOS ONE 8:1–11.

Mueller, R. C., L. Gallegos-Graves, D. R. Zak, and C. R. Kuske. 2016. Assembly of Active Bacterial and Fungal Communities Along a Natural Environmental Gradient. Microbial Ecology 71:57–67.

Navarrete, A. A., A. M. Venturini, K. M. Meyer, A. M. Klein, J. M. Tiedje, B. J. M. Bohannan, K. Nüsslein, S. M. Tsai, and J. L. M. Rodrigues. 2015. Differential Response of Acidobacteria Subgroups to Forest-to-Pasture Conversion and Their Biogeographic Patterns in the Western Brazilian Amazon. Frontiers in Microbiology 6.

Nelson, D. a, and L. Sommers. 1983. Total carbon, organic carbon, and organic matter. Methods of soil analysis: Part 2 chemical and microbiological properties 9:539–579.

Nguyen, N. H., Z. Song, S. T. Bates, S. Branco, L. Tedersoo, J. Menke, J. S. Schilling, and P. G. Kennedy. 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20:241–248.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2013. Community ecology package. R package version 2:321–326.

Pajares, S., B. J. M. Bohannan, and V. Souza. 2016. Editorial: The Role of Microbial Communities in Tropical Ecosystems. Frontiers in Microbiology 7.

Pansu, M., L. Sarmiento, M. A. Rujano, M. Ablan, D. Acevedo, and P. Bottner. 2010. Modeling organic transformations by microorganisms of soils in six contrasting ecosystems: Validation of the MOMOS model. Global Biogeochemical Cycles 24.

Petersen, I. A. B., K. M. Meyer, and B. J. M. Bohannan. 2019. Meta-Analysis Reveals Consistent Bacterial Responses to Land Use Change Across the Tropics. Frontiers in Ecology and Evolution 7.

Pfister, C. A., F. Meyer, and D. A. Antonopoulos. 2010. Metagenomic Profiling of a Microbial Assemblage Associated with the California Mussel: A Node in Networks of Carbon and Nitrogen Cycling. PLOS ONE 5:e10518.

Poorter, L., D. M. A. Rozendaal, F. Bongers, J. S. de Almeida-Cortez, A. M. Almeyda Zambrano, F. S. Álvarez, J. L. Andrade, L. F. A. Villa, P. Balvanera, J. M. Becknell, T. V. Bentos, R. Bhaskar, V. Boukili, P. H. S. Brancalion, E. N. Broadbent, R. G. César, J. Chave, R. L. Chazdon, G. D. Colletta, D. Craven, B. H. J. de Jong, J. S. Denslow, D. H. Dent, S. J. DeWalt, E. D. García, J. M. Dupuy, S. M. Durán, M. M. Espírito Santo, M. C. Fandiño, G. W. Fernandes, B. Finegan, V. G. Moser, J. S. Hall, J. L. Hernández-Stefanoni, C. C. Jakovac, A. B. Junqueira, D. Kennard, E. Lebrija-Trejos, S. G. Letcher, M. Lohbeck, O. R. Lopez, E. Marín-Spiotta, M. Martínez-Ramos, S. V. Martins, P. E. S. Massoca, J. A. Meave, R. Mesquita, F. Mora, V. de Souza Moreno, S. C. Müller, R. Muñoz, R. Muscarella, S. N. de Oliveira Neto, Y. R. F. Nunes, S. Ochoa-Gaona, H. Paz, M. Peña-Claros, D. Piotto, J. Ruíz, L. Sanaphre-Villanueva, A. Sanchez-Azofeifa, N. B. Schwartz, M. K. Steininger, W. W. Thomas, M. Toledo, M. Uriarte, L. P. Utrera, M. van Breugel, M. T. van der Sande, H. van der Wal, M. D. M. Veloso, H. F. M. Vester, I. C. G. Vieira, P. M. Villa, G. B. Williamson, S. J. Wright, K. J. Zanini, J. K. Zimmerman, and M. Westoby. 2019. Wet and dry tropical forests show opposite successional pathways in wood density but converge over time. Nature Ecology & Evolution 3:928–934.

Powers, J. S., M. D. Corre, T. E. Twine, and E. Veldkamp. 2011. Geographic bias of field observations of soil carbon stocks with tropical land-use changes precludes spatial extrapolation. Proceedings of the National Academy of Sciences 108:6318–6322.

Powers, J. S., and E. Marín-Spiotta. 2017. Ecosystem Processes and Biogeochemical Cycles in Secondary Tropical Forest Succession. Annual Review of Ecology, Evolution, and Systematics 48:497–519.

Powers, J. S., and S. Salute. 2011. Macro- and micronutrient effects on decomposition of leaf litter from two tropical tree species: inferences from a short-term laboratory incubation. Plant and Soil 346:245–257.

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41:D590–D596.

Ranjan, K., F. S. Paula, R. C. Mueller, E. da C. Jesus, K. Cenciani, B. J. M. Bohannan, K. Nüsslein, and J. L. M. Rodrigues. 2015. Forest-to-pasture conversion increases the diversity of the phylum Verrucomicrobia in Amazon rainforest soils. Frontiers in Microbiology 6.

Ren, C., J. Chen, J. Deng, F. Zhao, X. Han, G. Yang, X. Tong, Y. Feng, S. Shelton, and G. Ren. 2017. Response of microbial diversity to C:N:P stoichiometry in fine root and microbial biomass following afforestation. Biology and Fertility of Soils 53:457–468.

Romillac, N., and L. Santorufo. 2021. Transferring concepts from plant to microbial ecology: A framework proposal to identify relevant bacterial functional traits. Soil Biology and Biochemistry 162:108415.

Schilling, E. M., B. G. Waring, J. S. Schilling, and J. S. Powers. 2016. Forest composition modifies litter dynamics and decomposition in regenerating tropical dry forest. Oecologia 182:287–297.

Schimel, J., and S. Schaeffer. 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3.

Schneider, D., M. Engelhaupt, K. Allen, S. Kurniawan, V. Krashevska, M. Heinemann, H. Nacke, M. Wijayanti, A. Meryandini, M. D. Corre, S. Scheu, and R. Daniel. 2015. Impact of Lowland Rainforest Transformation on Diversity and Composition of Soil Prokaryotic Communities in Sumatra (Indonesia). Frontiers in Microbiology 6.

Semenov, M., E. Stolnikova, N. Ananyeva, and K. Ivashchenko. 2013. Structure of the microbial community in soil catena of the right bank of the Oka River. Biology Bulletin 40:266–274.

Shah, F., C. Nicolás, J. Bentzer, M. Ellström, M. Smits, F. Rineau, B. Canbäck, D. Floudas, R. Carleer, G. Lackner, J. Braesel, D. Hoffmeister, B. Henrissat, D. Ahrén, T. Johansson, D. S. Hibbett, F. Martin, P. Persson, and A. Tunlid. 2016. Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. New Phytologist 209:1705–1719.

Sommer, M., and E. Schlichting. 1997. Archetypes of catenas in respect to matter—a concept for structuring and grouping catenas. Geoderma 76:1–33.

Štursová, M., L. Žifčáková, M. B. Leigh, R. Burgess, and P. Baldrian. 2012. Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. FEMS Microbiology Ecology 80:735–746.

Taylor, D. L., W. A. Walters, N. J. Lennon, J. Bochicchio, A. Krohn, J. G. Caporaso, T. Pennanen, and D. Cullen. 2016. Accurate Estimation of Fungal Diversity and Abundance through Improved Lineage-Specific Primers Optimized for Illumina Amplicon Sequencing. Applied and Environmental Microbiology 82:7217–7226.

Templer, P. H., P. M. Groffman, A. S. Flecker, and A. G. Power. 2005. Land use change and soil nutrient transformations in the Los Haitises region of the Dominican Republic. Soil Biology and Biochemistry 37:215–225.

Townsend, A. R., G. P. Asner, and C. C. Cleveland. 2008. The biogeochemical heterogeneity of tropical forests. Trends in Ecology & Evolution 23:424–431.

Treseder, K. K., and J. T. Lennon. 2015. Fungal Traits That Drive Ecosystem Dynamics on Land. Microbiology and Molecular Biology Reviews 79:243–262.

Van Langenhove, L., L. T. Verryckt, C. Stahl, E. A. Courtois, I. Urbina, O. Grau, D. Asensio, G. Peguero, O. Margalef, V. Freycon, J. Peñuelas, and I. A. Janssens. 2021. Soil nutrient variation along a shallow catena in Paracou, French Guiana. Soil Research 59:130–145.

Vaughan, E., M. Matos, S. Ríos, C. Santiago, and E. Marín-Spiotta. 2019. Clay and climate are poor predictors of regional-scale soil carbon storage in the US Caribbean. Geoderma 354:113841.

Větrovský, T., K. T. Steffen, and P. Baldrian. 2014. Potential of Cometabolic Transformation of Polysaccharides and Lignin in Lignocellulose by Soil Actinobacteria. PLOS ONE 9:e89108.

Wadsworth, F. H. 1950. Notes on the climax forests of Puerto Rico and their destruction and conservation prior to 1900. Caribbean Forester 11:38–56.

Wang, G., Y. Liu, M. Cui, Z. Zhou, Q. Zhang, Y. Li, W. Ha, D. Pang, J. Luo, and J. Zhou. 2022. Effects of secondary succession on soil fungal and bacterial compositions and diversities in a karst area. Plant and Soil 475:91–102.

Wang, H., J. D. Cornell, C. A. S. Hall, and D. P. Marley. 2002. Spatial and seasonal dynamics of surface soil carbon in the Luquillo Experimental Forest, Puerto Rico. Ecological Modelling 147:105–122.

Wickham, H. 2016. Data analysis. Pages 189–201 ggplot2. Springer.

Wickham, H., R. François, L. Henry, and K. Müller. 2015. dplyr: A Grammar of Data Manipulation: https://CRAN. R-project. org/package= dplyr.

Wieder, W. R., G. B. Bonan, and S. D. Allison. 2013. Global soil carbon projections are improved by modelling microbial processes. Nature Climate Change 3:909–912.

Zanne, A. E., K. Abarenkov, M. E. Afkhami, C. A. Aguilar-Trigueros, S. Bates, J. M. Bhatnagar, P. E. Busby, N. Christian, W. K. Cornwell, T. W. Crowther, H. Flores-Moreno, D. Floudas, R. Gazis, D. Hibbett, P. Kennedy, D. L. Lindner, D. S. Maynard, A. M. Milo, R. H. Nilsson, J. Powell, M. Schildhauer, J. Schilling, and K. K. Treseder. 2020. Fungal functional ecology: bringing a trait-based approach to plant-associated fungi. Biological Reviews 95:409–433.

Zhang, Q., J. Yang, R. T. Koide, T. Li, H. Yang, and J. Chu. 2017. A meta-analysis of soil microbial biomass levels from established tree plantations over various land uses, climates and plant communities. CATENA 150:256–260.

Zhang, X., W. Li, Z. Zhong, Q. Zhang, X. Wang, X. Han, C. Ren, and G. Yang. 2020. Response of Soil Microbial Community to C:N:P Stoichiometry along a Caragana Korshinskii Restoration Gradient on the Loess Plateau, China. FORESTS 11.

Chapter 3: Defining carbon benchmark values for agroecosystems across diverse tropical soil environments for soil health assessment.

Introduction

Soil organic carbon (SOC) plays a crucial role in ecosystem functioning by affecting soil structure, water retention, nutrient availability, and rhizosphere and microbial processes (Rawls et al. 2003, Lal 2016, Kay 2018, Poirier et al. 2018). As one of the largest reservoirs of carbon in terrestrial ecosystems (Jobbágy and Jackson 2000), any changes in SOC can significantly impact atmospheric concentrations of carbon dioxide and methane. Therefore, it is important to understand the response of SOC to environmental and land use modifications in order to mitigate climate change. As a reservoir of nutrients in soils, understanding processes leading to the loss and recovery of soil organic matter is important for enhancing agricultural productivity. However, accurately predicting the effects of these changes on SOC poses challenges due to the complex interactions among climatic factors, soil conditions, and biotic properties (Lehmann and Kleber 2015, Rasmussen et al. 2018, Vaughan et al. 2019). Failure to consider these interactions when making SOC predictions can lead to substantial uncertainty in model outcomes (Luo et al. 2016). To overcome this challenge, the use of soil health tools, such as benchmarks, can establish a baseline for understanding SOC variability across different land uses, climates, soil conditions, and biotic properties.

Previous studies have demonstrated that the conversion of forests to agricultural land use has generally resulted in global losses of SOC, with the exception of certain pasture lands (Guo and Gifford 2002, Don et al. 2011, Powers et al. 2011). This conversion raises concerns about the potential increase in anthropogenic greenhouse gas emissions and the decline in soil health. These concerns are particularly relevant in tropical regions, where deforestation alone accounts

for a significant portion of soil carbon emissions resulting from land use change, ranging from 10% to 30% (Achard et al. 2004). However, research has shown that the impact of land use on SOC in tropical regions is highly variable (Don et al. 2011), which can be attributed to insufficient research coverage encompassing the diverse climates and soil conditions prevalent in these areas (Powers et al. 2011). To overcome these biases, large-scale assessments of SOC are needed.

Soil health scientists are actively working on defining indicators and benchmarks of soil properties to assess global and local environmental issues, providing insights into the effects of land use change on SOC and strategies to manage any losses in different regions (Allen et al. 2011, Bran Nogueira Cardoso et al. 2013). By recognizing SOC as a critical indicator of soil health, it becomes crucial to understand the impact of land use changes on SOC across various climate and soil environments in tropical regions. This understanding can help inform sustainable land management practices and policies to mitigate soil carbon losses and promote soil health in these areas.

Over the years, various benchmarks have been developed to evaluate soil health and gain insights into how different agricultural practices affect soil properties and function. One such benchmark is the Soil Health Gap, defined as the difference between soil health in undisturbed soil and the current soil health in cropland soil within a specific agroecosystem (Maharjan et al. 2020). However, the idea of undisturbed soil poses challenges in areas affected by extensive deforestation. In many parts of the tropics, forest cover is dominated by secondary growth, which can show the legacy effects of past land uses (FAO, 2010, Powers and Marín-Spiotta 2017). Secondary forests could be considered as proxies for undisturbed forests to understand carbon dynamics, based on data from previous studies demonstrating recovery of aboveground biomass

with forest succession (Jones et al. 2019). However, SOC recovery in such forests exhibits considerable variation, resulting in inconsistent outcomes (Marín-Spiotta and Sharma 2013, Powers and Marín-Spiotta 2017), which can result in incorrect assessment when using secondary forests as proxies in the Soil Health gap benchmark and significant challenges if there are no primary or undisturbed forests available for comparison.

Alternatively, benchmarks can be derived based on the distribution of SOC values found in specific regions or environmental conditions. Using the distribution of values, scores can be created to reflect the variability observed within a specific region, and these scores can be used to assess current SOC value in agricultural lands to evaluate if SOC is low compared to areas with similar environmental characteristics. Two examples of such benchmarks are the Comprehensive Assessment of Soil Health (CASH), which utilizes cumulative normal distribution of regional data sets from the northern United States (Idowu et al. 2009), and the Soil Management Assessment framework, which integrates biological, chemical, and physical soil health indicators by transforming measured values into 0-1 scores (Andrews et al. 2004). An updated version combining those two methods is the Soil Health Assessment Protocol and Evaluation (SHAPE), which interprets soil health based on peer soil groups defined by edaphic and climate factors (Nunes et al. 2021). By using these tools, we can improve soil health assessments to understand what drives SOC variability in tropical regions and to provide future land management recommendations. However, developing these benchmarks requires a comprehensive understanding of the factors influencing soil carbon dynamics and their interactions. Therefore, to establish a benchmark for agricultural soils in the tropics, it is crucial to understand what factors control SOC in soils of the tropics.

Climate and physicochemical soil properties have been widely recognized as significant drivers of (SOC) dynamics at both global and regional scales (Luo et al. 2017, Wiesmeier et al. 2019). Climate variables, such as temperature and precipitation, influence organic matter inputs through net primary productivity and decomposition, as well as soil biological, chemical, and physical characteristics that affect rates of accumulation and loss of organic carbon in soils (McGroddy and Silver 2000, Conant et al. 2011, Hobley et al. 2016). Soil texture - in particular, clay content - is commonly used as a primary predictor of SOC in many biogeochemical models; however, this relationship varies depending on climatic and other conditions (Oades 1988, Schimel et al. 1994, Rasmussen et al. 2018). A recent study across a wide precipitation range in the tropics found that climate, land use type, and clay content had limited predictability on soil carbon, whereas soil order classification, pH, and the fine silt plus clay fractions together explained a greater portion of the observed variation (Vaughan et al. 2019). However, our understanding of the factors influencing soil carbon within different land uses under diverse climatic and soil environmental conditions remains limited.

In this study, we aimed to 1) investigate the influence of agricultural land and vegetation cover on the variability of SOC content in a region with diverse climate and soil factors, 2) identify the factors that significantly affect SOC content within different agricultural land and vegetation covers, and 3) develop a benchmark for SOC assessment by using the Soil Health Gap benchmark and the Scores Benchmarks as conceptual models and evaluate their applicability and usefulness in our study context. We hypothesize that agricultural lands will lead to lower amounts and variability in SOC content compared to forests due to the high abundance of secondary forests in the tropics with inconsistent SOC responses. We also hypothesize that SOC variability at the regional scale will be modulated by climate, but within land use will be

dependent on soil physicochemical characteristics. Lastly, we hypothesize that the Soil Health Gap model will not be ideal for tropical regions due to the limited availability of primary forests as reference points and the secondary forests' high SOC variability. Hence, we expect that a Score benchmark representing the variation in the tropics will be a more practical approach. We use the island of Puerto Rico as a case study to investigate the factors influencing soil carbon dynamics and to establish a soil carbon benchmark specific to tropical regions given the island's well-documented land use history, diverse climate and soil environments, and richness of available data. We evaluated 586 pedons across a wide range of climatic conditions and 9 USDA-classified soil orders. By synthesizing available soil organic carbon concentrations and environmental and agricultural data at a regional scale, our work serves as guidance to improve predictions of SOC in tropical regions and to evaluate and assess agricultural impacts on SOC to develop soil conservation management plans.

Methods

A Case Study: Puerto Rico

The Caribbean Area Natural Resources Conservation Service (NRCS) has identified soil health as a priority for increased agricultural productivity and protection of soil from erosion and other disturbances in Puerto Rico (NRCS 2023). Several recent programs have addressed the potential for conservation investments to improve soil organic carbon. Historically, Puerto Rico experienced extensive deforestation for agriculture and livestock purposes, with approximately 78 percent of the island's land being deforested during the 1900s (Wadsworth 1950). However, in the 20th century, large portions of agricultural land were abandoned as the economy shifted towards industrialization and a service-based sector (Franco et al. 1997). Currently, potential agricultural lands encompass 42 percent of Puerto Rico (Gould et al. 2017). These include lands

well-suited for mechanized and non-mechanized agriculture, such as row and specialty crops, livestock, dairy, hay, pasture, and fruits, which occupy 23 percent of Puerto Rico, and areas suitable for forestry production, such as timber and non-timber products, agroforestry, and shade coffee, which occupy 19 percent.

Puerto Rico is an archipelago located between 17° 45' N and 18° 30' N and 65° 45' W to 67° 15' W. The main island occupies 8740 km^2 with vegetation zones ranging from dry, semi-deciduous forests in the southwest area to moist forests covering most of the island, to wet and rainy forests in the northeast and high elevation areas. Elevation ranges from sea level to the highest mountain peak at 1338 m in Cerro de Punta, located in the central region of the main island. The climate in Puerto Rico is tropical and predominantly maritime (Daly et al. 2003). The spatial pattern in temperature is linked to elevation, topographic position, and proximity to the ocean. Puerto Rico exhibits a diverse range of climate conditions. The mean annual temperature varies from 20 ºC in high elevations to 26 ºC in coastal areas. The island experiences a broad range of mean annual precipitation, ranging from 4500 mm in the northeast region to 850 mm in the south coast area. These climate gradients observed across Puerto Rico provide a relatively representative climate scenario for tropical regions.

Additionally, Puerto Rican soils encompass 10 of the 12 USDA soil orders, offering the advantage of various soil properties that can contribute to understanding spatial variability in soil carbon. Soils are classified as Alfisols, Entisols, Histosols, Inceptisols, Mollisols, Oxisols, Ultisols, Vertisols, Aridisols, and Spodosols. The diversity of soils in Puerto Rico is driven by the diverse parent materials, climatic conditions, and weathering processes found on the island (Lugo and Brown 1993, Muñoz et al. 2018). The suitability of land for agriculture and the last past use legacies makes, and the diverse soils and climate environments in Puerto Rico make it

an ideal location to examine land use on soil carbon dynamics. However, the limited availability of primary forests creates a challenge when evaluating different benchmarks, as the Soil Health Gap benchmarks are designed to have as a control undisturbed forests, which provides the opportunity to evaluate the potential of secondary forests as proxies of primary forest for soil health evaluations. Also, the diverse climates and soil environments provide a great opportunity to evaluate the variability of Score Benchmark at different scales.

Data collection

Part of the data was derived from samples submitted by the NRCS for soil characterization to the NRCS Kellogg Soil Survey Laboratory in Lincoln, NE (n of pedons = 234) using the "SoilDB" package in R (Beaudette et al. 2016) and extracting the data from the publicly available NRCS database. The pedons of this database contained measured chemical and physical properties of diverse geographical representations in Puerto Rico. Only latitude, longitude, soil series, soil order, soil suborders, SOC, sand, clay, and silt percentages were used from this dataset. The land use and management were recorded in the NRCS database so we extracted land use information from the National Land Cover Database (NLCD) circa 2001 for Puerto Rico using Google Earth Engine. The pedons were classified under "Developed, Open Space," "Developed, Low Intensity," "Developed, Medium Intensity," "Barren Land," "Evergreen Forest," "Shrub/Scrub," Grassland/Herbaceous," "Pasture/ Hay," "Cultivated Crops," and "Emergent Herbaceous Wetlands." To get more representation of land uses across different soil orders and climate regions, we reclassified all these categories into the following land use classifications "Agriculture," which indicates cultivated crops, "Pastures" which indicates Pastures, Hays, Grasslands, and Herbaceous lands that can be used or are being used for cattle, "Forests" which includes evergreen forests and woody vegetation areas, "Range" which is

described as herbaceous and shrubby vegetation, and “Wetlands”. We used aerial imagery for the categories classified as Developed to reclassify into one of the land use classes.

Data from the rest of the pedons (n = 352) were requested from the corresponding authors of Marin-Spiotta et al. 2008, Vaughan et al. 2019, and Acosta-Martinez et al. 2007. The data from these three publications contained land use, latitude, longitude, soil series, soil order, SOC concentration, sand, clay, and silt percentages for the first 10-30 cm of depth. Using the Taxonomic Classification of the Soils of Puerto Rico 2017 (Muñoz et al. 2018), we confirmed soil order and extracted soil suborder for each pedon.

The data from the NRCS database and the publications were combined to provide a total of 586 pedons representing 9 of 10 soil orders (Alfisols, Entisols, Histosols, Inceptisols, Mollisols, Oxisols, Ultisols, Vertisols, and Aridisols) in Puerto Rico and covering five different land uses (Agriculture, Pastures, Forest, Wetland, and Rangelands). Using WorldClim data, we extracted mean annual temperature (MAT) and mean annual precipitation (MAP) with a resolution of 1 km^2. The SOC concentration values for each pedon averaged by 0-30 depth ranged from 0.01 - 46.6% (Figure 1). For this study, we are using SOC concentration due to the lack of data on bulk density and carbon stocks in the NRCS databases We acknowledge that SOC concentrations are not standardized by soil volume and may result in biases when comparing sites (Don et al. 2007). However, our work objectives are to evaluate factors controlling SOC concentrations and different Benchmark models, which provide an opportunity to build up from our results for later researcher focus on assessing SOC stocks across diverse climates and environmental conditions.

Data analyses

We selected only the first 30 cm of the depth of each pedon for analysis due to high SOC carbon abundance and because it is the most active and most affected depth of the soil as a result of land use change (Jobbágy and Jackson 2000). Due to the variable depth of the horizon of each pedon, all variables were averaged by all horizons between 0 and 30 cm to standardize the depth among the pedons. To investigate the influence of agricultural land and vegetation cover on the variability of averaged SOC from 0-30 cm depth, we used an analysis of variance (ANOVA) to determine the significant difference among land uses or vegetation covers and Tukey HSD for pair comparisons. To identify the factors that affect SOC concentration within different land uses and vegetation covers, we sub-divided the dataset into Agriculture, Pasture, and Forest sub-datasets, and we evaluated the effect of soil order, soil suborder, texture class have on SOC concentration using an ANOVA and Tukey HSD for pair comparisons. We used simple linear regression to test for the effect of temperature, precipitation, clay, clay + silt, and soil pH on the averaged SOC from 0-30 cm depth. Note that we evaluated clay as a standard factor and reported it in tables, but we only discussed the effect of silt + clay in our results due to the low influence of clay alone on SOC, which has been previously reported (Rasmussen et al. 2018, Vaughan et al. 2019). Then, for each land use and vegetation cover type, we incorporated those factors that contributed to the variability of soil carbon into stepwise regression models backward and forward using the function “step” from the “MASS” R package (Ripley et al. 2013). We considered a significant value when the p-value <0.05.

For the development of the benchmarks, first, we performed a stepwise model backward and forward using the function “step” from the “MASS” R package to select the combination of variables that represented most of the variability across the whole island. We also performed a

Random Forest model using the package "randomForest" (Liaw and Wiener 2002) and determined variable importance using the function "varImPlot" from the package "caret" in R (Kuhn 2008). To apply the Score Benchmark, we trained a Random Forest model using 70% of the data and validated the model with 30% of the data. Then, we predicted all the values from our data and calculated the Empirical Cumulative Distribution by grouping the data into soil orders. To apply the Soil Health Gap Benchmark, we calculated the difference between the average SOC content in forest and agricultural lands and in forest and pasture lands grouped by soil order and soil order + climate. For climate, we classify precipitation into Dry (<1000 mm), Moist (1000–2500 mm), and Wet (>2500 mm) rainfall class and temperature into < 21 C, 22-25 C, >26, following the Koppen climate classification system criterion (Köppen 1900, Kottek and Hantel 2005). To test if soil order influenced the gap and to test if the gap was higher for agricultural lands or pastures, we used an ANOVA and Tukey HSD for pair comparisons. We considered a significant value when the p-value <0.05 unless otherwise noted.

Results

Land use effect on soil carbon

The average SOC concentrations from 0-30 cm depth varied significantly across land uses (p-value < 0.001; Table 1, Figure 1). A Tukey HSD test showed that all land uses were significantly different, except for Rangelands (Table 2.). SOC in Rangelands did not differ from Agricultural lands (p-value = 0.126), Forests s (p-value = 0.995), and Pastures (p-value = 0.165). Wetlands had the greatest carbon concentrations (7.75 ± 5.8 %), followed by Forests (which include evergreen forests and woody vegetation areas; 3.7 ± 3.51 %), Pastures (which indicates Pastures, Hays, Grasslands, and Herbaceous lands that can be used or are being used for cattle;

2.51 ± 1.36 %), Rangelands (which is described as herbaceous and shrubby vegetation 2.20 ± 0.68 %), and Agriculture (which indicated cultivated crops; 1.62 ± 0.7 %).

Factors affecting soil organic carbon in agricultural land and pastures.

We evaluated the effect of soil order, soil suborder, MAT, MAP, percentage of clay + silt, pH, and USDA soil texture classification for SOC % in agriculture and pasture to detect factors influencing their variability (Table 3, Table 4, Figure 2). In agricultural soils, we found that soil order ($p < 0.001$, $R^2 = 0.51$) and suborder ($p < 0.001$, $R^2 = 0.50$) had a strong effect on SOC%. Average temperature (p-value = 0.001, $R^2 = 0.1$) and precipitation (p-value < 0.001, $R^2 = 0.17$) significantly influence SOC% in agricultural lands. Similarly, soil properties, silt + clay percentage (p-value < 0.001, $R^2 = 0.32$), and texture class (p-value < 0.001, $R^2= 0.19$) were significantly related to agriculture SOC% and represented 20-32% of the variability, but pH (p-value = 0.003, $R^2 = 0.08$) only represented an 8%. A stepwise linear regression showed that the best model to predict agricultural SOC% includes soil order, percentage of silt + clay, average temperature, and pH (p-value < 0.001, $R^2 = 0.60$; Table 5).

In pasture soils (Figure 3), soil order ($p < 0.015$, $R^2 = 0.06$) and suborder ($p < 0.001$, $R^2 = 0.20$) significantly influenced SOC%. In contrast to agricultural soils, soil suborders represented more of the variability of the data suggesting that finer levels of taxonomic classification may be more informative than soil order. When evaluating climate conditions, we found no effect of average temperature (p-value = 0.36, $R^2 < 0.001$) nor precipitation (p-value = 0.509, $R^2 < 0.001$) on SOC% in pasture lands. When analyzing soil properties, silt + clay percentage (p-value < 0.001, $R^2 = 0.32$%) and texture class (p-value < 0.001, $R^2 = 0.32$) were significantly related to pastures SOC% and represented a third of the variability, but pH (p-value = 0.003, $R^2 = 0.10$)

only represented 10%. For pastures, the stepwise linear regression best model included soil suborder, percentage of silt + clay, and pH (p-value < 0.001, R^2 = 0.45; Table 5).

Factors affecting soil organic carbon in forest soils.

We evaluated the effect of soil order, soil suborder, average temperature, average precipitation, percentage of clay + silt, pH, and USDA soil texture classification for SOC % in forest soils to detect factors influencing its variability (Table 3, Table 4, Figure 4). We found that soil order (p = 0.003, R^2 = 0.05) and suborder (p < 0.001, R^2 = 0.46) significantly influence forest SOC %. Similar to pasture soils, these results suggest that soil suborders represented more of the variability of the data, pointing out that finer levels of taxonomic classification may be more informative than soil order. When looking at climate conditions, we found that both average temperature (p-value = 0.001, R^2 = 0.14) and precipitation (p-value < 0.001, R^2 = 0.11) affect SOC % in forest soils. For soil properties, silt + clay percentage (p-value < 0.001, R^2 = 0.19) and USDA texture classes (p-value < 0.001, R^2=0.20) were significantly related to forests SOC% and represented 20% of the variability, but pH (p-value = 0.452, R^2 < 0.001) in this case, was not significant. A stepwise regression running forward and backward showed that the best model to predict forest SOC% included the variables soil suborder, USDA texture classes, and average temperature (p-value < 0.001, R^2 = 0.60; Table 5).

Development of a benchmark

To be able to develop a benchmark, we used a stepwise regression to identify the most important factors influencing SOC% across the whole island of Puerto Rico. This stepwise regression was different from those above because it consolidated all land uses and considered the type of land use as a factor of interest. The stepwise model showed that the best model to describe SOC% included the soil suborder, land use type, USDA taxonomic classification,

average temperature, and pH (p-value <0.001, $R^2 = 0.55$; Table 5). However, due to our limited sample size within each soil suborder, we decided to continue developing the benchmark using the soil order taxonomic level. We also performed a Random Forest model to identify variable importance using the percentage increase in Mean Squared Error (IncMSE), and we found that all variables were above 20% of IncMSE, including average precipitation and soil order (Figure 5). We decided to keep average precipitation and soil order in our model.

To develop the Gap Benchmark, we subtracted the average SOC concentration of forests minus the SOC concentration from agricultural or pasture sites within each soil order and climate classification to calculate the gap. We found that forests on Alfisols, Inceptisols, Mollisols, Oxisols, and Ultisols have more carbon than agriculture or pastures, but forests in Aridisols and Vertisols have less. An ANOVA showed that soil order was relevant in controlling the gap between forest vs. agriculture or pastures (p-value = 0.012; Table 6). We also found a marginal difference between the pasture and agricultural gaps (SOC difference from forests; p-value 0.068; Table 6, Figure 8), suggesting that the gap is greater in agricultural lands compared to pastures. When we take into account climate, we found that in moist regions (rainfall of 100-2500 mm and temperature of > 26), agricultural lands in Alfisols and pasture lands in Aridisols and Mollisols have more carbon than forest, indicating that climate plays an important role in the difference of soil carbon from forests to agricultural lands (Figure 9).

To develop the Scoring Benchmark, we decided to exclude samples from Histosols, range, and wetlands due to their limited representation in the data. By training a Random Forest model with 70% of the data, we developed the scoring benchmark from 0 to 1 by grouping the prediction by soil order and calculating the Empirical Cumulative Distribution Function. Using the test data (that accounted for 30% of the total dataset), we predicted SOC% in which we could

assign a score, but the test data predictions resulted in less than 60% performance (RMSE= 0.49, R^2 = 0.59; Figure 6, See Score Benchmark in Figure 7).

Discussion

Our primary goal was to understand the factors influencing soil organic carbon (SOC) concentration in different soil types under various agricultural and vegetation covers in a wide range of climatic conditions. We developed a benchmark for SOC assessment using two conceptual models and evaluated their applicability in a tropical region with diverse land covers, soil types, and climates. We found that SOC variance differed among land uses, and the factors controlling SOC concentration varied within pastures, agricultural lands, and forests. For the whole island of Puerto Rico, several factors collectively accounted for half of the SOC variability, including soil suborder, land use type, USDA texture classification, average temperature, and pH. The differences in soil carbon between forests and agricultural or pasture lands varied depending on soil order and climate conditions, with a more pronounced gap observed in agricultural lands. Our study highlights that a Score benchmark model would be more suitable for diverse tropical soils, but improving the prediction of soil carbon would require evaluating a wider range of influencing factors. The implications of these findings are discussed further in the study.

Variability in soil carbon across different land uses

The results of our study highlight the influence of vegetation cover and land use change on SOC. We observed high variance in SOC concentration within forested areas and wetlands, with a standard deviation ranging from 3.51 to 5.8 %. In the case of forests, the variance in soil carbon can be attributed to a combination of factors, including land use legacies, climatic conditions, and soil characteristics. Forested areas may exhibit distinct patterns of SOC due to

historical land use practices, such as deforestation, grazing, and crop production, which can leave long-lasting impacts on carbon stocks (Powers and Marín-Spiotta 2017). Additionally, climatic conditions, such as temperature and precipitation, can influence the decomposition rates of organic matter and the overall carbon dynamics within forest soils (Conant et al. 2011). Furthermore, soil characteristics, such as texture, mineral composition, and nutrient availability, can vary across forest types, leading to variations in SOC concentrations (Oades 1988, Schimel et al. 1994, Rasmussen et al. 2018). We will go into more detail about how these factors influence our forest sites in the following section.

In our analysis of wetlands, we observed high variance in soil organic carbon (SOC) due to the diverse range of soil types and climates in our dataset. The wetland pedons included Entisols, Histosols, Inceptisols, Mollisols, Ultisols, Vertisols, and MAT, with temperatures ranging from 25.4-26.5°C and MAP ranging from 867-1864 mm. The variation in soil characteristics and the wide range of MAP can significantly influence SOC content, as the frequency of saturated or water-covered soils influences biogeochemical processes (Perez-Alegria 2001). For instance, soil orders like Vertisols, found in dry areas, exhibit SOC content ranging from 1-7%, while Histosols and Entisols, located in moist and wet areas such as mangrove swamps, marshes, lagoons along the coast, deposits along streams and in coastal floodplains exhibit SOC variation of 7-25% (Muñoz et al. 2018). This distinction in soil types and their associated water saturation levels can affect biogeochemical dynamics that control SOC.

Supporting our expectations, SOC concentration and its variance were found to be lower in agricultural land compared to forests and wetlands. The standard deviation of SOC in agricultural land was 0.7% in our study. This relatively low variance may be attributed to

management practices employed in these agricultural systems, which regulate the amount of crop residue incorporated into the soil (Lugo and Brown 1993). The return of crop residues to the soil has a direct impact on SOC levels (Graham et al. 2002), and intensive management and high biomass export in crop systems often result in lower SOC concentrations (Graham et al. 2002). However, it should be noted that our data resolution did not allow for detailed information on crop types or specific management practices, which limits our understanding of the observed low variability. Future research should incorporate such information to enhance our comprehension of the factors influencing SOC dynamics in agricultural systems.

In contrast, pasture lands exhibited a significant variance of SOC concentration, with a standard deviation of 1.36%. Previous research findings have also highlighted the high variance in SOC levels after forest conversion to pasture (Houghton 1995). The impact of this conversion on SOC levels has shown contrasting results in different studies, with some reporting increases (de Moraes et al. 1996, Neill et al. 1997) and others documenting decreases (Detwiler 1986, Fearnside and Imbrozio Barbosa 1998). The variance of SOC in pasture lands is influenced by management practices and climatic conditions, as suggested by (Guo and Gifford 2002). These factors will be discussed in greater detail in the subsequent section. Notably, grasslands, pastures, and perennial crops, which maintain permanent vegetation cover and experience high root turnover, contribute substantially to SOC inputs (Brown and Lugo 1990). This could explain the generally higher SOC concentrations observed in pasture lands compared to agricultural systems.

Soil carbon environmental controls vary within land use types.

The environmental controls on SOC vary within different land use types, revealing distinct patterns and factors influencing SOC concentrations. In forest systems, various factors contribute to the variability of SOC, including soil suborder, texture classes, and average

temperature. Interestingly, unlike in pasture and agriculture, pH did not play a significant role in SOC variability within forests. Many pedons of this study are karst-derived and contain free calcium carbonates, as evidenced by strong effervescence under acid in the field and in the lab found by Vaughan et al. (2019). These soils are rich in calcium and characterized by high pH, which can protect soil organic matter from decomposition through various mechanisms (Rasmussen et al. 2018). The presence of calcium and other base cations facilitates the formation of cation bridges between clay particles and organic matter, leading to stabilization in mineral-organic associations (Oades 1988, Lützow et al. 2006) while also promoting the formation of soil aggregates that potentially enhance the physical protection from decomposers (Six et al. 2004). However, in our data, soils with low pH, such as Oxisols, also had high concentrations of SOC, affecting a possible linear trend between pH and SOC content.

Precipitation had a significant though low effect on SOC in forest ecosystems, accounting for approximately 11% of the variability. Studies conducted in the Caribbean have demonstrated that SOC accumulation rates during succession are higher in moist and wet life zones than in dry forests (Brown and Lugo 1990). Additionally, other climate variables, such as temperature, during tropical succession have been found to be influential predictors of SOC in pantropical regions (Marín-Spiotta and Sharma 2013). These findings suggest that while the impact of climatic variables may be relatively low, they are crucial for comprehending the dynamics of SOC in forest soils.

Climate variables were also a significant predictor of SOC in agricultural lands. MAP explained nearly 20% of the variability in SOC within agricultural sites. MAT and soil characteristics such as soil order, the percentages of silt + clay, and pH also played a crucial role in controlling 60 % SOC concentration variability in agricultural soils. The relationship between

climate and soil properties in agricultural systems and their impact on SOC has been observed globally, particularly in tropical regions (Don et al. 2011). In a meta-analysis, Don et al. (2010) found that 55% of the SOC variability in agricultural systems was attributed to climatic and soil parameters, suggesting that the rest of the variability could be the result of management practices and crop residuals. Unfortunately, the resolution of our dataset did not account for management practices and the intensity of land use. Future analyses must consider management practices as they have been observed in other environments to have a significant effect on SOC within agricultural ecosystems (Alvarez 2005).

In contrast to agricultural lands, pasture lands were predominantly influenced by soil properties, such as soil suborder, percentage of silt + clay, and pH, accounting for 45% of the SOC variability. MAT and MAP did not affect soil C in our study, contrasting with a global meta-analysis by Dlamini et al. (2016), where MAT and MAP were the primary drivers of SOC variability in grasslands. In our study, soil texture, particularly the percentage of silt + clay, was more influential than climate. Fine-textured soils are expected to promote greater SOC content due to the stabilization of organic matter by clay and silt particles, protecting it from decomposition (Parton et al. 1987, Six et al. 2002). Although soil texture and soil pH may be good predictors of SOC in our analysis, there is still a 55% variability that was still not explained by the factors studied. This variability could be the result of management and grazing intensities influencing soil structure and compaction, affecting SOC dynamics (Dlamini et al. 2016, Byrnes et al. 2018).

Our study suggests that when considering pastures and forests, focusing on soil suborder classification provides a more informative perspective on SOC variability compared to soil orders. Soil orders inherently encompass substantial SOC variation within their categories

(Mayes et al. 2014). Contrasting to our results, Vaughan et al., (2019) found that soil order has a strong influence on SOC stocks. No effect of soil order could be the result of the uncertainty evaluating SOC concentration without the controls of soil area or bulk density (Don et al. 2007). Yet, soil suborders, which reflect major environmental controls on soil formation processes and indicate moisture or temperature regimes (Muñoz et al. 2018), appear to be more relevant for explaining SOC variability in forests and pastures, while in the case of agricultural soils can be more broadly explained across different soil orders.

Benchmark models for predicting SOC values regionally.

Benchmark models for predicting SOC concentration values at the regional level are essential for understanding soil health and informing agricultural and conservation practices. In this study, we assessed two benchmark models, the Soil Health Gap, and Scores Benchmarks, to determine their suitability in tropical regions characterized by diverse climates, soil environments, and vegetation composition. Both models serve distinct purposes and may not offer equal utility in all cases.

The Scores Benchmark offers the advantage of representing data variability and assigning scores to SOC, enabling individuals to assess their position within the SOC variability based on selected environmental conditions. This model has been successfully implemented in the United States. Notably, the Soil Management Assessment Framework (SMAF) developed by Andrews et al. (2004) integrates biological, chemical, and physical soil health indicators by transforming measured values into 0-1 scores. SMAF has demonstrated effectiveness in evaluating agricultural management styles in various regions, such as Georgia, California, Wisconsin, and Iowa, over time (Andrews et al. 2004); however, with some overestimations. Another tool, the Comprehensive Assessment of Soil Health (CASH), developed by Idowu et al. in (2009), utilizes

multiple soil health measurements in the northeastern United States and provides a good evaluation of soil health.

An updated model, the Soil Health Assessment Protocol, and Evaluation (SHAPE), developed by Nunes et al. in (2020), combines the SMAF and CASH methods. SHAPE interprets soil health based on peer groups defined by edaphic and climate factors, encompassing a wider sample size (n = 14,680) across the United States. This updated model improves upon the overestimation observed in SMAF while yielding results similar to those obtained with CASH (Nunes et al. 2021). These examples illustrate how Score benchmarks address the increasing demand for accessible, interpretable, and quantitative scoring curves, providing regionally relevant knowledge about soil status in response to agronomic and conservation initiatives. In our regional-scale study, the Scores Benchmark exhibited several advantages due to its ability to account for diverse factors influencing soil carbon.

It is important to acknowledge that the SOC concentration variation explained by benchmark models may not account for all factors influencing SOC, such as management practices, nutrient availability, plant organic matter inputs, topographic aspects, and organisms' metabolic processes. These factors should be considered in future developments of score benchmarks for tropical regions to enhance accuracy and applicability. Our dataset revealed that soil order, land use type, USDA texture classification, MAT, and pH accounted for 60% of the SOC variance. Developing score benchmarks require extensive data collection and representation of various environmental conditions influencing soil carbon.

On the other hand, the Soil Health Gap Benchmark is particularly suited for comparing specific agricultural sites with nearby forests. By utilizing appropriate control measures that enable the identification of land management practices known to decrease SOC, the Soil Health

Gap benchmark can effectively address site-level scale soil carbon loss while controlling for multiple contributing factors that influence SOC variability. In order to effectively implement the Gap Benchmark, it is crucial to possess prior knowledge regarding the impact of various land management practices, since they are known to affect SOC differently (Alvarez 2005). Conventional tillage, for instance, disrupts the soil structure and leads to the loss of SOC (Alvarez 2005). Conversely, the application of manure has been shown to increase SOC levels (Ozlu and Kumar 2018, Ozlu et al. 2019), while the practice of cover cropping enhances soil aggregation and reduces erosion (Alori et al. 2020).

Applying the Soil Health Gap Benchmark on a large scale can introduce complexities and potentially lead to the over- or under-representation of SOC gaps, primarily due to the influence of climate on SOC concentrations. Several meta-analyses have corroborated these findings, emphasizing the significance of factors such as temperature, precipitation, and ecological zones in comprehending the variability of SOC differences between managed lands and forests at a broader scale (Guo and Gifford 2002, Don et al. 2011). Critical Zone researchers have argued that a more universal measure of soil health may be possible by including climate indicators, but adding such indicators may also increase the uncertainty of already uncertain results (Yoder et al. 2022). This uncertainty arises from spatial climate variability, which impacts SOC at larger scales and influences the processes governing SOC inputs and outputs (McGroddy and Silver 2000, Conant et al. 2011, Hobley et al. 2016).

Furthermore, when applying this benchmark on a large scale, the predominance of secondary forests in the analyzed region introduces additional complexities. When looking at individual field studies investigating SOC dynamics across tropical secondary succession, varying outcomes emerge. Some studies report gains, losses, or no significant net change in bulk

soil carbon during tropical secondary forest succession (Hughes et al. 1999, de Koning et al. 2003, Bautista-Cruz and del Castillo 2005, Marin-Spiotta et al. 2009, Powers et al. 2011). Therefore, the use of secondary forests may not be suitable for Soil Health Gap Benchmark.

In general, both the Scores and Soil Health Gap benchmarks have their advantages and disadvantages. These approaches can be employed to model and predict SOC, a crucial dynamic soil property recognized by the U.S. Department of Agriculture Natural Resources Conservation Service (USDA NRCS) as essential for improving agricultural productivity and protecting soil from erosion and disturbances (NRCS 2023). It is recommended that benchmark models used to assess indices should be easily adaptable so not only scientists but also policymakers and farmers take advantage of it used to evaluate soil health. The Gap Benchmark can be relatively straightforward to manipulate, requiring limited controls. However, it may introduce biases depending on the scale employed. On the other hand, the Scoring Benchmark is more complex due to the need to understand the factors controlling SOC and its variations under specific environmental conditions. Nevertheless, further research is necessary to clarify the factors influencing the observed variability of SOC in tropical regions. Considering the high variability of SOC observed in our analyzed pedons, the Scores Benchmark is considered more appropriate for tropical regions due to its applicability at multiple scales and soil health goals. Its generalized results enable users to determine whether their soil is above or below the average conditions and goals of interest.

Conclusion

Our study aimed to investigate the influence of agricultural land and vegetation cover on SOC variability and to use this information to develop benchmark models for SOC assessment to inform soil health management programs and land-climate models. We found significant

variations in SOC across different land uses, with factors such as soil suborder, land use type, USDA texture classification, mean annual temperature, and pH collectively explaining 60% of SOC variability. The changes in SOC between forests and agricultural or pasture lands varied depending on soil order and climate conditions, indicating the importance of addressing the geographical gaps observed in the literature. Most research in the tropics focuses on highly weathered soils and wet climates (Powers et al. 2011, Marín-Spiotta and Sharma 2013, Díaz-Vallejo et al. 2021), but tropical regions are heterogeneous with a wide diversity of soils, climates, and vegetation (Townsend et al. 2008). Our work highlights that to be able to predict the response of tropical SOC to land use change and inform management practices, it is important to consider SOC response in these diverse environmental conditions.

Our analysis of benchmark models revealed that the Scores Benchmark offers advantages in representing SOC variability and assigning scores to SOC at regional scales. In contrast, the Soil Health Gap benchmark may be more suitable at site-level scales. However, challenges exist in implementing the benchmarks, particularly in tropical regions with diverse forest and agricultural systems and complex SOC controls. Generally, both benchmarks have advantages and disadvantages, but considering the ease of manipulation, a scoring benchmark would be more appropriate for tropical regions. Our research highlights the need to develop a benchmark for tropical soils to address the effects of land use change on soil carbon and establish guidelines or protocols.

Figures

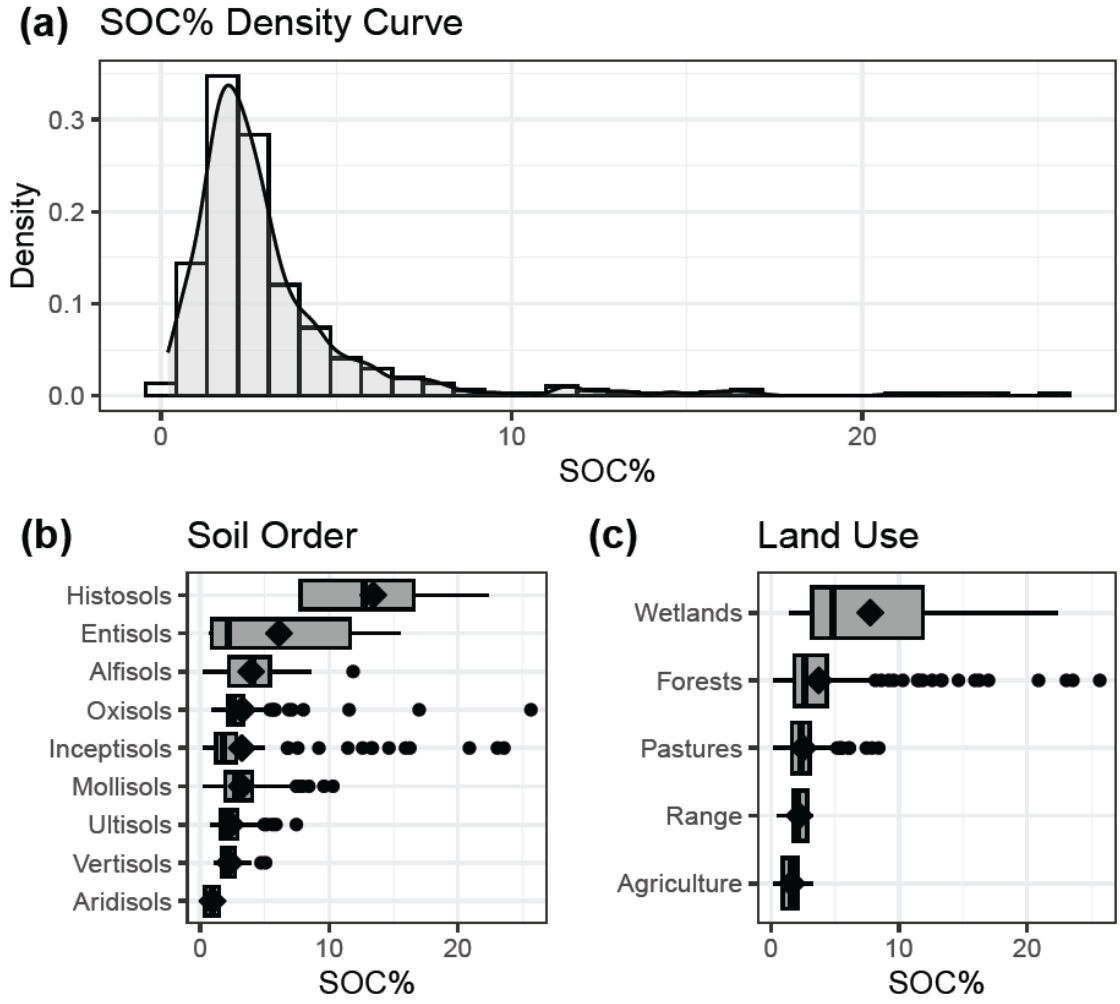

Figure 1. Soil organic carbon content from 0-30 cm depth a) density and distribution across b) soil orders and c) land uses.

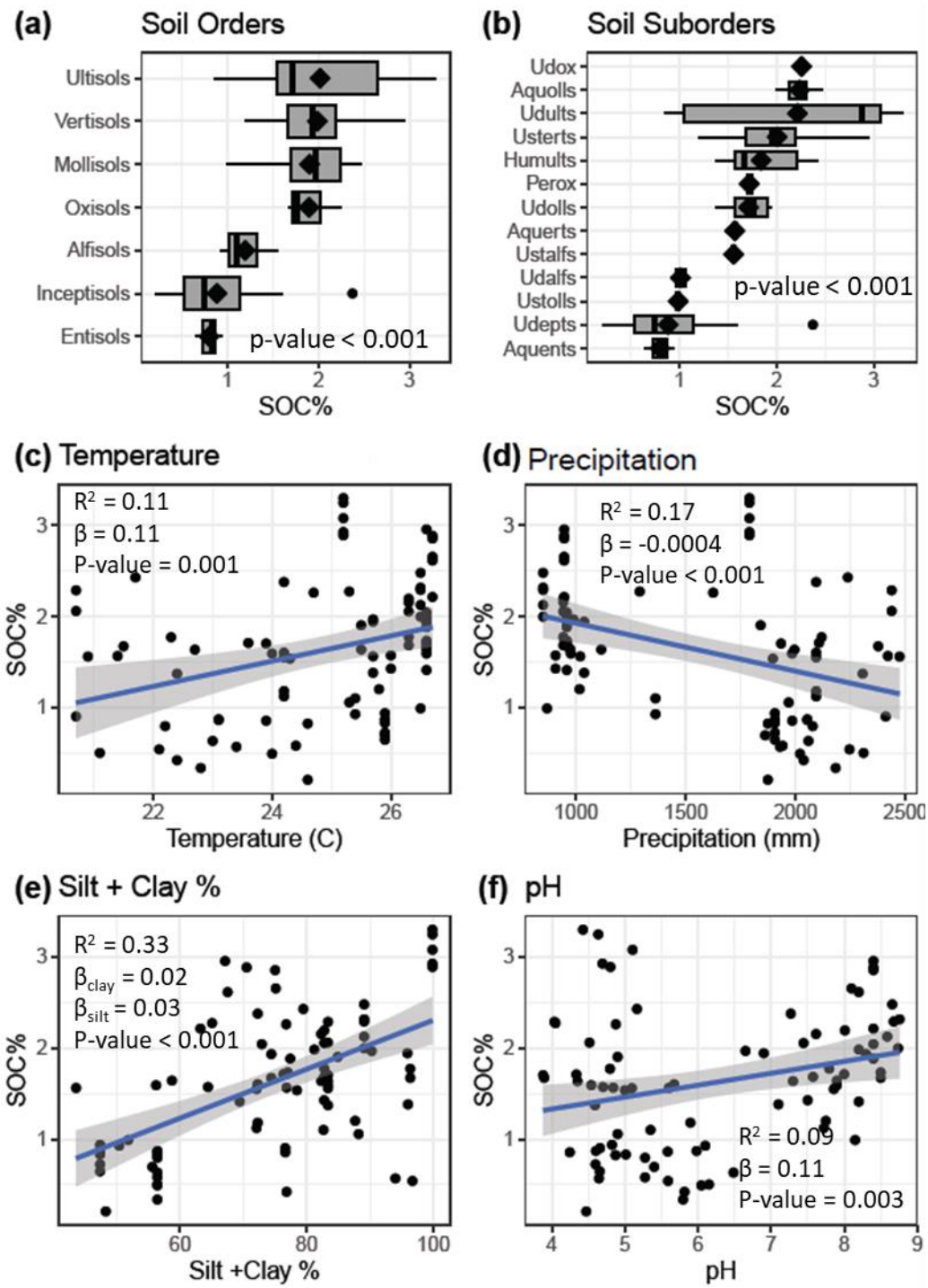

Figure 2. Agricultural lands soil organic content distribution across a) soil orders, b) soil suborders, c) Mean Annual Temperature, d) Mean Annual Precipitation, e) Silt + Clay, and d) pH.

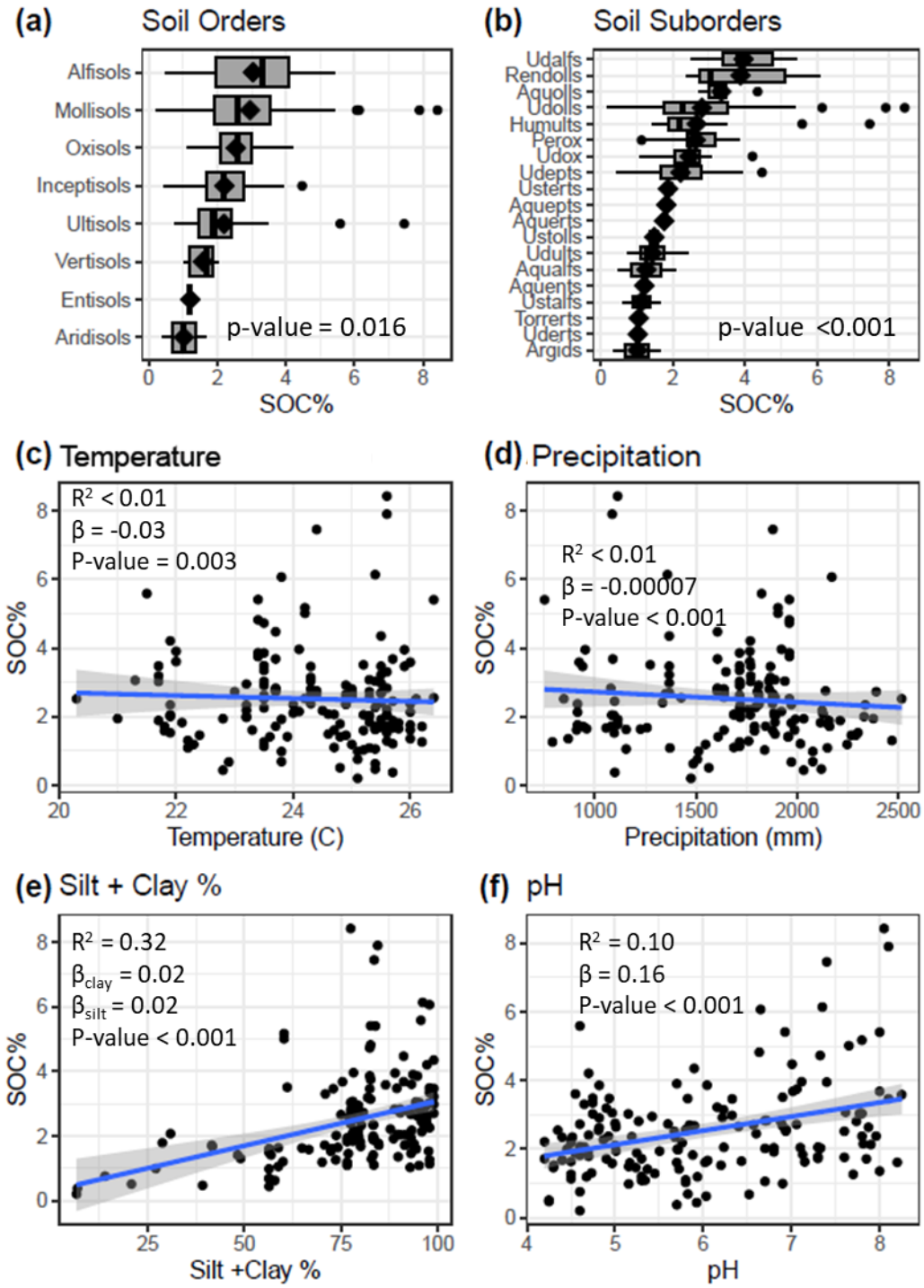

Figure 3. Pasture lands soil organic content distribution across a) soil orders, b) soil suborders, c) Mean Annual Temperature, d) Mean Annual Precipitation, e) Silt + Clay, and d) pH.

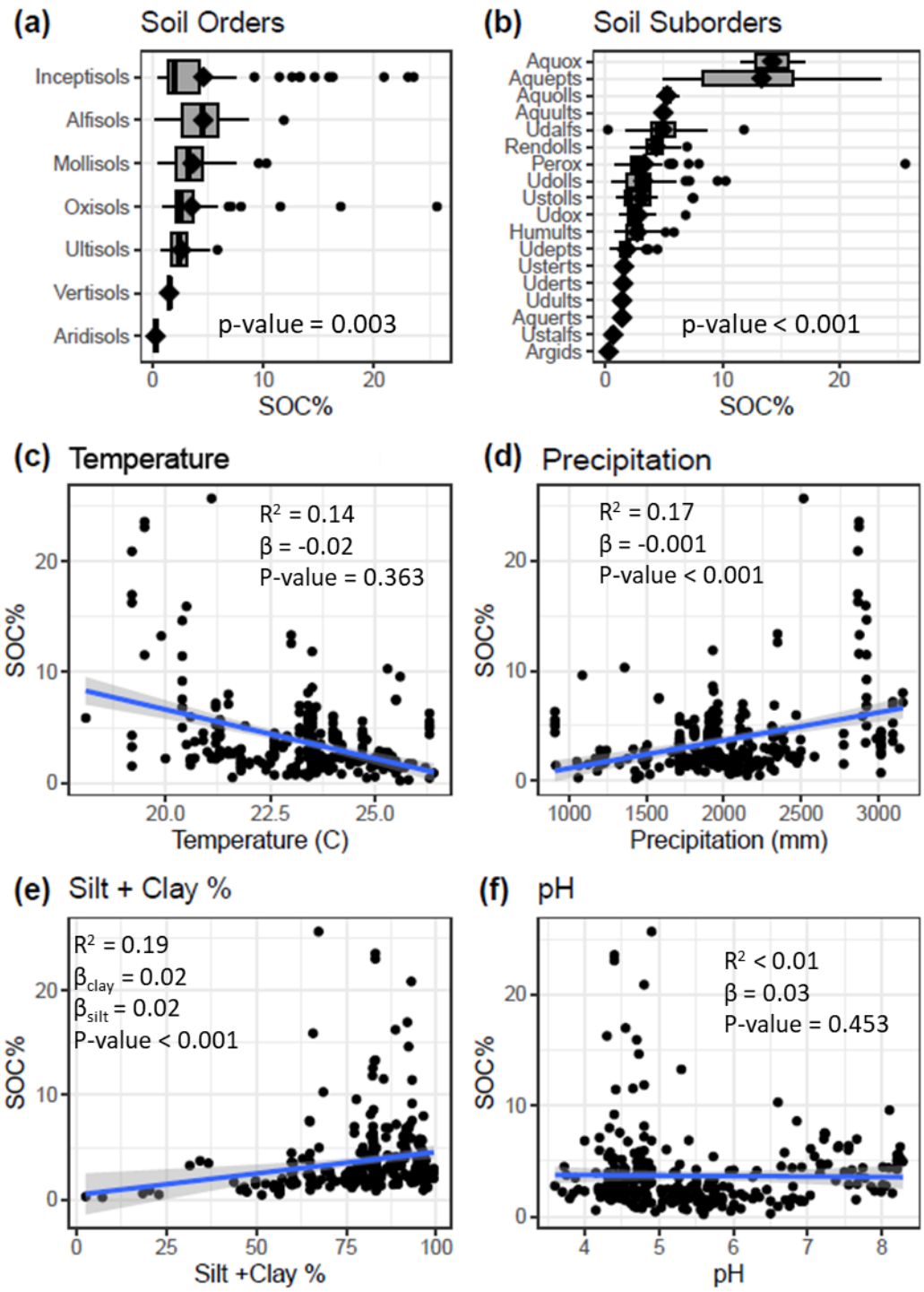

Figure 4. Forest soil organic content distribution across a) soil orders, b) soil suborders, c) Mean Annual Temperature, d) Mean Annual Precipitation, e) Silt + Clay, and d) pH.

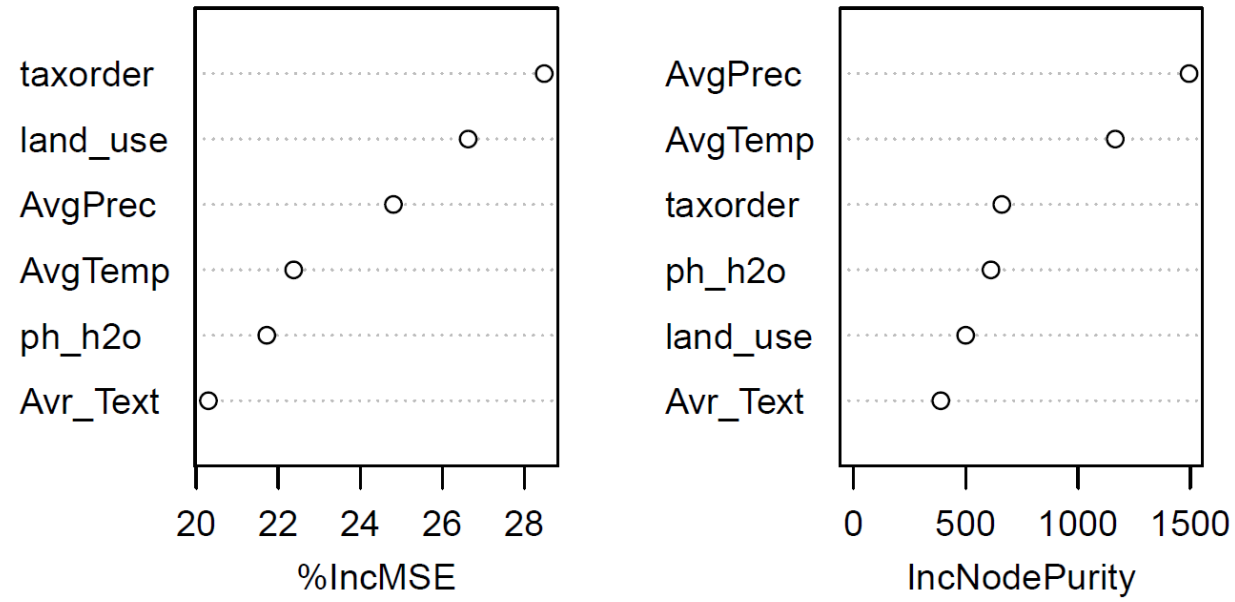

Figure 5. Random forest variable importance.taxorder= soil order, AbgPrec = Mean annual precipitation, AvgTemp = Mean annual temperature, Avr_Text = USDA texture classifications.

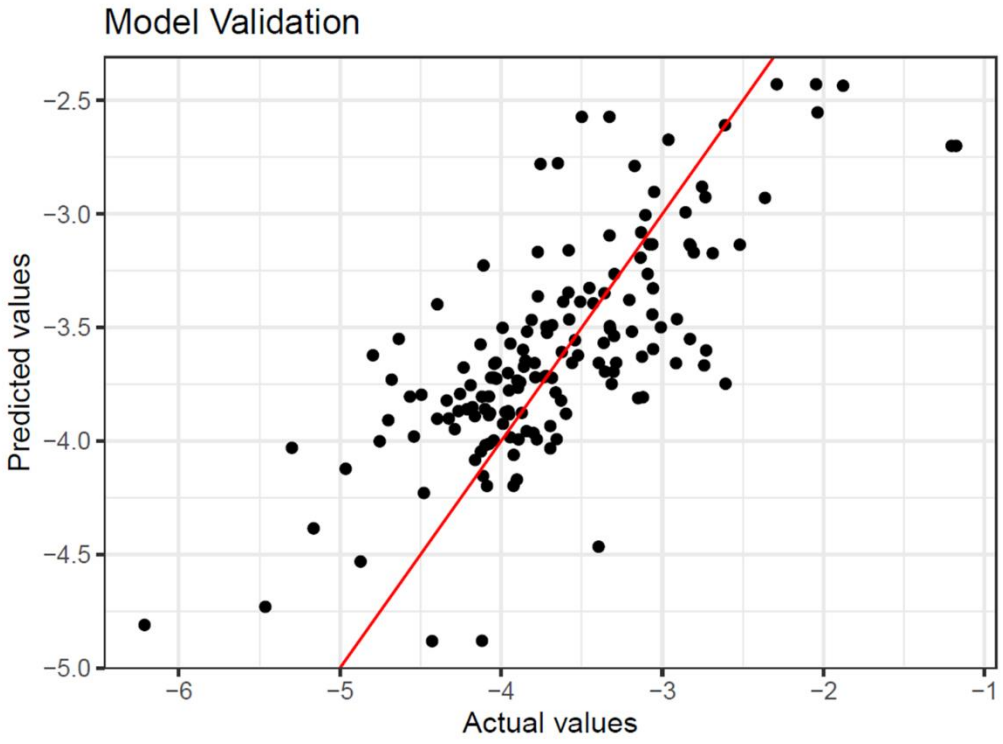

Figure 6. Random forest model validation using 30% of total data.

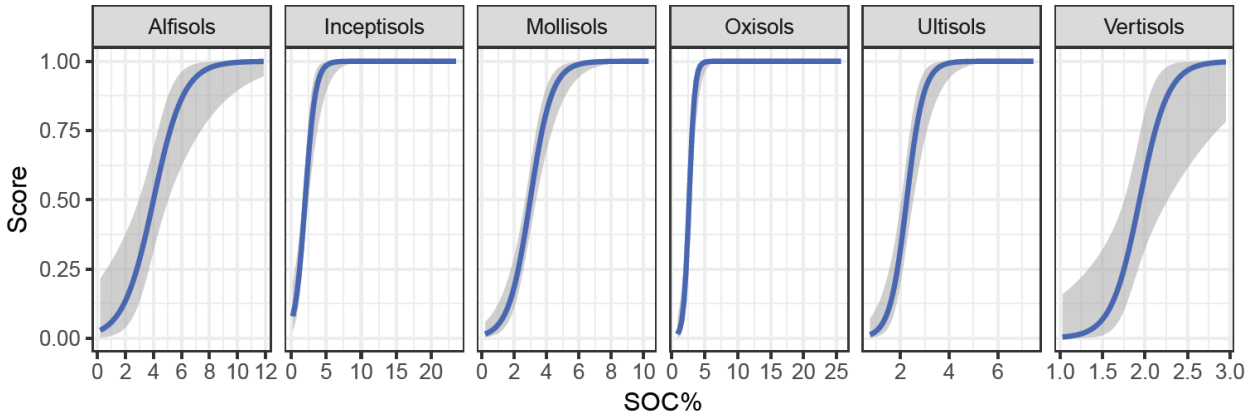

Figure 7. Soil organic carbon Score Benchmark by soil order.

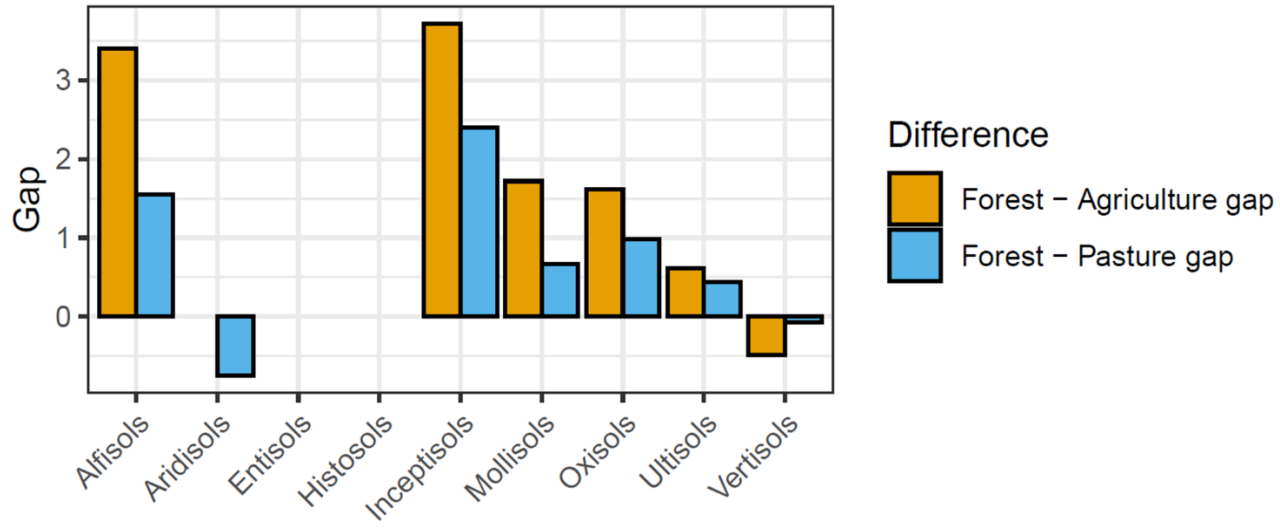

Figure 8. Soil organic carbon Soil Health Gap Benchmark by soil order. Gap = the difference of SOC in forest vs. agricultural lands or pastures.

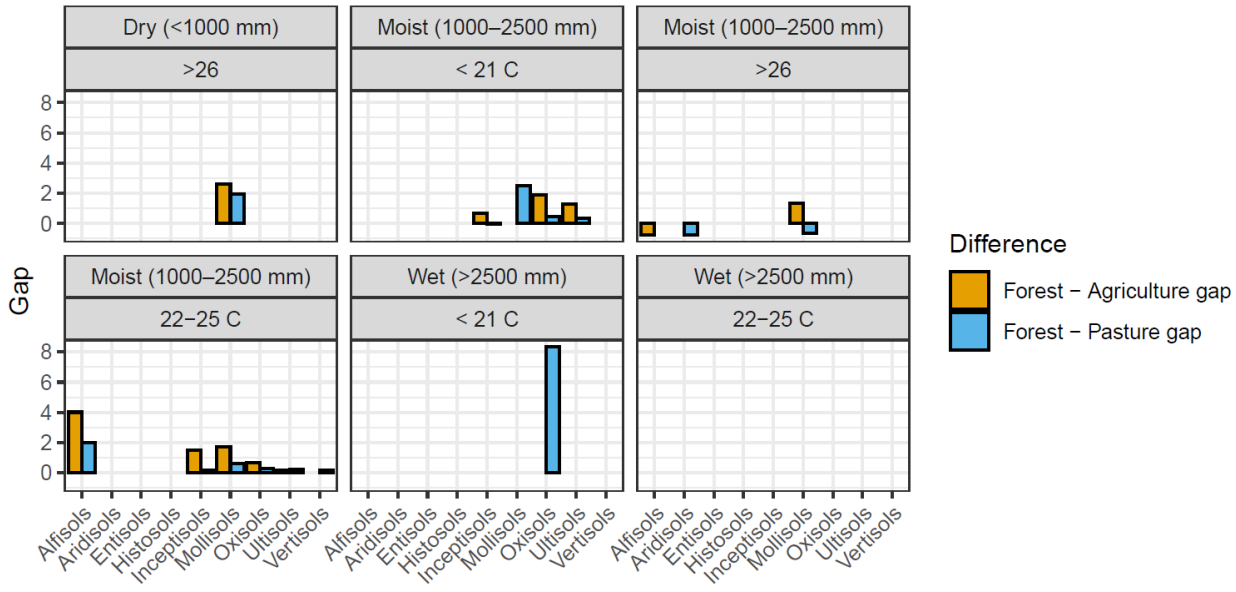

Figure 9. Soil organic carbon Soil Health Gap Benchmark by soil order and climate classes. Gap = the difference of SOC in forest vs. agricultural lands or pastures.

Tables

Table 1. Analysis of Variance Results for testing differences among land use types.

	df	Sum of Squares	Mean of Squares	F value	p-value
Land Use	4	54.61	13.651	29.5	<2e-16
Residuals	581	268.87	0.463		

Table 2. Tukey HDS to test pair comparisons among difference land use types.

	Difference	Lower CI	Upper CI	p-value
Forests-Agriculture	0.71	0.48	0.94	0.00
Pastures-Agriculture	0.44	0.19	0.69	0.00
Range-Agriculture	0.38	-0.06	0.82	0.13
Wetlands-Agriculture	1.47	1.03	1.92	0.00
Pastures-Forests	-0.27	-0.46	-0.09	0.00
Range-Forests	-0.33	-0.73	0.07	0.17
Wetlands-Forests	0.76	0.35	1.17	0.00
Range-Pastures	-0.06	-0.47	0.36	1.00
Wetlands-Pastures	1.04	0.61	1.46	0.00
Wetlands-Range	1.09	0.54	1.65	0.00

Table 3. Analysis of Variance testing difference of SOC among soil orders, suborders, and USDA texture class classification.

		df	Sum of Squares	Mean of Squares	F value	p-value
Agricultural lands						
	Soil Order	6	14.970	2.495	15.720	0.000
	Residuals	78	12.380	0.159		
	Soil Suborder	12	15.840	1.320	8.260	0.000
	Residuals	72	11.510	0.160		
	USDATexture Classification	7	7.241	1.034	3.962	0.001
	Residuals	77	20.104	0.261		
Pasture lands						
	Soil Order	7	5.690	0.812	2.575	0.016
	Residuals	152	47.950	0.315		
	Soil Suborder	18	15.660	0.870	3.230	0.000
	Residuals	141	37.980	0.269		
	USDATexture Classification	9	19.080	2.120	9.202	0.000
	Residuals	150	34.550	0.230		
Forest lands	Soil Order	6	11.060	1.844	3.386	0.003
	Residuals	289	157.370	0.545		
	Soil Suborder	17	82.930	4.878	15.860	<2e-16
	Residuals	278	85.510	0.308		
	USDATexture Classification	10	38.320	3.832	8.364	0.000
	Residuals	282	129.200	0.458		

Table 4. Regression analysis results from testing SOC relationship to Mean annual temperature, Mean annual precipitation, Silt+Clay%, and pH.

		Estimate	Standar Error	t-value	p-value	F value	R2
Agricultural lands							
	Intercept	-6.952	0.820	-8.483	0.000	11.050	0.107
	Mean Annual Temperature	0.109	0.033	3.324	0.001		
	Intercept	-3.550	0.168	-21.150	<2e-16	18.750	0.174
	Mean Annual Precipitation	0.000	0.000	-4.330	0.000		
	Intercept	-5.919	0.263	-22.534	<2e-16	21.500	0.328
	Clay%	0.018	0.003	5.231	0.000		
	Silt%	0.027	0.004	6.114	0.000		
	Intercept	-4.937	0.239	-20.660	<2e-16	9.178	0.089
	pH	0.112	0.037	3.030	0.003		
Pasture lands							
	Intercept	-3.045	0.829	-3.674	0.000	0.833	-0.001
	Mean Annual Temperature	-0.031	0.034	-0.913	0.363		
	Intercept	-3.670	0.201	-18.227	<2e-16	0.438	-0.004
	Mean Annual Precipitation	0.000	0.000	-0.662	0.509		
	Intercept	-5.222	0.167	-31.203	<2e-16	38.090	0.320
	Clay%	0.018	0.002	8.005	0.000		
	Silt%	0.017	0.002	7.227	0.000		
	Intercept	-4.735	0.226	-20.920	<2e-16	17.840	0.096
	pH	0.157	0.037	4.224	0.000		
Forest lands							
	Intercept	0.606	0.588	1.029	0.304	49.500	0.142
	Mean Annual Temperature	-0.177	0.025	-7.036	0.000		
	Intercept	-4.578	0.176	-25.967	<2e-16	37.900	0.112
	Mean Annual Precipitation	0.001	0.000	6.157	0.000		
	Intercept	-5.194	0.205	-25.345	<2e-16	34.670	0.187
	Clay%	0.019	0.003	7.172	0.000		
	Silt%	0.023	0.003	7.799	0.000		
	Intercept	-3.689	0.208	-17.738	<2e-16	0.566	-0.001
	pH	0.028	0.037	0.752	0.453		

Table 5. Stepwise model results for best model for SOC on each land use type and the whole region of Puerto Rico.

		R2	F value	p-value
Agricultural lands	$SOC = o\ \beta_o + \beta_1(\text{Soil Oder}) + \beta_2(\text{Silt+Clay}) + \beta_3(\text{Annual Temperature}) + \beta_4(\text{pH}) + e$	0.6	15.09	1.13E-13
Pasture lands	$SOC = \beta_o + \beta_1(\text{Soil Suborder}) + \beta_2((\text{Silt+Clay}) + \beta_3(\text{pH}) + e$	0.45	7.674	2.72E-14
Forest Lands	$SOC = \beta_o + \beta_1(\text{Soil Suborder}) + \beta_2(\text{Texture Classification}) + \beta_3(\text{Annual Temperature}) + e$	0.598	17.66	< 2.2e-16
Whole region	$SOC = \beta_o + \beta_1(\text{Soil Suborder}) + \beta_2(\text{Land use type}) + \beta_3(\text{Texture Classification}) + \beta_4(\text{pH}) + \beta_5(\text{Annual Temperature}) + e$	0.055	18.34	< 2.2e-16

Table 6. Analysis of Variance testing difference of SOC gap among soil orders and land use type.

	df	Sum of Squares	Mean of Squares	F value	p-value
Soil Order	6	19.231	3.205	9.708	0.0123
Land use type	1	1.774	1.774	5.372	0.0682
Residuals	5	1.651	0.33		

References

Achard, F., H. D. Eva, P. Mayaux, H.J. Stibig, and A. Belward. 2004. Improved estimates of net carbon emissions from land cover change in the tropics for the 1990s. Global Biogeochemical Cycles 18.

Allen, D. E., B. P. Singh, and R. C. Dalal. 2011. Soil Health Indicators Under Climate Change: A Review of Current Knowledge. Pages 25–45 *in* B. P. Singh, A. L. Cowie, and K. Y. Chan, editors. Soil Health and Climate Change. Springer Berlin Heidelberg, Berlin, Heidelberg.

Alori, E. T., A. O. Adekiya, and K. A. Adegbite. 2020. Impact of Agricultural Practices on Soil Health. Pages 89–98 *in* B. Giri and A. Varma, editors. Soil Health. Springer International Publishing, Cham.

Alvarez, R. 2005. A review of nitrogen fertilizer and conservation tillage effects on soil organic carbon storage. Soil Use and Management 21:38–52.

Andrews, S. S., D. L. Karlen, and C. A. Cambardella. 2004. The Soil Management Assessment Framework. Soil Science Society of America Journal 68:1945–1962.

Bautista-Cruz, A., and R. F. del Castillo. 2005. Soil Changes During Secondary Succession in a Tropical Montane Cloud Forest Area. Soil Science Society of America Journal 69:906–914.

Beaudette, D. E., J. Skovlin, S. Roecker, and M. D. Beaudette. 2016. Package 'soilDB.'

Bran Nogueira Cardoso, E. J., R. L. Figueiredo Vasconcellos, D. Bini, M. Y. Horta Miyauchi, C. A. dos Santos, P. R. Lopes Alves, A. M. de Paula, A. S. Nakatani, J. de M. Pereira, and M. A. Nogueira. 2013. Soil health: looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? SCIENTIA AGRICOLA 70:274–289.

Brown, S., and A. E. Lugo. 1990. Effects of forest clearing and succession on the carbon and nitrogen content of soils in Puerto Rico and US Virgin Islands. Plant and Soil 124:53–64.

Byrnes, R. C., D. J. Eastburn, K. W. Tate, and L. M. Roche. 2018. A Global Meta-Analysis of Grazing Impacts on Soil Health Indicators. Journal of Environmental Quality 47:758–765.

Conant, R. T., M. G. Ryan, G. I. Ågren, H. E. Birge, E. A. Davidson, P. E. Eliasson, S. E. Evans, S. D. Frey, C. P. Giardina, F. M. Hopkins, R. Hyvönen, M. U. F. Kirschbaum, J. M. Lavallee, J. Leifeld, W. J. Parton, J. Megan Steinweg, M. D. Wallenstein, J. Å. Martin Wetterstedt, and M. A. Bradford. 2011. Temperature and soil organic matter decomposition rates – synthesis of current knowledge and a way forward. Global Change Biology 17:3392–3404.

Daly, C., E. H. Helmer, and M. Quiñones. 2003. Mapping the climate of Puerto Rico, Vieques and Culebra. International Journal of Climatology 23:1359–1381.

D.C. Yoder, S. Jagadamma, S. Singh, A. Nouri, S. Xu, D. Saha, S.M. Schaeffer, N. Adotey, F.R. Walker, J. Lee, and M. Budipradigdo. 2022. Soil health: Meaning, measurement, and value through a critical zone lens. Journal of Soil and Water Conservation 77:88.

Detwiler, R. P. 1986. Land use change and the global carbon cycle: the role of tropical soils. Biogeochemistry 2:67–93.

Díaz-Vallejo, E. J., M. Seeley, A. P. Smith, and E. Marín-Spiotta. 2021. A meta-analysis of tropical land-use change effects on the soil microbiome: Emerging patterns and knowledge gaps. Biotropica 53:738–752.

Dlamini, P., P. Chivenge, and V. Chaplot. 2016. Overgrazing decreases soil organic carbon stocks the most under dry climates and low soil pH: A meta-analysis shows. Agriculture, Ecosystems & Environment 221:258–269.

Don, A., J. Schumacher and Freibauer. 2011. Impact of tropical land-use change on soil organic carbon stocks – a meta-analysis. Global Change Biology 17:1658–1670.

Don, A., J. Schumacher, M. Scherer-Lorenzen, T. Scholten, and E.-D. Schulze. 2007. Spatial and vertical variation of soil carbon at two grassland sites — Implications for measuring soil carbon stocks. Geoderma 141:272–282.

Fearnside, P. M., and R. Imbrozio Barbosa. 1998. Soil carbon changes from conversion of forest to pasture in Brazilian Amazonia. Forest Ecology and Management 108:147–166.

Franco, P. A., P. L. Weaver, and S. Eggen-McIntosh. 1997. Forest resources of Puerto Rico, 1990. Resour. Bull. SRS-22. Asheville, NC: US Department of Agriculture, Forest Service, Southern Research Station. 4.5 p. Resour. Bull. SRS-22. Asheville, NC: US Department of Agriculture, Forest Service, Southern Research Station. 4.5 p.

Gould, W. A., F. H. Wadsworth, M. Quiñones, S. J. Fain, and N. L. Álvarez-Berríos. 2017. Land Use, Conservation, Forestry, and Agriculture in Puerto Rico. Forests 8:242.

Graham, M. H., R. J. Haynes, and J. H. Meyer. 2002. Soil organic matter content and quality: effects of fertilizer applications, burning and trash retention on a long-term sugarcane experiment in South Africa. Soil Biology and Biochemistry 34:93–102.

Guo, L. B., and R. M. Gifford. 2002. Soil carbon stocks and land use change: a meta-analysis. Global Change Biology 8:345–360.

Hobley, E. U., J. Baldock, and B. Wilson. 2016. Environmental and human influences on organic carbon fractions down the soil profile. Agriculture, Ecosystems & Environment 223:152–166.

Houghton, R. A. 1995. Soils and global change. London, L ew is Publisher:45–65.

Hughes, R. F., J. B. Kauffman, and V. J. Jaramillo. 1999. Biomass, carbon and nutrient dynamics of secondary forests in a humid tropical region of Mexico. Ecology 80:1892–1907.

Idowu, O. J., H. M. van Es, G. S. Abawi, D. W. Wolfe, R. R. Schindelbeck, B. N. Moebius-Clune, and B. K. Gugino. 2009. Use of an integrative soil health test for evaluation of soil management impacts. Renewable Agriculture and Food Systems 24:214–224.

Jobbágy, E. G., and R. B. Jackson. 2000. The Vertical Distribution of Soil Organic Carbon and Its Relation to Climate and Vegetation. Ecological Applications 10:423–436.

Jones, I. L., S. J. DeWalt, O. R. Lopez, L. Bunnefeld, Z. Pattison, and D. H. Dent. 2019. Above- and belowground carbon stocks are decoupled in secondary tropical forests and are positively related to forest age and soil nutrients respectively. Science of The Total Environment 697:133987.

Kay, B. D. 2018. Soil structure and organic carbon: a review. Soil processes and the carbon cycle:169–197.

de Koning, G. H. J., E. Veldkamp, and M. López-Ulloa. 2003. Quantification of carbon sequestration in soils following pasture to forest conversion in northwestern Ecuador. Global Biogeochemical Cycles 17.

Köppen, W. 1900. Versuch einer Klassifikation der Klimate, vorzugsweise nach ihren Beziehungen zur Pflanzenwelt. Geographische Zeitschrift 6:593–611.

Kottek, M., and M. Hantel. 2005. 17 Global climate maps (Part 12/12). Observed global climate:124–144.

Kuhn, M. 2008. Building predictive models in R using the caret package. Journal of statistical software 28:1–26.

Lal, R. 2016. Soil health and carbon management. Food and Energy Security 5:212–222.

Lehmann, J., and M. Kleber. 2015. The contentious nature of soil organic matter. Nature 528:60–68.

Liaw, A., and M. Wiener. 2002. Classification and regression by randomForest. R news 2:18–22.

Lugo, A. E., and S. Brown. 1993. Management of tropical soils as sinks or sources of atmospheric carbon. Plant and soil 149:27–41.

Luo, Y., A. Ahlström, S. D. Allison, N. H. Batjes, V. Brovkin, N. Carvalhais, A. Chappell, P. Ciais, E. A. Davidson, A. Finzi, K. Georgiou, B. Guenet, O. Hararuk, J. W. Harden, Y. He, F. Hopkins, L. Jiang, C. Koven, R. B. Jackson, C. D. Jones, M. J. Lara, J. Liang, A. D. McGuire, W. Parton, C. Peng, J. T. Randerson, A. Salazar, C. A. Sierra, M. J. Smith, H. Tian, K. E. O. Todd-Brown, M. Torn, K. J. van Groenigen, Y. P. Wang, T. O. West, Y. Wei, W. R. Wieder, J. Xia, X. Xu, X. Xu, and T. Zhou. 2016. Toward more realistic projections of soil carbon dynamics by Earth system models. Global Biogeochemical Cycles 30:40–56.

Luo, Z., W. Feng, Y. Luo, J. Baldock, and E. Wang. 2017. Soil organic carbon dynamics jointly controlled by climate, carbon inputs, soil properties and soil carbon fractions. Global Change Biology 23:4430–4439.

Lützow, M. v., I. Kögel-Knabner, K. Ekschmitt, E. Matzner, G. Guggenberger, B. Marschner, and H. Flessa. 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. European Journal of Soil Science 57:426–445.

Maharjan, B., S. Das, and B. S. Acharya. 2020. Soil Health Gap: A concept to establish a benchmark for soil health management. Global Ecology and Conservation 23:e01116.

Marín-Spiotta, E., and S. Sharma. 2013. Carbon storage in successional and plantation forest soils: a tropical analysis: Carbon in reforested and plantation soils. Global Ecology and Biogeography 22:105–117.

Marin-Spiotta, E., W. L. Silver, C. W. Swanston, and R. Ostertag. 2009. Soil organic matter dynamics during 80 years of reforestation of tropical pastures. Global Change Biology 15:1584–1597.

Mayes, M., E. Marin-Spiotta, L. Szymanski, M. Akif Erdoğan, M. Ozdoğan, and M. Clayton. 2014. Soil type mediates effects of land use on soil carbon and nitrogen in the Konya Basin, Turkey. Geoderma 232–234:517–527.

McGroddy, M., and W. L. Silver. 2000. Variations in Belowground Carbon Storage and Soil CO2 Flux Rates along a Wet Tropical Climate Gradient1. Biotropica 32:614–624.

de Moraes, J. F. L., B. Volkoff, C. C. Cerri, and M. Bernoux. 1996. Soil properties under Amazon forest and changes due to pasture installation in Rondônia, Brazil. Geoderma 70:63–81.

Muñoz, M. A., W. I. Lugo, C. Santiago, M. Matos, S. Ríos, and J. Lugo. 2018. Taxonomic classification of the soils of Puerto Rico, 2017.

Neill, C., J. M. Melillo, P. A. Steudler, C. C. Cerri, J. F. L. de Moraes, M. C. Piccolo, and M. Brito. 1997. Soil carbon and nitrogen stoks following forest clearing for pastures in the southwestern Brazilian Amazon. Ecological Applications 7:1216–1225.

Nunes, M. R., K. S. Veum, P. A. Parker, S. H. Holan, D. L. Karlen, J. P. Amsili, H. M. van Es, S. A. Wills, C. A. Seybold, and T. B. Moorman. 2021. The soil health assessment protocol and evaluation applied to soil organic carbon. Soil Science Society of America Journal 85:1196–1213.

Oades, J. M. 1988. The retention of organic matter in soils. Biogeochemistry 5:35–70.

Ozlu, E., and S. Kumar. 2018. Response of Soil Organic Carbon, pH, Electrical Conductivity, and Water Stable Aggregates to Long-Term Annual Manure and Inorganic Fertilizer. Soil Science Society of America Journal 82:1243–1251.

Ozlu, E., S. S. Sandhu, S. Kumar, and F. J. Arriaga. 2019. Soil health indicators impacted by long-term cattle manure and inorganic fertilizer application in a corn-soybean rotation of South Dakota. Scientific Reports 9:11776.

Parton, W. J., D. S. Schimel, C. V. Cole, and D. S. Ojima. 1987. Analysis of Factors Controlling Soil Organic Matter Levels in Great Plains Grasslands. Soil Science Society of America Journal 51:1173–1179.

Perez-Alegria, L. 2001. Atmospheric carbon sequestration in tropical watersheds. Pages 306–310 *in* F. Zazueta and J. Xin, editors. University of Puerto Rico.

Poirier, V., C. Roumet, and A. D. Munson. 2018. The root of the matter: Linking root traits and soil organic matter stabilization processes. Soil Biology and Biochemistry 120:246–259.

Powers, J. S., M. D. Corre, T. E. Twine, and E. Veldkamp. 2011. Geographic bias of field observations of soil carbon stocks with tropical land-use changes precludes spatial extrapolation. Proceedings of the National Academy of Sciences 108:6318–6322.

Powers, J. S., and E. Marín-Spiotta. 2017. Ecosystem Processes and Biogeochemical Cycles in Secondary Tropical Forest Succession. Annual Review of Ecology, Evolution, and Systematics 48:497–519.

Rasmussen, C., K. Heckman, W. R. Wieder, M. Keiluweit, C. R. Lawrence, A. A. Berhe, J. C. Blankinship, S. E. Crow, J. L. Druhan, C. E. Hicks Pries, E. Marin-Spiotta, A. F. Plante, C. Schädel, J. P. Schimel, C. A. Sierra, A. Thompson, and R. Wagai. 2018. Beyond clay: towards an improved set of variables for predicting soil organic matter content. Biogeochemistry 137:297–306.

Rawls, W. J., Y. A. Pachepsky, J. C. Ritchie, T. M. Sobecki, and H. Bloodworth. 2003. Effect of soil organic carbon on soil water retention. Quantifying agricultural management effects on soil properties and processes 116:61–76.

Ripley, B., B. Venables, D. M. Bates, K. Hornik, A. Gebhardt, D. Firth, and M. B. Ripley. 2013. Package 'mass.' Cran r 538:113–120.

Schimel, D. S., B. H. Braswell, E. A. Holland, R. McKeown, D. S. Ojima, T. H. Painter, W. J. Parton, and A. R. Townsend. 1994. Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils. Global Biogeochemical Cycles 8:279–293.

Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Advances in Soil Structure Research 79:7–31.

Six, J., R. T. Conant, E. A. Paul, and K. Paustian. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil 241:155–176.

Townsend, A. R., G. P. Asner, and C. C. Cleveland. 2008. The biogeochemical heterogeneity of tropical forests. Trends in Ecology & Evolution 23:424–431.

Vaughan, E., M. Matos, S. Ríos, C. Santiago, and E. Marín-Spiotta. 2019. Clay and climate are poor predictors of regional-scale soil carbon storage in the US Caribbean. Geoderma 354:113841.

Wadsworth, F. H. 1950. Notes on the climax forests of Puerto Rico and their destruction and conservation prior to 1900. Caribbean Forester 11:38–56.

Wiesmeier, M., L. Urbanski, E. Hobley, B. Lang, M. von Lützow, E. Marin-Spiotta, B. van Wesemael, E. Rabot, M. Ließ, N. Garcia-Franco, U. Wollschläger, H.-J. Vogel, and I. Kögel-Knabner. 2019. Soil organic carbon storage as a key function of soils - A review of drivers and indicators at various scales. Geoderma 333:149–162.

Conclusion

This work underscores the importance of considering the vast diversity of climates, vegetation, management practices, and soil conditions in tropical regions to understand their potential influence on a global-scale response to environmental changes. Specifically, it reveals trends that indicate distinct responses of soil microbial communities and associated ecosystem functions to land-use conversions. Furthermore, the research highlights the complex dynamics of microbial communities during forest succession and emphasizes the urgent need for further studies to enhance our understanding of microbial functionalities in disturbed tropical forest soils. Importantly, the research emphasizes the need to develop a benchmark for tropical soils to address the effects of land use change on soil carbon and establish guidelines or protocols. To achieve this, further investigations are required to explore the factors influencing soil carbon variability.

Future research should enhance our understanding of microbial functionalities and their roles in disturbed tropical forest soils. Our work reveals a significant bias towards moist and wet areas of the tropics. Little is known about dry systems and how land uses influence microbial communities and their functionalities. Also, incorporating other molecular techniques, such as metagenomics and metatranscriptomics, would enable a more in-depth exploration of microbial functional potentials and their responses to environmental changes. Furthermore, considering the influence of specific soil characteristics, such as pH, texture, and nutrient availability, on microbial communities and functionality would enhance our understanding of the complex interactions between soil properties, forest succession, and microbial dynamics at local scales. Additionally, incorporating a broader range of soil types found in tropical regions would provide

a more comprehensive understanding of the impact of land use changes on microbial communities and ecosystem functioning at global scales.

Future research should focus on enhancing our understanding of SOC dynamics and improving SOC assessment in agricultural and forested lands. By investigating the long-term effects of different land management practices, we will be able to understand SOC accumulation and stability variability found in the tropics. Additionally, considering the interactions between SOC and other soil properties, such as nutrient availability, topography, and macro and microorganisms, will contribute to a more comprehensive understanding of SOC dynamics. Incorporating technologies, such as remote sensing and molecular analysis techniques, can also help to improve spatial mapping and quantification of SOC at larger scales. Furthermore, collaboration among researchers, policymakers, and land managers is crucial for implementing sustainable soil management practices and promoting the adoption of appropriate SOC assessment tools. By addressing these future directions, we can advance our knowledge of SOC dynamics and contribute to developing effective strategies for soil health management and climate change mitigation.

Overall, the findings of this research provide valuable insights into the complex relationships between land use changes, soil dynamics, and ecosystem functioning, emphasizing the need for further investigations and interdisciplinary collaborations to tackle the challenges associated with tropical soil management and conservation.