

**Ecology of soil microbial communities in degraded
logged-over tropical rainforests**

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Abstract

Tropical rainforests are affected by selective logging and experience varying degrees of forest degradation, resulting in changes in forest structures (e.g., tree species composition and canopy density). This process may further affect soil ecosystems. Microbial communities are important components of soil ecosystems and play vital roles in various biogeochemical processes. Previous studies report the possible effects of forest degradation on below-ground ecosystem and provide a theoretical basis for the ecology of microbial communities in soil. However, I wonder how microbial community attributes (e.g., biomass, community composition, and functional structures) respond to changes in the below-ground ecosystem caused by forest degradation in tropical rainforests. Therefore, three research questions were proposed and carefully investigated. This doctoral dissertation consists of five chapters.

In Chapter 1, I conducted a literature review, including forest structure changes caused by forest degradation in the tropics, soil microbial communities respond to substrate variations in degraded forests, and the ways of soil microbial communities participate in nutrient cycling. I realized a close link between above- and below-ground ecosystems, that the forest degradation caused by selective logging leads to changes in litter input, may affect soil organic carbon (SOC), and SOC as a substrate further influences the microbial attributes (e.g., biomass, community compositions, and functional structures), while these changes in microbial communities may be transmitted to the activities of extracellular enzymes. However, few studies have analyzed the complex relationships between litter, substrate, soil physicochemical properties, extracellular enzymes, soil microbial biomass, and soil microbial communities. Also, the previous studies on forest degradation are coarse and lack a spatial-temporal gradient to accurately depict the forest degradation processes. Therefore, I pointed out the necessity to establish a forest degradation gradient through appropriate vegetation metrics and to analyze the changes in soil physicochemical properties, extracellular enzymes, soil microbial biomass, and soil bacterial and fungal communities along the forest degradation gradient. “Forest degradation” was defined

here as changes in forest attributes (e.g., carbon storage, biological productivity, species compositions, and forest structures) caused by anthropogenic activities.

In Chapter 2, I conducted a field survey and analyzed how soil physicochemical properties, soil microbial biomass, and extracellular enzyme activities change along a forest degradation gradient. Initially, a forest degradation gradient was established and represented as forest degradation indexes. Concentrations of SOC, total nitrogen (TN), soil microbial biomass carbon (SMBC), and soil microbial biomass nitrogen (SMBN) as well as C/N ratio decreased with decreasing forest degradation indexes, while soil pH values increased. The activities of acid phosphatase (ACP), β -glucosidase (BG), leucine aminopeptidase (LAP), and N-acetyl-glucosaminidase (NAG) all decreased with decreasing forest degradation indexes. ACP hydrolyzes organic P and influences P availability. BG occupies a central position in the global-scale C cycling because it catalyzes the final step in cellulose decomposition. LAP releases N-terminal leucine residues from proteins and peptide. NAG hydrolyzes N-acetyl-glucosides mainly targeting chitin and peptidoglycan. The SOC explained the greatest variation of soil microbial biomass was also observed. The results suggested that above-ground forest degradation has a direct effect on soil organic matter, which further affects the soil microbial biomass. Moreover, the combined effects of reduced microbial biomass and reduced soil organic matter as a substrate for enzymes reduce microbial enzyme activities.

In Chapter 3, I analyzed the bacterial and fungal community compositions and enzyme efficiency (the quotient of enzyme activity to SMBC) through a series of experiments and statistical analyses. The data obtained in Chapter 2 were still utilized. The PCoA axis-1 values for the absolute abundances of bacterial and fungal dominant phyla were chosen to represent their community compositions, respectively. Bacterial community composition did not change with forest degradation gradient, while fungal community composition shifted. Reduced SOC in degraded forests influenced bacterial and fungal community compositions, but only fungal community composition affected the activities of ACP, BG, and LAP. Additionally, ACP activity/SMBC and LAP

activity/SMBC increased with decreasing SOC. My result showed distinctions in the shifts of bacterial and fungal community compositions along forest degradation gradient, highlighting shifts in fungal community composition may reflect preferences of different fungal phyla for changes in soil organic matter quality and/or quantity during litter input reduction, and such changes further influence enzyme activities. Moreover, microbial communities might be limited by soil N and P in highly degraded forests.

In Chapter 4, I predicted the functional potentials of bacterial and fungal communities using PICRUST2 and analyzed how bacterial and fungal functional structures change with forest degradation. In addition, the relative abundances of potential functional genes for specific extracellular enzymes were also analyzed to investigate how their changes relate to enzyme activities. The PCoA axis-1 values for the relative abundances of bacterial and fungal E.C. numbers (i.e., the relative abundances of potential functional genes) were chosen to represent their functional structures, respectively. Fungal functional structure did not change with forest degradation indexes, while bacterial functional structure shifted. Soil physicochemical properties including SOC, TN, TP, and pH influenced bacterial functional structure, whereas TN, TP, and pH influenced fungal functional structure. Besides, the relative abundance of ACP gene (for bacteria and fungi) decreased with decreasing forest degradation indexes, while those of BG (for bacteria and fungi) increased. The relative abundance of NAG gene increased in bacterial communities whereas decreased in fungal communities. My results showed clear differences in the changes of bacterial and fungal functional structures along forest degradation gradients. Directional changes in the relative abundance of some bacterial phyla with forest degradation may cause the shift in bacterial functional structures. The unchanged fungal functional structure may suggest that fungi have functional redundancy. Also, bacterial functional potentials are more susceptible to altered soil physicochemical properties. Furthermore, the increased relative abundances of BG and NAG genes during forest degradation suggest improved microbial potential for cellulose and chitin degradation, which may contribute to the

maintenance of BG and NAG efficiencies in highly degraded forests.

In Chapter 5, I discussed the ecology of microbial communities in degraded forests in the tropics based on the results of Chapter 2 to Chapter 4. Forest degradation caused by logging in Malaysian Borneo has formed degraded logged-over tropical rainforest. In this substrate and nutrients limited (e.g., P and N) forest, microbial communities, especially fungi, promote nutrient access by regulating extracellular enzyme activities such as ACP and LAP. Compared with the primary forest, the bacterial and fungal α -diversities in degraded forest are lower, while fungal β -diversity is higher. The changes in substrates (from recalcitrant litter in the primary forest to SOC in degraded logged-over forest) lead to turnover of saprophytic fungi and changes in fungal community composition. However, due to functional redundancy, fungal communities in degraded forests maintain the functional reservoir with genes coding similar metabolic functions as those in primary forests. Bacterial community composition of degraded logged-over tropical rainforest is not much different from that of primary forest because the substrate availability for bacteria is buffered and stabilized by the fungal community, but *r*-strategists (e.g., Proteobacteria and Bacteroidetes) are mainly selected in bacterial communities in degraded forest. In addition, I recognized that my findings in the mixed dipterocarp lowland tropical rainforests of Malaysian Borneo may be unique compared to research on microbial ecology in other degraded tropical forests. Therefore, three explore topics were proposed as future plans to form a complete profile of microbial ecology in degraded forests.

My study supplements the data on below-ground ecosystem and establishes a close relationship between above-ground and below-ground ecosystems by analyzing the ecology of soil microbial communities. The mechanisms of the Bornean ecosystem response to anthropogenic disturbance (e.g., selective logging) are further completed from a soil microbial perspective. I concluded that decreased quality and quantity of litter can lead to SOC decline. Changed substrate affects fungal community composition and transmits to the extracellular enzyme activities. Substrate decrease does not affect fungal functional structures as fungi have functional redundancy.

Chapter 1 General introduction

1.1 Forest degradation in tropical rainforests

Forest degradation is a serious global environmental issue, exerting a profound impact on the Earth's ecosystem (Thompson et al., 2003; Sasaki et al., 2009; Ghazoul et al., 2015; Vásquez-Grandón et al., 2018). The Food and Agriculture Organization of the United Nations (FAO) defines forest degradation as the decline in the capacity of a forest to provide goods and services (FAO, 2011). This definition has a broad connotation and is based on the role of forests in providing ecosystem services for human wellbeing. The most well-used definition in terms of scientific research is the one by the IPCC (Inter-governmental Panel of Climate Change), which defines forest degradation as the decline of forest biomass over a certain period (IPCC, 2003). The magnitude of forest degradation can be quantitatively evaluated by several metrics using this IPCC definition (IPCC, 2003; Simula, 2009; Bustamante et al., 2016), but both reference levels and reference periods vary considerably among studies (Simula, 2009; Thompson et al. 2013; Gao et al., 2020). In addition to these two most cited definitions, there are more than 50 definitions that are proposed by different researchers and multiple stakeholders (Lund 2009). It should be noted that forest degradation can be context-dependent and the following my review on forest degradation in tropical countries is largely pertinent to the IPCC definition.

Tropical rainforests continue to suffer from negative impacts of forest degradation no matter how forest degradation is defined. Anthropogenic activities such as unsustainable selective logging of timber, overgrazing, over-hunting, and wildfires are the known drivers of forest degradation (Asner et al., 2006; Asner et al., 2009; Murdiyarso et al., 2008; Putz et al., 2010). Hosonuma et al. (2012) indicate that selective logging is identified as the primary cause of degradation in production forests, accounting for 52% of all contributing factors. Selective logging is a method of timber harvesting in which only the largest, most commercially valuable specific trees are cut and removed from the forest, rather than clear-cutting the entire area (Asner, 2005). Intensive selective logging driven by the escalating demand for timber products results

in severe stand damages (Lambin, 1999; Pimm and Raven, 2000; Huth and Ditzer, 2001; Lewis, 2006; Grainger, 2013; Stanturf et al., 2014; de Andrade et al., 2017). In Brazilian Amazon rainforests, the disturbances of forests are primarily attributed to selective logging (60.4%), with the majority (approximately 90%) of degraded forests concentrated in Mato Grosso and Pará (Matricardi et al., 2013). Landsat images reveal that selective logging has disturbed over 31% of natural forests in the Brazilian Amazon between 1992 and 2004 (Matricardi et al., 2010). At least 76% of harvesting practices cause significant levels of canopy damage (Asner et al., 2005). In Indonesia, selective logging since the 1970s has resulted in relatively high levels of forest degradation, leading to a notable reduction in tree diversity (FWI, 2002; FAO, 2010; Margono et al., 2012; Tsujino et al., 2016). Research in West Kalimantan, Indonesia, shows that forest degradation results in more than 50% loss of important species (e.g., *Gonystylus bancanus*, *Shorea belangeran*, *Shorea teijsmanniana*), and the abundance of trees in highly degraded forests is reduced by about 40% compared with that in primary forests (Astiani, 2016). Selective logging has been practiced in Malaysian Borneo since 1978 (Thang, 1987). Dipterocarps (Dipterocarpaceae) are considered the main commercial trees and are usually removed (Pinard and Putz, 1996). Forest degradation caused by selective logging destroys the integrity of the primary forests. Of the total remaining forest cover in Malaysian Borneo (109,877 km² in 2009), only 38% remains relatively intact, and the remainder is either degraded (39%) or severely degraded (23%) (Bryan et al., 2013). Besides, such forest degradation can also exert a detrimental influence on biodiversity and tree compositions (Aoyagi et al., 2017).

Plant litter can be considered as the “pump” in the C cycling of terrestrial ecosystems, as the quality and quantity of litter inputs are key drivers of decomposition processes and soil organic carbon (SOC) formation (Paul 2016). Forest degradation caused by selective logging has impacts on litter characteristics (e.g., litterfall rate, litter compositions, and litter chemistry) by reducing litter production and changing tree species composition in addition to the direct impacts on stand structure (Berry et al., 2008). Selective logging in Sabah of Malaysian Borneo primarily harvests commercial

trees such as large individuals of dipterocarp, leaving behind logged-over forests dominated by the pioneer species of *Macaranga* (Howlett and Davidson, 2003; Aoyagi et al., 2013). Burghouts et al. (1992) find that in Sabah, the annual litterfall rate is lower in logged forests than in primary forests. Junaedi et al. (2020) also demonstrate that the litterfall rate in *Macaranga* stands (i.e., logged forests; 4.74 to 7.04 ton ha⁻¹ yr⁻¹) is lower than dipterocarp stands (i.e., primary forests; 9.18 ton ha⁻¹ yr⁻¹). Pfeifer et al. (2016) also suggest that above-ground biomass (AGB) decreases progressively with the increase of logging intensity in Sabah from 413.4 t ha⁻¹ on average in a primary forest to 267.3 t ha⁻¹ in a lightly logged forest to 110.6 t ha⁻¹ in a twice-logged forest and to 42.4 t ha⁻¹ in an intensively harvested forest. Moreover, litter quality may also change as the primary forests become open lands during forest degradation process (Paudel et al., 2015). Litter chemistry varies among species in carbon (C) and nitrogen (N) content, lignin content, and C/N ratio (McGroddy et al., 2004). Yeong et al. (2016) demonstrate that the total amount of C, N, and P provided by litter in degraded forests may be lower than in primary forests in Malaysian Borneo. The simultaneous changes in both quality and quantity of litter during forest degradation process could limit soil C and nutrient input, thereby directly impacting soil physicochemical properties.

Hereafter, I define forest degradation as changes in forest attributes (e.g., C storage, biological productivity, species compositions, and forest structures) caused by anthropogenic activities (Bustamante et al., 2016; Lapola et al., 2023). Because I deal with selective logging in Bornean tropical rainforests, forest degradation is a change in canopy species composition and structure caused by selective logging. The magnitude of forest degradation will be expressed as the dissimilarity of canopy species composition of a given logged-over forest with a primary forest as will be shown in Chapters 2-4.

1.2 Effects of altered litter inputs on soil physicochemical properties

Litter is the primary source of C input to the soil through decomposition (Berg and

McClaugherty, 2008). Theoretically, an increase in plant detritus inputs to the soil should promote the formation of SOC, since litter contains a large amount of C (Sayer et al., 2021). In contrast, the formation of SOC may be limited by reducing the input of plant detritus. A study in African tropical forests shows that the SOC concentration in a logged forest decreases by about 20 g C kg⁻¹ compared to an unlogged forest in Ghana (Chiti et al., 2016). Such SOC loss is generally associated with a decreased C supply rate, limiting SOC accumulation (Cao et al., 2020; Gao et al., 2020). Sayer et al. (2021) show that in Panama, forests treated with litter removal for 13 years have less C storage than undisturbed forests, with a 37±6% reduction in total SOC. Furthermore, a decreased litter input through forest degradation may affect SOC fractions, because a labile SOC fraction is consumed by decomposers first and a passive fraction (i.e., a more recalcitrant fraction) is successively consumed if the overall turnover time of SOC is decreased (Chiti et al., 2018). The decline in SOC storage may also be due to the chemical release of chelated soil C protected by the OM mineral association (Qin et al., 2024) in addition to reduced organic matter (OM) input. Overall, forest degradation caused by selective logging seems to cause a SOC loss in previous studies.

Litter is considered a source and reservoir of soil fertility in forests, and contains major minerals such as nitrogen (N) and phosphorus (P) as well as trace elements for biota (Song et al., 2023). Wieder et al. (2013) show that removal of litter input can reduce soil N availability and decrease N cycling rates in Malaysian lowland tropical rainforest. Sayer and Tanner (2010) also show that both nitrate and ammonium are reduced, and total inorganic N is decreased by about 50% after litter reduction in Panama. In addition, shifts in forest species composition can lead to changes in the stoichiometry of litter (e.g., C/N ratio and C/P ratio). Dipterocarp contributes less to the annual litterfall rate (15%) while *Macaranga* mainly contributes 34% in a logged forest of Malaysian Borneo (Burghouts et al., 1994). The soil C/N ratio may also be affected by a shift in litter C/N ratio during forest degradation, because fast growing *Macaranga* in degraded forests may supply litter with a lower C/N ratio to the ground (Jinggut, 2015). Another critical element in tropical rainforests is P because P often limits the net

primary productivity in tropical rainforests due to soil P deficiency (Alvarez-Clare et al., 2013; Dalling et al., 2016; Mori et al., 2018; Cunha et al., 2022). P in litter is mineralized by soil microbes and mineralized P is released to soils to enter P cycling (i.e., net P mineralization) or absorbed by microbes. The magnitude of net P mineralization is controlled by litter and soil C/P ratios. Selective logging disturbs P mineralization by changing both/either litter C/P ratio and/or environmental conditions for P-mineralization enzymes (Cleveland et al., 2002; Dalling et al., 2016; Schaap et al., 2021). In summary, forest degradation caused by selective logging may disrupt the balance of soil C, N, and P by changing the quality and quantity of the litter on the surface.

Additionally, the removal of trees that previously occupied the main canopy and the damage caused to the remaining canopies by harvested neighboring trees change canopy structure, which subsequently impacts microclimate and light availability (Pfeifer et al., 2015). Alterations in humidity and temperature of topsoils may occur as a consequence, indirectly influencing soil physicochemical properties such as soil moisture content, pH value, SOC content, and nutrient levels (McCauley et al., 2009; Chiti et al., 2016; Vaglio Laurin et al., 2016).

1.3 Responses of soil microbes to changes in soil critical substrate

The aforementioned variations in above-ground forest structure (Section 1.1) and below-ground soil physicochemical properties (Section 1.2) in forest degradation both ultimately point to a reduction in soil SOC. Soil organic carbon serves as a critical substrate to support microbial metabolic activities (Sturner and Elser, 2002; Zechmeister-Boltenstern et al., 2015). The quality and availability of SOC may directly influence soil microbial biomass and the viability of microbial communities (Schutter and Dick, 2001; Song et al., 2015; Nguyen and Marschner, 2016; Mayer et al., 2022; Wang et al., 2003).

For soil microbial biomass, Shahbaz et al. (2017) indicate that soil microbial

communities may enter a dormant state by adopting spore forms or perish, resulting in a decrease in microbial biomass in situations where substrates are depleted or their supply is interrupted (Liddle et al., 2020). In Borneo, the reduced SOC in a logged forest results in much lower soil microbial biomass C ($589\text{--}641\ \mu\text{g C g soil}^{-1}$) than in a primary forest ($840\text{--}985\ \mu\text{g C g soil}^{-1}$), suggesting that reduced substrate limits microbial activities (Mori et al., 2017).

Catabolic diversity refers to the variety of C sources that microbial communities in an environment can utilize or metabolize. Reduced SOC has been found to result in reduced catabolic diversity of soil microbial communities because SOC reduction is always accompanied by a preferential loss of the more readily decomposable organic C fraction (microbial biomass and potentially mineralized C) (Degens et al., 2000). Fungal communities (especially saprophytic fungi) dominate litter decomposition, and can utilize the recalcitrant lignocellulosic substrate through several extracellular enzymes, such as cellulase and ligninase; these substrates are difficult for other organisms to degrade (Voříšková and Baldrian, 2013; Brzostek et al., 2013; Cline and Zak, 2015). On the other hand, substrates of bacterial communities are often by-products (inorganic compounds) of fungal degradation, such as simple sugars and organic acids (Boer et al., 2005; Purahong et al., 2016). Besides, the fungal communities and their diversity undergo rapid succession during litter decomposition (Voříšková and Baldrian, 2013; Bani et al., 2018). The abundance of Ascomycota may decrease as they are gradually replaced by Basidiomycota during a late phase of decomposition (Frankland, 1998; Osono, 2007; Voříšková and Baldrian, 2013). While the relationship between litter quality and bacterial communities is not as close as that between litter quality and fungal communities (Bender et al., 2014; Treseder and Lennon, 2015). Substrate quality such as C/N ratio is also found to affect microbial community compositions. A higher soil C/N ratio tends to increase the relative abundance of Actinomycetes and fungi (Eskelinen et al., 2009), whereas a lower soil C/N ratio causes detrimental impacts on fungal biomass and richness (Bader et al., 1995; Holden and Treseder, 2013).

In summary, SOC is an essential substrate of microbial metabolism, driving the activities and composition of microbial communities, which further affects the process of microbial participation in soil nutrient cycling (Liu et al., 2015; Abdul Rahman et al., 2021; Moreno et al., 2023).

1.4 Nutrient cycling in tropics and roles of soil microbial communities

Bacterial and fungal communities play different but mutually interrelated roles in the breakdown of complex organic components and the release of essential nutrients back into the soil (Glassman et al., 2018). Extracellular enzymes, as molecular proteins produced by both bacterial and fungal communities, are considered to play critical roles in the transformation and mineralization of C, N, and P (Sinsabaugh, 2005; Burns et al., 2013). Cellulose and hemicellulose are the most abundant carbohydrates among the chemical components of litter, which can be degraded by extracellular enzymes such as β -glucosidases (BG) and β -xylosidases (Sinsabaugh and Moorhead, 1994). β -glucosidase (BG) can catalyze the final step of hydrolysis and convert cellobiose (an intermediate in cellulose hydrolysis) into glucose (Sinsabaugh et al., 2002), while β -xylosidases hydrolyzes xylobiose and short xylooligosaccharides to xylose (López-Mondéjar et al., 2016). During N cycling, Leucine-aminopeptidase (LAP) and N-acetyl-Glucosaminidase (NAG) regulate the turnover of N mineralization-immobilization through releasing N-terminal leucine residues and hydrolyzing N-acetyl-glucosides, respectively (Sayer et al., 2012; Cheng et al., 2019; Kurniawan et al., 2019). In P mineralization, acid phosphatase (ACP) produced by soil microbes catalyzes the cleavage of phosphate bonds and phosphate is released as available P during P cycling (Deubel and Merbach, 2005; Billah et al., 2019). Despite the fact that both bacterial and fungal communities secrete the aforementioned extracellular enzymes, the completely diverse taxonomic groups may cause bacterial and fungal communities to participate in C, N, and P cycling in different ways (Waring et al., 2013). Understanding different roles of bacterial and fungal communities in ecosystems helps

to more accurately understand nutrient cycling.

1.4.1 Roles of bacterial communities

Soil bacterial communities play an important role in the services and functioning of terrestrial ecosystems (Falkowski et al., 2008; Zhou et al., 2023), and their diversity is closely linked to multiple functions in biogeochemical processes (Jing et al., 2015; Delgado-Baquerizo et al., 2016). Bacteria can be divided into a copiotrophic group and an oligotrophic group based on their C mineralization potential and growth rate (Fierer et al., 2007). The copiotrophic group refers to bacteria taxa that primarily decompose labile soil C pools, including Bacteroidetes, Alphaproteobacteria, and Betaproteobacteria. The oligotrophic group refers to bacterial taxa that primarily decompose recalcitrant soil C pools, including Acidobacteria, Planctomycetes, and Chloroflexi. In addition, some members of Chloroflexi can also participate in C cycling through photoautotrophic C fixation (Klatt et al., 2013).

Bacterial communities are found to participate in N mineralization by facilitating the conversion of organic N into bioavailable forms like ammonium (NH_4^+) and nitrate (NO_3^-) (Li et al., 2023a). Certain bacterial taxa with specific physiological capabilities have been implicated in N cycling, such as rhizobia, nitrifying bacteria and denitrifying bacteria (Li et al., 2023b; Hayatsu et al., 2008). Moreover, the functional genes such as nitrogen fixation genes (*nifD*, *nifH*, *nifK*), nitrate reduction genes (*narG*, *narH*), nitrate respiration (*napB*), nitrification functional genes (AOA *amoA* and AOB *amoA*) and denitrification functional genes (*nirS*, *nirK*, and *nosZ*) are found in bacterial communities (Li et al., 2023a; Li et al., 2023b).

During P cycling, specific bacterial taxa harboring *pho-C* and *pho-D* phosphatase genes are known as phosphate-solubilizing bacteria (PSB), such as Proteobacteria (especially Gammaproteobacteria, Betaproteobacteria, and Alphaproteobacteria), Firmicutes, and Actinobacteria (Zheng et al., 2019). These PSBs participate in regulating the turnover of P solubilization and immobilization (Zheng et al., 2019; Wan et al., 2021). Also, functional genes involved in the solubilization of inorganic P, such

as the gene encoding quinoprotein glucose dehydrogenase (PQQGDH), are also identified in bacterial communities (Oliverio et al., 2020).

1.4.2 Roles of fungal communities

Fungi are often thought to be able to produce a wider range of extracellular enzymes than bacteria, and secrete multiple enzymes involved in the decomposition of particular C sources such as glycine, sucrose, lignin, and cellulose (Sinsabaugh, 2005; Allison et al., 2007). Saprotrophic fungi such as Basidiomycota and Ascomycota are the most important contributors to C cycling (Juan-Ovejero et al., 2020). Saprotrophic fungi dominate the decomposition of litter and organic matter by breaking down relatively recalcitrant compounds (e.g., cellulose) into simpler compounds (Lombard et al., 2014; Zhang et al., 2019). These simpler compounds can be metabolized by other fungi, thereby facilitating greater fungal diversity and faster decomposition (Tiunov & Scheu, 2005). Mycorrhizal fungi, especially ectomycorrhizal (EcM) fungi and ericoid mycorrhizal (ErM) fungi, can increase soil C uptake by influencing the nutrient uptake capacity of plant roots (Parihar et al., 2020; Hawkins et al., 2023). Moreover, functional genes linked to lignin and cellulose degradation within fungal communities have been identified in association with C cycling (Kameshwar et al., 2019; Zhang et al., 2019). *Lepiota* (order *Agaricales*, class *Agaricaceae*) is widely recognized as the most representative taxon in studies on litter decomposition and C cycling due to its expression of genes encoding carbohydrate-active enzymes (CAZymes) (Graf et al., 2014). Within the CAZyme auxiliary activity family 3 (AA3), the Pdh1 gene belonging to the glucose-methanol-choline superfamily of oxidoreductases exhibits a remarkable ability to oxidize a diverse range of carbohydrates generated during lignocellulose degradation (Graf et al., 2015).

Fungal communities have the ability to immobilize readily available N in their biomass, thereby influencing the mineralization of N in the soil ecosystem (Waring et al., 2016; Wang et al., 2021). Research in China proves that the relative abundances of Ascomycota demonstrate a positive relationship with potential N mineralization (Li et

al., 2022). Additionally, decomposition pathways dominated by fungi can exert an influence on rates of N mineralization, while the proportion of inorganic to organic N is associated with variations in fungal community composition (de Vries 2009; Waring et al., 2014). However, fungi may potentially exhibit lower N mineralization rates *per* unit biomass compared to bacteria due to their higher C assimilation efficiencies (de Vries, 2009). Arbuscular mycorrhiza (AM) fungi also involve in N uptake and mineralization, and the identified functional genes related to primary N nutrition such as glutamine synthetase and some nitrate reductases are found in AM fungi (Veresoglou et al., 2012).

The production of phosphatase, solubilization of P, and mineralization can also be influenced by fungal communities. The findings of Della et al. (2018) indicate that the relative abundance of P solubilizing fungi, such as *Talaromyces flavus*, *T. helicus* L, *T. helicus* N, *T. diversus* and *Penicillium purpurogenum* can influence phosphatase release patterns and impact the efficiency of both P solubilization and mineralization processes. Besides, the elevated abundance of symbiotrophs and saprotrophs can expedite soil P turnover and facilitate the maintenance of a heightened P availability (Liu et al., 2022; Yang et al., 2024).

1.5 Limitation of prior studies

Soil physicochemical properties are crucial for assessing the response of forest soils to external changes, and providing a basis for studying extracellular enzymes, microbial attributes, and nutrient cycling (Schoenholtz et al., 2000). Selective logging has the potential to increase pH in tropical rainforests (Olander et al., 2005; Li et al., 2013; Huang et al., 2022). Whereas losses of SOC, N, and P are observed in different research sites (Ganeshamurthy, 2009; Liu et al., 2010; Suhaili et al., 2023). A previous study also indicates that soil P deficiency may enhance acid phosphatase (ACP) activity (Kitayama 2013). Different soil physicochemical properties may play distinct roles in nutrient cycling by regulating extracellular enzyme activities (Ushio et al., 2010). Soil

microbial community compositions are found sensitive to variations in soil N availability and soil pH (Rousk et al., 2010; Zechmeister-Boltenstern et al., 2015; Zhou et al., 2017). The community composition, nutrient requirements, and metabolic activity of fungi are highly susceptible to the influence exerted by C substrates (Hanson et al., 2008). Aforementioned previous studies separately demonstrate the direct or indirect influence of soil physicochemical properties on extracellular enzyme activities and microbial community characteristics. However, the lack of data on the relationships between soil physicochemical properties (e.g., pH, SOC, N, and P) and extracellular enzyme activities in forest degradation caused by selective logging is the most serious shortcoming. Only with a comprehensive understanding of the dynamic changes of each factor in soil physicochemical properties and extracellular enzyme activities, as well as their interrelationships, I can elucidate how soil extracellular enzyme activities and soil physicochemical properties respond to forest degradation. Also, I can further explain how soil microbial communities participate in nutrient cycling by combining the data of soil microbial communities.

In addition, owing to the intricate nature of microbial communities, many studies only solely concentrate on individual either bacterial or fungal communities. Tripathi et al. (2012) investigate which dominant environmental factors most influence the bacterial community composition and diversity in Malaysia. Rodrigues et al. (2013) study how anthropogenic disturbances affect the taxonomic and phylogenetic diversity of soil bacteria in the Amazon region. Chen et al. (2019) research how historical logging alters soil fungal community composition and network. Such separate study of bacterial and fungal communities overlooks the complex and distinct relationships between bacterial and fungal communities in biogeochemical processes. Therefore, it is necessary to consider both bacterial and fungal communities simultaneously in a forest degradation process.

The limitations in the research design of previous studies are also noteworthy. For researching “forest degradation” or “selective logging”, *in situ* fieldwork or remote sensing techniques are always conducted (Sodhi et al., 2009; Costantini et al., 2016;

Gao et al., 2020; Takeshige et al., 2022). Most previous fieldworks compared representative primary forests with logged forests without considering the spatial-temporal variation in landscapes influenced by logging activities (Sahani and Behera, 2001; Villela et al., 2006). In contrast, remote sensing enables continuous monitoring of terrestrial vegetation changes over a specific time period (Gao et al., 2020; Miettinen et al., 2014; Takeshige et al., 2022); however, this technique does not facilitate comprehensive investigations into soil ecosystems. Hence, it is necessary to consider establishing a spatial-temporal trajectory or gradient of forest degradation processes in order to accurately depict the alterations in soil biochemical factors in subterranean ecosystems.

To my knowledge, to date, there is no study that provides comprehensive data on soil physicochemical properties, soil extracellular enzymes, and microbial communities (both bacterial and fungal communities) during a forest degradation process. A forest degradation gradient also needs to be developed with adequate vegetation metrics.

1.6 Study objectives

In this thesis, I aimed to investigate the changes in soil physicochemical properties, extracellular enzyme activities, and microbial communities during the forest degradation process, and to elucidate the effects of environmental changes on microbial involvement in nutrient cycling. Comprehending the ecology of microbial communities in tropical degraded forests is crucial for understanding ecosystem function, guiding the restoration of degraded forests, and predicting long-term impacts, which ultimately contribute to the development of sustainable forest management strategies. Therefore, I address the following presented three research questions in this study and answer them in Chapter 2 to Chapter 4.

Research question 1 (Chapter 2): How do soil physicochemical properties, microbial biomass, and enzyme activities change along a degradation gradient?

The magnitude and extent of forest degradation in logged-over tropical rainforests

are spatially and temporally variable in the contemporary landscapes (Asner et al., 2009), contingent upon the cumulative impacts of historical and recent logging activities (Kitayama et al., 2018; Imai et al., 2019). Quantitatively investigating the effects of selective logging on soil ecosystems and microbial ecology in such spatial-temporally heterogeneous landscapes is challenging. Following the suggestions of Mertens and Lambin (2000) and Ouedraogo et al. (2011), I proposed the utilization of the concept of a “forest degradation gradient” to constrain logging histories and intensities.

In order to address the data gap in the relationships between soil physicochemical properties and selective logging, I investigated the changes in soil physicochemical properties along the forest degradation gradient. Soil organic carbon (SOC) substrate can provide energy to support substantial basal metabolism of soil microbial biomass, and alterations in soil C fractions (SOC and microbial biomass C), further influence the extracellular enzyme activities (Sayer et al., 2012). Additionally, soil N and P contents can influence microbial involvement in N and P mineralization (Ndabankulu et al., 2022; Liu et al., 2022). Therefore, I also analyzed the alterations in microbial biomass, and activities of extracellular enzymes which were associated with C, N, and P cycling under the disturbance caused by forest degradation.

Research question 2 (Chapter 3): How do bacterial and fungal community compositions as well as enzyme efficiency change along a degradation gradient?

Bacterial versus fungal communities show differential preferences for different substrates (Nicolardot et al., 2007; Kallenbach et al., 2016). Also, bacteria typically exhibit higher growth rates and turnover rates compared to fungi under identical environmental conditions (Rousk and Bååth, 2011). In Chapter 3, I investigated how bacterial and fungal community composition change along the forest degradation gradient in relation to substrate (i.e., SOC) changes. Alterations in soil microbial community compositions in turn lead to subsequent changes in extracellular enzyme activities. I tested how the effects of community composition were transmitted to the changes in enzymes using a mathematical model. Additionally, variations in enzyme

activities *per* unit of microbial C in can be explained by substrate variability, suggesting substrate (i.e., SOC) is the key points regarding enzyme efficiency. Therefore, I also analyzed how enzyme efficiencies change with changing substrate during forest degradation.

Research question 3 (Chapter 4): How do bacterial and fungal functional structures as well as the relative abundance of potential functional genes (i.e., the relative abundance of E.C. numbers for specific extracellular enzymes) change along a degradation gradient?

In Chapter 4, I aimed to study the effects of forest degradation on microbial functional structures. Additionally, I examined changes in the relative abundance of potential functional genes for extracellular enzymes along a forest degradation gradient to reveal the correlation between potential genes and enzyme activities. The functional structures of bacterial and fungal communities are found to be influenced by soil physicochemical properties, particularly the SOC substrate in previous studies (Xue et al., 2018; Prescott et al., 2013; Louisson et al., 2023). However, previous studies also suggest that fungi may not be affected by soil physicochemical properties, as fungi may have functional redundancy (Wilson et al., 2011; Zeng et al., 2023). If so, bacterial and fungal functional structures may show different patterns along a forest degradation gradient. Moreover, forest degradation has been demonstrated to reduce the abundance of genes involved in chitin decomposition, lignocellulose degradation, and N cycling, while increasing the expression of genes associated with carbohydrate metabolism and aromatic compound degradation (Lladó et al., 2017). Therefore, changes in the relative abundance of potential functional genes (i.e., a specific biological process with a functional role) for several specific extracellular enzymes in my samples would also be predicted, which can be represented by the relative abundance of E.C. numbers.

Forest degradation caused by selective logging is widespread in tropical rainforests, which results in increased C emissions and thereby contributes to global warming (Lewis et al., 2015; Wright, 2010). Forest degradation directly alters the above-ground ecosystem and further affects the below-ground ecosystem. The response

of soil microbial communities to forest degradation is the most important concern in this study because they regulate C dynamics and associated mineral cycles in forest ecosystems (Lladó et al., 2017).

1.7 Study sites

I used Deramakot and Tangkulap Forest Reserve in Sabah, Malaysian Borneo to research how forest degradation affects soil physicochemical properties, extracellular enzyme activities, soil microbial community compositions and functional structures. Deramakot (5°14'-28', 117°20'-38'E) covers an area of approximately 55,083 ha and Tangkulap (5°18'-31'N, 117°11'-22'E) covers about 27,550 ha (Fig. 1-1). These sites have a humid equatorial climate. The annual rainfall in these areas ranges from 1700 to 5100 mm, as well as the annual average temperature is 28 °C (Huth and Ditzer 2001). The soils are largely Ultisols derived from Tertiary sedimentary rocks (Sabah Forestry Department 2005). Two forest reserves are adjacent to one another. The terrain in both reserves is undulating, with the altitude in the large parts of the reserve ranging between 20-300m above sea level, and an average altitude of 100 m (Ashton and Hall 1992). The original vegetation in Deramakot and Tangkulap is the mixed dipterocarp lowland tropical rainforest dominated by dipterocarp species (Ong and Kleine 1996).

Deramakot and Tangkulap Forest Reserve experience different logging histories (Sabah Forestry Department 2005). The initial logging activities in Deramakot commenced as conventional logging was started in 1956. Logging was suspended in 1989 to allow the forest to regenerate. Then, in 1995, a long-term management plan was put in place with reduced impact logging (RIL). In 1997, Deramakot was certified by the Forest Stewardship Council (FSC), an international certification organization, as the first tropical natural forest to be certified as a "well-managed forest." In contrast, Tangkulap began conventional logging in the 1970s and continued until 2002 (Ong et al., 2013). To allow the forest to regrow, all logging activities were suspended after 2002. An earlier study suggested that the logging activities in Tangkulap were much

more intensive than those in Deramakot, especially during the 1990s (Imai et al., 2009).

In the study site, secondary succession after moderate selective logging proceeds as in the typical successional sere of the disturbed mixed dipterocarp forest described in the previous studies (Imai et al., 2019). Once dipterocarp trees are selective logged, pioneer tree species (e.g., *Macaranga* spp.) immediately occupy the logged patches. However, some intensively logged forest patches are dominated by ferns (*Dicranopteris* spp.) and/or vines (liana or climbing bamboo). Such forests dominated by ferns/vines do not seem to follow the typical secondary successional sere because the number of trees is still low with a wide-open canopy even after several decades had elapsed since the last logging (Takeshige et al., 2022).

Overall, landscapes of varying degrees of degradation *in situ* with widely different compositions of extant tree communities in Deramakot and Tangkulap are formed, which was caused by differences in logging histories and intensity (i.e., the amount of logging and associated environmental impacts). The dominant tree species range from climax dipterocarp species in primary forests to pioneer *Macaranga* species and ferns/vines in highly degraded forests (Imai et al., 2012, 2019; Kitayama et al., 2018) (See Figs. 1-2 to 1-4). In these partially and temporally heterogeneous landscapes, remnant forests with different degradation statuses are juxtaposed with each other (Imai et al., 2012; Aoyagi et al., 2017; Kitayama et al., 2018; Takeshige et al., 2022). Therefore, the sequence of tree communities from a primary forest to a degraded forest can be defined as the degradation gradient in terms of community compositions. Through this gradient, I can reconstruct the process of forest degradation using a space-for-time approach (Mertens and Lambin 2000; Ouedraogo et al., 2011). It is believed that by establishing a degradation gradient, the continuous changes in soil physicochemical properties and soil microbial activities in forests with different logging intensities can be better measured and understood.

Figures

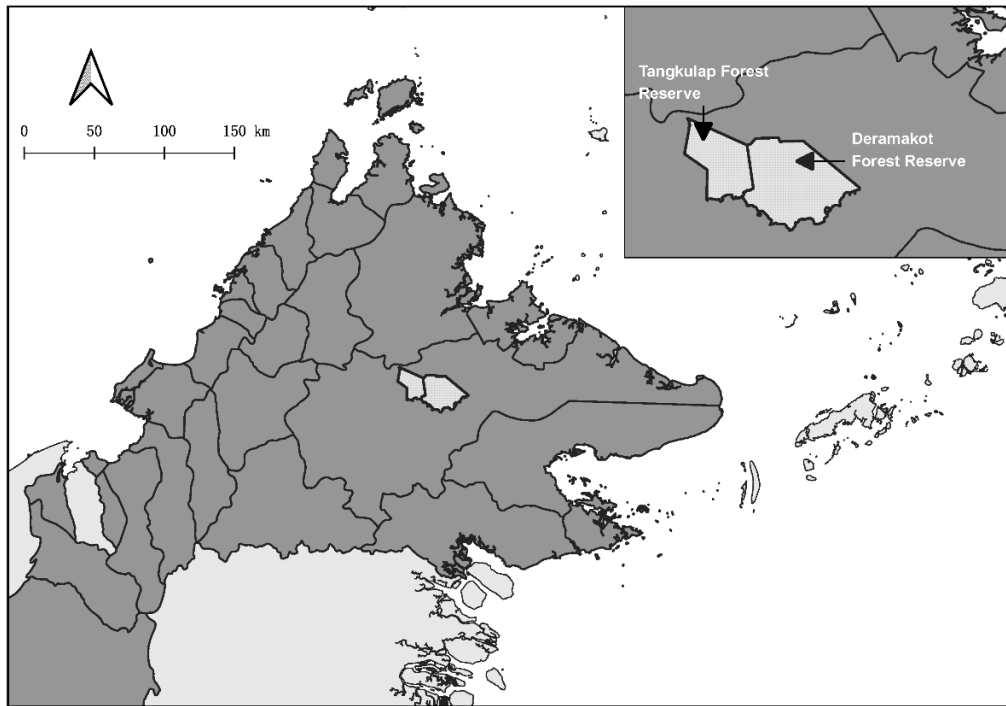


Fig. 1-1 Location of study sites: Deramakot Forest Reserve and Tangkulap Forest Reserve, in Sabah, Malaysian Borneo.



Fig. 1-2 Dipterocarpaceae (e.g., *Shorea* spp.) are the dominant tree species in the primary forest of Sabah, Malaysian Borneo. Photos were taken by Jiang in 2019.

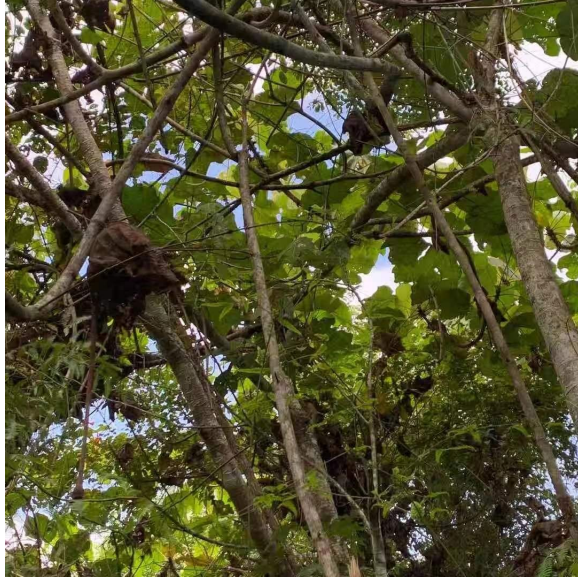


Fig. 1-3 Pioneer tree species (e.g., *Macaranga* spp.) immediately occupy the logged forest of Sabah, Malaysian Borneo. Photos were taken by Jiang in 2019.



Fig. 1-4 Some intensively logged forests are dominated by ferns (*Dicranopteris* spp.). Photos were taken by Jiang in 2019.

Chapter 2 Changes of soil physicochemical properties, microbial biomass, and enzymatic activities along a forest degradation gradient in logged-over tropical rainforests, Malaysian Borneo

Abstract

Forest degradation leads to changes in above-ground vegetation characteristics and below-ground environmental factors, further affecting soil microbial biomass and microbial activities. Besides, extracellular enzymes produced by microbes can be influenced by changes in microbial communities/biomass *per se* and soil organic carbon. Malaysian Borneo has experienced a long period of selective logging and formed landscapes with varying degradation degrees *in situ*, which can be established as a forest degradation gradient. Therefore, soil physicochemical properties, soil microbial biomass, and soil enzyme activities along a forest degradation gradient were investigated. The hypothesis was put forward that the changes in above-ground biomass (AGB) could affect soil organic matter, which further influenced soil microbial biomass and enzyme activities.

A total of 35 plots (i.e., forest plots) with varying magnitude of logging influences were investigated for vegetation composition and soil biochemical parameters. The 35 plots were sorted based on tree genus compositions by a Principal Co-ordinates analysis (PCoA). The PCoA axis-1 values of canopy trees were called “forest degradation indexes” and represented a gradient of forest degradation. Soil physicochemical properties (pH, soil organic carbon (SOC), total nitrogen (TN) and C/N ratio), soil microbial biomass carbon (SMBC), and nitrogen (SMBN), as well as the activities of extracellular enzymes (acid phosphatase, β -glucosidase, leucine aminopeptidase, and N-acetyl-glucosaminidase) were measured. Both aforementioned soil biochemical parameters were regressed with forest degradation indexes. The correlations between pH, SOC, TN, SMBC, and SMBN with enzymes were measured. Structural equation modeling (SEM) analysis was conducted to explore the relationship among AGB, soil pH, SOC, and SMBC.

Linear regression analyses showed that SOC and TN concentrations decreased with decreasing forest degradation indexes, while soil pH values increased. Soil microbial biomass carbon and nitrogen, and the activities of four extracellular enzymes all decreased with decreasing forest degradation indexes. The activities of four enzymes were correlated with soil microbial biomass. The SEM showed that SOC explained the greatest variation of soil microbial biomass.

Above-ground forest degradation directly affects soil organic matter, which then affects soil microbial biomass. The combined actions of reduced microbial biomass and reduced soil organic matter as the substrate for the enzymes decrease microbial enzyme activities during the process of forest degradation. These changes may in turn feed back to the regrowth or succession of degraded forest ecosystems.

Keywords: Logged-over tropical rainforests; Soil physicochemical properties; Soil microbial activities; Soil extracellular enzymes; Magnitude of degradation

2.1 Introduction

Deforestation and forest degradation are widespread throughout the world's tropical rainforests (Houghton, 2012; Pearson et al., 2014). Forest covers are completely removed after deforestation, while thinned forests still remain after forest degradation. Commercial selective logging is largely responsible for forest degradation in the tropics because it harvests above-ground biomass (timber) on a large scale leaving behind a huge tract of degraded forests (Houghton, 2012; Kissinger et al., 2012). The magnitude of forest degradation in logged-over tropical rainforests varies in space and time in the landscape (Asner et al., 2009) depending on the cumulative impacts of past to recent logging events (Imai et al., 2009; Kitayama et al., 2018; Langner et al., 2012; Pfeifer et al., 2016). Pfeifer et al. (2016) select a primary forest, a lightly logged forest, a twice-logged forest and an intensively harvested forest to establish a degradation gradient of tropical forest in East Sabah, Malaysian Borneo, and find that above-ground biomass (AGB) decrease progressively along the degradation gradient (413.4 t ha^{-1} on average to 267.3 t ha^{-1} to 110.6 t ha^{-1} and to 42.4 t ha^{-1}). Besides, C stock (Blanc et al., 2009; de Andrade et al., 2017; Saner et al., 2012), canopy tree communities (Aoyagi et al., 2017; Imai et al., 2012b, 2014), decomposer fauna (Hasegawa et al., 2014), mammal communities (Jati et al., 2018; Samejima et al., 2012) in the above-ground system of remnant logged-over forests also change proportionately with logging intensity.

Degradation not only affects the above-ground system, but may also affect below-ground system. The decline of above-ground biomass and associated changes in tree communities may influence soil physicochemical properties, particularly the stock of soil organic matter and its quality as indexed by element stoichiometry (such as soil C/N ratio) (Xu et al., 2018). Changes in soil physicochemical properties may alter soil microbial processes primarily through two pathways, one through the change in resources for the microorganisms (i.e., soil organic matter) and the other through the direct change in microbial biomass and community compositions due to soil perturbations (e.g., Ushio et al., 2010). Degradation in tropical primary forests cause a

loss of soil organic matter (organic C, N, and P) and/or the reduction of available P (Don et al., 2011; Imai et al., 2012a; Aoyagi and Kitayama, 2016; Ngaba et al., 2020). The decline of soil organic matter may influence microbial activities because these elements are the substrate for microbial growth and metabolisms. Changes in microbial communities/biomass *per se* as well as in soil organic matter would influence soil enzyme activities (Sinsabaugh, 2010; Ushio et al., 2013), which then feeds back to the above-ground processes because soil enzyme activities determine nutrient (particularly N and P) availability for trees (Ushio et al., 2017). However, how the above-ground forest degradation affects soil physicochemical properties, soil microbial biomass, and enzymatic activities remains unknown to date in logged-over tropical rainforests. The lack of knowledge may hinder the establishment of sustainable management of tropical rainforests including soil processes.

My aim is to assess how the increasing magnitude of above-ground forest degradation influences soil processes in this study. A gradient of forest degradation in Sabah, Malaysian Borneo has been established, where the composition of extant tree communities varies greatly due to different logging intensities (i.e., harvest volume and associated environment impacts) in the past. The dominant tree species range from climax dipterocarp species in primary forests to pioneer *Macaranga* species in heavily degraded forests (Imai et al., 2012b, 2019; Kitayama et al., 2018). The sequence of tree communities from a primary to a degraded forest can define the degradation gradient in terms of community composition. Through this gradient, I can reconstruct the process of forest degradation by a space for time approach (Mertens and Lambin, 2000; Ouedraogo et al., 2011). I believe that by establishing a gradient of degradation, the continuous changes of soil physicochemical properties and soil microbial activities in forests with different logging intensities can be better measured and understood.

I hypothesized that the changes of above-ground vegetation could affect soil physicochemical properties, which further influences soil enzyme activities. These changes may show linear trends with a gradient of degradation. Specifically, the following two hypotheses were tested: (1) The concentration of soil organic matter and

microbial biomass decrease linearly with the magnitude of above-ground forest degradation. (2) Soil enzyme activities, affected by soil microbial biomass and soil physicochemical properties, decrease linearly with the magnitude of above-ground forest degradation.

2.2 Materials and methods

2.2.1 Study sites

The study sites were selected in Deramakot Forest Reserve and Tangkulap Forest Reserve, Sabah, Malaysian Borneo (See Chapter 1, Fig. 1-1), which have a humid equatorial climate. The annual rainfall ranges from 1700 to 5100 mm and the annual average temperature is 28 °C (Huth and Ditzer, 2001). Deramakot covers an area of approximately 55,083 ha, and Tangkulap covers about 27,550 ha. The terrain in the two reserves is undulating and the mean altitude is 100 m (Ashton and Hall, 1992). The original vegetation in Deramakot and Tangkulap is the mixed dipterocarp lowland tropical rainforests dominated by dipterocarp species (Ong and Kleine, 1996).

Both Deramakot Forest Reserve and Tangkulap Forest Reserve have been designated as production forests to produce timber. In Deramakot Forest Reserve, commercial logging started from 1956 to 1989 (Sabah Forestry Department, 2005). In these years, intense logging caused serious damage to this area. To achieve the sustainable utilization of forests, intense logging was suspended in 1989 and reduced-impact logging (RIL) was introduced since 1995 (Seino et al., 2006). RIL used a strictly planned and carefully controlled timber harvesting method to minimize impacts on forest stands and the soil environment. On the other hand, Tangkulap Forest Reserve was intensively logged from the 1970s until 2002 (Ong et al., 2013); logging activities were suspended after 2002 to allow vegetation recovery. Reflecting the past severer logging impacts, Tangkulap had poorer aboveground biomass compared with Deramakot at the time of my investigation.

2.2.2 Tree community census and soil sampling

I made use of the existing plots that were established by earlier researchers such as Imai et al. (2014) and Kitayama et al. (2018) for sampling soils and investigating vegetation. These existing plots were placed following the stratified random design as explained in Kitayama et al. (2018). For stratification, a Land-sat Thematic Mapper (TM) band-7 image was used to divide the area into six degradation strata ranging from the primary forest (stratum 1) to a highly degraded open stand without trees (stratum 6). The categorization of six strata was given in detail by Imai et al. (2014). Except for stratum 6 (i.e., the open stand without trees), 50 plots were established by earlier researchers for stratum 1–5 with the distance between any two plots greater than 100 m to minimize spatial autocorrelation. These existing plots had different sizes, including 0.9 ha rectangular plots and approximately 0.13 ha circular plots.

Among the 50 plots, 7 plots for each of the stratum 1–5 were selected for sampling, yielding a total of 35 plots (i.e., forest plots). These 35 plots were placed on gentle slopes to avoid the effect of topography. If the plots were chosen as rectangular plots (i.e., 0.9 ha) of 30 m by 300 m, I selected 4 subplots within the rectangular plot for our sampling, subplots were selected to include large canopy trees of the dominant species (*Shorea* or *Macaranga*), and the spacing between any two subplots was more than 20 m to minimize spatial autocorrelation. Therefore, 35 plots also followed the stratified random design. The AGB decreased gradually from stratum 1 to 5 (Aoyagi et al., 2017; Imai et al., 2014; Kitayama et al., 2018). These five strata were juxtaposed with each other to form the present landscape (Fig. 2-1). Because these strata reflected either the difference in the past logging intensity or the time elapsed after the last logging, the effects of the past logging were inseparable from the effects of regrowth. Hence, I considered that the current six strata (five strata for sampling) formed a sequence of above-ground forest degradation and I defined this sequence as a gradient of forest degradation. The degradation gradient was actually derived based on the tree community composition as will be explained later.

In each of the 35 plots, trees greater than 10 cm diameter at breast height (DBH)

were enumerated, and their DBH were measured. Subsequently, soil cores each with 10 cm depth, were collected from a surface soil layer at four points in each plot in February 2020, and then four cores were uniformly mixed to form a composite sample. Soil sampling was conducted generally 3 days after rain. Finally, a total of 35 samples (seven samples from each of the five different strata) were obtained, and all samples were divided into two parts, one was refrigerated at 4 °C for studying soil physicochemical properties and soil microbial biomass. The other one was freeze-dried as archive samples.

2.2.3 Chemical and microbial analyses

2.2.3.1 Soil Physicochemical Properties

Soil pH, soil organic carbon (SOC), soil total nitrogen (TN), and C/N ratio were measured through experiments. Soil pH was determined by suspending the soil with the ratio of soil to water 1:2.5. After mixing and standing for 1 hour, pH of soil suspension was measured by a pH meter (HORIBA pH/Cond Meter D-54). Each sample was measured three times and an average value was calculated. The values of SOC, TN, and C/N ratio were measured at the same time after air-drying. Each air-dried soil sample was wrapped with tinfoil (total 35 samples). In addition, added 2 apple leaf standards (NIST SRM 1515) and 2 blank samples were added. All of them were burned on a CHNS-O analyzer (Thermo Fisher Scientific Inc.) and the obtained data were used to calculate total C and total N in the soil. Due to its geology and low pH, the value of total C could represent soil organic C. Subsequently, the C/N ratio was calculated.

2.2.3.2 Soil microbial biomass

Soil microbial biomass C (SMBC) and soil microbial biomass N (SMBN) were determined by the chloroform fumigation extraction method (Carter and Gregorich, 2008). Each refrigerated soil sample was divided into two parts each with 10 g: fumigation (experimental group) and non-fumigation (control group). Soils were fumigated in a desiccator, with a beaker (containing zeolite) containing 25 ml of chloroform (ethanol removed), and a beaker (containing zeolite) with 300 ml of water

at 70–80 °C. The desiccator was depressurized with an aspirator for 2 minutes and then closed. The fumigation was continued at 25 °C for 24 hours in the dark. After fumigation, the beakers with water and chloroform were removed, and refilled with 300 ml water. Then the desiccator was depressurized again for two minutes. This procedure was repeated several times until there was no smell of chloroform in the soil. Fifty ml of 0.5 M K₂SO₄ was added into each fumigated and non-fumigated soil. The mixture was vibrated for 30 min, and then filtered. These filtrates were cryopreserved in 50 ml plastic solution bottles until analysis. Total C and N concentrations in the thawed filtrates were determined on a total-C and N analyzer (Shimadzu Total Organic Carbon Analyzer with Total Nitrogen Measuring Unit) to derive SMBC and SMBN. I used C₆H₄(COOK)(COOH) and KNO₃ as standard for C and N in this analysis, respectively.

2.2.3.3 Soil extracellular enzymes

The activities of acid phosphatase (ACP, E.C. 3.1.3.2), β -glucosidase (BG, E.C. 3.2.1.21), leucine aminopeptidase (LAP, E.C. 3.4.11.1), and N-acetyl-glucosaminidase (NAG, E.C. 3.2.1.30) as representative soil extracellular enzymes were assessed. ACP hydrolyzes organic P and influences P availability (Kieliszewska-Rokicka, 1999). BG occupies a central position in the global-scale C cycling because it catalyzes the final step in cellulose decomposition (Dick, 2011; Knight and Dick, 2004; Moscatelli et al., 2012). LAP releases N-terminal leucine residues from proteins and peptides (Wang et al., 2020). NAG hydrolyzes N-acetyl-glucosides mainly targeting chitin and peptidoglycan (Sistla and Schimel, 2013; Stone et al., 2014). These extracellular enzymes are key enzymes for maintaining the nutrient cycling of forest ecosystems (Allison and Vitousek, 2005; Luo et al., 2017; Moorhead et al., 2012; Sinsabaugh et al., 2008; Sinsabaugh, 2010).

The activity of acid phosphatase (ACP) was measured by the colorimetric method following Kitayama (2013). Each soil sample was weighed into a test tube and cultured in 4 ml 20 mM modified universal buffer (MUB buffer) solution (20 mM trisaminomethane, 20 mM maleic acid, 15 mM citric acid, 2 mM boric acid, and

98 mM NaOH adjusted to pH 6.0). The value of pH 6.0 was employed for all soil samples measured in the assay because based on peak ACP activity was observed at this pH across several soils in nearby forest in a previous study (Kitayama, 2013). Before incubation, 1 ml of 200 mM p-nitrophenyl phosphate (pNPP) was added as the substrate and then each sample was incubated in the dark at 20 °C for one hour. After incubation, each test tube was put into ice water to stop the reaction, then 4 ml 0.5 M NaOH and 1 ml 0.5 M CaCl₂ were added. The incubated soil and buffer solution was filtrated with filter paper (ADVAN-TEC No.6), and each filtrate was diluted 100 times. The absorbance of the diluted sample was measured at 410 nm on a spectrophotometer by a microplate reader (SH-9000Lab, Corona Electric). In the control group, 4 ml MUB buffer was added before incubation and 1 ml subtraction solution was added after incubation. The absorbance from the control group was also measured by this spectrophotometer to correct the enzyme activity.

The activities of BG, LAP, and NAG were measured by high-throughput fluorometric measurement (Bell et al., 2013). Briefly, I weighed 2.75 g soil into a blender and added 100 ml of 50 mM sodium acetate buffer, then blended at high speed for 1 min. Mixed soil slurry was stirred at a low speed, then 800 µL slurry was taken by an 8-channel pipette into a deep-well plate, and 200 µL substrate was added. The substrate was 4-methylumbelliferyl β-D-glucopyranoside for BG, 4-methylumbelliferyl N-acetyl-β-D-glucosaminide for NAG, and L-Leucine-7-amido-4-methylcoumarin hydrochloride for LAP. The concentration of each substrate was 1 mM. All deep-well plates were incubated at 25 °C for 4 hours. After incubation, 0.2 M NaOH was added and frozen for 30 min to stop the reaction. Then 250 µL supernatant was moved to the 96-well black microplate. Fluorescence data were read on a microplate reader. The standard curves of BG and NAG were made of 4-methylumbelliferone (MUB), while that of LAP was made of 7-amino-4-methylcoumarin (MUC).

2.2.4 Statistical analysis

The forest degradation gradient was assessed using vegetation intactness, which

involves calculating a similarity distance matrix based on the relative abundance of plant genera, as described by Aoyagi et al. (2017). Initially, the relative abundances of tree genera were computed for each of the 35 plots, and a distance matrix was calculated using the Bray-Curtis dissimilarity. Subsequently, the 35 plots were ordinated using a Principal Coordinates Analysis (PCoA) with the "vegan" package in R (Oksanen et al., 2020). The results of the PCoA suggested that the magnitude of forest degradation in the 35 plots corresponded to the order of PCoA axis-1 values (Fig. S2-1), and thus I considered that PCoA axis-1 values as forest degradation indexes. Previous studies have also found that a coordinate axis after dimensional reduction techniques effectively represents the forest degradation gradient (Aoyagi et al., 2017; Imai et al., 2014). The degradation became more aggravated when the axis-1 value changed from positive to negative.

To validate the estimation of forest degradation, a regression analysis of forest degradation indexes with remnant above-ground (AGB) values across the 35 plots was conducted. I assumed that the AGB values of the original vegetation were similar among the 35 plots and that greater logging impacts would result in proportionally smaller remnant AGB values. The remnant AGB of each plot was calculated as the sum of the biomass of all remaining trees with a diameter at breast height (DBH) greater than 10 cm. The biomass of each tree was determined using the equation proposed by Chave et al. (2014):

$$AGB(kg) = \exp(-1.803 - 0.976 \times E + 0.976 \times \log(WD) + 2.673 \times \log(DBH) - 0.0299 \times (\log(DBH))^2)$$

In the equation, WD represented wood density, while E denoted a measurement of environmental stress derived from the coordinates of each plot. AGB *per* plot was converted to the value on a hectare basis (Mg ha⁻¹). Four plots from stratum 5 and 1 plots from stratum 3 were 0.13 ha, and the remaining 30 plots were all 0.9 ha.

The calculated remnant AGB values exhibited a significant correlation with forest degradation indexes (Fig. S2-2), suggesting that the extent of forest degradation assessed based on tree genus composition was consistent with forest structural

properties. Consequently, variation of the composition of tree genera along the axis-1 of PCoA was explained by logging intensity, and forest degradation indexes (i.e., the PCoA axis-1 values of canopy trees) were indeed considered to represent the magnitude of forest degradation. It is noteworthy that although the forest degradation indexes are significantly positively correlated with AGB values, these two metrics may represent different aspects of forest composition. Since the forest degradation indexes are considered indicators based on tree community structure, changes in the forest degradation indexes should be accompanied by changes in tree community structure. Furthermore, since forest degradation indexes represent the compositional distance between tree communities and the primary forest, the degradation gradient can be standardized to a range of -1 to 1 for any research site using the aforementioned method. Given its ability to quantify forest degradation across diverse forest ecosystems, this indicator could have universal applicability.

Subsequently, a linear regression analysis was performed to analyze the relationship between soil physicochemical properties, extracellular enzymatic activities, and soil microbial biomass with forest degradation indexes (i.e., the PCoA axis-1 values of canopy trees). Additionally, a bivariate correlation was tested between soil extracellular enzymes versus soil physicochemical properties and soil microbial biomass using a Pearson correlation analysis. The statistical analyses were carried out using IBM SPSS Statistics 25.

To investigate the impact of above-ground forest degradation on soil and microbial processes, a structural equation modeling (SEM) analysis was conducted to explore the relationship among AGB, soil pH, SOC, and SMBC (see Fig. S2-3 and S2-4). Two scenarios (models) were considered, one with SMBC as the dependent variable and SOC and soil pH as independent variables, and the other with SOC as the dependent variable and SMBC and soil pH as independent variables. In both cases, AGB was the topmost independent variable. I selected AGB instead of forest degradation indexes to represent above-ground forest degradation because forest degradation indexes contained multiple factors such as logging disturbance, including logging intensity,

temporal aspects, and frequency of interventions. Utilizing such a comprehensive indicator as the primary causal factor would render the interpretation of results exceedingly complex, making it difficult to determine which specific factor directly influences SOC. Consequently, AGB can be used as a singular, representative indicator of forest degradation in SEM. Before being imported into the inner model through IBM SPSS Amos 26, all data were normalized to have a zero mean and a unit standard deviation. The goodness of the models was evaluated using the root mean square error of approximation (RMSEA), Akaike information criteria (AIC), and Bayesian information criteria (BIC), and the best model was selected.

2.3 Results

2.3.1 Soil physicochemical properties and soil microbial biomass

Soil physicochemical properties, including pH value, SOC, TN, and C/N ratio, exhibited significant linear changes along the forest degradation gradient (Fig. 2-2, $p < 0.001$). The concentration of SOC ranged from 1.04 to 48.89 mg g⁻¹ and decreased with decreasing forest degradation indexes (i.e., the PCoA values of canopy trees; increasing magnitude of forest degradation). Similarly, the concentration of soil TN ranged from 0.75 to 2.76 mg g⁻¹ and decreased with decreasing forest degradation indexes. During the forest degradation process, the highest SOC decreased by 97.8% compared to the lowest SOC, while the highest TN decreased by about 72% compared to the lowest TN, suggesting that the reduction of SOC was more pronounced than that of soil TN, and the C/N ratio also decreased linearly with decreasing forest degradation indexes. In contrast, soil pH values increased by approximately 2 units with decreasing forest degradation indexes.

Soil microbial biomass changed dramatically along the forest degradation gradient. Both the concentrations of SMBC and SMBN significantly decreased with decreasing forest degradation indexes (i.e., PCoA axis-1 values of canopy trees; increasing magnitude of forest degradation) (Fig. 2-3; $p < 0.001$ for SMBC, $p < 0.01$ for SMBN).

Results of structural equation modeling (SEM) showed that the model with SMBC as the dependent variable was selected as the best model with the lowest BIC (Fig. S2-3 versus Fig. S2-4). Furthermore, the path coefficient from SOC to SMBC was 0.72, while the path coefficient from SMBC to SOC was 0.53, suggesting that SOC could account for a greater variation in SMBC.

2.3.2 Soil extracellular enzymes

The activity levels of ACP ranged from 4.46 to 51.27 $\mu\text{mol g soil}^{-1} \text{ hr}^{-1}$, BG ranged from 0.85 to 16.19 $\text{nmol MUB g soil}^{-1} \text{ hr}^{-1}$, NAG ranged from 0.40 to 10.08 $\text{nmol MUB g soil}^{-1} \text{ hr}^{-1}$, and LAP ranged from 0.14 to 0.93 $\text{nmol MUB g soil}^{-1} \text{ hr}^{-1}$. All four enzymes showed a significant decrease with decreasing forest degradation indexes (i.e., PCoA axis-1 values of canopy trees; increasing magnitude of forest degradation) (Fig. 2-4; $p < 0.01$ for ACP, $p < 0.001$ for BG, $p < 0.05$ for NAG, $p < 0.001$ for LAP).

The correlations between soil physicochemical properties and soil microbial biomass with soil extracellular enzymes were measured (Table 2-1). All four extracellular enzymes showed a positive correlation between SOC and SMBC. The activities of ACP, BG, and LAP exhibited a positive correlation with TN and a negative correlation with pH values. Only BG and LAP displayed a positive correlation with SMBN.

2.4 Discussion

The forest degradation indexes (i.e., PCoA axis-1 values of canopy trees) of the 35 plots have been considered to represent a degradation gradient. Imai et al. (2014) find that a coordinate axis is positively correlated with above-ground biomass after dimension reduction. My research has the same research area with Imai et al. (2014) and yield similar results (Fig. S2-2). Additionally, the response of pioneer species to forest degradation is linear, and their abundance increase with increasing degradation, while climax species decreasing linearly (Imai et al. 2014; Aoyagi et al. 2017). Therefore, forest degradation involves both the reduction of above-ground biomass and

shifts in tree community composition. In the following, I discuss how these changes in the above-ground system would affect the below-ground system addressing my two hypotheses.

2.4.1 Changes in soil properties under forest degradation

First of all, my first hypothesis (the concentration of soil organic matter and microbial biomass decrease linearly with the magnitude of above-ground forest degradation) is supported. All soil physicochemical properties have been found to change with forest degradation. The most distinctive change is the linear reduction of SOC and TN with decreasing forest degradation indexes (i.e., the PCoA axis-1 values of canopy trees; increasing magnitude of forest degradation). SOC exhibits a 3-fold reduction from a primary forest to a highly degraded forest based on the slope of the linear regression in Fig. 2-2, while soil TN shows a 2.5-fold reduction. This drastic reduction of soil organic matter (C and N) in the topsoils may be related to the reduction of litterfall caused by degradation. Imai et al. (2014) demonstrate that the AGB of remaining stands decreases with the intensification of degradation. Additionally, dominant tree species also change in association with the reduction in AGB, from dipterocarp species to *Macaranga* species. These changes can collectively lead to a decline in the litterfall supply rate, which further affects soil organic matter. Junaedi et al. (2020) measure the litter productivity of all tree species in tropical peatland forests and confirm that *Macaranga* stands (4.74 to 7.04 ton ha⁻¹ yr⁻¹) have a lower litterfall rate than dipterocarp stands (9.18 ton ha⁻¹ yr⁻¹). Moreover, enhanced decomposition may be involved in the decline of soil organic matter in topsoils because microclimate conditions on the forest floor under thinned crowns may favor faster organic matter decomposition, leading to the reduction of soil organic matter stabilization. In line with this possibility, the soil C/N ratio became disproportionately smaller with increasing magnitude of degradation, indicating that C mineralization is enhanced. However, the dominance of fast growing *Macaranga* in degraded forests may also contribute to the reduction of the soil C/N ratio because litter with a lower C/N ratio must have been

supplied to the ground. Intuitively, both/either the decline of litterfall productivity and/or enhanced decomposition in degraded forests are involved in the decline of soil organic matter (SOC and TN; Giweta, 2020). Other factors such as the interaction between root-derived compounds and fungal necromass, microbial necromass chemistry, and plant biodiversity may also influence the C pool (e.g., SOC) and alter soil organic matter stabilization (Adamczyk, 2021).

The second distinct pattern is the decline of soil microbial biomass in degraded forests. Both SMBC and SMBN decrease with decreasing forest degradation indexes (i.e., the PCoA axis-1 values of canopy trees; the increasing magnitude of forest degradation) in Fig. 2-3, which may be related to the decline of soil organic matter. Since soil organic matter serves as the substrate for soil microbes, the decline of soil organic matter may directly influence microbial biomass. Indeed, we find a significant positive correlation between SMBC and SOC ($r^2 = 0.61$; Fig. S2-5). Alternatively, the changes in soil microbial biomass as a consequence of above-ground forest degradation may also explain the variation of soil organic matter (e.g., SOC). However, the coefficient of soil microbial biomass to SOC is smaller than that of SOC to soil microbial biomass in our SEM analysis (see Figs. S2-3 and S2-4). Also, the SEM model explaining soil microbial biomass shows a lower BIC. Therefore, I suggest that the decline of soil microbial biomass is a consequence of the decline of SOC.

2.4.2 The activity of soil extracellular enzymes

My second hypothesis (soil enzyme activities, affected by soil microbial biomass and soil physicochemical properties, decrease linearly with the magnitude of above-ground forest degradation) is also supported. The activities of all extracellular enzymes (ACP, BG, LAP, and NAG) exhibit a linear decrease with decreasing forest degradation indexes (i.e., the PCoA axis-1 values of canopy trees; increasing magnitude of forest degradation). The activities of the four extracellular enzymes decrease by 2 to 3-fold from a primary forest to a highly degraded forest. These changes might reflect the metabolisms of microbial communities concerning the amount of available substrate,

such as soil organic C, organic N, and organic P (Akinyemi et al., 2020; Bai et al., 2021; Bayranvand et al., 2021). Indeed, the activities of all four extracellular enzymes show a positive correlation with SOC (Table 2-1). These results suggest that the amount of available substrate for soil microbes regulates enzyme activities during forest degradation, which is following the findings of Wang et al. (2022).

In addition, soil pH and soil TN are negatively and positively correlated with enzyme activities except for NAG (Table 2-1), respectively, suggesting that these may reflect changes in microbial resource acquisition strategies (Liu et al., 2022). Besides, the negative correlation of pH may indicate the overall pH regulation on enzymatic rates as seen in ACP, and/or pH specific activities of certain microbial guilds (e.g., pH dependent Acidobacteria activity/abundance); however, I still do not have direct evidence for this. The positive correlation of soil TN again suggests the effects of organic-matter substrate on enzyme activities because SOC and TN covary with each other.

Findings by Adamczyk et al. (2015) agree with my results that extracellular enzymes are related to remnant forest stands. During the process of forest degradation, the mineralization of critical biophilic nutrients (N and P) is slowed due to the decreasing extracellular enzyme activities, which may negatively affect the regrowth of the remnant forest stands. My study shows that soil chemical and microbial properties in below-ground ecosystems change concomitantly with above-ground vegetation during the process of forest degradation in Bornean tropical rainforests. Earlier studies primarily focus on the above-ground ecosystem properties as consequences of forest degradation. My study demonstrates that soil processes are equally susceptible to forest degradation, and the changes in soil processes can influence the subsequent forest regeneration.

2.5 Conclusion

In this study, I have elucidated the main biological and environmental factors

influencing the activities of soil extracellular enzymes along a forest degradation gradient in Sabah, Borneo. My findings suggest that above-ground forest degradation directly influences soil physicochemical properties (particularly soil organic matter), which subsequently affect soil microbial biomass. The combined actions of altered soil physicochemical properties and soil microbial biomass further influence microbial enzyme activities. These changes in soil processes may in turn feed back to the regrowth or succession of degraded rainforest ecosystems.

Tables and Figures

Table

Table 2-1. Correlations between soil extracellular enzymes and soil physicochemical properties. SOC, soil organic carbon; TN, total nitrogen; SMBC, soil microbial biomass carbon; SMBN, soil microbial biomass nitrogen; ACP, acid phosphatase; BG, β -glucosidase; LAP, leucine aminopeptidase; and NAG, N-acetyl-glucosaminidase. ***p < 0.001; ** p < 0.01; and * p < 0.05.

	ACP	BG	NAG	LAP
pH	-0.44**	-0.56***	-0.32	-0.60***
SOC	0.53**	0.65***	0.35*	0.59***
TN	0.51**	0.51**	0.27	0.49**
SMBC	0.57***	0.72***	0.54**	0.72***
SMBN	0.32	0.49**	0.32	0.56**

Figures



Fig. 2-1 Photos presenting the appearance of representative plot in each of the five forest degradation strata. Photos are arranged in increasing magnitude of forest degradation from the left to the right stratum. Taken by Jiang in the year of 2020.

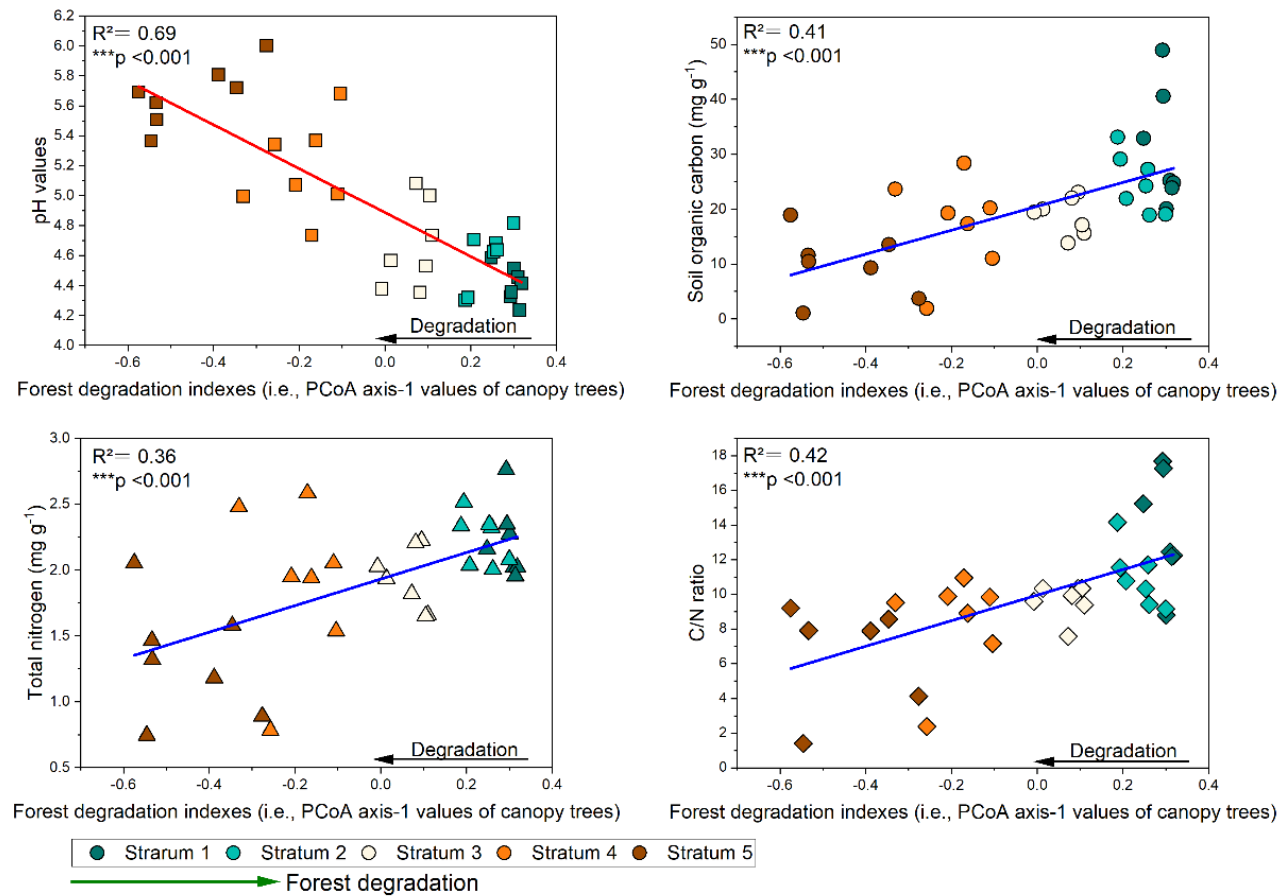


Fig. 2-2 Correlation between forest degradation indexes (i.e., PCoA axis-1 values of canopy trees) and soil physicochemical properties. The direction of the arrow indicates the aggravation of degradation and different color points indicate different degradation strata. SOC, soil organic carbon; and TN, total nitrogen.

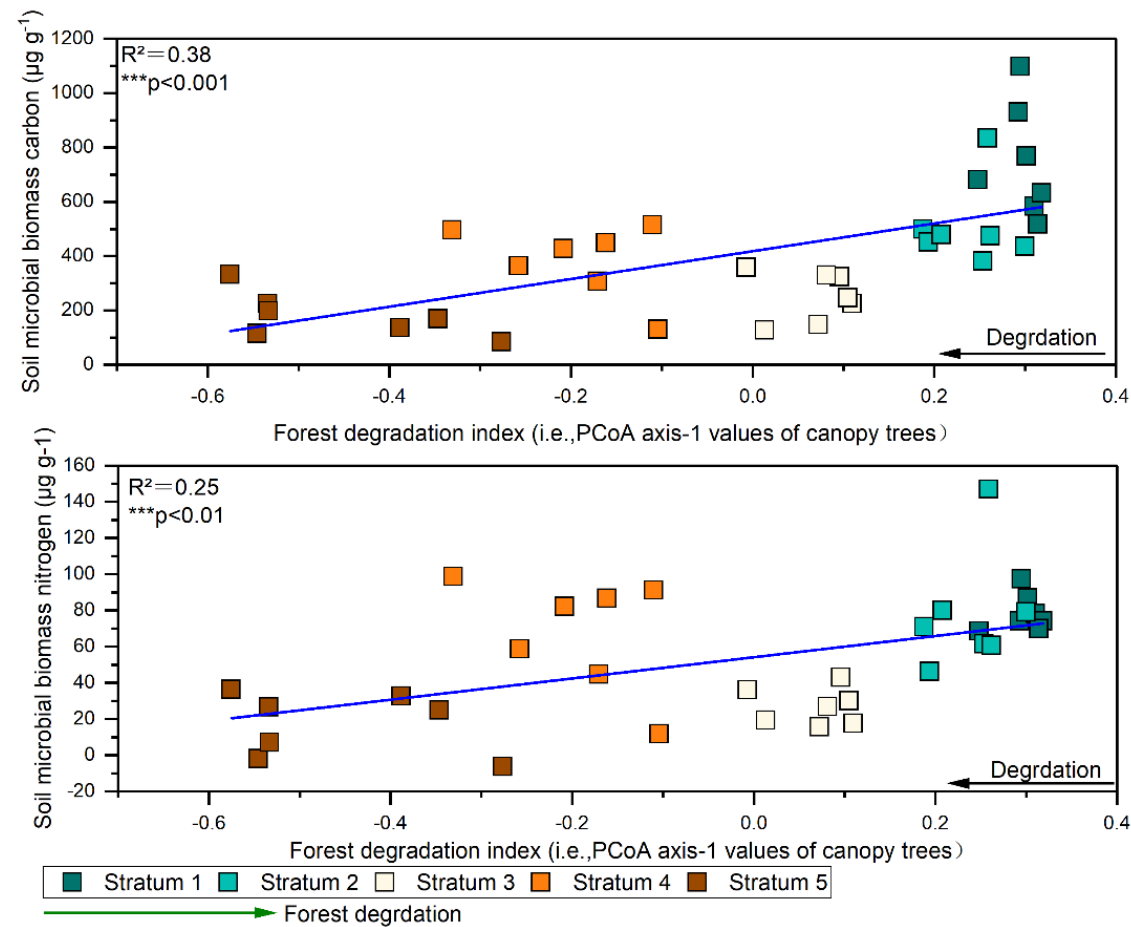


Fig. 2-3 Correlation between forest degradation indexes (i.e., PCoA axis-1 values of canopy trees) and soil microbial biomass (carbon and nitrogen). The direction of the arrow indicates the aggravation of degradation and different color points indicate different degradation strata. SMBC, soil microbial biomass carbon; and SMBN, soil microbial biomass.

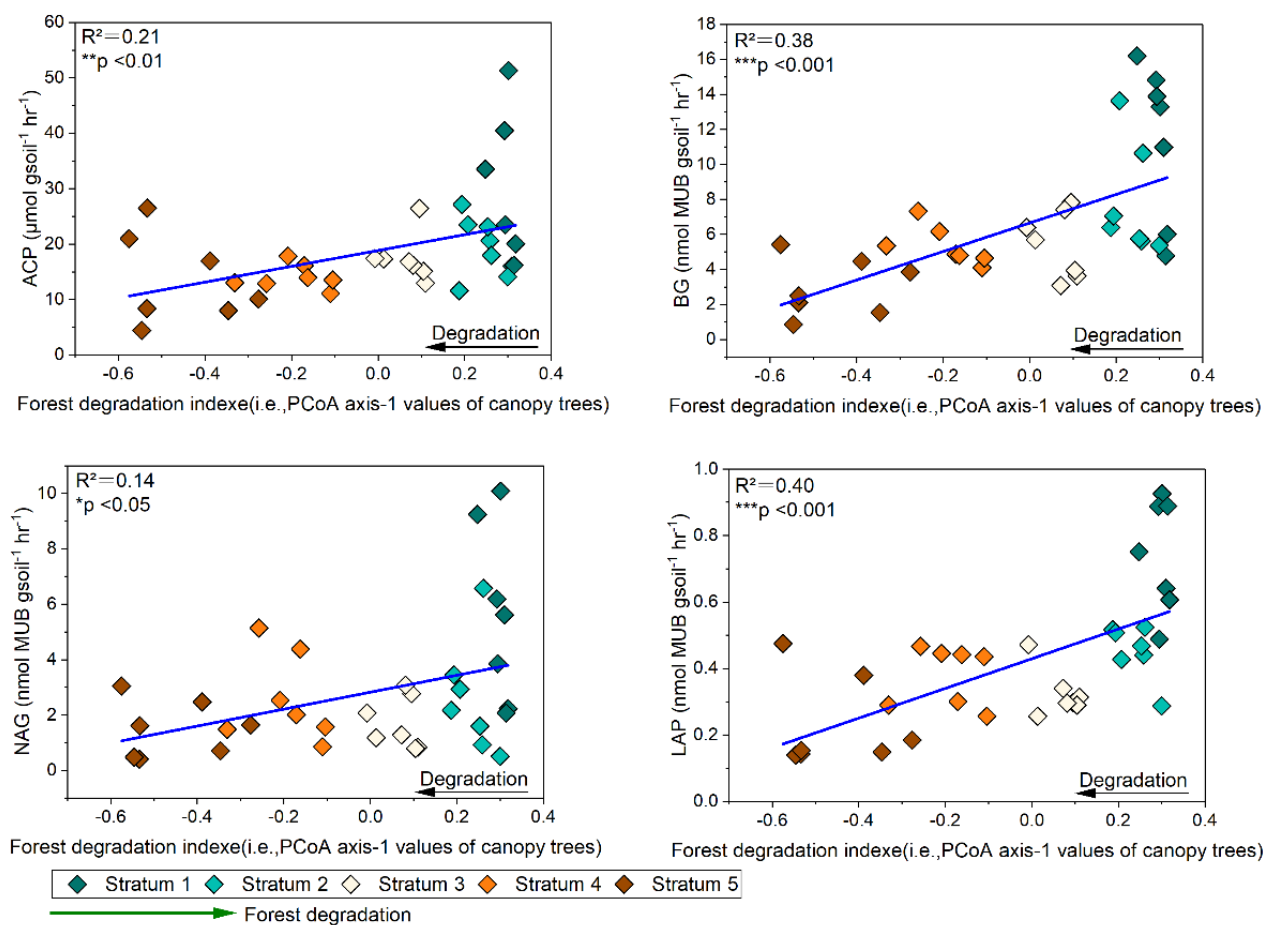


Fig. 2-4 Correlation between forest degradation indexes (i.e., PCoA axis-1 values of canopy trees) and soil extracellular enzymes. The direction of the arrow indicates the aggravation of degradation and different color points indicate different degradation strata. ACP, acid phosphatase; BG, β -glucosidase; LAP, leucine aminopeptidase; and NAG, N-acetyl-glucosaminidase.

Supplementary information

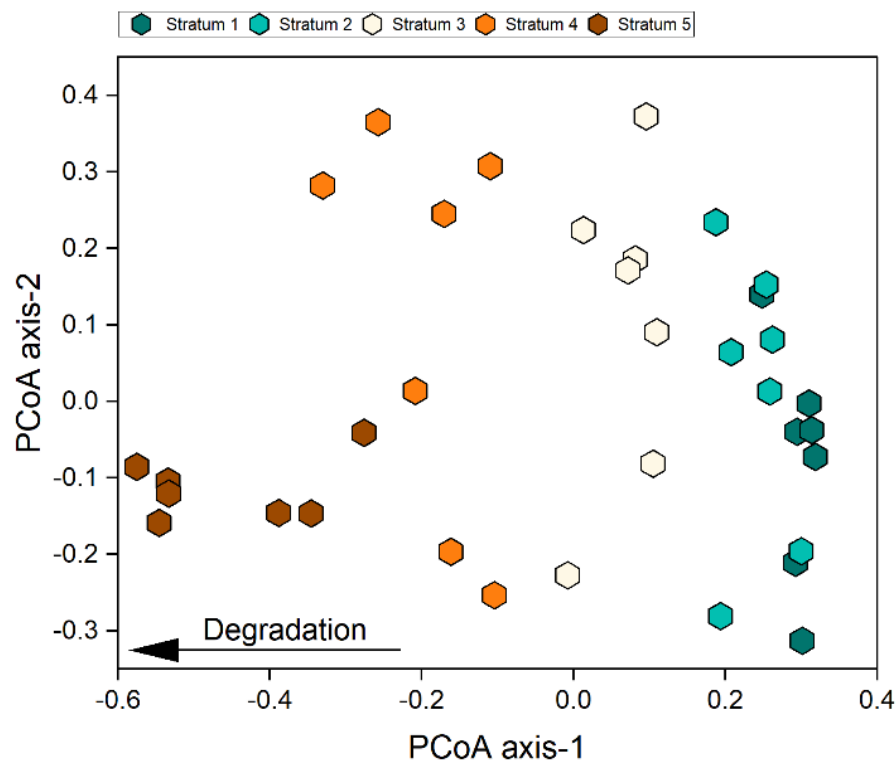


Fig. S2-1 Results of a PCoA analysis on tree genus composition for the 35 study plots in Deramakot and Tangkulap, Sabah, Malaysian Borneo. According to the above-ground vegetation condition in Deramakot and Tangkulap, forest degradation was divided into five stages from Stratum 1 (Primary Forest) to Stratum 5 (Degraded Forest). The five degradation strata were sorted along the PCoA axis-1. Consequently, axis-1 values of the 35 plots were considered to represent a magnitude of forest degradation. The arrow indicates the aggravation of forest degradation.

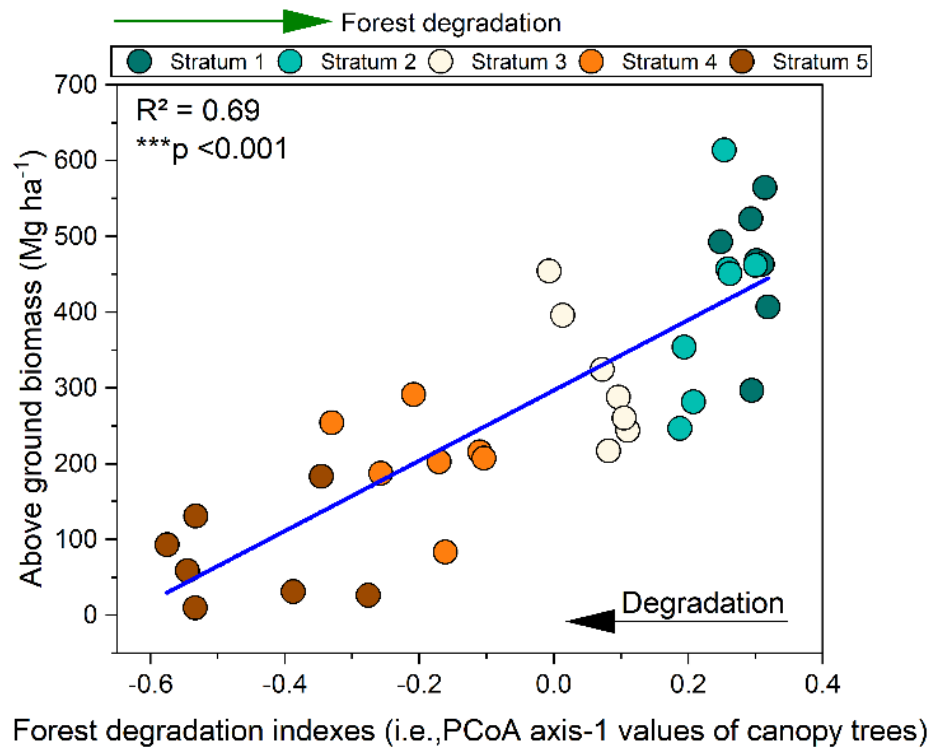


Fig. S2-2 The correlation between forest degradation indexes (i.e., PCoA axis-1 values of canopy trees) of the 35 plots and their above-ground biomass (AGB). Remnant AGB significantly decreased with increasing magnitude of forest degradation that was based on tree genus composition. Five strata of forest degradation are indicated with different colors. The arrow indicates the aggravation of forest degradation.

P=.000, Df=2 ,GFI=.833,
RMSEA<0.001, AIC=33.497, BIC=45.940

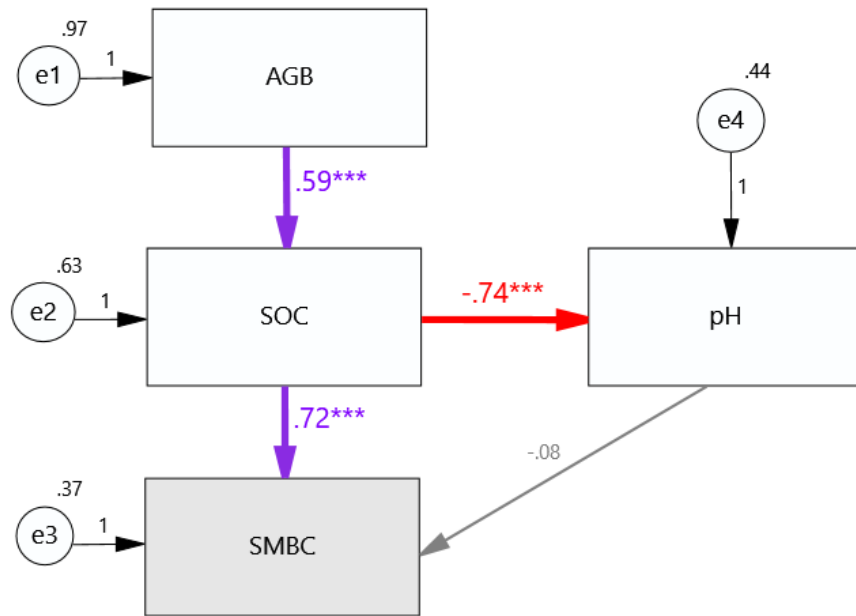


Fig. S2-3 Results of the structural equation model (SEM) with SMBC as dependent variable. The final SEM is shown to represent the casual relationships among AGB, SOC, SMBC, and pH values. The variable in grey represents the dependent variables (SMBC) and the variables in white represent the independent variables. Small circles e1, e2, e3, e4 correspond to the residuals of each variable. Purple arrows represent positive effects, red arrows have negative effects, and grey arrow have weak relations. AGB, above-ground biomass; SOC, soil organic carbon; and SMBC, soil microbial biomass carbon.

P=.000, Df=2 ,GFI=.820,
RMSEA<0.001, AIC=35.578, BIC=48.021

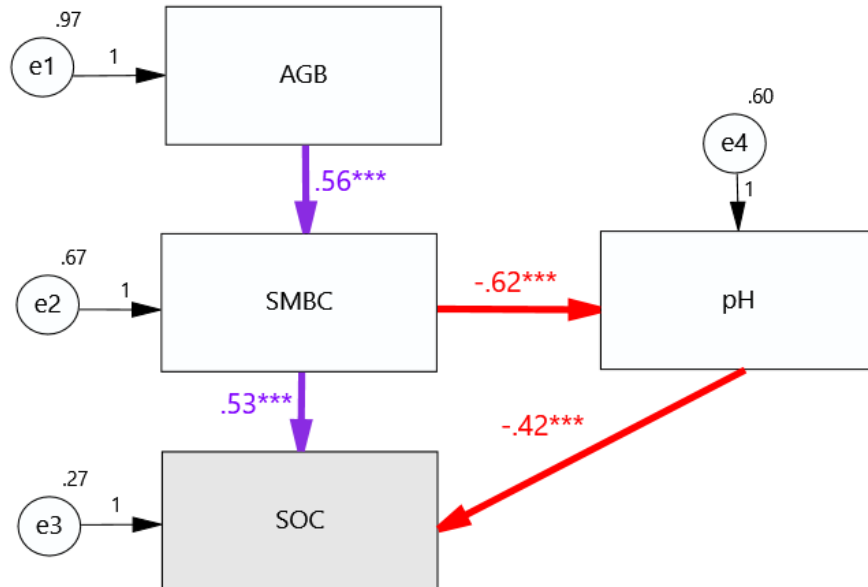


Fig. S2-4 Results of the structural equation model (SEM) with SOC as dependent variable. The final SEM is shown to represent the causal relationships among AGB, SOC, SMBC, and pH values. The variable in grey represents the dependent variables (SOC) and the variables in white represent the independent variables. Small circles e1, e2, e3, e5 correspond to the residuals of each variable. Purple arrows represent positive effects, and red arrows have negative effects. AGB, above-ground biomass; SOC, soil organic carbon; and, SMBC, soil microbial biomass carbon.

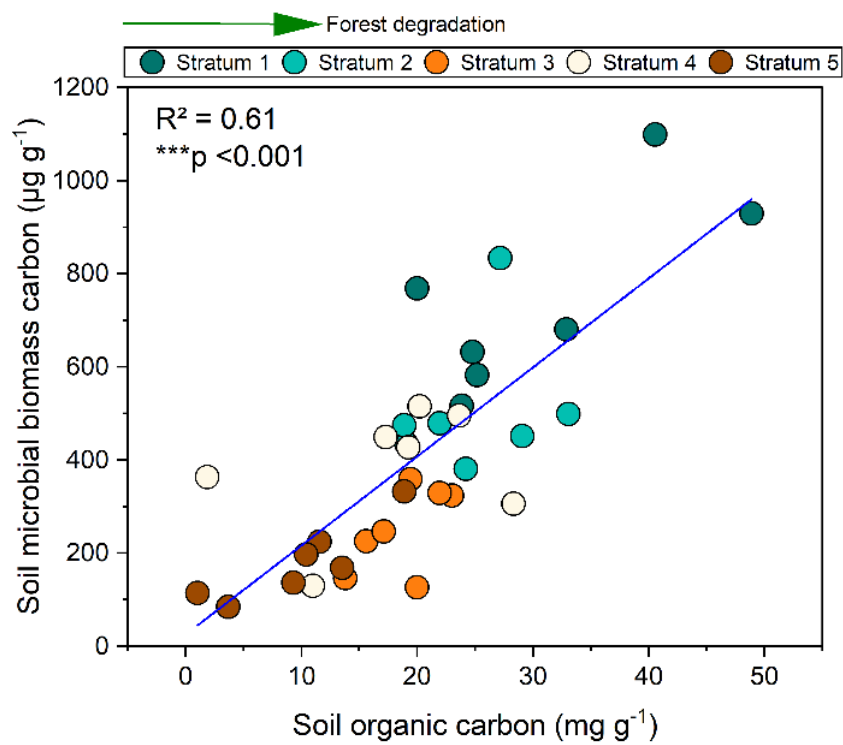


Fig. S2-5 The correlation between the concentration of soil organic carbon and soil microbial biomass carbon. Five stages of forest degradation are indicated with different colors.

Chapter 3 Changes of bacterial versus fungal community composition along a forest degradation gradient of logged-over tropical rainforests, and their consequences on soil enzyme activities in Malaysian Borneo

Abstract

Soil bacterial and fungal communities play different but mutually interrelated roles in releasing enzymes to catalyze organic matter decomposition. In Chapter 2, my discussion suggests that declining soil organic carbon (SOC), above-ground biomass (AGB), and C/N ratio may show an increase in C mineralization during the forest degradation process due to decreasing litter inputs. Besides, enzyme activities also decrease with forest degradation indexes. However, changes in microbial community compositions are still unclear despite their importance in regulating ecosystem processes. Therefore, I decided to investigate how compositions of bacterial and fungal communities change along a forest degradation gradient and how their changes affect enzyme activities.

Previous data including SOC, soil microbial biomass carbon (SMBC), and extracellular enzyme activities from Chapter 2 were still utilized in this study. Initially, I used 16S and ITS amplicon sequencing and ergosterol extraction to reveal bacterial and fungal absolute and relative abundances. Principal coordinates analyses (PCoA) were performed on their absolute abundances, and the PCoA axis-1 values of bacteria and fungi were considered as their community compositions, respectively. Then I regressed bacterial and fungal PCoA axis-1 with forest degradation indexes (i.e., the gradient of forest degradation; was established in Chapter 2), respectively. Structural equation modeling (SEM) was performed with SOC as an independent variable, while bacterial and fungal community compositions as well as extracellular enzyme activities as dependent variables. Additionally, I used the enzyme activity/SMBC ratio to indicate the potential efficiency of the enzyme *per unit* of microbial biomass under a given environment. The linear regression was conducted between enzyme activity/SMBC ratio and SOC.

My results showed that fungal community composition shifted more distinctly than bacterial community composition along the forest degradation gradient. The SEM suggested that reduced SOC in degraded forests influenced bacterial and fungal community compositions, and fungal community composition affected activities of acid phosphatase, β -glucosidase, and leucine aminopeptidase. Acid phosphatase activity/SMBC and leucine aminopeptidase activity/SMBC increased with decreasing SOC.

Greater changes in fungal community composition during forest degradation could be caused by phylum-specific responses to changing bulk organic matter substrate quality with decreasing litter inputs. The stable composition of bacterial community may reflect buffering effects by fungal decomposition activities since bacteria rely on fungal decomposition by-products. Variations in fungal community composition could subsequently induce changes in enzyme activities. Besides, soil microbial communities in the forests are generally limited by P and N in degraded forests.

Keywords: Amplicon sequencing; Selective logging; Structural equation modeling; Soil organic matter quality; P and N limitation

3.1 Introduction

Soil microbial communities, including bacterial and fungal communities, play an important role in soil organic matter decomposition and nutrient mineralization in forest ecosystems (van der Heijden et al., 2008; You et al., 2014; De Gannes et al., 2016). The major phyla in soil bacterial communities include Proteobacteria, Acidobacteria, Actinobacteria, Verrucomicrobia, and Bacteroidetes, which have been identified as predominant groups in different soil types under various environmental conditions (Lauber et al., 2009; Nemergut et al., 2010; Wei et al., 2018; Li et al., 2022). Functional groups such as pathogens, saprophytes, and symbionts occur in soil fungal communities (Bender et al., 2014, Treseder and Lennon, 2015), and most of them participate in soil organic matter decomposition and/or affect plant nutrient status (Setälä et al., 2004; Frey et al., 2019). For example, arbuscular mycorrhizal fungi (such as Glomeromycota) and ectomycorrhizal fungi (such as Basidiomycota) are plant symbionts that mediate nutrient acquisition to plants, while soil saprophytic fungi (such as Ascomycota) break down soil organic matter substrates and enhance soil nutrient cycling (Bender et al., 2014, Treseder and Lennon, 2015). Because soil bacterial and fungal communities consist of different phyla and are often functionally distinct, they will exhibit complex and differential responses to a given disturbance event.

Bacterial and fungal communities play different but mutually interrelated roles in the breakdown of complex organic substances in litter and the release of essential nutrients back into the soil (Glassman et al., 2018). Fungal communities are considered the primary decomposers of dead plant biomass in terrestrial ecosystems, and several enzymes (e.g., cellulases and ligninases) produced by fungal communities enable them to efficiently attack the recalcitrant lignocellulosic matrix which other organisms struggle to decompose (Voříšková and Baldrian, 2013; Brzostek et al., 2013, Cline and Zak, 2015). Ascomycota, together with some Basidiomycota fungi (Osono, 2007; Voříšková and Baldrian, 2013), are found to dominate during the early stages of litter decomposition. However, the fungal community and its diversity undergo rapid succession during litter decomposition (Voříšková and Baldrian, 2013; Bani et al.,

2018). Ascomycota abundance may decrease as they are gradually replaced by Basidiomycota fungi, especially saprotrophic cord formers, during later stages of decomposition (Frankland, 1998; Osono, 2007; Voříšková and Baldrian, 2013). The bacterial communities are involved in the degradation of the simpler compounds that are produced as a result of fungal activities (Purahong et al., 2016). Bacteria further degrade the by-products of fungal degradation, such as simple sugars and organic acids, into inorganic compounds, thereby completing the degradation process (Boer et al., 2005). Due to their relatively high intrinsic growth rates and unicellular nature, bacterial communities tend to be less directly linked to litter quality than fungal communities (Bender et al., 2014, Treseder and Lennon, 2015). The composition of fungal communities is expected to shift with degrading litter, while that of bacterial communities may be decoupled.

In addition to the differences in litter-decomposition function, the responses to environmental changes may also differ between bacterial and fungal communities. While soil microbial community composition is generally susceptible to environmental changes (He et al., 2023), fungal communities show distinct responses to the changes in plant species richness, soil moisture, soil organic carbon, as well as soil nitrogen and phosphorus availability than bacteria communities do (Xue et al., 2021; Geng et al., 2023). The impacts of forest degradation on fungi are more pronounced than on bacteria (Zhou et al., 2018; Liu et al., 2019). On the other hand, bacterial communities are more resilient in composition to environmental changes than fungal communities are (Girvan et al., 2005).

Extracellular enzymes secreted by soil microbes catalyze the decomposition of organic matter and nutrient cycling and are closely associated with bacterial and fungal communities. The activities of extracellular enzymes may be influenced by the composition and the diversity of microbial communities, as well as other environmental factors (Strickland et al., 2009). Therefore, shifts in soil bacterial and fungal communities are subsequently transmitted to changes in extracellular enzyme activities.

Here, I study how bacterial and fungal community compositions shift along a

gradient of forest degradation in Malaysian-Borneo, and how microbial community compositions relate to soil enzyme activities. Selective logging is one of the major land-use types in Borneo, and creates spatially and temporally heterogeneous landscapes where remnant forests with different degradation statuses are juxtaposed with each other (Imai et al., 2012; Imai et al., 2014; Aoyagi et al., 2017; Kitayama et al., 2018; Takeshige et al., 2022). The sustainability and recovery potentials of these remnant forests are dependent on nutrient availability (Aoyagi et al., 2013). Therefore, the responses of microbial community structures and compositions to selective logging have a significant role in determining the fate of logged-over forests. I previously investigated the changes of soil physicochemical properties and four types of soil enzymes (β -glucosidase, leucine aminopeptidase, N-acetyl-glucosaminidase, and acid phosphatase) along a gradient of forest degradation in Bornean logged-over tropical rainforests in Chapter 2, and found that soil organic carbon and the activity of each of the four extracellular enzymes decline monotonously with the increasing magnitude of forest degradation. However, responses of microbial community compositions to forest degradation and their relations with soil enzyme activities have not been investigated despite that understanding their responses is essential to predict how/if degraded forests recover.

The following three research questions were asked in this study. 1) How do bacterial and fungal communities change along a forest degradation gradient in terms of community composition? 2) How do they relate to the change in soil enzyme activities? 3) How does the shift of soil microbial communities relate to enzyme efficiency (indexed by the ratio of enzyme activity to microbial biomass) along the forest degradation gradient? The ratio of enzyme activity to microbial biomass has been used to represent enzyme efficiency in my study because microbial biomass is another important factor affecting extracellular enzyme activities (Ndabankulu et al., 2022) and an enhanced efficiency may involve a shift in fungal or bacterial communities. In the present study, I used 16S and ITS amplicon sequencing and ergosterol extraction to reveal the absolute and relative abundances of bacteria and fungi and performed

principal coordinate analyses on the absolute abundances to represent the bacterial and fungal community compositions.

3.2 Material and methods

3.2.1 Study sites description

The research sites are Deramakot Forest Reserve and Tangkulap Forest Reserve, Sabah, Malaysian Borneo (See Chapter 1, Fig. 1-1). The original vegetation in research areas are mainly dipterocarp species (Ong and Kleine, 1996), and have humid equatorial climate with the average temperature is 28 °C (Huth and Ditzer, 2001). Soil are predominantly Acrisols (Sabah Forestry Department 2005).

The logging history in these two forest reserves has been described in detail in Chapter 2 (see section 2.2.1). Briefly, both Deramakot and Tangkulap Forest Reserves were designed as production forests and underwent selective logging. In Deramakot, commercial selective logging took place from 1956 to 1989, after which reduced-impact logging (RIL) was implemented in 1995 (Sabah Forestry Department 2005). While Tangkulap experienced intensive selective logging from the 1970s to 2002 (Ong et al., 2013). Aforementioned different logging histories formed heterogeneous landscapes with more degraded remnant forests in Tangkulap (Kitayama et al., 2018; Takeshige et al., 2022).

3.2.2 Definition of the gradient of forest degradation, soil physicochemical properties, and enzyme activities

The forest degradation gradient in the Deramakot and Tangkulap Forest Reserves has been established in Chapter 2. Briefly, the Landsat Thematic Mapper (TM) Band-7 image was used to divide the research areas into five strata ranging from the primary forest (stratum 1) to a highly degraded forest (stratum 5), and dominant tree species were found ranged from climax dipterocarp species in stratum 1 to pioneer *Macaranga* species in stratum 5 (Kitayama et al., 2018; Imai et al., 2019) (see Chapter 2, Fig. 2-1).

Seven research plots were selected in each stratum, yielding a total of 35 plots. The relative abundances of tree genera in 35 plots were calculated, and then were ordinated through a principal coordinate analysis (PCoA) using the Bray-Curtis dissimilarity index. The PCoA result showed that the magnitude of forest degradation in the 35 plots corresponded to the order of PCoA axis-1 values (see Chapter 2, Fig. S2-1) suggesting that PCoA axis-1 values of canopy tree as forest degradation indexes. Moreover, above-ground biomass (AGB) of 35 plots were calculated and found correlated with the forest degradation indexes ($R^2 = 0.69$, $p < 0.001$, see Chapter 2, Fig. S2-2), confirming that forest degradation in terms of tree community composition well corresponded to structural deterioration of the above-ground vegetation. Mean ($\pm$ SD) AGB decreased predictably from stratum 1 to 5 as follows: stratum 1, $459.33 \pm 80.73 \text{ Mg ha}^{-1}$; stratum 2, $409.50 \pm 116.19 \text{ Mg ha}^{-1}$; stratum 3, $311.82 \pm 79.54 \text{ Mg ha}^{-1}$; stratum 4, $205.84 \pm 59.78 \text{ Mg ha}^{-1}$; and stratum 5, $76.24 \pm 58.52 \text{ Mg ha}^{-1}$.

The 35 soil samples collected from 35 plots in Chapter 2 were still used in this study. Briefly, each sample was taken from the surface soil layer (10 cm depth) by four-point method, and then uniformly mixed to form a composite sample. Total samples were divided into two parts. I still used 4°C refrigerated sample for chemical analysis, and used freeze-dried sample for DNA analysis.

I cited values of pH, soil organic carbon (SOC), total nitrogen (TN), C/N ratio and soil microbial biomass carbon (SMBC) from Chapter 2 because my current study was conducted using the same forest plots (i.e., 35 plots) and soil samples. SOC and TN were measured by burning air-dried samples (wrapped with tinfoil) in a CHNS-O analyzer (Thermo Fisher Scientific Inc.), and pH was measured with a pH meter (HORIBA pH/Cond Meter D-54). SMBC was determined by the chloroform fumigation extraction method. I determined total phosphorus (TP) in the current study using a wet digesting method with concentrated sulfuric acid and H_2O_2 . After digestion, the concentration of TP was determined by ICPS-7510 (Shimadzu Corp., Japan). Combined with the results from Chapter 2, concentrations of SOC, TN, and C/N decreased with increasing magnitude of forest degradation, suggesting that soil organic

matter was less decomposed underneath primary forests and became more decomposed with degradation (see Table 3-1). While pH increased with forest degradation due to changes in soil redox potentials (Qin et al., 2024). TP did not vary with forest degradation. Mean ($\pm$ SD) TP from stratum 1 to 5 as follows: stratum 1, $181.82 \pm 25.36 \mu\text{g g}^{-1}$; stratum 2, $236.20 \pm 40.60 \mu\text{g g}^{-1}$; stratum 3, $159.24 \pm 20.68 \mu\text{g g}^{-1}$; stratum 4, $184.24 \pm 19.82 \mu\text{g g}^{-1}$; and stratum 5, $154.36 \pm 40.42 \mu\text{g g}^{-1}$.

The activities of four extracellular enzymes have been determine in Chapter 2 through the colorimetric method (Kitayama, 2013) for acid phosphatase (ACP; can hydrolyze organic P) (Kieliszewska-Rokicka 1999), and high-throughput fluorometric measurement (Bell et al., 2013) for β -glucosidase (BG; target cellulose decomposition) (Knight and Dick, 2004; Dick 2011), leucine aminopeptidase (LAP; can releases N-terminal leucine residues) (Wang et al., 2020), and N-acetyl-glucosaminidase (NAG; target chitin) (Sistla and Schimel, 2013; Stone et al., 2014). These extracellular enzymes have been considered key enzymes for maintaining C, N, and P cycling in forest ecosystems (Allison and Vitousek, 2005; Luo et al., 2017; Moorhead et al., 2012), and were found to decrease monotonously with increasing magnitude of forest degradation (see Table S3-1).

3.2.3 Ergosterol extraction

Ergosterol was extracted from each soil sample to determine fungal biomass. Two g of freeze-dried soil sample was placed in each test tube, which contained 2 g KOH, three zeolites, 25 ml methanol and 5 ml ethanol. Test tubes were placed in a water bath at 80°C for 1 hour. After heat treatment, 10 ml distilled water was added and stirred thoroughly before being transferred to centrifuge tubes. Subsequently, 10 ml ethanol was added to each centrifuge tube, which was then vortexed for 30 sec and centrifuged at 2000 rpm for 5 min. Supernatants were collected in new test tubes. This procedure was repeated until 30 ml of supernatant was collected in each test tube. The liquid in the test tubes was evaporated by using a nitrogen evaporator (Nitrogen Evaporator SC10-1, Biobase Biodustry (Shandong) Co., Ltd.) heated to 40 °C at 0.2 MPa. Then, 2

ml of 1:1 mixture of chloromethane and methanol was added, and the solution was sonicated for 1 minute to completely dissolve the dried solid in the test tubes. Finally, the solution was filtered through a 13-mm hydrophobic PTFE filter and transferred to vials. Ergosterol concentration was determined on high-performance liquid chromatography (HPLC) (Shimadzu Corp., Japan). An ergosterol standard (FUJIFILM, JAPAN) was used to construct a standard curve.

3.2.4 DNA extraction, amplification, and sequencing

DNA was extracted from the freeze-dried soil samples using the QIAGEN DNeasy PowerSoil Pro Kit (QIAGEN, Hilden, Germany). Each soil sample was homogenized in the PowerBead Pro tube, which contained beads. Total genomic DNA was captured on a silica membrane in the form of a spin column, purified and eluted from the membrane for subsequent detection. The final elution volume was 100 μ l, and the extracted DNA was stored at -20°C until further processing.

The 16S and ITS amplicon sequencing were performed to characterize soil bacterial and fungal communities, respectively. The detailed protocol for library preparation was described in the Supplementary methods. Briefly, the target regions, 16S for bacteria and ITS for fungi, were amplified using 515F–806R (Caporaso et al., 2011) and ITS_F_KYO1/ITS2_KYO2 (Toju et al., 2012), respectively. To improve the quality of the sequence in the library, 0-18 N (random base) was added outside the universal primer. In the first-round PCR (1st PCR), the target regions were amplified and then purified using AMPure XP. The purified 1st PCR products were used as templates for the second round (2nd PCR), during which sample-specific index sequences and adapter sequences were appended. The purified and concentration-adjusted library was sequenced on the iSeq system (Illumina, San Diego, CA, USA) using a 300-cycle single-end mode. The 16S and ITS amplicon sequencing was conducted in the laboratory of the Clockmics Co. (Osaka, Japan).

After the sequencing, the low-quality sequence was initially removed using fastp (Chen et al., 2018), followed by the removal of Ns and primer sequences using seqkit

(Shen et al., 2016) and cutadapt (Martin, 2011). Subsequently, DADA2 (amplification sequence variant, ASV; Callahan et al., 2017) was employed to infer ASVs, and Claident was used to assign taxa information to each ASV (Tanabe and Toju, 2013). To mitigate the effects of sequence depth on the community compositions detected, coverage-based rarefaction was performed before the subsequent statistical analysis (Chao et al., 2014) using the iNEXT package of R (Hsieh et al., 2020). More specific information on amplicon sequencing can be found in the supplementary materials. The nucleotide data associated with this study is publicly accessible at the National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov/>) under accession number PRJNA1082715.

3.2.5 Statistical analysis

3.2.5.1 The relative abundance and absolute abundance of each microbial phylum

Although the 16S primer set which I used was actually for prokaryotes, I only focused on bacterial ASVs in this study because Archaea ASVs were minor components. To mitigate the effects of sequence depth on the detected community compositions, the coverage-based rarefaction was performed using the “iNEXT” package in R for bacterial and fungal communities so that each sequence depth covers 97% of total diversity of each sample. Before rarefaction, 3 samples for bacteria and 2 samples for fungi were removed because these samples contained extremely low sequence reads. The relative abundance of each ASV was then calculated based on rarefied sequence reads.

The absolute abundances of bacteria and fungi were calculated from the results of the ergosterol analysis according to Montgomery et al., (2000).

$$FB(\mu g\ g^{-1}soil) = Ergosterol(\mu g\ g^{-1}soil) \times f \times R_f$$

$$FBC = FB \times C$$

$$BBC = \frac{SMBC - FBC}{\text{proportion of prokaryote}} \times \text{proportion of bacteria}$$

, where f is conversion factor (i.e., $[1\text{ mg } FB/4\ \mu g\text{ ergosterol}] \times 1000\ [\mu g\text{ mg}^{-1}]$), and $R_f=1.61$ (correction factor for average percent recovery, $1/0.62$), FBC = fungal

biomass carbon, BBC = bacterial biomass carbon and $C = 0.43$, average C fraction in fungal species. The proportions of bacteria and prokaryotes were calculated based on the rarefied ASV tables, and the influence of Archaea was excluded.

The absolute abundances of bacterial and fungal phyla in each sample were also calculated by the following formula.

$$\begin{aligned} \text{Absolute abundance of bacterial phylum } A \\ &= BBC \times \text{Relative abundance of bacterial phylum } A \\ \text{Absolute abundance fungal phylum } A \\ &= FBC \times \text{Relative abundance of fungal phylum } A \end{aligned}$$

A logarithmic transformation was applied to all bacterial and fungal absolute abundances to account for the significant difference between the maximum and minimum absolute abundances. The purpose of this transformation was to reduce noise and bias of the overall data towards a normal distribution (Verma et al., 2019). Subsequently, I compared the skewness, kurtosis, and Z-score of the original data and the logarithmic transformed data, respectively. And under significance level of $\alpha = 0.05$, the Z-score after logarithmic transformation was within the range of ± 1.96 , which was closer to a normal distribution.

I also calculated the relative abundance of bacterial and fungal dominant phyla, respectively, which was the sum of ASVs belonging to the same dominant phylum divided by the total ASVs of all forest plots, and presented as a bar chart.

3.2.5.2 Analysis of microbial community compositions

A distance matrix was calculated using the Bray-Curtis dissimilarity index each for bacterial and fungal communities based on log-transformed absolute abundances of phyla using the "vegan" package (Oksanen et al., 2020) in R. A principal coordinate analysis (PCoA) was applied to each distance matrix to ordinate the forest plots. The greatest variation of community composition should occur along the PCoA axis-1 in both bacterial and fungal communities. Subsequently, PCoA axis-1 values of microbial communities were regressed with forest degradation indexes (i.e., PCoA axis-1 values of canopy trees) for bacterial and fungal communities, respectively, which was to

examine how microbial communities changed along the forest degradation gradient. In the following, PCoA axis-1 values for bacteria are denoted as PCoA axis-1 (bacteria), and those for fungi are denoted PCoA axis-1 (fungi). I also used bar charts to display the absolute and relative abundance of bacteria and fungi in each forest plot, respectively.

In addition, I examined how life-history traits of bacterial communities changed along the forest degradation gradient. For this purpose, we followed the definitions in the previous studies; Acidobacteria and Actinobacteria are assigned to K-strategy phyla, and Proteobacteria and Bacteroidetes are assigned to r-strategy phyla (Fierer et al., 2007; Zechmeister-Boltenstern et al., 2015; Yan et al., 2022). Subsequently, the proportions of the absolute abundances of K-strategy phyla to those of r-strategy phyla *per* forest plot were regressed with forest degradation indexes (i.e., PCoA axis-1 values of canopy trees).

3.2.5.3 A structural equation modeling

To examine how SOC (i.e., the substrate for soil microbes) affected extracellular enzyme activities via altering the composition of microbial communities, a structural equation modeling (SEM) was performed. The topmost independent variable was SOC, while the dependent variables were set to bacterial community composition (i.e., PCoA axis-1 (bacteria)), fungal community composition (i.e., PCoA axis-1 (fungi)), and four enzyme activities (ACP, BG, LAP and NAG). All data were normalized to have a mean of zero and a standard deviation of one before import into the internal model through the “lavaan” and “semPlot” packages in R (Rosseel, 2012; Epskamp et al., 2017). P-value, root mean square residual (RMR), and Akaike information criteria (AIC) were used to evaluate the goodness of the model.

Here, SOC was chosen as the topmost independent variable in SEM rather than the forest degradation indexes or AGB, despite the fact that forest degradation indexes and AGB may represent the impact of above-ground forest degradation and affect community composition. This decision was made to emphasize that among the many factors influenced by forest degradation, the SOC substrate might be the most effective

for soil microbial communities. In Chapter 2, I have pointed out that forest degradation indexes can be considered as composite variables that cannot be included in SEM. Additionally, although AGB may be associated with both forest degradation and SOC, it is the quality and quantity of litter, rather than the biomass of tree species, that directly affects microbial communities. Consequently, SOC was considered to be the factor most closely related to microbial activities.

Although some studies indicate that SOC can be also influenced by microbial communities and/or soil enzyme activities (Kallenbach et al., 2016; Yang et al., 2023), the SEM analyses in Chapter 2 have confirmed that the coefficient of soil microbial biomass to SOC was smaller than that of SOC to soil microbial biomass, suggesting that the decline of soil microbial biomass was a consequence of the decline of SOC along a forest degradation in logged-over tropical rainforests, Malaysian Borneo. Soil microbial biomass is closely linked with microbial activities and community compositions (Beugnon et al., 2021). Therefore, I considered that the shifts in microbial community compositions are also a result of the reduced SOC along the forest degradation gradient in my study sites, and the setting of SOC as a topmost independent variable is reasonable.

3.2.5.4 Analysis of microbial resources-use

Extracellular enzyme activities are a consequence of microbial metabolisms. The ratio of enzyme activity to SMBC indicates the potential efficiency of the enzyme per unit of microbial biomass under a given environment. Ratios of each soil enzyme to SMBC were regressed with concentrations of SOC to examine how the resource uses by microbial metabolism changed with declining substrates for microbes during the process of forest degradation. I examined the linear relationship between SOC and enzyme activity *per* unit microbial biomass carbon (i.e., ACP activity/SMBC, BG activity/SMBC, LAP activity/SMBC, or NAG activity/SMBC).

3.3 Results

3.3.1 Soil microbial biomass based on ergosterol analysis

The concentrations of soil microbial biomass carbon (SMBC), fungal biomass carbon (FBC), and bacterial biomass carbon (BBC) were shown in Table 3-1. All of the mean SMBC and FBC showed the highest value in the primary forest (stratum 1) and the lowest value in the most degraded forest (stratum 5). Mean FBC monotonously decreased with increasing magnitude of forest degradation. Mean SMBC and BBC monotonously decreased except for stratum 4. FMBC comprised 40 to 78% of total SMBC.

3.3.2 Overview of the 16S and ITS amplicon sequencing

In total, I obtained 1,496,361 reads and 37,627 ASVs for bacteria and 1,443,290 reads and 18,241 ASVs for fungi. When all forest plots were merged based on the rarefied sequence reads (see coverage-based rarefaction in Figs. S3-1 and S3-2), the most abundant phyla based on the relative abundance were as follows (Figs. S3-3–S3-5): for bacteria, Actinobacteria (32.63%), Proteobacteria (25.25%), Planctomycetes (15.81%), Unidentified-bacteria (10.58%), Acidobacteria (5.64%), Firmicutes (4.18%), Verrucomicrobia (3.14%), Chloroflexi (2.53%), Gemmatimonadetes (0.15%), and the other bacteria (0.09%) including Bacteroidetes, Chlamydiae, Candidatus Melainabacteria, Nitrospirae, Cyanobacteria, Tenericutes, and Chlorobi (relative abundance for each phylum was less than 0.1%); and for fungi, Ascomycota (76.26%), Basidiomycota (15.15%), Mucoromycota (8.32%), Unidentified-fungi (0.17%), Chytridiomycota (0.08%), and Zoopagomycota (0.01%). Note that the proportion of unidentified phylum was relatively high due to the conservative taxa assignment criteria implemented in Claident (Tanabe and Toju, 2013). The use of other pipelines may decrease the proportion of unidentified phylum, but I adopted this approach to avoid the misassignment of microbial taxa (i.e., the possibility of false positive taxa assignment was low in this study).

3.3.3 Soil bacterial and fungal communities

The results of PCoA for bacterial and fungal communities were shown in Fig. 3-1. Points represented the forest plots and the different colors represented the forest degradation strata. In the bacterial PCoA, the first axis and second axis explained 79.4% and 18.3% of the total variation, respectively (Fig. 3-1 a). In the fungal PCoA, the first axis and second axis explained 56.9% and 26.0% of the total variation, respectively (Fig. 3-1 b). The shape of the bacterial two-dimension coordinate appeared as a horseshoe effect (Morton et al., 2017), but I considered this was not a consequence of a horseshoe effect. The very high proportion of the explained variation by the bacterial PCoA axis-1 as well as the arch shape has been a natural consequence of the strong effect of bacterial biomass to derive absolute abundances (Fig. S3-4 a). Because bacterial biomass dictated absolute abundances of bacterial phyla, while relative abundances were stable (Fig. S3-4 b), the forest plots were aligned in the order of bacterial biomass in the bacterial PCoA. Consequently, the PCoA axis-1 had a high proportion of variation explained (i.e., 79.4%).

PCoA axis-1 values were chosen to represent community composition for both bacteria and fungi. A linear regression indicated that PCoA axis-1 values of bacterial communities did not correlate with forest degradation indexes (i.e., PCoA axis-1 values of canopy trees). PCoA axis-1 values of fungi correlated with forest degradation indexes ($R^2 = 0.54$, $p < 0.001$; Fig. 3-2).

Acidobacteria to Proteobacteria ratio was shown in Fig. S3-6, as previous studies suggested that Acidobacteria and Proteobacteria are in general K-strategist and r-strategist, respectively (Fierer et al., 2007; Zechmeister-Boltenstern et al., 2015; Zhou et al., 2017; Yan et al., 2022). The ratio of Acidobacteria absolute abundance to Proteobacteria absolute abundance significantly decreased with forest degradation indexes (i.e., PCoA axis-1 values of canopy trees) (linear regression, $R^2 = 0.13$, $p < 0.05$), suggesting that the ratio of K-strategists to r-strategists decreased with forest degradation (Fig. S3-6).

3.3.4 The SEM result

The result of SEM was shown in Fig. 3-3. The effects of SOC on bacterial community composition and fungal community composition (quantified as coefficients of the SEM model) were significant (effect of SOC on bacterial community = -0.612 , $p < 0.001$; on fungal community = -0.687 , $p < 0.001$). The effects of fungal community composition on ACP (coefficient = -0.358 , $p < 0.01$), BG (coefficient = -0.380 , $p < 0.05$) and LAP (coefficient = -0.518 , $p < 0.001$) were significant (Fig. 3-3), while those of bacterial community composition on enzymes were not significant.

3.3.5 Enzyme efficiency along the forest degradation gradient

The quotient of enzyme activity to SMBC increased with decreasing SOC for ACP (ACP activity/SMBC, $r^2 = 0.16$, $p < 0.05$) and LAP (LAP activity/SMBC, $r^2 = 0.18$, $p < 0.05$) (Fig. 3-4). BG activity/SMBC and NAG activity/SMBC did not show a significant correlation with SOC.

3.4 Discussion

3.4.1 Overall patterns of soil bacterial and fungal community compositions along the forest degradation gradient

As to the first research question (changes in soil bacterial versus fungal communities along a forest degradation gradient), fungal communities change significantly in biomass and composition with increasing magnitude of forest degradation (Fig. 3-1 b and Fig. S3-5), and bacterial communities change in biomass but not in composition (Fig. 3-1 a and Fig. S3-4). About 56.9% of the fungal community PCoA axis-1 values are explained by forest degradation indexes (Fig. 3-1b), meaning that 30.7% of the total variation in fungal community composition is ascribed to forest degradation indexes (i.e., the fungal community PCoA axis-1 explains 56.85% of the total variation, multiplied with 0.54). By contrast, little variation of the bacterial community compositions can be explained by the forest degradation indexes (Fig. 3-1

a). Although the soil bacterial and fungal biomass decrease with the forest degradation, the bacterial communities show more stable community composition than fungal communities do. My findings are consistent with a global meta-analysis (Zhou et al., 2018), and a study of tropical forests in Sumatra, Indonesia (Brinkmann et al., 2019), both of which find that the impacts of forest degradation on fungal communities are more pronounced than on bacterial communities.

In the bacterial PCoA, an arch shape aligns with axis-1 with its high proportion of the explained variation (i.e., 79.4%) can be attributed to the use of absolute abundances prorated to bacterial biomass that changed greatly among the forest plots (Fig. S3-4 a). The linear regression of bacterial PCoA axis-1 by BBC ($p < 0.001$) supports my view because the variance in microbial community composition may not be explained by the forest degradation but can be explained primarily by total bacterial biomass.

3.4.2. Possible mechanisms behind the changes in the bacterial and fungal community compositions

Different responses between bacteria and fungi might be related primarily to their differences in substrate use. The quantity and quality of soil organic matter in topsoil change in a predictable way along our forest degradation gradient. The concentration of topsoil SOC linearly decreases with the increasing magnitude of forest degradation, while its C/N ratio decreases, indicating that more C relative to N has been mineralized in the degraded forests (Chapter 2). Soil organic matters underneath primary forests are less decomposed (inferred by a higher C/N ratio) due to continuous fresh litterfall, while soil organic matters underneath degraded forests are more decomposed (a lower C/N ratio) due to decreased litterfall input. In terrestrial ecosystems, fungi have been considered to be the primary decomposers of fresh, dead plant biomass, and cellulases and ligninases produced by the fungal communities allow them to efficiently degrade the recalcitrant lignocellulosic matrix (Voříšková and Baldrian, 2013; Brzostek et al., 2013, Cline and Zak, 2016). During the early stages of litter decomposition, Ascomycota dominates together with some Basidiomycota fungi (Osono, 2007;

Voříšková and Baldrian, 2013). Due to the rapid succession of fungal communities during litter decomposition (Voříšková and Baldrian, 2013; Bani et al., 2018), the abundances of Ascomycota may decrease as they are gradually replaced by Basidiomycota, such as saprotrophic cord-forming fungi, which are active in the later stages of the decomposition process (Frankland, 1998; Osono, 2007; Voříšková and Baldrian, 2013). Such a successional pattern should be elucidated along our forest degradation gradient although caution must be needed because my gradient does not directly represent a successional sere.

In contrast, bacterial communities may generally utilize more labile compounds, which are by-products of fungal decomposition activities (Purahong et al., 2016). The primary reason why bacterial communities are not explained by forest degradation may be their insensitivity to the quality of bulk soil organic matter, as well as to the buffering effects of soil fungi. Previous studies suggest that soil pH significantly affects bacterial community compositions via optimal bacterial growth among different phyla (Lladó et al., 2017; Mod et al., 2021; Rousk et al., 2010; Wang et al., 2019). Soil pH has been shown to exert a strong influence on the composition of soil bacterial communities across different spatial scales and soil types, indicating the universal significance of soil pH in shaping bacterial community composition (Lauber et al. 2009; Fierer and Jackson, 2006). In tropical forests, the abundances of Acidobacteria and Proteobacteria decrease with increasing soil pH, whereas Bacteroidetes and Actinobacteria increase with increasing soil pH (Lladó et al., 2017; Wei et al., 2018; Yu et al., 2021). In my case, soil pH increases with forest degradation ($R^2 = 0.69$, $p < 0.001$) as a consequence of soil redox changes (Qin et al. 2024). However, I cannot find significant changes in overall bacterial community composition probably because of the relatively narrow range of soil pH in our study (4.41–5.67; Table 3-1).

The only pattern that I find in bacterial communities is the decline of the ratio of Acidobacteria to Proteobacteria abundance with forest degradation (Fig. S3-6). This can imply a directional shift from K-strategist to r-strategist bacteria. A meta-analysis also supports our results that the composition of soil bacterial communities shifts from

K-strategists to r-strategists with forest degradation. (Zhou et al., 2018). My finding is reasonable because microclimatic environments underneath the degraded forests with large canopy gaps are temporally more fluctuating than microclimatic environments underneath the primary forests, which favors r-strategists. Such a change, however, may not influence overall bacterial community composition probably because other dominant bacterial phyla such as Actinobacteria ($R^2 = 0.05$, $p > 0.05$), Planctomycetes ($R^2 = 0.05$, $p > 0.05$) and some unidentified-bacteria ($R^2 = 0.03$, $p > 0.05$) do not change directionally along the forest degradation gradient. In addition, Liu et al. (2019) show that the interaction network in bacterial communities becomes more complex and stable to resist external disturbances under high degradation stress. I speculate that the interrelationships within bacterial communities might become more stable and probably more resilient reflecting high intrinsic growth rates after logging disturbances compared with fungal communities.

3.4.3 Soil microbial community compositions in relation to soil enzyme activities

As to the second research question (how bacterial and fungal community compositions relate to the change in soil enzyme activities along the degradation gradient), my SEM model clearly shows that only fungal community composition is related to the changes in ACP, BG and LAP, while bacterial community composition does not explain the changes in soil enzyme activities during the forest degradation.

Previous studies have shown that the quality and availability of SOC are closely linked to microbial community compositions (Don et al., 2017; Zhu et al., 2024). My SEM results confirm that SOC significantly affected bacterial and fungal community compositions. However, only fungal community compositions explain ACP, BG, and LAP, suggesting that a reduction in SOC may cause decreased ACP, BG, and LAP activities only through changes in fungal community composition. As shown in Table 3-1 and Fig. 3-2, SOC and fungal community compositions change along the forest degradation gradient, suggesting that forest degradation may have caused changes in soil enzyme activities through its effects on SOC and fungal community compositions.

Although my study design does not allow for a detailed explanation of how changes in fungal community compositions induce changes in soil enzyme activities, my observations may be explained by variations in decomposition activities among different fungal phyla. For example, ACP, which mineralizes organic phosphorus and increases the phosphorus availability for plants, may often be associated with mycorrhizal symbiosis (Yadav and Tarafdar, 2003; Hidayat et al., 2006). BG is playing a major role in converting celooligosaccharides into glucose and filamentous fungi, such as *Aspergillus niger*, *Aspergillus oryzae* and *Penicillium brasilianum* have been found to have BG activities (Pathan et al., 2017; Zang et al., 2018; Keller et al., 2020). Some *Aspergillus* fungi, as well as Mucoromycota and Basidiomycota have been shown to have strong LAP activities, and LAP is involved in peptides and proteinaceous leucine hydrolysis and can facilitate the release of N for plant uptake and microbial utilization (Nampoothiri et al., 2005; Tedersoo et al., 2021; Leonhardt et al., 2019).

Although bacterial communities can also secrete ACP, BG, and LAP (Zang et al., 2017; Zhang et al., 2021; Zheng et al., 2021), the SEM model does not show a correlation between bacterial community composition and extracellular enzyme activities in our results. This may be because the fungal communities contributed to the overall soil decomposition processes greater than the bacterial communities in our forest plots and because the bacterial communities only rely on the by-products of fungal decomposition processes as discussed previously. However, my study provides no direct evidence for this inference. Manipulative experiments using selective inhibitors (e.g., bactericide and fungicide) will provide the valuable information about how much bacteria and fungi contribute to the overall soil enzyme activities (Rousk et al., 2007; Ushio et al., 2013) and whether bacteria rely on the fungal activities under different forest degradation intensities.

In summary, my findings suggest that fungal community compositions may have a greater impact on extracellular enzyme activities than bacterial community compositions. Forest degradation can lead to a decrease in the availability of SOC, which in turn affects fungal community composition, resulting in changes to soil

enzyme activities.

3.4.4 The shift of enzyme efficiency along the forest degradation gradient

As to the third research question (how the shift of soil microbial community composition relates to the potential enzyme efficiency), my results show that ACP activity/SMBC and LAP activity/SMBC increase with decreasing SOC (Fig. 3-4). ACP is related to the utilization of phosphorus by microbial communities (Huang et al., 2011), while LAP is related to the utilization of nitrogen (Huang et al., 2021; Uwituze et al., 2022). The increase in the efficiency of extracellular enzymes is thus related to P and N acquisition efficiency *per* microbial biomass in degraded forests.

In Bornean lowland tropical rainforests, heterotrophic microbial communities are frequently limited by P (Kitayama and Aiba, 2002; Imai et al., 2010; Yokoyama et al., 2017; Mori et al., 2017) because intense weathering in tropical forests leads to the reduction of soil total P and available inorganic P. Similarly, Liu et al., (2015) point out that continuous logging is a major cause of P limitation in microbial communities. In addition, it has been suggested that N also limits the net primary productivity of secondary tropical rainforests (Davidson et al., 2004). P and N deficiency aggravated by selective logging may select fungal communities and influences overall microbial enzyme efficiency in our study sites. Enhanced ACP activity/SMBC and LAP activity/SMBC indicate that soil microbial communities in the forests are generally limited by P and N.

3.5 Conclusion

Soil bacterial communities and fungal communities show different patterns in terms of community composition along a forest degradation gradient in logged-over tropical rainforests in Malaysian-Borneo. Shifts in fungal community composition are more distinct than those in bacterial community composition along the gradient, probably reflecting differential substrate preferences among fungal phyla with changing

soil organic matter quality and quantity. Soil bacterial community compositions are more stable, possibly because bacteria are utilizing by-products of fungal decomposition processes (i.e., more labile organic matter), and because the substrate availability for bacteria is buffered and stabilized by the fungal community. The changes in fungal community composition along the degradation gradient might in turn influence soil enzyme activities (i.e., ACP, BG, and LAP activities). Although the reduction of soil microbial biomass primarily explains the decrease in soil enzyme activities, fungal community composition likely controls soil enzyme activities. In addition, soil enzyme efficiency (i.e., the quotient of enzyme activity to microbial biomass) is enhanced for ACP (P mineralization) and LAP (N mineralization) with increasing forest degradation. I suggest that the availabilities of soil C as well as P and N, affected by the forest degradation, select fungal communities in Bornean logged-over tropical rainforests, which further feeds back to enhance the efficiency of P- and N-degrading soil enzymes.

Tables and Figures

Tables

Table 3-1 Summary of the soil properties and microbial biomass. SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; SMBC, soil microbial biomass carbon; FBC, fungal biomass carbon; and BBC, bacterial biomass carbon. Stratum 1 to 5 represents the aggravation of forest degradation (i.e., primary forest to highly degraded forest).

	pH	SOC (mg g ⁻¹)	TN (mg g ⁻¹)	C/N ratio	TP (μg g ⁻¹)	SMBC (μg g ⁻¹)	FBC (μg g ⁻¹)	BBC (μg g ⁻¹)	FBC/SMBC%	BBC/SMBC%
St.1	4.41±0.11	30.87±9.71	2.22±0.26	13.70±3.19	181.82±25.36	743.93±190.83	362.43±11.78	377.10±182.13	48.71	50.69
St.2	4.58±0.18	24.76±4.91	2.19±0.15	11.01±1.69	236.20±40.60	507.61±137.64	351.04±26.33	154.76±117.74	69.16	30.49
St.3	4.66±0.27	18.70±3.11	1.93±0.22	9.65±0.99	159.24±20.68	250.92±84.31	194.05±64.32	56.21±25.48	77.34	22.40
St.4	5.17±2.89	17.37±8.04	1.90±0.56	9.38±2.89	184.24±19.82	383.70±123.51	156.30±22.34	224.78±113.35	40.73	58.58
St.5	5.67±0.19	9.78±5.54	1.32±0.41	6.71±2.85	154.36±40.42	179.56±76.59	120.36±23.23	58.51±58.08	67.03	32.59

Figures

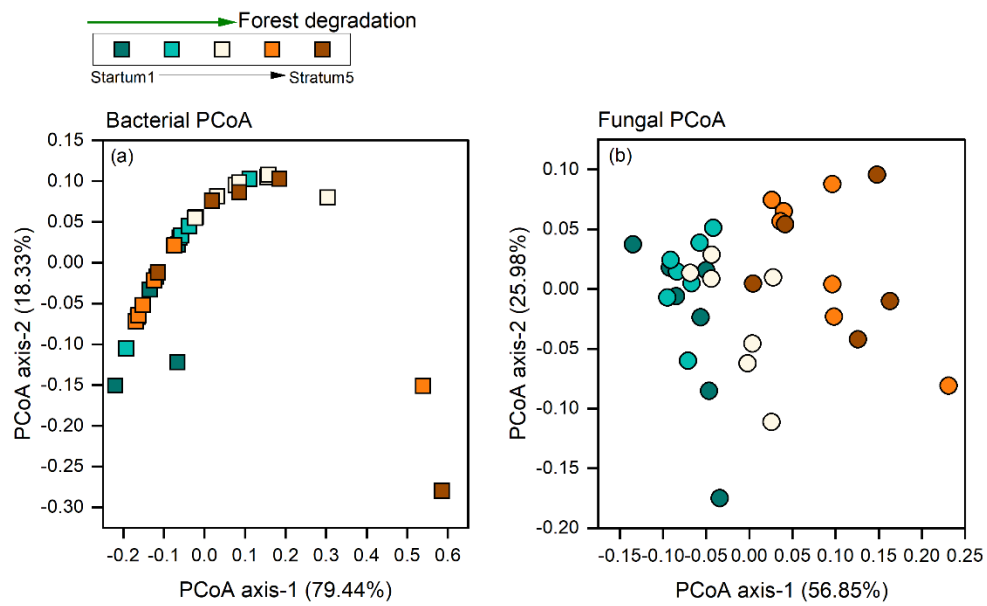


Fig. 3-1 Principal coordinate analysis (PCoA) for bacterial and fungal logarithmic absolute abundances. Symbols colors from dark green to brown indicate the aggravation of forest degradation (i.e., stratum 1 primary forest to stratum 5 highly degraded forest).

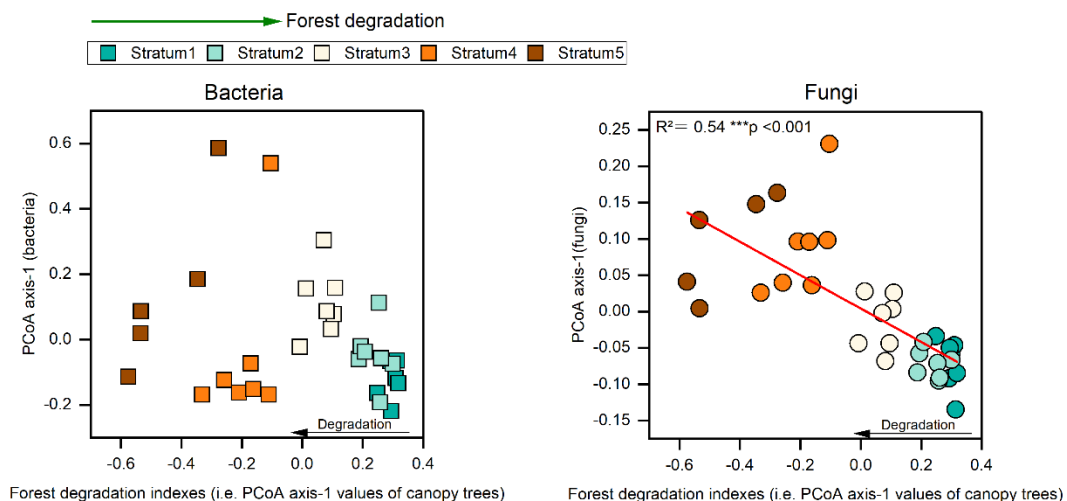
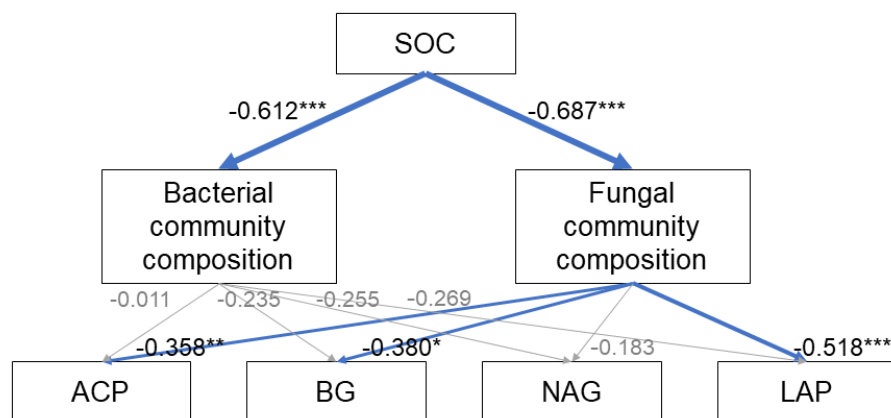


Fig. 3-2 Linear regressions of PCoA axis-1 values of bacterial communities and fungal communities with the forest degradation indexes (i.e., PCoA axis-1 values of canopy trees). The direction of the arrow indicates the aggravation of degradation. Symbol colors from dark green to brown indicate the aggravation of forest degradation (i.e., stratum 1 primary forest to stratum 5 highly degraded forest).



P < 0.001 RMR < 0.05 AIC = 113.517

Fig. 3-3 Results of the structural equation model (SEM) with SOC as a dependent variable. The SEM is shown to represent the casual relationships among SOC, bacterial community composition, fungal community composition, and the activities of ACP, BG, NAG, and LAP. Blue arrows represent negative effects and grey arrows show weak relations. SOC, soil organic carbon; ACP, acid phosphatase; BG, β -glucosidase; LAP, leucine aminopeptidase; and NAG, N-acetyl-glucosaminidase.

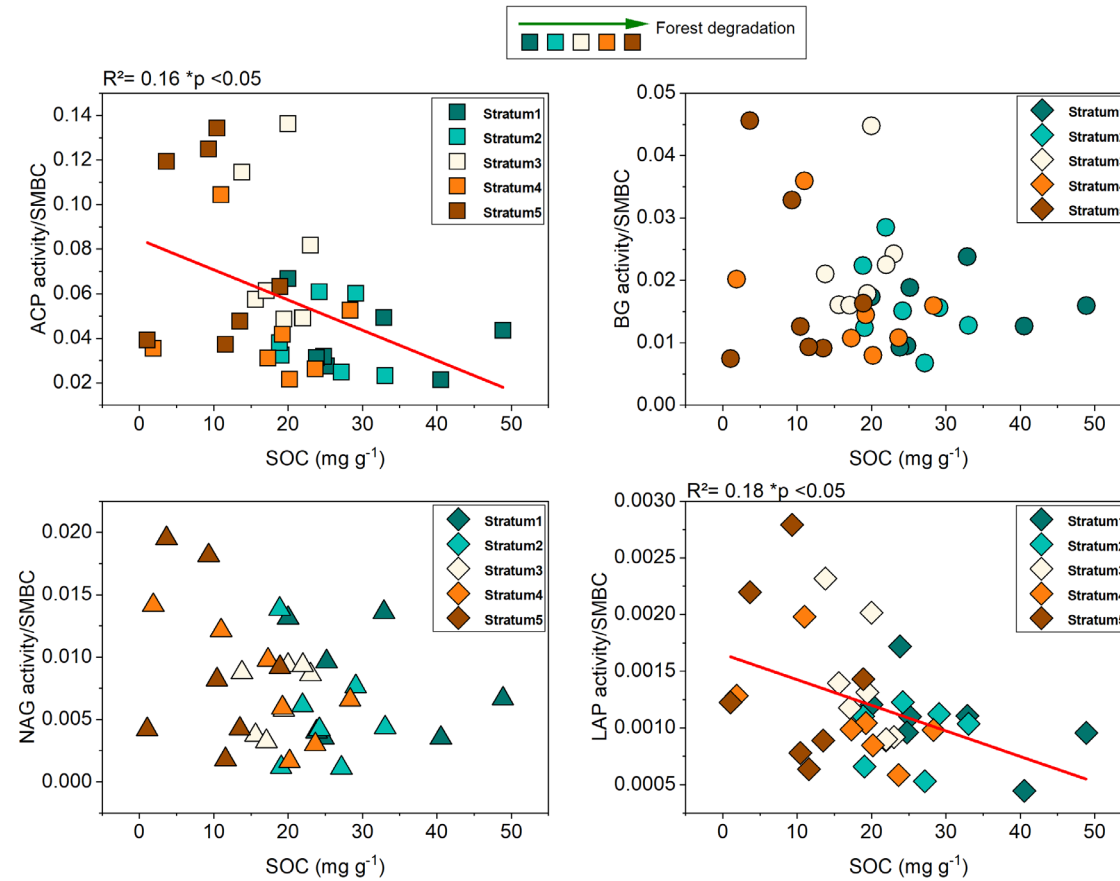


Fig. 3-4 Linear regression of SOC and ACP activity /SMBC ratio; SOC and BG activity /SMBC ratio; SOC and NAG activity /SMBC ratio; SOC and NAG activity /SMBC ratio. Symbol colors from dark green to brown indicate the aggravation of forest degradation (i.e., stratum 1 primary forest to stratum 5 highly degraded forest). SOC, soil organic carbon; SMBC, soil microbial biomass carbon; ACP, acid phosphatase; BG, β -glucosidase; LAP, leucine aminopeptidase; and NAG, N-acetyl-glucosaminidase.

Supplementary information

Tables

Table S3-1 Mean ($\pm$ SD) of four extracellular enzyme activities per forest degradation stratum. ACP, acid phosphatase; BG, β -glucosidase; LAP, leucine aminopeptidase; and NAG, N-acetyl-glucosaminidase. Stratum 1 represents primary forest and stratum 5 represents highly degraded forest; increasing numbers indicate the aggravation of forest degradation.

	ACP ($\mu\text{mol gsoil}^{-1}$ hr^{-1})	BG (nmol MUB $\text{gsoil}^{-1} \text{hr}^{-1}$)	NAG (nmol MUB $\text{gsoil}^{-1} \text{hr}^{-1}$)	LAP (nmol MUB $\text{gsoil}^{-1} \text{hr}^{-1}$)
Stratum1	28.72 $\pm$ 12.49	11.42 $\pm$ 4.10	5.61 $\pm$ 2.94	0.74 $\pm$ 0.16
Stratum2	19.73 $\pm$ 5.11	7.78 $\pm$ 2.91	2.60 $\pm$ 1.89	0.45 $\pm$ 0.07
Stratum3	17.45 $\pm$ 3.94	5.42 $\pm$ 1.76	1.72 $\pm$ 0.86	0.32 $\pm$ 0.06
Stratum4	14.05 $\pm$ 2.06	5.32 $\pm$ 1.01	2.57 $\pm$ 1.48	0.38 $\pm$ 0.08
Stratum5	13.63 $\pm$ 7.43	2.96 $\pm$ 1.54	1.48 $\pm$ 0.94	0.23 $\pm$ 0.13

Figures

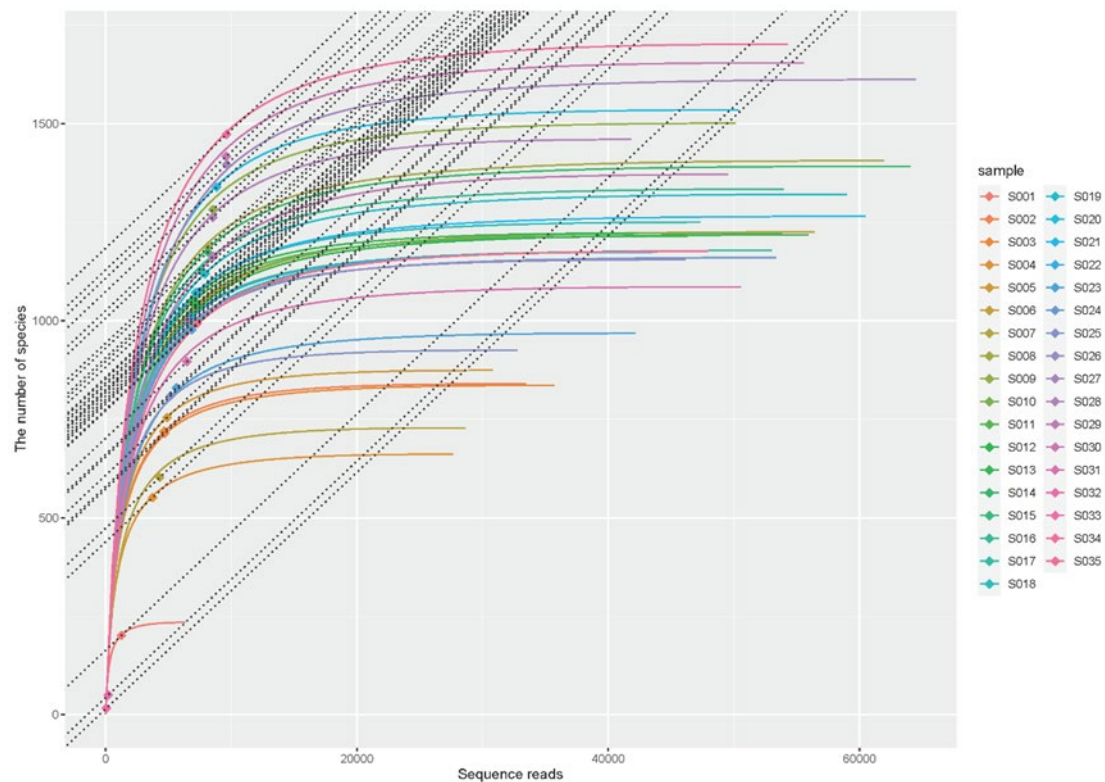


Fig. S3-1 Results of coverage-based rarefaction (bacteria) on 35 samples in Sabah-Malaysian Borneo. Lines indicate rarefaction curves calculated by the iNEXT package of R. Colors, points and dotted slope indicate samples, rarefied sequence reads and slopes at the rarefied sequence reads (i.e., coverage = 97%), respectively.

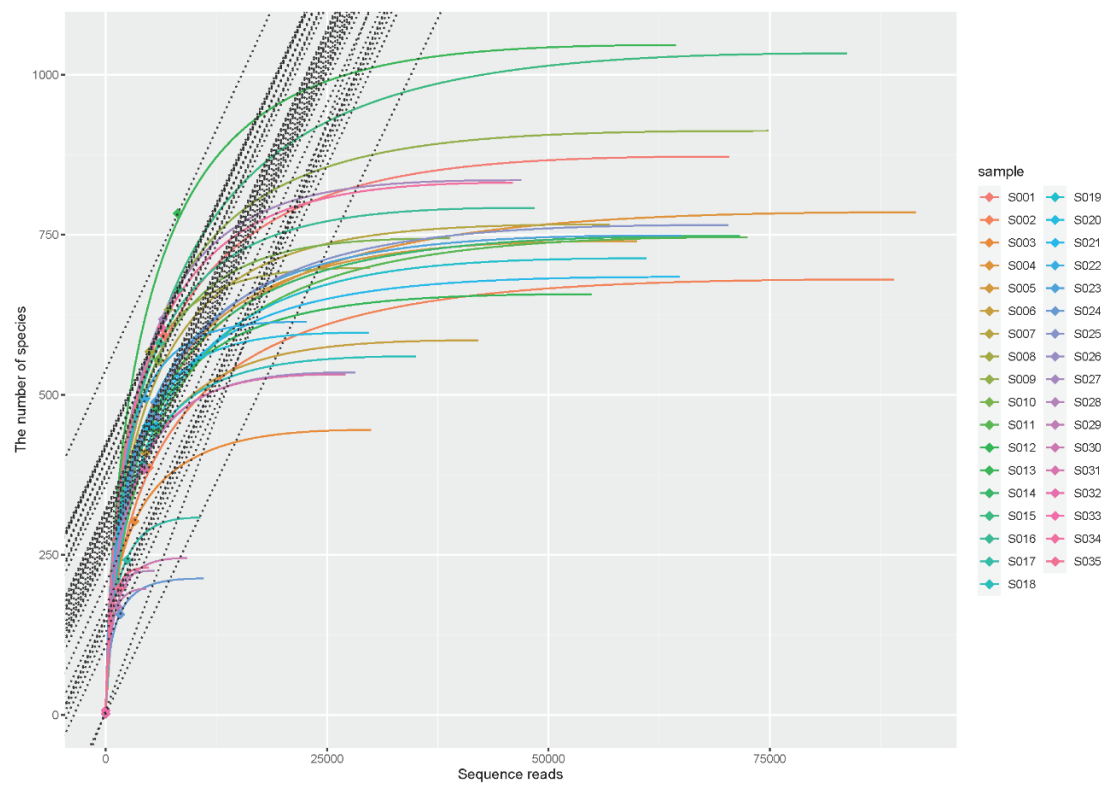


Fig. S3-2 Results of coverage-based rarefaction (fungi) on 35 samples in Sabah-Malaysian Borneo. Lines indicate rarefaction curves calculated by the iNEXT package of R. Colors, points and dotted slope indicate samples, rarefied sequence reads and slopes at the rarefied sequence reads (i.e., coverage = 97), respectively.

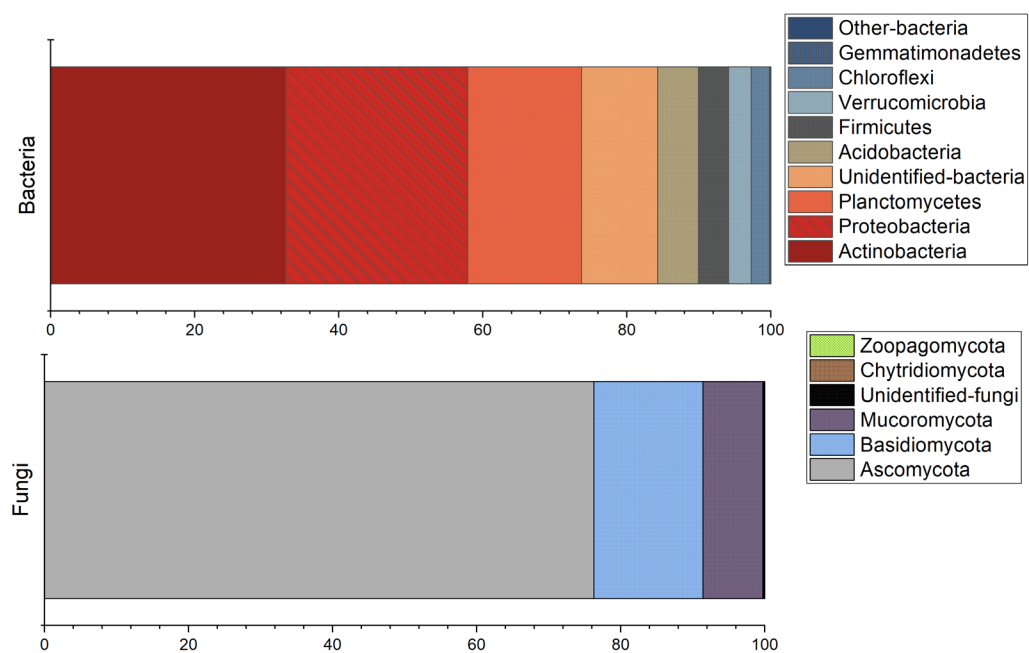


Fig. S3-3 Relative abundances of dominant phyla in all research plots combined for bacteria and fungi.

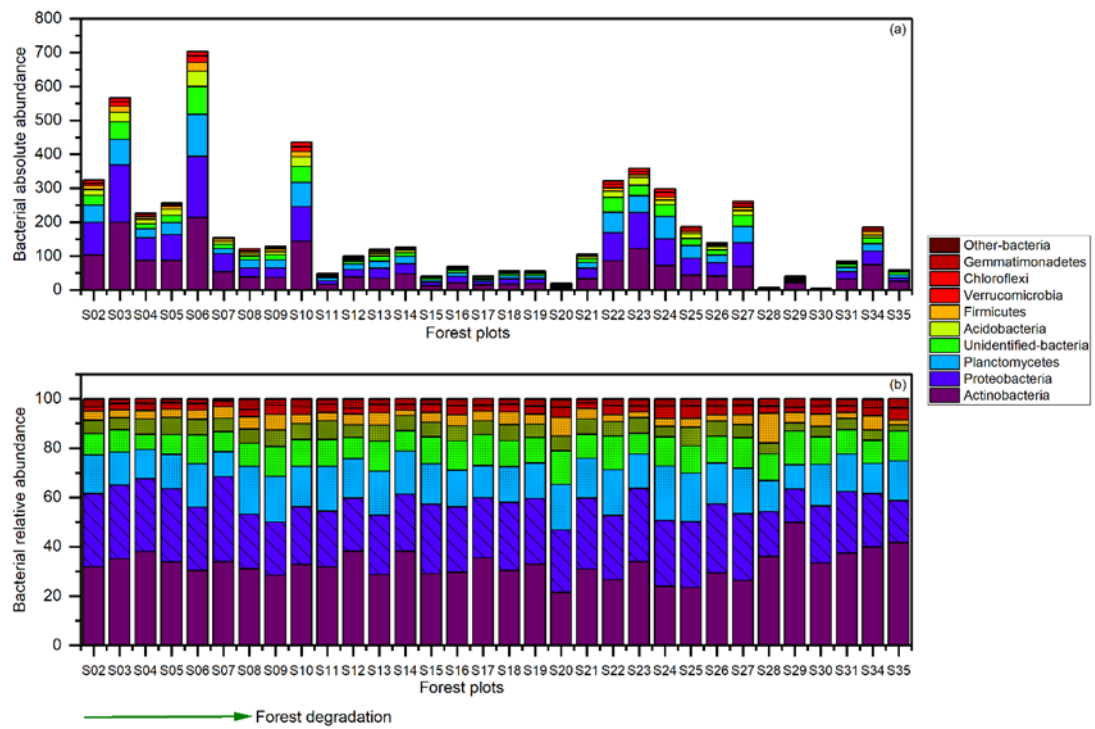


Fig. S3-4 Absolute and relative abundances of bacterial phyla in each of the forest plots. The direction of the arrow indicates the sequence of forest degradation from primary to highly degraded forests.

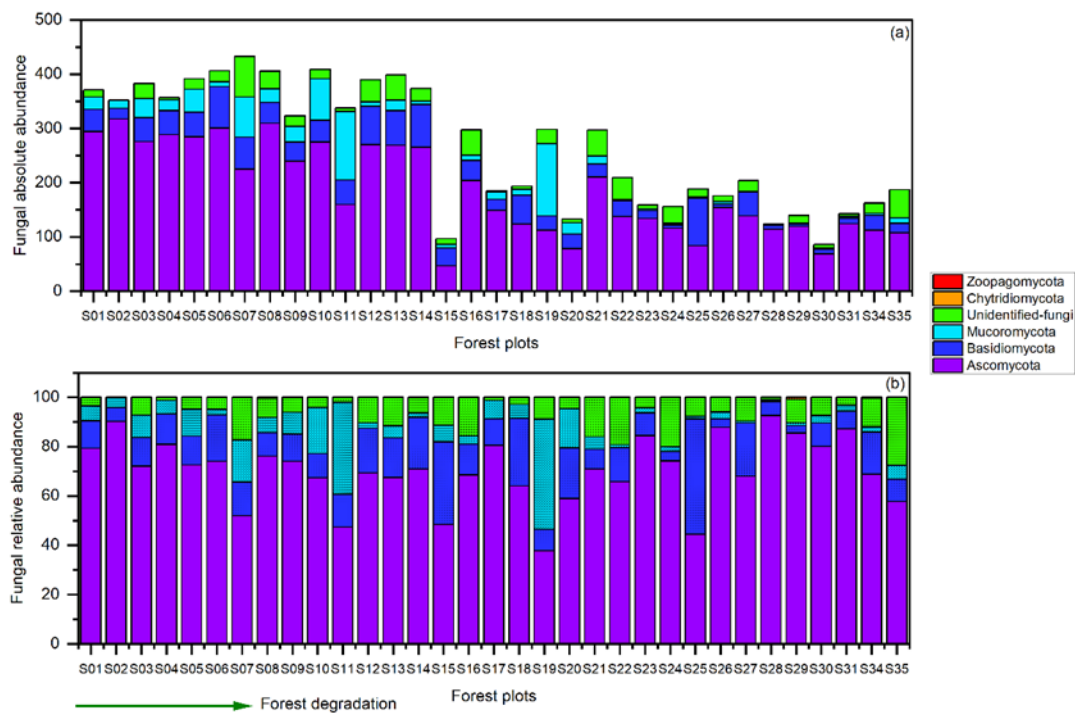


Fig. S3-5 Absolute and relative abundances of fungal phyla in each of the forest plots. The direction of the arrow indicates the sequence of forest degradation from primary to highly degraded forests.

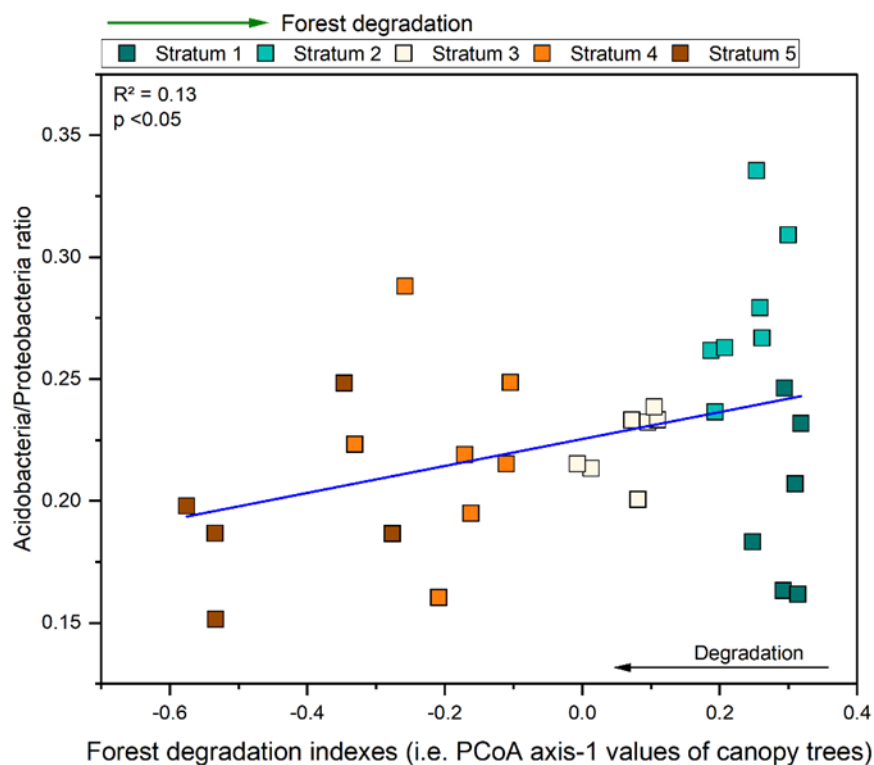


Fig. S3-6 Linear regressions of the forest degradation indexes (i.e., PCoA axis-1 values of canopy trees) and the ratio of Acidobacteria/Proteobacteria. The direction of the arrow indicates the aggravation of degradation. Symbol colors from dark green to brown indicate the aggravation of forest degradation (i.e., stratum 1 primary forest to stratum 5 highly degraded forest).

Supplementary Methods

Library preparation protocol for iSeq sequencing

The first-round PCR (first PCR) was carried out with a 10- μ l reaction volume containing 5.0 μ l of 2 $\times$ KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, WA, USA), 2.0 μ l of forward primer, 2.0 μ l of reverse primer (515F–806R or ITS_F_KYO1/ITS2_KYO2; each primer at 1.5 μ M used in the reaction; with adaptor and 0-18 random bases), 1.0 μ l of DNA template. For 16S, the thermal cycle profile after an initial 3 min denaturation at 95°C was as follows (35 cycles): denaturation at 98°C for 20 sec; annealing at 60°C for 15 sec; and extension at 72°C for 30 sec, with a final extension at the same temperature for 5 min. For ITS, the thermal cycle profile after an initial 3 min denaturation at 95°C was as follows (35 cycles): denaturation at 98°C for 20 sec; annealing at 55°C for 15 sec; and extension at 72°C for 30 sec, with a final extension at the same temperature for 5 min. Four technical replicates were prepared for each sample, and these replicate products were pooled to mitigate the PCR dropouts. The pooled first PCR products were purified using AMPure XP (PCR product: AMPure XP beads = 1:0.8; Beckman Coulter, Brea, California, USA). The pooled, purified, and diluted first PCR products were used as templates for the second-round PCR.

The second-round PCR (second PCR) was carried out with a 20- μ l reaction volume containing 10 μ l of 2 $\times$ KAPA HiFi HotStart ReadyMix, 4.0 μ l of forward/reverse primer (each primer includes Illumina sequencing primer [Rd1SP/Rd2SP], sample index [dual-unique index], and P5/P7 adapter sequence; each primer at 1.5 μ M in the reaction volume), and 2.0 μ l of template. The thermal cycle profile after an initial 3 min denaturation at 95 °C was as follows (12 cycles): denaturation at 98 °C for 20 sec; annealing at 68°C for 15 sec; and extension at 72 °C for 15 sec, with a final extension at 72 °C for 5 min. Then, the indexed 2nd PCR products were mixed, and the combined library was again purified using AMPure XP (PCR product: AMPure XP beads = 1:0.8). Target-sized DNA of the purified library was excised using E-Gel SizeSelect (ThermoFisher Scientific, Waltham, MA, USA).

The double-stranded DNA concentration of the library was quantified and adjusted using MilliQ water and the DNA was applied to the iSeq and sequenced using 300-cycle single-end mode (Illumina, San Diego, CA, USA).

Chapter 4 Changes of bacterial versus fungal functional structures and the relative abundances of potential functional genes for specific extracellular enzymes along a forest degradation gradient in logged-over tropical rainforest, Malaysian Borneo

Abstract

Soil bacterial and fungal communities are involved in litter and organic matter decomposition. However, they may play mutually different roles in organic matter decomposition. The previous chapter (Chapter 3) demonstrates that fungal community composition compared with bacterial community composition more distinctly shifts along a forest degradation gradient, where soil organic carbon (SOC) and soil C/N ratio decrease with increasing magnitude of forest degradation. I anticipate that their functional structures also shift along the forest degradation gradient because the relative abundances of potential functional genes may be influenced by changes in soil C, N, and P. In addition, changes in the relative abundances of some potential functional genes may also be reflected in extracellular enzyme activities, which may have important implications for soil nutrient dynamics. However, how microbial functional structures change along the forest degradation gradient in tropical rainforests and how/if bacterial versus fungal functional structures differ from each other are still unknown. Therefore, I investigated how bacterial versus fungal functional structures shifted and how the relative abundances of potential functional genes for specific extracellular enzymes changed along the forest degradation gradient.

The PICRUST2 analyses were performed using ASVs obtained from 16S and ITS amplicon sequencing. Enzyme Commission (E.C.) numbers were annotated to represent the potential functional genes. Principal coordinates analyses (PCoA) were performed on the relative abundances of E.C. numbers. Obtained axis-1 values were considered to represent functional structures and regressed with the forest degradation indexes derived in Chapters 2 and 3. The top 20 relative abundances of E.C. numbers were selected and rank-abundance curves were constructed to investigate the

differences between bacterial versus fungal functional structures. Also, Pearson correlation analyses were performed between the top 20 relative abundances of E.C. numbers and forest degradation indexes. Redundancy analyses were conducted to show the effects of soil physicochemical properties on microbial functional structures. Further, the relative abundances of E.C. numbers for specific extracellular enzymes were regressed with forest degradation indexes.

Bacterial functional structure shifted more obviously compared with fungal functional structure along the forest degradation gradient. The rank-abundance curves showed that there were obvious differences between the bacterial and fungal functional structures, with a few potential functional genes in bacterial functional structure occupying very large relative abundances. While the relative abundances of potential functional genes in fungal functional structures were more even. Most of the relative abundances of bacterial E.C. numbers correlated with forest degradation indexes, while only one relative abundance of fungal E.C. numbers correlated with forest degradation indexes. Soil pH, soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) affected bacterial functional structure, while soil pH, TN, and TP affected fungal functional structure. In both bacterial and fungal communities, the relative abundance of acid phosphatase (ACP) gene decreased along the forest degradation gradient, while that of β -glucosidase (BG) gene increased. The relative abundance of leucine aminopeptidase (LAP) gene did not show any change. The relative abundance of N-acetyl-glucosaminidase (NAG) gene increased with forest degradation in bacterial communities while decreasing in fungal communities.

Directional changes in the relative abundance of some bacterial phyla with forest degradation may cause the shift in bacterial functional structures. Despite the dramatic change observed in the fungal community composition at the phylum level (see Chapter 3), fungal functional structure did not change along the degradation gradient due to high functional redundancy. The potential genes with very high relative abundance are occupied in bacterial functional structure, while the relative abundances of potential functional genes are more even in fungal functional structure, suggesting a sharp

difference between bacterial versus fungal functional structures. Besides, bacterial functional structure is susceptible to altered soil physicochemical properties, and fungal functional structure exhibits functional redundancy because potential functional genes related to litter and organic matter decomposition are widely distributed in various fungal taxa. Furthermore, the increased relative abundances of BG and NAG genes during forest degradation suggest improved microbial potential for cellulose and chitin degradation, which may contribute to the maintenance of BG and NAG efficiencies in highly degraded forests.

Keywords: PICRUSt2; microbial functional potentials; relative abundances of E.C. numbers

4.1 Introduction

Microbial communities mediate most soil biogeochemical processes through their specific roles as reflected by taxonomic composition, biotic interactions, and functional potentials (Nannipieri et al., 2013; Bahram et al., 2018). The composition and functional structure of soil microbial communities control carbon (C) and nutrient cycling (Berg et al., 2003; Kunito et al., 2023). Microbial community compositions focus on the taxonomic diversity and abundance of microorganisms, while microbial functional structures address the metabolic functions and interactions that drive ecosystem processes (Fuhrman, 2009). Microbial functional structures refer to the metabolic capabilities and functional roles of microorganisms within communities, which are always predicted by tools such as PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Douglas et al., 2019; Douglas et al., 2020). Previous study indicates that the intensity and frequency of environmental perturbations caused by anthropogenic activities play important roles in determining bacterial functional structure, with a higher intensity and frequency generally resulting in a greater shift in bacterial functional structure (Ager et al., 2010; Berga et al., 2012). However, a similar magnitude of environmental perturbations may not cause the shift of fungal functional structures due to their functional redundancy (Falkowski et al., 2008; Nelson et al., 2016). Here, functional redundancy refers to the occurrence the same functional potential across multiple microbial taxa.

Changes in soil properties must affect bacterial and fungal functional structures because soil properties directly relate to microbial metabolic processes. Previous studies confirm that various soil physicochemical properties, such as soil pH, soil organic carbon (SOC), total nitrogen (TN) total phosphorus (TP), and C/N ratio can influence microbial community composition and functional structure (Berg et al., 2003; Lauber et al., 2006; Bahram et al., 2018). Research in a pine forest in northeast China indicates that soil nutrient contents (SOC and C/N), pH, and root biomass together accounted for 37.9% of the variance in bacterial community compositions, while pH and nutrients contributed to 63.1% of the variance in bacterial functional structures

(Teng et al., 2021). Functional structure of fungi may not show significant differences in response to variations in physicochemical characteristics because of the functional redundancy (Zeng et al., 2023). In Malaysian Borneo, forest degradation causes a decrease in above-ground biomass (AGB), soil organic carbon (SOC), total nitrogen (TN) and C/N ratio, and an increase in soil pH (Chapter 2). However, how bacterial versus fungal functional structures change along the forest degradation gradient, and whether they are differently influenced by changing soil physicochemical properties remain unknown in my study areas.

In addition, soil extracellular enzymes such as acid phosphatase (ACP), β -glucosidase (BG), leucine aminopeptidase (LAP), and N-acetyl-glucosaminidase (NAG) play indispensable roles in P, C, and N cycling, and their activities are strongly influenced by soil microbial community composition (Cui et al., 2020). In Chapter 3, the result of the structural equation model (SEM) shows that reduced SOC influences bacterial and fungal community compositions and that variations in fungal community composition only are subsequently transmitted to changes of ACP, BG, and LAP activities. However, gene abundance may also be another important factor influencing extracellular enzyme activities. Moore et al. (2020) suggest that there are also potential relationships between functional gene abundances and specific extracellular enzymes. Changes in some microbial gene abundances can be reflected in extracellular enzyme activities, and these may have important implications for soil nutrient dynamics (Chen et al., 2018).

Therefore, I investigated how bacterial versus fungal functional structures shift and how gene abundances of ACP, BG, LAP, and NAG change along the forest degradation gradient. Two hypotheses were put forth: 1) Functional structure of bacterial communities compared with fungal communities shows a more distinct shift because of the greater functional redundancy of fungal communities. 2) The relative abundances of potential functional genes for extracellular enzymes in bacterial and fungal communities change along the forest degradation gradient, and these variations can be reflected in extracellular enzyme activities.

4.2 Materials and methods

4.2.1 Study sites and the forest degradation gradient

My research sites are the Deramakot and Tangkulap Forest Reserves, Malaysian Borneo consistent with Chapter 2 and Chapter 3, where the climate is humid equatorial climate and the soil is mainly Acrisols.

The Landsat Thematic Mapper (TM) Band-7 image was used to divide the research sites into five degradation strata ranging from a primary forest (stratum 1) to a highly degraded forest (stratum 5) (See Chapter 2, Fig. 2-1). Seven plots were placed in each stratum, yielding a total of 35 sample plots. Relative abundance of tree genera in each sample plot were calculated, and ordinated by principal coordinate analysis (PCoA) using the Bray-Curtis dissimilarity index. The gradient of forest degradation is represented by axis-1 values of PCoA of the tree canopy composition, and axis-1 values are defined as "forest degradation indexes", as have been used also in chapters 2 and 3.

4.2.2 Soil samples and PICRUST2

Soil samples were taken from the 35 sample plots mentioned above, yielding a total of 35 soil samples. Each soil sample was collected simultaneously from four 10-cm depth soil cores and then uniformly mixed to form a composite sample. These 35 samples were divided into two parts with one set refrigerated for soil biogeochemical parameter analyses and the other set freeze-dried for DNA analyses. Soil biogeochemical properties including pH, SOC, TN, TP, and activities of ACP, BG, LAP, and NAG were analyzed as described in Chapters 2 and 3. DNA were analyzed as described in Chapter 3; DNA was extracted from freeze-dried samples using the QIAGEN DNeasy PowerSoil Pro Kit (QIAGEN, Hilden, Germany), and the 16S and ITS amplicon sequencing were conducted to characterize soil bacterial and fungal communities, respectively.

The PICRUST2 was used to predict potential metabolic functions based on microbial communities (Douglas et al., 2019; Douglas et al., 2020). Briefly, ASVs (derived from Chapter 3) were aligned to reference sequence (16S and ITS) and placed

in a reference phylogenetic tree containing genomes with known functions. Then, the evolutionary model was used to predict gene family copy numbers for each placed sequence based on their phylogenetic relationships to the reference genomes. Finally, the predicted gene family abundances were quantitatively derived by being weighted by 16S or ITS copy numbers (i.e. multiplied by their respective ASV abundances based on read numbers) (Fig. 4-1). In my analysis, a total of 37,627 ASVs for bacteria and 18,241 ASVs for fungi were used as input for the 16S and ITS PICRUST2 pipelines, respectively. The abundances of Enzyme Commission numbers (E.C. numbers) were obtained and annotated to represent the predicted potential functional gene profiles of the whole microbiome.

4.2.3 Statistical analysis

Before performing other statistical analyses, the abundances of E.C. numbers in each of the 35 soil samples were transformed to relative abundances, where the sum of the relative abundances of all E.C. numbers equals 1.0 in each sample. To analyze the functional structures of bacteria and fungi, PCoA analyses were conducted using Bray-Curtis dissimilarity through the relative abundances of E.C. numbers, and the microbial functional structure was represented by the PCoA axis-1 for bacteria and fungi, respectively. To study the effect of forest degradation on microbial functional structure, PCoA axis-1 values of bacterial and fungal functional structures were respectively regressed with forest degradation indexes (i.e., PCoA axis-1 values of the tree canopy composition; see Chapters 2 and 3). The top 20 relative abundances of E.C. numbers were selected and rank-abundance curves were constructed for each sample (i.e., research plot) to investigate the differences between bacterial versus fungal functional structure. Here, the log-transformed relative abundances of E.C. numbers were shown along the vertical axis and the top 20 E.C. numbers were arranged in descending order along the horizontal axis (1.0 was added to each relative abundance prior to logarithmic transformation). Pearson correlation analyses were performed between each of the top 20 relative abundances of potential functional genes and forest degradation indexes to

verify whether the relative abundance of E.C. numbers changed along a forest degradation gradient. Redundancy analyses (RDA) were conducted to clarify the effects of soil physicochemical properties on microbial functional structures respectively for bacteria and fungi, and the significances of soil physicochemical properties were tested by envifit, R-package 'vegan' (Oksanen et al., 2020). Linear regressions were also conducted for the relative abundances of E.C. numbers for each of the four extracellular enzymes (i.e., gene abundances of ACP, BG, LAP, and NAG) in bacterial and fungal communities with forest degradation indexes, respectively. Besides, the relative abundance of each bacterial phylum (derived from Chapter 3) was calculated, and linear regressed with forest degradation indexes respectively.

4.3 Results

4.3.1 Microbial functional structures

For bacterial communities, a total of 2305 E.C. numbers were predicted. For fungal communities, a total of 905 E.C. numbers were predicted. The results of PCoA for bacterial and fungal functional structures were shown in Fig. 4-2. Points represent 35 plots and different colors represent five forest degradation strata. In the bacterial functional structure PCoA, 41.0% of the overall variance was explained by the axis-1 and 29.1% by the axis-2. In the fungal functional structure PCoA, 51.7% of the overall variance was explained by the axis-1 and 29.1% by the axis-2. PCoA axis-1 values were chosen to represent microbial functional structures for both bacteria and fungi. A linear regression showed that PCoA axis-1 values of fungal functional structure did not correlate ($p > 0.05$) with forest degradation indexes (i.e., PCoA axis-1 values of canopy trees), while PCoA axis-1 values of bacterial functional structure correlated with forest degradation indexes ($R^2 = 0.24$, $p < 0.01$; Fig. 4-3).

The differences between bacterial versus fungal functional structures were shown in rank-abundance curves (Fig. 4-4). In bacterial functional structures, the top 4 potential functional genes occupied very large relative abundances, which were

E.C:2.2.1.6 (acetolactate synthase), E.C:2.1.2.1 (glycine hydroxymethyltransferase), E.C:2.7.9.3 (selenide, water dikinase), and E.C:2.8.4.5 (tRNA (N6-L-threonylcarbamoyladenosine37-C2)-methylthiotransferase) (See Table S4-1). However, the relative abundances of potential functional genes after the top 7 were very small. In fungal functional structures, despite the relative abundances of the top 5 potential functional genes were high in the samples, which were E.C:1.2.4.1 (pyruvate dehydrogenase (acetyl-transferring)), E.C:1.5.1.20 (methylenetetrahydrofolate reductase [NAD(P)H]), E.C:6.1.1.5 (isoleucine---tRNA ligase), E.C:4.1.3.27 (anthranilate synthase), and E.C: 3.4.11.9 (Xaa-Pro aminopeptidase) (See Table S4-2), the rest of the potential functional genes also remained certain relative abundances, and the relative abundances of rest E.C. numbers were close in each sample (See Table S4-2).

Results of correlation analysis showed that in bacterial functional structure, except for E.C:1.1.1.48 (D-galactose-1-dehydrogenase), E.C:4.1.1.55 (4,5-dihydroxyphthalate decarboxylase) and E.C:2.3.1.48 (histone acetyltransferase), the relative abundances of other E.C. numbers were significantly correlated with forest degradation indexes (Table 4-1). In contrast, in fungal functional structure, only the relative abundance of EC:1.2.4.1(pyruvate dehydrogenase (acetyl-transferring)) was correlated with forest degradation indexes (Table 4-2).

4.3.2 Soil physicochemical properties affecting microbial functional structures

The results of soil physicochemical properties affecting the functional structures of bacteria and fungi in RDA were presented respectively in Fig. 4-5 and Fig. 4-6. Points represented 35 plots and different colors represented five forest degradation strata. In the bacterial functional structure RDA, 59.7% of the overall variance was explained by the axis-1 and 27.5% by the axis-2; all soil physicochemical properties including SOC, TN, TP, and pH were significant. In the fungal functional structure RDA, 71.8% was explained by the axis-1 and 15.0% by the axis-2; TN, TP, and pH were significant.

4.3.3 The relative abundances of E.C. numbers for four important extracellular enzymes

Linear regressions of the relative abundances of E.C. numbers (i.e., relative abundance of potential functional genes) for each of the four extracellular enzymes in bacterial and fungal communities with forest degradation indexes (i.e., PCoA axis-1 values of canopy trees) were shown in Fig. 4-7 and Fig. 4-8, respectively. For bacterial communities, the relative abundance of ACP gene ($R^2 = 0.23$, $p < 0.01$) significantly decreased with decreasing forest degradation indexes, while those of BG ($R^2 = 0.21$, $p < 0.01$) and NAG ($R^2 = 0.24$, $p < 0.01$) increased. For fungal communities, the relative abundances of ACP ($R^2 = 0.27$, $p < 0.01$) and NAG genes ($R^2 = 0.23$, $p < 0.01$) significantly decreased with decreasing PCoA axis-1 values of canopy trees, while those of BG ($R^2 = 0.13$, $p < 0.05$) increased.

4.4 Discussion

4.4.1 Changes of soil bacterial versus fungal functional structures

First of all, my hypothesis (functional structures of bacterial communities compared with fungal communities show a more distinct shift because of the greater functional redundancy of fungal communities) is supported. Bacterial functional structure changed significantly with increasing magnitude of forest degradation ($R^2 = 0.24$, $p < 0.01$), and 9.9% of the total variation in bacterial functional structure is ascribed to forest degradation (i.e., the bacterial community PCoA axis-1 explains 41.0% of the total variation, multiplied with 0.24). The low explanation power (i.e., 9.9%) may be due to the presence of many bacterial metabolic functions that do not directionally change with forest degradation; in the other word, metabolic functions that change directionally with forest degradation must be limited in number in bacteria although the overall functional structure shifts with forest degradation. Moreover, only the relative abundances of Proteobacteria, Acidobacteria, Verrucomicrobia, and Chloroflexi change with forest degradation indexes (Fig. S4-1), suggesting that the directionally altered metabolic functions may be mainly present in these bacterial phyla. By contrast, fungal

functional structure does not change although their relative abundances of phyla such as Ascomycota and Basidiomycota change dramatically in samples (see Chapter 3, Fig S3-5 b), suggesting that fungi might have functional redundancy. Previous study in the Brazilian Amazon region demonstrates that logging has a negative impact on bacterial diversity and functional potentials (Pedrinho et al., 2023). Zeng et al. (2023) indicate that the functional structure of soil fungal communities in primary forests is stable and able to resist the pressures of environmental changes due to ecological redundancy.

In bacterial functional structure, the relative abundances of E.C:2.2.1.6 (acetolactate synthase), E.C:2.1.2.1 (glycine hydroxymethyltransferase), E.C:2.7.9.3 (selenide, water dikinase), and E.C:2.8.4.5 (tRNA (N6-L-threonylcarbamoyladenosine37-C2)-methylthiotransferase) occupy very large relative abundances. Acetolactate synthase catalyzes the first step in the synthesis of the branched-chain amino acids (valine, leucine, and isoleucine) (Schloss, 1994). Glycine hydroxymethyltransferase converts glycine into serine by one-carbon transfer from 5,10-methylenetetrahydrofolate (Schirch and Szebenyi, 2005). Selenide, water dikinase participates in selenoamino acid metabolism (Veres et al., 1992). tRNA (N6-L-threonylcarbamoyladenosine37-C2)-methylthiotransferase catalyzes the methylthiolation of the N6-L-threonylcarbamoyladenosine37 residue in certain tRNA molecules (Arragain et al., 2010). There are other potential genes for some oxidoreductases (E.C:1.-), transferases (E.C:2.-), and lyases (E.C: 4.-) among the identified E.C. numbers in the bacterial communities. However, their relative abundances are extremely low, which means that their functions may not play major roles in the ecosystem. Therefore, in my research sites, high relative abundances of transferases (E.C: 2.-) related to amino acids may mainly support energy production and metabolic regulation of bacterial communities.

In fungal functional structure, the relative abundances of E.C:1.2.4.1 (pyruvate dehydrogenase (acetyl-transferring)), E.C:1.5.1.20 (methylenetetrahydrofolate reductase [NAD(P)H]), E.C:6.1.1.5 (isoleucine---tRNA ligase), E.C:4.1.3.27 (anthranilate synthase), and E.C: 3.4.11.9 (Xaa-Pro aminopeptidase) are high in each

sample. Pyruvate dehydrogenase (acetyl-transferring) is an enzyme that catalyzes the reaction of pyruvate and a lipoamide (Patel and Korotchkina, 2006). Methylenetetrahydrofolate reductase [NAD(P)H] catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Friso et al., 2002). Isoleucine--tRNA ligase participates in valine, leucine, isoleucine biosynthesis, and aminoacyl-tRNA biosynthesis (Hughes and Mellows, 1980). Anthranilate synthase can promote the production of the amino acid tryptophan by regulating the formation of anthranilate (Zalkin, 1973). Xaa-Pro aminopeptidase can release any N-terminal amino acid (Wang et al., 2020). Several hydrolases (E.C: 3.-), transferases (E.C: 2.-), ligases (E.C: 6.-), and lyases (E.C: 4.-) also maintain certain relative abundances, although they are lower than the top 5 potential genes mentioned above. In my research sites, these abundant enzymes may collectively support the basic metabolic activities and nutrient cycling of fungal communities. Moreover, E.C: 3.2.1.24 (alpha-mannosidase) is found in Table S4-2. Alpha-mannosidase plays a role in the breakdown of mannose-containing polysaccharides in plant cell walls (Bail et al., 2022). This result further supports my view in Chapter 3 that fungal communities mainly secrete extracellular enzymes for litter decomposition.

The results of Pearson correlation analyses are consistent with the results of Fig. 4-3. Most of the relative abundances of bacterial E.C. numbers correlated with forest degradation indexes, while the relative abundances of a few fungal E.C. numbers only correlated with forest degradation indexes, suggesting that forest degradation may affect bacterial functional structure rather than fungal functional structure.

The reduced quantity and quality of fresh litter and changing soil physicochemical properties during forest degradation might be a factor that caused the difference in bacterial versus fungal functional structures (Giweta, 2020; Peng et al., 2020; Lin et al., 2021). RDA figures show all soil physicochemical properties including pH value, SOC, TN, and TP affect bacterial functional structure, while only TN, TP, and pH affect fungal functional structure. Fungal communities have been considered to be the primary decomposers of fresh and dead plant biomass (Voříšková and Baldrian, 2013). In

Malaysian Borneo, biomass of remnant forests as well as soil C/N ratio decreased with forest degradation (Chapter 2). However, the fungal functional structure is not affected by reduced SOC, suggesting that enzyme genes involved in SOC decomposition are widespread in multiple taxa of fungi, which leads to a functional redundancy in fungal functional structure (Wilson et al., 2011). The relative abundance of E.C: 3.2.1.24 (alpha-mannosidase; participates in the breakdown of mannose-containing polysaccharides in plant cell walls) in fungal functional structure also supports this view. Despite the dramatic changes in the relative abundances of fungal phyla in each sample (See Chapter 3, Fig. S-5 b), the relative abundance of alpha-mannosidase in each sample is basically the same (Table S4-2) and does not change with forest degradation (Table 4-2). Previous studies also demonstrate that litter heterogeneity may not affect litter degrading enzyme genes (CAZymes) because the function of litter decomposition is redundant in the fungal communities (Purahong et al., 2014; Guerreiro et al., 2023).

In bacterial functional structures, key functional potentials of Proteobacteria, Acidobacteria, and Actinobacteria, which are involved in C and N cycling (Zhou et al., 2017), can be affected by the reduction of TN and SOC during forest degradation. Despite that the concentration of total phosphorus (TP) does not change along my forest degradation gradient (see Chapter 3), previous studies have shown that organic P and label inorganic P (Aoyagi et al., 2013; Swinfield et al., 2020) are reduced during forest degradation. The concentration of these P fractions may decrease with forest degradation in my sites, which in turn can affect the bacterial communities that are involved in P transformation such as Proteobacteria and P-solubilizing bacteria, and consequently their functional potentials (Pedrinho et al., 2023). Soil pH has also been found to affect bacterial functional structure (Wang et al., 2019). These previous studies suggest that variation of bacterial functional structure is mainly related to the changes in soil physicochemical properties such as soil pH, bulk density, SOC, and TN contents, which agrees with my results.

In my study sites, fungal functional structure is influenced by pH, TN, and TP, which is also consistent with some previous studies (Rousk et al., 2009; Bhalla et al.,

2021; Moore et al., 2021). Nevertheless, previous studies point out that only some functional potentials in saprophytic fungi and/or ectomycorrhizas have significant correlations with soil nutrients due to the functional redundancy (Rousk et al., 2009; Meeds et al., 2012; Wu et al., 2019). Such a variation of functional structure is a minor fraction only in the overall fungal functional structure.

4.4.2 The changes in the relative abundances of E.C. numbers for four extracellular enzymes along the forest degradation gradient

My results partially support the second hypothesis (the relative abundances of potential functional genes for extracellular enzymes in bacterial and fungal communities change along the forest degradation gradient, and these variations can be reflected in extracellular enzyme activities). In both bacterial and fungal communities, the relative abundance of ACP gene decreases with decreasing forest degradation indexes (i.e. increasing magnitude of forest degradation), while those of BG gene increase, and those of LAP gene do not show a clear correlation with forest degradation. Only the relative abundance of NAG gene exhibits differences between bacteria and fungi, where the relative abundance of NAG gene in bacterial communities increases with decreasing forest degradation indexes, while that in fungal communities decreases.

BG is considered an important extracellular enzyme involved in C cycling, and it completes the final step of cellulose hydrolysis by converting the disaccharide cellobiose to glucose molecules (Knight and Dick, 2004; Pathan et al., 2017). The increase in the relative abundance of BG genes during forest degradation may suggest an improved microbial potential to cellulose degradation (Zhang et al., 2022), although I did not find increasing BG/SMBC with forest degradation in Chapter 3. The improved microbial potential to cellulose degradation may indirectly contribute to maintaining the C metabolism efficiency for soil microbial communities.

There are inconsistent changes in the relative abundance of NAG gene between bacterial and fungal communities, which may suggest that bacteria and fungi play distinct roles in chitin and other complex carbohydrates decomposition (Swiontek

Brzezinska et al., 2004). Despite both bacteria and fungi are involved in chitin degradation, bacteria are believed to be major mediators of chitin degradation in soil systems (Beier and Bertilsson, 2013). The increase in the relative abundance of NAG gene in bacterial communities may suggest that bacteria maintain the efficiency of chitin degradation by improving the potential for chitin decomposition.

The variations of the relative abundances of ACP and LAP genes with forest degradation indexes are not in accordance with the conclusion in Chapter 3. In Chapter 3, the quotient of ACP activity to soil microbial biomass as well as the quotient of LAP activity to soil microbial biomass increase with decreasing SOC, suggesting the ACP and LAP efficiencies *per* microbial biomass are enhanced in degraded forests. However, I find a decreasing trend of relative abundances of ACP and LAP genes in this chapter. These inconsistencies may suggest that the reduction of microbial substrate during forest degradation, but not the relative abundance of potential functional genes *per se*, directly affects ACP and LAP efficiency as a proximate cause under the limitation of P and/or N, since the changes in enzyme efficiency are consequences of short-term induction/repression responses.

Changes in relative abundance of gene (functional potential) cannot directly translate to the changes in enzyme activity, as various regulatory mechanisms at different levels (i.e., transcription, translation, post-translational modification, allosteric regulation) regulate the final enzyme activity (Rocca et al., 2015; Vanella et al., 2024). Additional studies and analyses related to gene expression might be required to validate and strengthen my findings in the future.

4.4.3 Limitations of PICRUSt2

PICRUSt2 is a method for inferring functions based on phylogenetic relationships, which has been found to have the following limitations (Toole et al., 2021; Matchado et al., 2024): PICRUSt2 can only predict functions present in known genomes, potentially missing unknown or rare functions; Prediction results are highly dependent on the quality and completeness of the reference database; PICRUSt2 assumes

phylogenetic relationships correlate with function, but this relationship does not always hold true.

In Chapters 2 and 3, I investigated changes in the activities and efficiencies of enzymes related to C, N, and P cycling along the forest degradation gradient. In Chapter 4, I examined changes in gene abundances associated with C, N, and P cycling enzymes as forests degraded. The increased gene abundances of BG and NAG with forest degradation may potentially validate that BG and NAG efficiencies do not change significantly with forest degradation, as the functional potentials for cellulose and chitin decomposition may be maintained by the microbial communities. However, changes in ACP and LAP gene abundances differ from actual observations. This suggests that in my study sites, although there might be a correlation between phylogeny and function, it is limited to some common, conserved core functions such as C and N decomposition.

Furthermore, predicted gene abundances do not necessarily equate to protein expression levels or enzyme activities (Douglas et al., 2019). Environmental factors, regulatory mechanisms, and other elements that may influence actual gene expression and enzyme activity are not considered in PICRUSt2. Therefore, I suggest that the results of this chapter should be viewed as preliminary considerations on how microbial functional potentials change with forest degradation, providing possible directions for future exploration of microbial functions, but not as final conclusions. The accuracy of functional predictions will be improved in future studies through experimental validation, such as RT-qPCR and proteomics (Schneider and Riedel, 2010; Gao et al., 2011).

4.5 Conclusion

Soil bacterial functional structure versus fungal functional structure along a forest degradation gradient in logged-over tropical rainforests in Malaysian Borneo show very different patterns. The potential genes with very high relative abundance are occupied in bacterial functional structure, while the relative abundances of potential functional

genes are more even. Combined with the RDA results, shifts in bacterial functional structure are more evident than fungal functional structure along the gradient of forest degradation, reflecting that bacterial functional potentials are more susceptible to the influences of altered soil physicochemical properties. Fungal functional structures exhibit functional redundancy because the functional potentials involved in litter and organic matter decomposition are widely distributed in different fungal taxa. The functional potentials derived from saprophytic fungi and ectomycorrhiza may be affected by soil physicochemical properties, but partial changes do not affect the stability of the overall fungal functional structure. Moreover, relative abundances of potential functional genes for extracellular enzymes are partially reflected in the changes in extracellular enzyme activities. During forest degradation, the increased relative abundance of BG and NAG genes may contribute to the maintenance of cellulose and chitin metabolism efficiencies in highly degraded forests.

Tables and Figures

Tables

Table 4-1 Correlations between the top 20 relative abundance of bacterial E.C. numbers soil extracellular enzymes and forest degradation indexes. F.D. indexes, forest degradation indexes.

Function	E.C. numbers	F.D. indexes	
		Pearson	Sig.
Acetolactate synthase	EC:2.2.1.6	0.624***	P <0.001
Glycine hydroxymethyltransferase	EC:2.1.2.1	-0.596***	P <0.001
Selenide, water dikinase	EC:2.7.9.3	-0.524**	P <0.01
tRNA (N6-L-threonylcarbamoyladenine37-C2)-methylthiotransferase	EC:2.8.4.5	0.475**	P <0.01
D-amino-acid transaminase	EC:2.6.1.21	0.435**	P <0.001
Trans-2,3-dihydro-3-hydroxyanthranilate isomerase	EC:5.3.3.17	0.537**	P <0.001
3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione monooxygenase	EC:1.14.14.12	0.661***	P <0.001
5-oxoprolinase (ATP-hydrolysing)	EC:3.5.2.9	0.442**	P <0.01
Gentamicin 2'-N-acetyltransferase	EC:2.3.1.59	0.712***	P <0.001
D-galactose 1-dehydrogenase	EC:1.1.1.48	0.284	0.098
4,5-dihydroxyphthalate decarboxylase	EC:4.1.1.55	0.285	0.098
Dolichyl-diphosphooligosaccharide---Protein glycotransferase	EC:2.4.99.18	0.487**	P <0.001
Maleylacetate reductase	EC:1.3.1.32	0.289	0.093
Histone acetyltransferase	EC:2.3.1.48	0.511**	P <0.001
tRNA (cytidine56-2'-O)-methyltransferase	EC:2.1.1.206	0.523**	P <0.01
Archaetidylinositol phosphate synthase	EC:2.7.8.39	0.525**	P <0.01
Phosphoglycerol geranylgeranyltransferase	EC:2.5.1.41	0.525**	P <0.01
tRNA-guanine15	EC:2.4.2.48	0.525**	P <0.01
Transglycosylase	EC:2.3.3.15	0.793***	P <0.001
sulfoacetaldehyde Acetyltransferase	EC:1.2.1.83	0.554**	P <0.01
-succinoylsemialdehyde-pyridine dehydrogenase			

Table 4-2 Correlations between the top 20 relative abundance of fungal E.C. numbers soil extracellular enzymes and forest degradation indexes. F.D. indexes, forest degradation indexes.

Function	E.C. numbers	F.D. indexes	
		Pearson	Sig.
Pyruvate dehydrogenase (acetyl-transferring)	EC:1.2.4.1	0.355*	P <0.05
Methylenetetrahydrofolate reductase [NAD(P)H]	EC:1.5.1.20	0.299	0.086
Isoleucine---tRNA ligase	EC:6.1.1.5	0.258	0.141
Anthranilate synthase	EC:4.1.3.27	0.133	0.453
Xaa-Pro aminopeptidase	EC:3.4.11.9	0.137	0.439
Glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)	EC:1.2.1.12	0.058	0.743
ATP adenyllyltransferase	EC:2.7.7.53	0.048	0.786
Protein-S-isoprenylcysteine O-methyltransferase	EC:2.1.1.100	0.303	0.081
Alpha-mannosidase	EC:3.2.1.24	0.306	0.078
Riboflavin kinase	EC:2.7.1.26	0.299	0.086
5-aminolevulinate synthase	EC:2.3.1.37	0.298	0.087
dUTP diphosphatase	EC:3.6.1.23	0.298	0.087
Phosphoenolpyruvate carboxykinase (ATP)	EC:4.1.1.49	0.298	0.087
Phosphoacetylglucosamine mutase	EC:5.4.2.3	0.298	0.087
Adenosine kinase	EC:2.7.1.20	0.298	0.087
Pantoate---beta-alanine ligase (AMP-forming)	EC:6.3.2.1	0.298	0.087
Aspartyl aminopeptidase	EC:3.4.11.21	0.298	0.087
Mitochondrial intermediate peptidase	EC:3.4.24.59	0.298	0.087
dCMP deaminase	EC:3.5.4.12	0.298	0.087
Diamine N-acetyltransferase.	EC:2.3.1.57	0.286	0.101

Figures

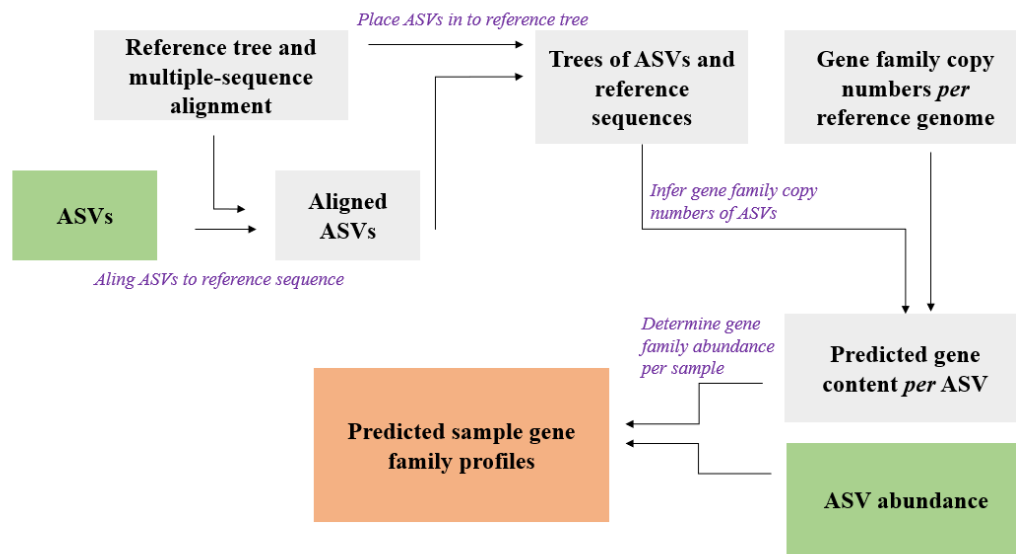


Fig. 4-1 A flowchart of PICRUST2. The green squares refer to the input data from Chapter3 in my study, while the grey squares represent the products of the parsing process, and the orange square represents the final result.

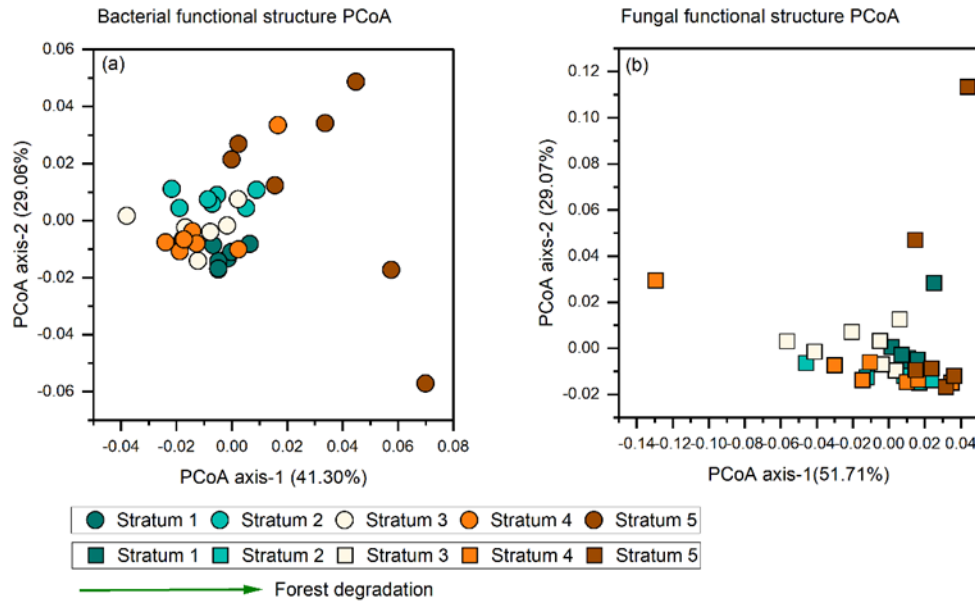


Fig. 4-2 Principal coordinate analysis (PCoA) for the relative abundances of E.C. numbers in bacterial and fungal communities, respectively. Point colors from dark green to brown indicate the aggravation of forest degradation (i.e., stratum 1 primary forest to stratum 5 highly degraded forest).

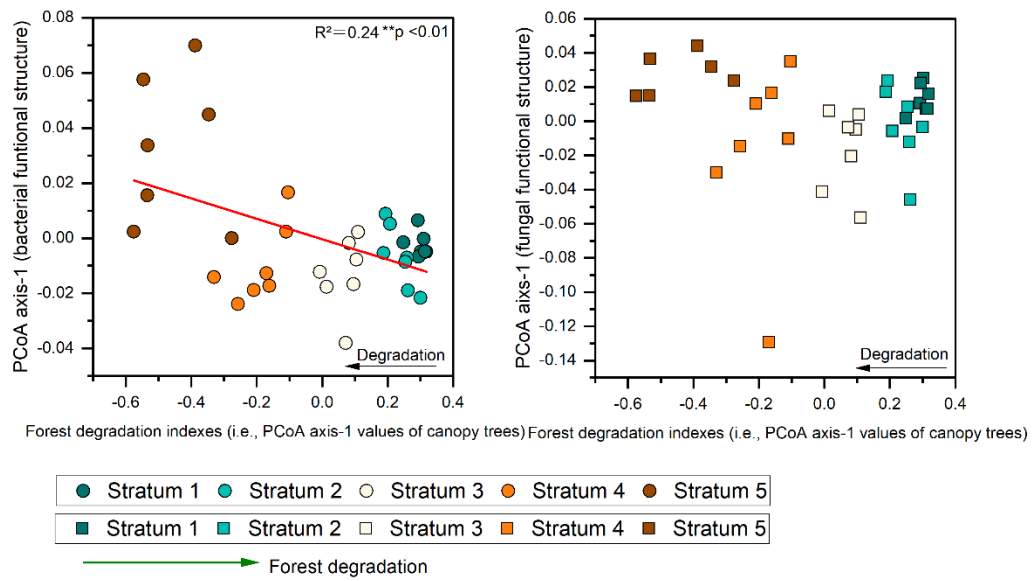


Fig. 4-3 Linear regressions of forest degradation indexes (i.e., PCoA axis-1 values of canopy trees) and microbial functional structure PCoA axis-1 (bacterial and fungal). The direction of the arrow indicates the aggravation of degradation. Point colors from dark green to brown indicate the aggravation of forest degradation (i.e., stratum 1 primary forest to stratum 5 highly degraded forest).

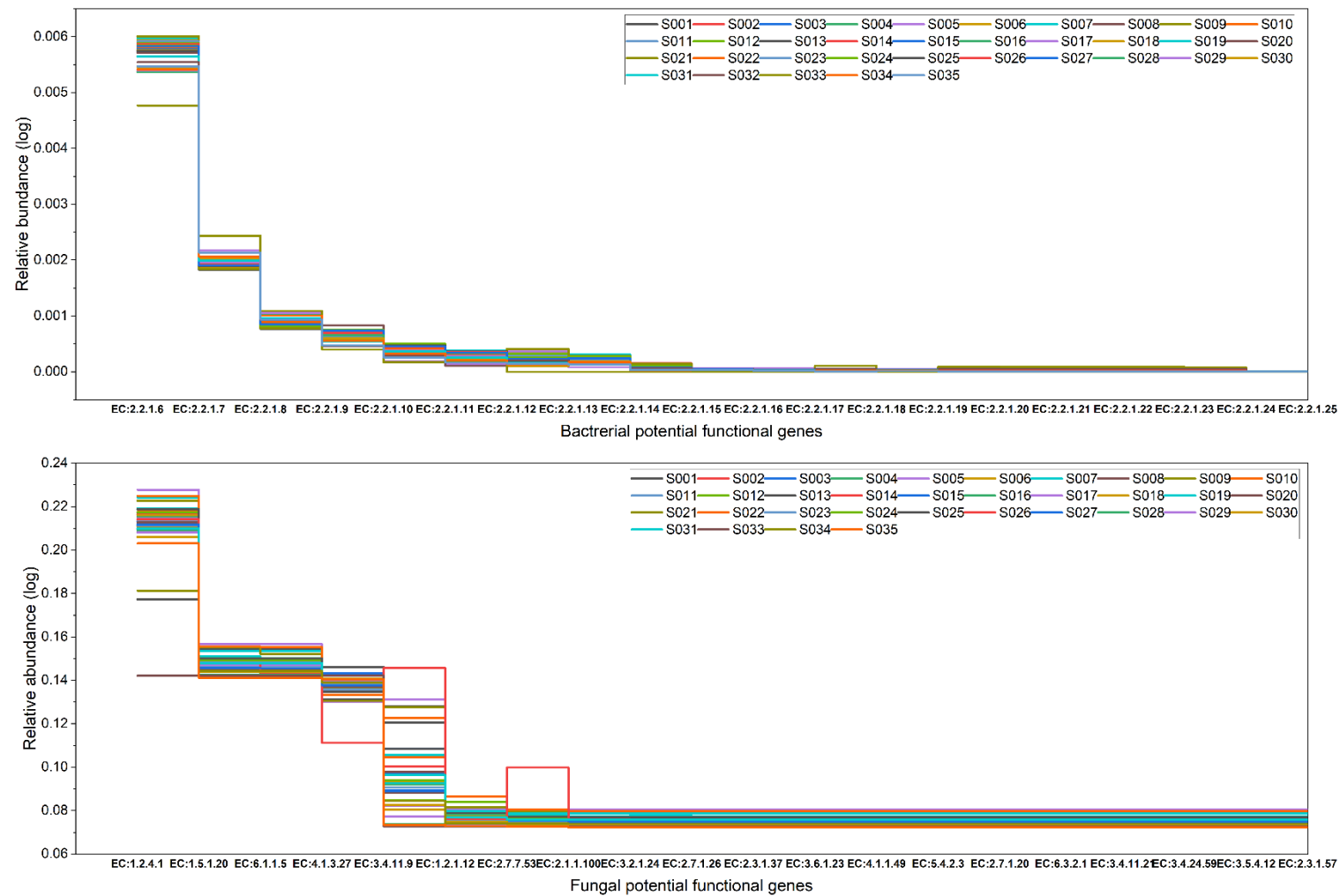


Fig. 4-4 Rank-abundance curves of relative abundance of potential functional genes (top 20).

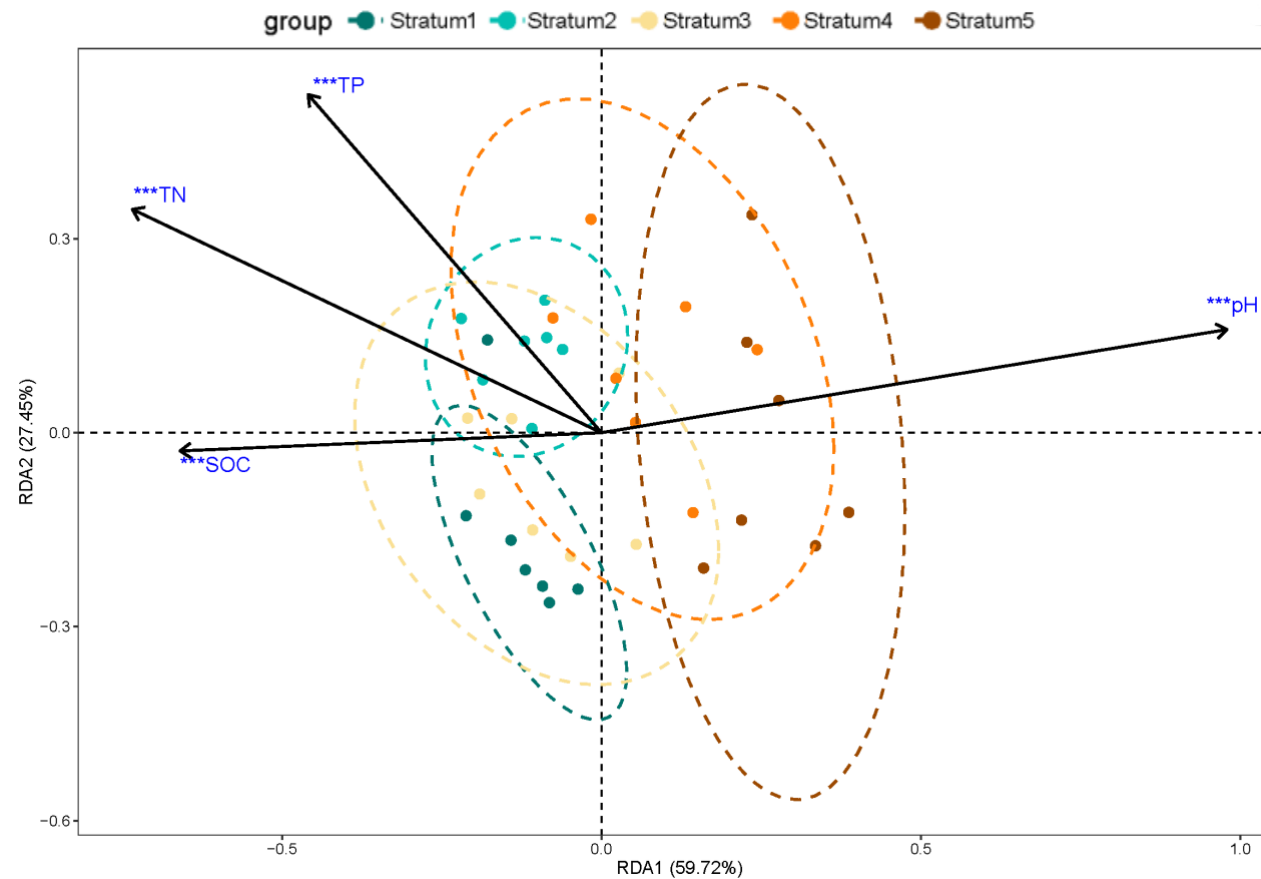


Fig. 4-5 Redundancy analysis (RDA) plot showing the relationship between bacterial functional structures and the soil physicochemical properties. Point colors from dark green to brown indicate the aggravation of forest degradation (i.e., stratum 1 primary forest to stratum 5 highly degraded forest). The dashed circles represent 95% confidence intervals and the arrows represent environment variables. *** $p < 0.001$; ** $p < 0.01$; and * $p < 0.05$. SOC, soil organic carbon, TN, total nitrogen, TP, total phosphorus.

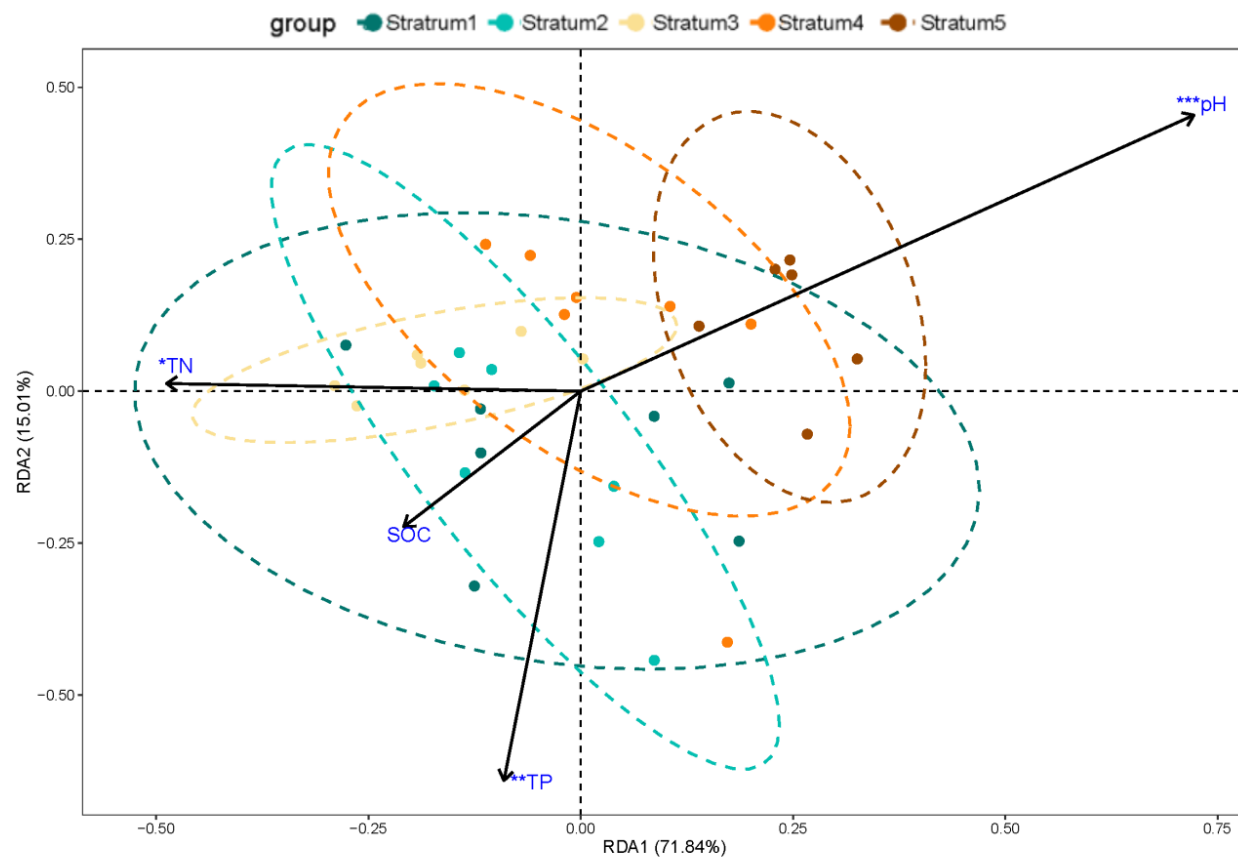


Fig. 4-6 Redundancy analysis (RDA) plot showing the relationship between fungal functional structures and the soil physicochemical properties. Point colors from dark green to brown indicate the aggravation of forest degradation (i.e., stratum 1 primary forest to stratum 5 highly degraded forest). The dashed circles represent 95% confidence intervals and the arrows represent environment variables. *** $p < 0.001$; ** $p < 0.01$; and * $p < 0.05$. SOC, soil organic carbon, TN, total nitrogen, TP, total phosphorus.

Bacteria

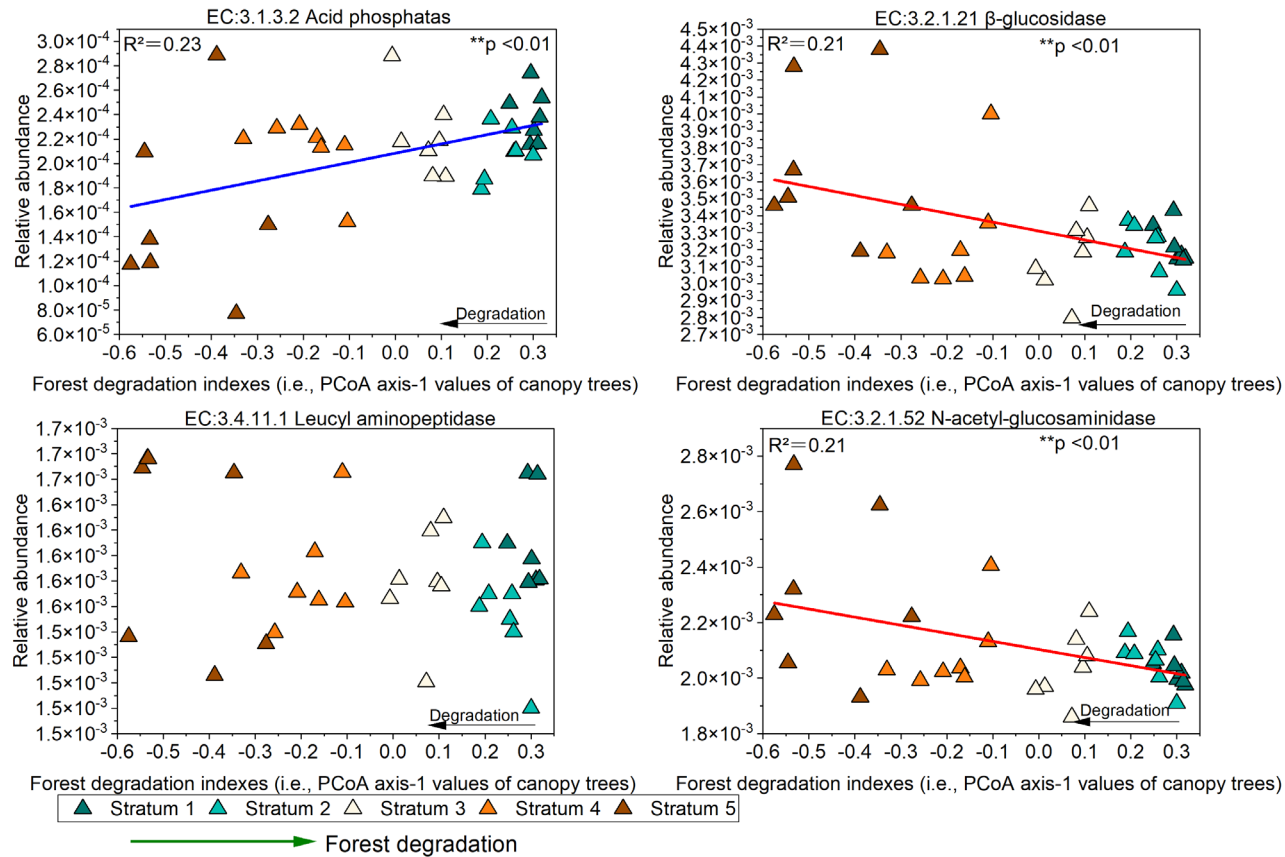


Fig. 4-7 Linear regression of forest degradation indexes (i.e., PCoA axis-1 values of canopy trees) and the relative abundances of E.C. numbers for four extracellular enzymes in bacterial communities. The direction of the arrow indicates the aggravation of degradation. Point colors from dark green to brown indicate the aggravation of forest degradation (i.e., stratum 1 primary forest to stratum 5 highly degraded forest).

Fungi

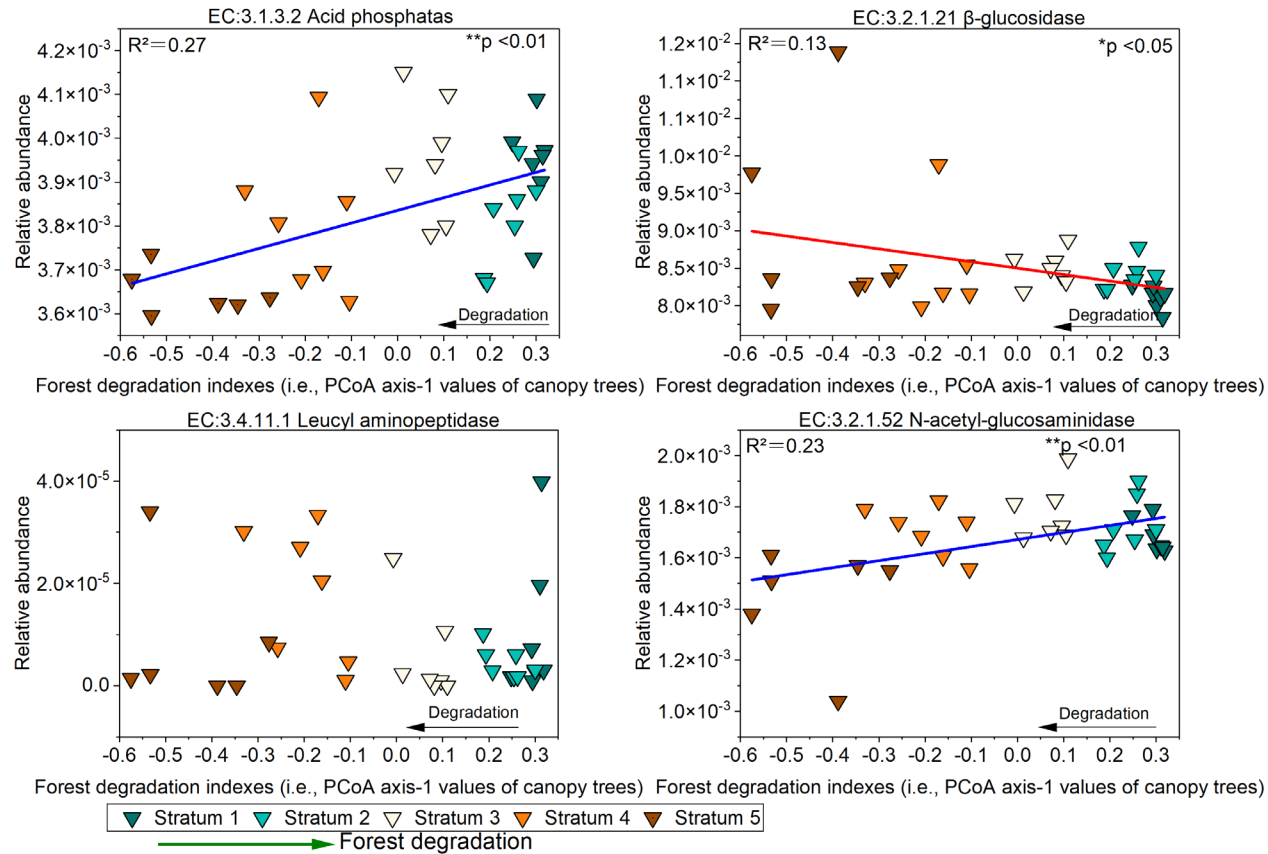


Fig. 4-8 Linear regression of forest degradation indexes (i.e., PCoA axis-1 values of canopy trees) and the relative abundances of E.C. numbers for four extracellular enzymes in fungal communities. The direction of the arrow indicates the aggravation of degradation. Point colors from dark green to brown indicate the aggravation of forest degradation (i.e., stratum 1 primary forest to stratum 5 highly degraded forest).

Supplementary information

Tables

Table S4-1 The top 20 relative abundances of bacterial functional genes in each sample. EC:2.2.1.6, acetolactate synthase; EC:2.1.2.1, glycine hydroxymethyltransferase; EC:2.7.9.3, selenide, water dikinase; EC:2.8.4.5, tRNA (N6-L-threonylcarbamoyladenine37-C2)-methylthiotransferase; EC:2.6.1.21, D-amino-acid transaminase; EC:5.3.3.17, trans-2,3-dihydro-3-hydroxyanthranilate isomerase; EC:1.14.14.12, 3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione monooxygenase; EC:3.5.2.9, 5-oxoprolinase (ATP-hydrolysing); EC:2.3.1.59, gentamicin 2'-N-acetyltransferase; EC:1.1.1.48, D-galactose 1-dehydrogenase; EC:4.1.1.55, 4,5-dihydroxyphthalate decarboxylase; EC:2.4.99.18, dolichyl-diphosphooligosaccharide---protein glycotransferase; EC:1.3.1.32, maleylacetate reductase; EC:2.3.1.48, histone acetyltransferase; EC:2.1.1.206, tRNA (cytidine56-2'-O)-methyltransferase; EC:2.7.8.39, tRNA (cytidine56-2'-O)-methyltransferase; EC:2.5.1.41, phosphoglycerol geranylgeranyltransferase; EC:2.4.2.48, tRNA-guanine15 transglycosylase; EC:2.3.3.15, sulfoacetaldehyde acetyltransferase; EC:1.2.1.83, 3-succinoylsemialdehyde-pyridine dehydrogenase.

	EC:2.2.1.6	EC:2.1.2.1	EC:2.7.9.3	EC:2.8.4.5	EC:2.6.1.21	EC:5.3.3.17	EC:1.14.14.12	EC:3.5.2.9	EC:2.3.1.59	EC:1.1.1.48	EC:4.1.1.55	EC:2.4.99.18	EC:1.3.1.32	EC:2.3.1.48	EC:2.1.1.206	EC:2.7.8.39	EC:2.5.1.41	EC:2.4.2.48	EC:2.3.3.15	EC:1.2.1.83
S1	4.15 ×10 ⁻³	1.27 ×10 ⁻³	5.34 ×10 ⁻⁴	4.72 ×10 ⁻⁴	3.46 ×10 ⁻⁴	2.58 ×10 ⁻⁴	2.37 ×10 ⁻⁴	1.73 ×10 ⁻⁴	9.48 ×10 ⁻⁵	2.68 ×10 ⁻⁵	2.14 ×10 ⁻⁵	4.33 ×10 ⁻⁶	1.71 ×10 ⁻⁵	4.33 ×10 ⁻⁶	4.33 ×10 ⁻⁶	4.33 ×10 ⁻⁶	4.33 ×10 ⁻⁶	4.33 ×10 ⁻⁶	4.33 ×10 ⁻⁶	1.13 ×10 ⁻⁶
S2	4.11 ×10 ⁻³	1.30 ×10 ⁻³	5.52 ×10 ⁻⁴	4.88 ×10 ⁻⁴	3.25 ×10 ⁻⁴	2.23 ×10 ⁻⁴	2.21 ×10 ⁻⁴	1.58 ×10 ⁻⁴	7.72 ×10 ⁻⁵	2.73 ×10 ⁻⁵	2.07 ×10 ⁻⁵	5.52 ×10 ⁻⁶	1.65 ×10 ⁻⁵	5.52 ×10 ⁻⁶	5.52 ×10 ⁻⁶	5.52 ×10 ⁻⁶	5.52 ×10 ⁻⁶	5.52 ×10 ⁻⁶	2.52 ×10 ⁻⁶	2.58 ×10 ⁻⁷
S3	4.09 ×10 ⁻³	1.34 ×10 ⁻³	5.70 ×10 ⁻⁴	4.50 ×10 ⁻⁴	3.17 ×10 ⁻⁴	2.15 ×10 ⁻⁴	1.72 ×10 ⁻⁴	1.70 ×10 ⁻⁴	5.81 ×10 ⁻⁵	2.73 ×10 ⁻⁵	1.67 ×10 ⁻⁵	7.25 ×10 ⁻⁶	1.40 ×10 ⁻⁵	7.25 ×10 ⁻⁶	7.25 ×10 ⁻⁶	7.25 ×10 ⁻⁶	7.25 ×10 ⁻⁶	7.25 ×10 ⁻⁶	3.35 ×10 ⁻⁶	3.31 ×10 ⁻⁷
S4	4.15 ×10 ⁻³	1.31 ×10 ⁻³	5.49 ×10 ⁻⁴	4.22 ×10 ⁻⁴	3.00 ×10 ⁻⁴	2.19 ×10 ⁻⁴	2.24 ×10 ⁻⁴	1.49 ×10 ⁻⁴	8.85 ×10 ⁻⁵	2.83 ×10 ⁻⁵	2.43 ×10 ⁻⁵	3.53 ×10 ⁻⁶	2.35 ×10 ⁻⁵	2.70 ×10 ⁻⁶	2.70 ×10 ⁻⁶	2.70 ×10 ⁻⁶	2.70 ×10 ⁻⁶	2.70 ×10 ⁻⁶	4.55 ×10 ⁻⁶	1.02 ×10 ⁻⁶
S5	4.11 ×10 ⁻³	1.29 ×10 ⁻³	5.43 ×10 ⁻⁴	4.63 ×10 ⁻⁴	3.03 ×10 ⁻⁴	2.46 ×10 ⁻⁴	2.49 ×10 ⁻⁴	1.72 ×10 ⁻⁴	8.16 ×10 ⁻⁵	4.15 ×10 ⁻⁵	4.36 ×10 ⁻⁵	9.26 ×10 ⁻⁶	3.35 ×10 ⁻⁵	7.17 ×10 ⁻⁶	7.17 ×10 ⁻⁶	7.23 ×10 ⁻⁶	7.23 ×10 ⁻⁶	7.17 ×10 ⁻⁶	3.87 ×10 ⁻⁶	2.30 ×10 ⁻⁷
S6	4.13 ×10 ⁻³	1.30 ×10 ⁻³	5.92 ×10 ⁻⁴	4.79 ×10 ⁻⁴	2.93 ×10 ⁻⁴	2.28 ×10 ⁻⁴	2.29 ×10 ⁻⁴	1.72 ×10 ⁻⁴	8.21 ×10 ⁻⁵	2.69 ×10 ⁻⁵	2.04 ×10 ⁻⁵	1.30 ×10 ⁻⁵	1.72 ×10 ⁻⁵	1.28 ×10 ⁻⁵	1.23 ×10 ⁻⁵	1.23 ×10 ⁻⁵	1.23 ×10 ⁻⁵	1.23 ×10 ⁻⁵	4.92 ×10 ⁻⁶	2.45 ×10 ⁻⁷
S7	4.15	1.33	5.40	4.96	3.46	2.63	1.93	2.19	7.17	2.26	1.38	1.96	1.47	1.85	1.87	1.85	1.85	1.85	3.56	1.04

	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-6}$
S8	4.04	1.36	6.56	4.37	2.40	1.70	1.72	1.12	5.93	1.34	1.22	3.31	1.01	3.15	3.15	3.15	3.15	3.14	2.36	4.86
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-7}$
S9	3.97	1.36	6.34	4.74	2.25	1.82	1.69	1.32	5.91	1.49	1.18	7.45	9.85	5.97	5.96	5.78	5.78	5.75	2.73	5.94
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-8}$
S10	4.03	1.34	6.38	4.41	2.53	1.94	1.75	1.24	6.35	1.43	9.77	3.70	8.51	3.54	3.55	3.54	3.54	3.53	3.61	3.30
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$
S11	3.98	1.35	6.34	4.25	2.27	1.88	1.97	1.30	7.05	1.61	1.06	3.76	8.91	3.38	3.39	3.37	3.37	3.36	3.12	1.38
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-7}$
S12	4.07	1.36	6.52	3.89	2.25	1.66	2.20	1.12	8.47	1.24	6.32	3.15	5.86	3.04	3.04	3.03	3.03	3.03	5.02	1.36
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-6}$
S13	4.01	1.34	6.42	4.79	2.62	2.01	1.50	1.38	4.96	1.81	1.24	2.61	1.10	2.44	2.40	2.38	2.38	2.37	2.45	1.38
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-7}$
S14	4.08	1.33	5.99	3.82	2.38	1.70	2.79	1.31	1.0	2.32	1.31	1.37	1.24	1.32	1.32	1.31	1.31	1.31	3.14	7.59
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-7}$
S15	4.02	1.33	5.86	4.88	3.15	2.36	1.61	1.75	5.48	2.93	2.33	3.08	2.24	2.80	2.79	2.78	2.78	2.77	3.51	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	
S16	4.05	1.34	5.97	4.91	2.89	2.23	1.42	1.56	4.36	3.31	2.47	2.96	2.31	2.87	2.81	2.81	2.81	2.80	2.64	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	
S17	4.04	1.36	6.59	4.51	2.63	1.92	1.33	1.38	4.34	2.22	1.30	1.49	1.19	1.31	1.27	1.27	1.27	1.27	3.49	5.21
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-8}$
S18	4.00	1.32	5.74	4.91	3.15	2.25	1.63	1.51	5.77	3.58	2.04	8.73	1.92	6.09	6.12	6.03	6.03	5.94	3.05	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	
S19	4.04	1.33	6.06	4.72	2.98	2.25	1.44	1.59	4.80	2.32	1.89	1.52	1.60	1.42	1.41	1.40	1.40	1.40	2.99	3.22
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-7}$
S20	3.97	1.33	6.37	5.75	3.08	2.28	1.00	1.62	3.03	2.63	1.87	3.82	1.59	3.74	3.58	3.59	3.59	3.58	1.32	0

	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	
S21	4.17	1.28	5.40	4.90	3.18	2.44	2.84	1.93	9.68	2.70	1.83	9.78	1.72	8.52	8.26	8.26	8.26	8.26	1.53	3.19
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-7}$
S22	4.04	1.33	6.13	4.94	2.90	2.27	1.41	1.55	4.51	2.43	2.36	7.35	1.89	8.12	7.35	7.35	7.35	7.35	1.82	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	
S23	4.11	1.33	5.94	4.64	3.14	2.39	1.68	1.49	5.58	2.43	2.00	6.11	1.71	5.17	5.17	5.17	5.17	5.17	1.49	2.97
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-7}$
S24	4.09	1.31	5.68	5.18	3.45	2.29	1.73	1.86	5.96	2.41	1.42	3.68	1.16	2.80	2.42	2.42	2.42	2.42	2.01	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	
S25	3.99	1.31	5.93	5.03	3.25	2.49	1.41	1.68	4.54	3.30	2.69	2.92	2.59	1.70	1.13	1.13	1.13	1.13	1.92	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	
S26	4.08	1.33	5.92	4.85	3.06	2.43	1.56	1.66	4.92	2.63	2.04	1.75	1.88	1.55	1.50	1.50	1.50	1.50	1.26	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$
S27	4.05	1.32	5.91	5.11	3.16	2.37	1.60	1.69	5.03	2.97	2.30	7.95	2.21	5.88	5.30	5.28	5.28	5.04	2.66	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	
S28	3.73	1.43	7.09	4.54	2.02	1.47	9.84	9.04	1.99	2.47	1.90	1.36	2.03	7.66	8.57	7.60	7.60	7.17	2.45	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	
S29	3.75	1.51	7.29	3.22	1.26	9.63	9.73	5.73	2.12	7.91	1.10	2.91	4.78	1.78	1.73	1.73	1.73	1.73	9.43	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-7}$
S30	3.79	1.41	6.60	4.15	2.26	1.49	7.19	1.31	1.22	1.76	1.32	6.56	1.11	4.18	2.76	2.76	2.76	2.76	1.63	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	
S31	3.92	1.38	6.63	3.77	2.55	1.74	1.14	1.17	2.72	1.69	1.14	2.68	1.09	7.31	4.06	4.06	4.06	4.06	4.06	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-7}$	$\times 10^{-7}$	$\times 10^{-7}$	$\times 10^{-7}$	$\times 10^{-7}$	$\times 10^{-7}$	
S32	3.85	1.48	6.41	3.18	2.00	7.43	1.42	1.17	4.73	1.18	0	0	0	0	0	0	0	0	0	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$										

S33	3.31	1.69	7.55	2.76	1.15	1.26	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$														
S34	3.76	1.43	6.98	3.84	2.27	1.42	9.17	1.17	1.80	2.41	1.77	1.09	1.41	9.66	8.65	8.60	8.60	8.47	1.96	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$	
S35	3.80	1.48	6.49	3.26	1.77	1.14	9.91	8.90	2.31	2.09	1.06	1.71	9.28	8.53	3.70	3.70	3.70	3.70	9.67	0
	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-7}$	$\times 10^{-7}$	$\times 10^{-7}$	$\times 10^{-7}$	$\times 10^{-7}$	$\times 10^{-7}$	

Table S4-2 The top 20 relative abundances of fungal functional genes in each sample. EC:1.2.4.1, pyruvate dehydrogenase (acetyl-transferring); EC:1.5.1.20, methylenetetrahydrofolate reductase [NAD(P)H]; EC:6.1.1.5, isoleucine---tRNA ligase; EC:4.1.3.27, anthranilate synthase; EC:3.4.11.9, Xaa-Pro aminopeptidase; EC:1.2.1.12, glyceraldehyde-3-phosphate dehydrogenase (phosphorylating); EC:2.7.7.53, ATP adenyltransferase; EC:2.1.1.100, protein-S-isoprenylcysteine O-methyltransferase; EC:3.2.1.24, alpha-mannosidase; EC:2.7.1.26, riboflavin kinase; EC:2.3.1.37, 5-aminolevulinate synthase; EC:3.6.1.23, dUTP diphosphatase; EC:4.1.1.49, phosphoenolpyruvate carboxykinase (ATP); EC:5.4.2.3, phosphoacetylglucosamine mutase; EC:2.7.1.20, adenosine kinase; EC:6.3.2.1, pantoate---beta-alanine ligase (AMP-forming); EC:3.4.11.21, aspartyl aminopeptidase; EC:3.4.24.59, mitochondrial intermediate peptidase; EC:3.5.4.12, dCMP deaminase; EC:2.3.1.57, diamine N-acetyltransferase.

	EC:1. 2.4.1	EC:1.5. 1.20	EC:6. 1.1.5	EC:4.1 .3.27	EC:3.4 .11.9	EC:1.2 .1.12	EC:2.7 .7.53	EC:2.1. 1.100	EC:3.2 .1.24	EC:2.7 .1.26	EC:2.3 .1.37	EC:3.6 .1.23	EC:4.1 .1.49	EC:5. 4.2.3	EC:2.7 .1.20	EC:6. 3.2.1	EC:3.4. 11.21	EC:3.4. 24.59	EC:3.5 .4.12	EC:2.3 .1.57
S1	1.31 ×10 ⁻³	1.08 ×10 ⁻³	1.08 ×10 ⁻³	1.07 ×10 ⁻³	8.72 ×10 ⁻⁴	5.42 ×10 ⁻⁴	5.40 ×10 ⁻⁴	5.40 ×10 ⁻⁴	5.40 ×10 ⁻⁴	5.40 ×10 ⁻⁴	5.40 ×10 ⁻⁴	5.40 ×10 ⁻⁴	5.40 ×10 ⁻⁴	5.40 ×10 ⁻⁴	5.40 ×10 ⁻⁴	5.40× 10 ⁻⁴	5.40 ×10 ⁻⁴	5.40 ×10 ⁻⁴	5.40 ×10 ⁻⁴	5.40 ×10 ⁻⁴
S2	1.63 ×10 ⁻³	1.09 ×10 ⁻³	1.09 ×10 ⁻³	1.04 ×10 ⁻³	6.48 ×10 ⁻⁴	5.45 ×10 ⁻⁴	5.50 ×10 ⁻⁴	5.45 ×10 ⁻⁴	5.45 ×10 ⁻⁴	5.45 ×10 ⁻⁴	5.45 ×10 ⁻⁴	5.45 ×10 ⁻⁴	5.45 ×10 ⁻⁴	5.45 ×10 ⁻⁴	5.45 ×10 ⁻⁴	5.45 ×10 ⁻⁴	5.45 ×10 ⁻⁴	5.45 ×10 ⁻⁴	5.45 ×10 ⁻⁴	5.45 ×10 ⁻⁴
S3	1.59 ×10 ⁻³	1.08 ×10 ⁻³	1.07 ×10 ⁻³	1.04 ×10 ⁻³	6.32 ×10 ⁻⁴	5.38 ×10 ⁻⁴	5.38 ×10 ⁻⁴	5.38 ×10 ⁻⁴	5.38 ×10 ⁻⁴	5.38 ×10 ⁻⁴	5.38 ×10 ⁻⁴	5.38 ×10 ⁻⁴	5.38 ×10 ⁻⁴	5.38 ×10 ⁻⁴	5.38 ×10 ⁻⁴	5.38 ×10 ⁻⁴	5.38 ×10 ⁻⁴	5.38 ×10 ⁻⁴	5.38 ×10 ⁻⁴	5.38 ×10 ⁻⁴
S4	1.61 ×10 ⁻³	1.09 ×10 ⁻³	1.08 ×10 ⁻³	1.02 ×10 ⁻³	6.58 ×10 ⁻⁴	5.59 ×10 ⁻⁴	5.52 ×10 ⁻⁴	5.44 ×10 ⁻⁴	5.44 ×10 ⁻⁴	5.44 ×10 ⁻⁴	5.44 ×10 ⁻⁴	5.44 ×10 ⁻⁴	5.44 ×10 ⁻⁴	5.44 ×10 ⁻⁴	5.44 ×10 ⁻⁴	5.44 ×10 ⁻⁴	5.44* ×10 ⁻⁴	5.44 ×10 ⁻⁴	5.44 ×10 ⁻⁴	5.39 ×10 ⁻⁴
S5	1.61 ×10 ⁻³	1.07 ×10 ⁻³	1.07 ×10 ⁻³	1.05 ×10 ⁻³	5.87 ×10 ⁻⁴	5.40 ×10 ⁻⁴	5.37 ×10 ⁻⁴	5.36 ×10 ⁻⁴	5.36 ×10 ⁻⁴	5.36 ×10 ⁻⁴	5.36 ×10 ⁻⁴	5.36 ×10 ⁻⁴	5.36 ×10 ⁻⁴	5.36 ×10 ⁻⁴	5.36 ×10 ⁻⁴	5.36 ×10 ⁻⁴	5.36 ×10 ⁻⁴	5.36 ×10 ⁻⁴	5.36 ×10 ⁻⁴	5.36 ×10 ⁻⁴
S6	1.57 ×10 ⁻³	1.05 ×10 ⁻³	1.05 ×10 ⁻³	1.02 ×10 ⁻³	5.73 ×10 ⁻⁴	5.26 ×10 ⁻⁴	5.25 ×10 ⁻⁴	5.25 ×10 ⁻⁴	5.25 ×10 ⁻⁴	5.25 ×10 ⁻⁴	5.25 ×10 ⁻⁴	5.25 ×10 ⁻⁴	5.25 ×10 ⁻⁴	5.25 ×10 ⁻⁴	5.25 ×10 ⁻⁴	5.25 ×10 ⁻⁴	5.25 ×10 ⁻⁴	5.25 ×10 ⁻⁴	5.25 ×10 ⁻⁴	5.25 ×10 ⁻⁴
S7	1.64 ×10 ⁻³	1.10 ×10 ⁻³	1.06 ×10 ⁻³	1.02 ×10 ⁻³	6.66 ×10 ⁻⁴	5.53 ×10 ⁻⁴	5.54 ×10 ⁻⁴	5.52 ×10 ⁻⁴	5.53 ×10 ⁻⁴	5.52 ×10 ⁻⁴	5.52 ×10 ⁻⁴	5.52 ×10 ⁻⁴	5.52 ×10 ⁻⁴	5.52 ×10 ⁻⁴	5.52 ×10 ⁻⁴	5.52 ×10 ⁻⁴	5.52 ×10 ⁻⁴	5.52 ×10 ⁻⁴	5.52 ×10 ⁻⁴	5.52 ×10 ⁻⁴
S8	1.58 ×10 ⁻³	1.06 ×10 ⁻³	1.06 ×10 ⁻³	1.01 ×10 ⁻³	6.30 ×10 ⁻⁴	5.34 ×10 ⁻⁴	5.33 ×10 ⁻⁴	5.29 ×10 ⁻⁴	5.29 ×10 ⁻⁴	5.29 ×10 ⁻⁴	5.29 ×10 ⁻⁴	5.29 ×10 ⁻⁴	5.29 ×10 ⁻⁴	5.29 ×10 ⁻⁴	5.29 ×10 ⁻⁴	5.29 ×10 ⁻⁴	5.29 ×10 ⁻⁴	5.29 ×10 ⁻⁴	5.29 ×10 ⁻⁴	5.29 ×10 ⁻⁴

[illegible]

[illegible]

Figures

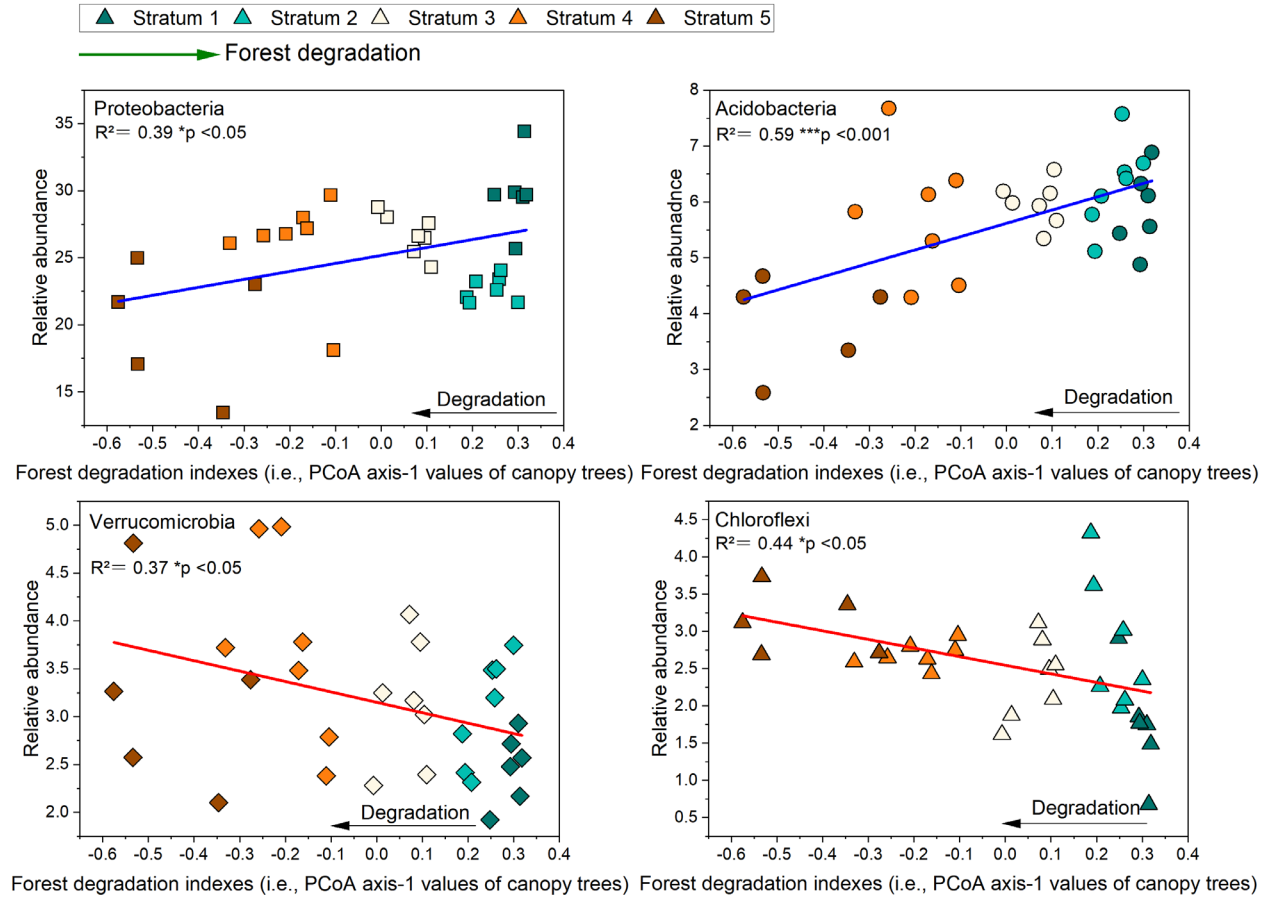


Fig. S4-1 Linear regression of forest degradation indexes (i.e., PCoA axis-1 values of canopy trees) and the relative abundances of bacterial phyla (Proteobacteria, Acidobacteria, Verrucomicrobia, and Chloroflexi). The direction of the arrow indicates the aggravation of degradation. Point colors from dark green to brown indicate the aggravation of forest degradation (i.e., stratum 1 primary forest to stratum 5 highly degraded forest).

Chapter 5 General discussion

In this thesis, I focused on the variations of soil physicochemical properties, extracellular enzyme activities, bacterial and fungal community compositions, as well as bacterial and fungal functional structures along a forest degradation gradient in lowland tropical rainforests in Sabah, Malaysian Borneo. Three research questions were asked:

Research question 1 (Chapter 2): How do soil physicochemical properties, microbial biomass, and enzyme activities change along a degradation gradient?

Research question 2 (Chapter 3): How do bacterial and fungal community compositions as well as enzyme efficiency change along a degradation gradient?

Research question 3 (Chapter 4): How do bacterial and fungal functional structures as well as the relative abundance of potential functional genes (i.e., the relative abundance of E.C. numbers for specific extracellular enzymes) change along a degradation gradient?

In Chapter 2, I investigated the first research question by measuring soil pH, soil organic carbon (SOC), total nitrogen (TN), C/N ratio, soil microbial biomass carbon (SMBC), soil microbial biomass nitrogen (SMBN), and extracellular enzymes including acid phosphatase (ACP), β -glucosidase (BG), leucine aminopeptidase (LAP) and N-acetyl-glucosaminidase (NAG). A forest degradation gradient was established and represented as forest degradation indexes (i.e., the PCoA axis-1 values of canopy trees). The concentration of SOC, TN, SMBC, and SMBN as well as C/N ratio decreased with decreasing forest degradation indexes, while soil pH values increased. The activities of ACP, BG, NAG, and LAP all decreased with decreasing forest degradation indexes. The SOC explained the greatest variation of soil microbial biomass was also observed. My results successfully demonstrate that above-ground forest degradation has a direct effect on soil organic matter, which further affects the soil microbial biomass. During the process of forest degradation, the combined effects of reduced microbial biomass and reduced soil organic matter as a substrate for enzymes reduce microbial enzyme activities. In turn, the regrowth or succession of

degraded forest ecosystems may be affected by these changes.

In Chapter 3, I investigated the second research question by measuring the soil microbial community compositions (bacteria and fungi) and enzyme efficiencies. The primary axis of the PCoA analyses based on the absolute abundances of each of bacterial and fungal dominant phyla was considered to represent the community compositions. Bacterial community composition did not correlate with forest degradation indexes, while fungal community composition correlated with forest degradation indexes. Reduced SOC in degraded forests influenced bacterial and fungal community compositions, but only fungal community composition affected the activities of ACP, BG, and LAP. Additionally, the quotient of enzyme activity to SMBC was used to indicate enzyme efficiency. Acid phosphatase activity/SMBC and LAP activity/SMBC increased with decreasing SOC. Absolute abundance, rather than relative abundance, was chosen as the indicator for analyzing community composition in my study because absolute abundance can capture the true changes in population sizes of taxa. However, selecting only relative abundance to analyze community composition may result in false positives or false negatives. Because relative abundance is constrained to a constant (e.g., 100%), an increase in a taxon may inevitably lead to a decrease in other taxa (Morton et al., 2019). Therefore, by selecting the absolute abundance, the actual situation of the community composition changes with forest degradation gradient in my research sites can be restored as much as possible. My findings clearly demonstrate distinctions in the shifts of bacterial and fungal community compositions along forest degradation gradient, highlighting shifts in fungal community composition may reflect preferences of different fungal phyla for changes in soil organic matter quality and/or quantity during litter input reduction, and such changes further influence enzyme activities. Moreover, microbial communities might be limited by soil N and P in highly degraded forest.

In Chapter 4, I investigated the third research question by measuring soil microbial functional structures (bacteria and fungi) and relative abundances of potential functional genes for specific enzymes (i.e., the relative abundance of ACP, BG, NAG,

and LAP genes). The PCoA axis-1 values for the relative abundances of bacterial and fungal E.C. numbers were chosen to represent their functional structures because the functional structures were predicted based on the relative abundance in either bacterial or fungal community. Overall fungal functional structure did not correlate with forest degradation indexes, while bacterial functional structure correlated with forest degradation indexes. Directional changes in the relative abundance of some bacterial phyla with forest degradation may cause the shift in bacterial functional structures. The non-significant overall fungal functional structure with forest degradation may suggest that fungi have functional redundancy, especially the potential functional genes involved in litter decomposition are widespread in fungal communities. Soil physicochemical properties including SOC, TN, TP, and pH were correlated with bacterial functional structure, whereas TN, TP, and pH were correlated with fungal functional structure. When I analyzed individual putative functional genes, in both bacteria and fungi, the relative abundance of ACP gene decreased with decreasing forest degradation indexes, while those of BG gene increased. On the other hand, the relative abundance of NAG gene increased in bacterial communities whereas decreased in fungal communities. If the relative abundance of potential functional genes is partially reflected by the variation of their actual activity, bacterial and fungal communities may maintain the efficiency of cellulose metabolism by increasing its metabolic potential, and bacterial communities may maintain the efficiency of chitin metabolism by increasing its metabolic potential in highly degraded forests.

The discrepancies between the patterns of microbial community composition and functional structure along the degradation gradients in Chapters 3 and 4 are caused by differences in statistical methods. In Chapter 3, absolute abundances were used to analyze community compositions, while in Chapter 4, relative abundances were used to analyze functional structure. Another reason for discrepancies between community compositions and functional structures may be related to the resolution of the analysis. Community compositions are analyzed at the phylum level in Chapter 3, whereas functional structures are analyzed at the ASV level in Chapter 4. There are directional

changes in absolute abundances (phylum level) in fungal communities along the forest degradation gradient. The directional change in the quantity and quality of soil organic matter must have worked as a strong selection on fungal phyla, whereas such a selection force does not work on bacterial phyla. Instead, selection operates on the relative abundances of bacterial E.C. numbers that show directional changes. Such fine-tuned changes in the abundance of potential functional genes are not reflected in entire bacterial phyla, probably because the β -diversity of ASVs is greater within a given bacterial phylum. The relative abundance of E.C. numbers does not change directionally in fungal communities, but fungal potentially functional genes associated with critical C-, N-, and P-degrading enzymes may exhibit different metabolic potentials among different fungal phyla along the gradient, these genes may further expression and subsequently fungal community shifts.

5.1 The ecology of soil microbial communities in degraded logged-over tropical rainforests

The degraded logged-over tropical rainforest in Malaysian Borneo is a consequence of forest degradation caused by selective logging, leading to reduced canopy cover and larger gaps in the understory compared to primary forests (Bryan et al., 2013; Astiani, 2016). The removal of commercially valuable species and the entry of pioneer species have also altered the tree species composition (Howlett and Davidson, 2003; Aoyagi et al., 2013). In addition, changes in litter quantity and quality as well as microclimate simultaneously affect the soil organic matter (Cao et al., 2020; Gao et al., 2020), resulting in more limited soil nutrients than in the primary forest (Cleveland et al., 2002; Wieder et al., 2013; Dalling et al., 2016; Schaap et al., 2021).

As an important component of soil ecosystems, soil microbial communities are affected by substrate changes and nutrient limitation in their metabolic activities (Glassman et al., 2018). In P- and N-limited degraded tropical rainforest (Kitayama and Aiba, 2002; Davidson et al., 2004; Imai et al., 2010; Yokoyama et al., 2017; Mori et al.,

2017), microbial communities can improve the efficiency of acid phosphatase (ACP) and leucine aminopeptidase (LAP) to promote P and N acquisition. Furthermore, fungal communities may contribute more to soil organic matter decomposition than bacterial communities in degraded tropical rainforest. Extracellular enzymes related to P, C and N have been secreted to varying degrees by mycorrhizal fungi, *Aspergillus* spp. and Basidiomycetes (Yadav and Tarafdar, 2003; Nampoothiri et al., 2005; Hidayat et al., 2006; Zang et al., 2018; Tedersoo et al., 2021).

The bacterial and fungal diversity and community composition of the degraded logged-over tropical rainforest also differ greatly from the original forest due to changes in the soil environment. Previous study (Li et al., 2022) shows that both bacterial and fungal α -diversity in degraded forest is significantly lower than primary forest, as their α -diversity is sensitive to changes in stand density, soil pH, soil moisture, and soil organic C. However, β -diversity in degraded forest, especially for fungi (Zhou et al., 2020; Li et al., 2022), is always higher than in undisturbed primary forest, because decreased litter quality and quantity enhances saprophytic fungi turnover.

In Malaysian lowland tropical rainforests, Acidobacteria and Proteobacteria of bacteria as well as Ascomycota and Basidiomycota of fungi are considered as dominant phyla. Fungal community composition in degraded logged-over tropical rainforests is different from that in the primary forest because substrate preferences are different among fungal phyla. The decreases in the quantity and quality of litter cause the substrate change from recalcitrant litter to soil organic matter during forest degradation. This process also leads to increased C mineralization in degraded logged-over tropical rainforest. For bacterial communities, their substrate is mainly by-products of fungal decomposition (Boer et al., 2005). Therefore, the bacterial community composition of degraded logged-over tropical rainforest is not much different from that of primary forest because the substrate availability for bacteria is buffered and stabilized by the fungal community. However, r-strategists are selected in bacterial communities in degraded logged-over tropical rainforest (e.g., Proteobacteria and Bacteroidetes) rather than K-strategists (e.g., Acidobacteria and Actinobacteria) (Fierer et al., 2007;

Zechmeister-Boltenstern et al., 2015; Zhou et al., 2017; Yan et al., 2022), because environments in degraded forests are temporally more fluctuating.

In spite of the changes in community composition at phylum level, fungal communities in degraded forests maintain the functional reservoir with genes coding similar metabolic functions as those in primary forests. Bacterial communities also maintain the functional reservoir in degraded forests although relative abundances of genes coding some metabolic functions change. The potential complementarity between fungi and bacteria seems to be maintained in degraded logged-over tropical forests. Whether/how this plausible functional robustness (i.e., the maintenance of functional reservoir as well as the maintenance of complementarity) of soil microbial communities affects the resilience of degraded forest ecosystems needs to be investigated in the future.

5.2 Outstanding research questions in the microbial ecology of degraded tropical forests

My research focuses on the microbial ecology of mixed dipterocarp lowland tropical rainforests in Malaysia after selective logging, comprehensively analyzing the interactions between aboveground vegetation, soil physicochemical properties, extracellular enzymes, soil microbial community composition, and potential functional gene during forest degradation process. This study provides novel insights into soil microbial community ecology and forest degradation in tropical region. However, composition and diversity of soil microbial communities can be different at various scales, which are mainly influenced by the combination of forest type, soil physicochemical properties such as soil moisture content, soil temperature, pH value, organic matter, and available P. Tripathi et al., (2016) focus on the variation in the composition and diversity of soil bacterial and fungal communities in several different types of lowland tropical rainforest habitats in northwest Borneo and suggests that the relative abundance of ectomycorrhizal (EcM) fungi varied significantly between forest

types, with the highest relative abundance of *Russula* observed in primary mixed dipterocarp forest. Despite the ubiquitous presence of some bacterial phyla such as Proteobacteria, Acidobacteria, and Planctomycetes as well as fungal phyla such as Ascomycota and Basidiomycota, across different types of tropical forests, their relative abundance may vary substantially between forest types (Miyashita et al., 2013; Tripathi et al., 2016). Furthermore, microbial functions may also differ among forest types. Research comparing the functional profiles of the Atlantic Forest (humid tropical) and Caatinga (a semi-arid tropical forest) in Brazil suggest that sequences related to amino acid metabolism, nitrogen and DNA metabolism, and stress resistance are more frequent in Caatinga soil, while phosphorus metabolism, defense mechanisms, and aromatic degradation subsystems are more frequent in Atlantic Forest soil (Pacchioni et al., 2014). Therefore, I speculate that my research results in the lowland mixed dipterocarp tropical rainforests of Sabah, Malaysian Borneo might be unique in comparison to other regions. On the one hand, aforementioned previous studies have mentioned that lowland mixed dipterocarp show different community compositions with other types of tropical forest. On the other hand, forest landscapes of varying magnitude of degradation in my research are derived from the special logging history in Malaysia. This means that in addition to focusing on the lowland mixed dipterocarp tropical rainforests of Malaysia, other research regions should also be studied in the future to form a complete profile of microbial ecology in degraded forests.

I propose the following three research themes on the ecology of microbial communities in the process of forest degradation to be explored in the future; (1) Expand research to various forest types in Malaysian Borneo, (2) Large-scale ecology of soil microbial communities; (3) Technical improvements in evaluating the soil microbial functions.

(1) Expand research to various forest types in Malaysian Borneo

Malaysian Borneo has not only tropical rainforest but also other types of forest including extensive peat swamp forests and heath forest (Kerangas) (Alias and Hamzah, 1998; Yule 2010; Wan Mohd Jaafar et al., 2020). In peat swamp forests,

overexploitation and repeated fires cause forest degradation (Tata et al., 2016). Previous studies have found that tropical peat swamp forests are relatively distinctive habitats compared to mixed dipterocarp forests in Borneo in terms of species composition and diversity (Brünig, 1974; MacKinnon, 1996; Cannon and Leighton, 2004). Heath forests (Kerangas), an unusual variant of tropical forests in Borneo, characterized by open scrubby vegetation, low productivity, and distinctive plant species composition and soil microbial community, are regarded as a stressful low-pH and/or nutrient environment (Kerfahi et al., 2019). In recent decades, such forests have been disturbed by anthropogenic activities (e.g., non-traditional shifting cultivation and fires), resulting in forest degradation (Alias and Hamzah, 1998; Hattori et al., 2019). However, it is still unclear how the ecology of microbial communities in degraded peat swamp forests and heath forest (Kerangas) forests. Therefore, understanding the microbial communities of these forests in Malaysian Borneo can provide a new perspective and theoretical support for forest conservation and restoration.

(2) Large-scale ecology of soil microbial communities

Global atlases of the abundance or biodiversity of bacteria (Delgado-Baquerizo et al., 2018; Delgado-Baquerizo et al., 2019), fungi (Egidi, et al., 2019), mycorrhizal fungi, and N fixer organisms (Steidinger et al., 2019) have been made. A diverse range of soil characteristics and climatic conditions has been used to predict and map the global distributions of a myriad of soil organisms at regional (Ferrier et al., 2007; Rodriguez-Brito et al., 2010), national (Terrat et al., 2017, Karimi et al., 2018), continental (Griffiths et al., 2016; Jiao et al., 2019), and global scales (Tedersoo et al., 2014, Delgado-Baquerizo et al., 2018). Besides, a microbial-explicit model (i.e., the CLM-Microbe) has been established to investigate the dynamics of C in vegetation, litter, soil, and microbes (Xu et al., 2022). Previous studies have investigated the macroecological differences between bacteria and fungi in U.S. topsoil based on this model (He et al., 2023). Therefore, by collecting and analyzing microbial community data from other tropical regions, it may be possible to summarize some general patterns of microbial community response to forest degradation.

(3) Technical improvements in evaluating the soil microbial functions.

It is technically very difficult to extract mRNA from soil in an *in situ* experiment and perform the transcriptome sequencing due to the fast degradability of mRNA. Therefore, ASVs from amplicon sequencing is used to predict microbial functions using analytical platforms such as PICRUSt2. Since the predicted functional profiles neither wholly represents the patterns of mRNA nor functions, the results of such platforms are considered as just predictions and do not necessarily reconstruct the actual situation of the ecosystem. Therefore, methodological advances to extract high-quality mRNA from forest soils should be extremely important to advance our understanding of soil functions.

5.3 Conclusion

Forest degradation caused by selective logging in tropical rainforests leads to changes in forest characteristics, especially tree composition, and subsequently affects the below-ground ecosystem. My study demonstrates the comprehensive effects of tropical rainforest degradation on soil physicochemical properties, soil microbial biomass, extracellular enzyme activities, microbial community compositions, and microbial functional structure. Variations from above- to below-ground ecosystems are evident. Changes in above-ground vegetation can affect SOC, and SOC as a substrate further affects the attributes (e.g., biomass, community compositions, and functional structures) of soil microbial communities. These changes in microbial communities transmit to the activities of extracellular enzymes.

The knowledge obtained in this thesis may contribute to the understanding of how soil microbial communities respond to forest degradation. While it is known that forest degradation can alter soil properties, and stimulate microbial activities (McCauley et al., 2009; Liu et al., 2015; Chiti et al., 2016; Vaglio Laurin et al., 2016; Abdul Rahman et al., 2021; Moreno et al., 2203), and shift soil microbial community compositions from K-strategist dominated to r-strategist dominated (Fierer et al., 2007; Zechmeister-

Boltenstern et al., 2015; Zhou et al., 2017; Yan et al., 2022), previous studies have not comprehensively addressed the complex relationship between vegetation, soil parameters, and soil microbial communities. In this thesis, I have successfully elucidated how soil bacterial and fungal communities respond to changed ecosystems from the perspective of community compositions and functional structures. Soil microbial communities are important indicators of forest ecosystem health. Understanding the changes of soil microbial communities in forest degradation, and their important relationships with soil environment factors and vegetation (litter) can provide the theoretical basis for guiding degraded forest ecological restoration.

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