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Soil Microbial Diversity and Litter Decomposition Increase with Time Since Land Use Disturbance in Tropical Montane Forests of Malaysian Borneo

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Soil Microbial Diversity and Litter Decomposition Increase with Time Since Land Use Disturbance in Tropical Montane Forests of Malaysian Borneo

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Abstract

Logging and forest conversion are occurring at alarming rates in the tropical forests of Southeast Asia. These disturbances alter soil chemistry, microbial diversity, and disrupt carbon cycling through shifts in litter decomposition. Direct links between microbial diversity and soil properties such as pH are well established; however, the indirect impacts of logging and forest conversion on microbial diversity and litter decomposition are poorly understood. We used surface (5 cm) soil to assess soil physicochemical properties, next-generation DNA sequencing to assess soil microbial diversity, and standardized litterbags to assess litter decomposition stabilization at five sites along a land use disturbance gradient in the tropical montane forests of Sabah state in eastern Malaysia. We used a hierarchical approach to explore how land use disturbance affects soil properties; how those soil properties in conjunction with land use disturbance affect soil microbial diversity; and how each of those factors affects litter decomposition and stabilization. We show that soil pH, total nitrogen, and bacterial diversity increase with time since disturbance; and sulfur, total carbon, percent sand, soil organic matter, and fungal diversity display a peaked response with peak around 100 years since the last disturbance. Fungal diversity is positively correlated with onsite forest cover, litter decomposition rate, and litter stabilization. Bacterial diversity shows a positive linear relationship with litter decomposition rates, but a peaked relationship with litter stabilization with peak around the mid-range of bacterial diversity. In summary, soils of the older forests harbor significantly greater microbial diversity and stabilize greater amounts of litter than soils of the younger forests and converted sites. Our results suggest that logging and forest conversion significantly affect soil microbial diversity and have lasting effects on carbon cycling in tropical montane forests.

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1. Introduction

Tropical forests are the most biodiverse ecosystems (Dirzo and Raven, 2003), store the largest amount of living carbon (C) (Pan et al., 2011), and account for over half of the global annual net primary production (Melillo et al., 1993). Logging and clear-cutting are a major concern in the tropical forests, with an estimated 27.2 million hectares cleared between 2000 and 2005 (Hansen et al., 2008). These activities can have lasting effects on canopy structure (Cole et al., 2014; Foody and Cutler, 2003; Okuda et al., 2003), microclimate (Meijide et al., 2018), soil pH (Waldrop et al., 2000), soil nutrient content (McGrath et al., 2001; Murty et al., 2002), biomass C sequestration, productivity (DeFries et al., 2002; Pinard and Cropper, 2000), and biodiversity (Barlow et al., 2016; Barnes et al., 2014; Gibson et al., 2011). Southeast Asia is particularly vulnerable to this rapid loss of forests where logged areas are often converted to more profitable rubber and oil palm plantations (Abood et al., 2015; Meijide et al., 2018). We chose to study the effects of disturbance by logging and forest conversion on soil physical, chemical and microbial properties; and how these changes affect ecosystem functions in the tropical montane forests of Malaysian Borneo.

Disturbances such as logging and forest conversion can significantly impact soil through decreasing soil C and nitrogen (N) content, pH, and a variety of soil nutrients (Waldrop et al., 2000). Disturbance-induced changes can significantly impact soil microbial communities because of the physical and chemical changes in soils (Lee-Cruz et al., 2013, Moon et al., 2016, 2019). Microbes such as bacteria and fungi make up a significant portion of the biodiversity of tropical forests (Arnold and Lutzoni, 2007; Whitman et al., 1998). While land use practices such as logging and forest conversion are capable of significantly impacting microbial community composition and overall biomass (Kerfahi et al., 2014; Maharjan et al., 2017), little is known

about the role of logging and forest conversion in shaping the soil microbial diversity in tropics; while a number of studies found no difference in microbial diversity between logged and unlogged tropical forests (Lee-Cruz et al., 2013; Tripathi et al., 2016a), others found an increase in microbial diversity along a tropical land use intensification gradient (Carvalho et al., 2016; Mendes et al., 2015). Further, Zhu et al. (2010) found that microbial diversity increased with successional age until ~100 years of regeneration, supporting the intermediate disturbance hypothesis. Despite the recent progress in our understanding of soil microbiome, we still lack a clear understanding of how logging and forest conversion alter soil microbial diversity in tropical ecosystems.

Soil microbial community composition affects some soil processes (e.g., nitrification, methanogenesis, and sulfur reduction) over the smaller temporal scales due to the specificity of the microbial groups required to perform these functions (Schimel and Gulledge, 1998), and some soil processes (e.g., decomposition and stabilization) over longer temporal scales where microbial resource allocation drives system dynamics (Schimel and Schaeffer, 2012). For example, fungi are expected to metabolize complex substrates more directly than bacteria (Holland and Coleman, 1987), and fungi are more resistant to decomposition than bacterial residues (Guggenberger et al., 1999). However, the lack of scalable links between these microorganisms and ecosystem functions make it difficult to integrate microbes into the Earth System Models that inform citizens and policy makers of C dynamics and exchanges between the biosphere and the atmosphere (Wieder et al., 2013, 2015). Thus, it is critical to understand the links between soil microbial communities and ecosystem processes to establish effective policies and land management practices (Bardgett and van der Putten, 2014; Singh et al., 2010).

Given the complexity of direct and indirect relationships linking physical structure, biological structure, and biological function, we take a hierarchical approach to our questions (**Figure 1**). *First, we ask, does time since disturbance affect soil physical and chemical properties in tropical montane forests?* We expect to see changes in soil physical and chemical properties based on the number of years since the last major disturbance. Many prior studies have found disturbance events to significantly reduce soil pH and soil nutrients such as C and N (Alele et al., 2014; Templer et al., 2005; Waldrop et al., 2000). With time as the ecosystem recovers, these properties often return to near pre-disturbance levels (Templer et al., 2005; Zhu et al., 2012). Given the consensus among prior studies, it is likely that time since disturbance will result in an increase in soil nutrients and pH, and a return to near-disturbance levels of other measured properties.

Second, we ask, what factors affect soil microbial diversity in tropical montane forests? We expect microbial diversity to increase with time since disturbance and pH. Microbial diversity has previously been shown to increase with time since disturbance in both tropical and temperate climates (Wallander et al., 2010; Zhu et al., 2010). Changes in fungal diversity are often attributed to successional changes in the plant community (Zhu et al., 2012), while bacteria are often more strongly linked to soil properties such as pH (Tripathi et al., 2012). For example, Tripathi et al. (2012) found bacterial diversity in tropical soils to increase with soil pH up to near-neutral values.

Finally, we ask what factors drive changes in litter decomposition and stabilization in tropical montane forests? We expect mature forests to have higher litter decomposition rates and litter stabilization compared to recently disturbed sites (Ribolzi et al., 2017; Sarneel et al., 2020). For example, changes in microclimate, as a result of selective logging, have been shown to affect

litter decomposition, with faster litter decomposition in undisturbed tropical forests than selectively logged forests (Both et al., 2017). Soil microbial communities are one of the main drivers of soil C cycling, mediated through litter decomposition and stabilization which regulate soil-atmosphere C exchange (Gleixner, 2013; Schimel and Schaeffer, 2012). Therefore, it is likely that disturbance-induced changes in microbial diversity may have lasting impacts on C cycling.

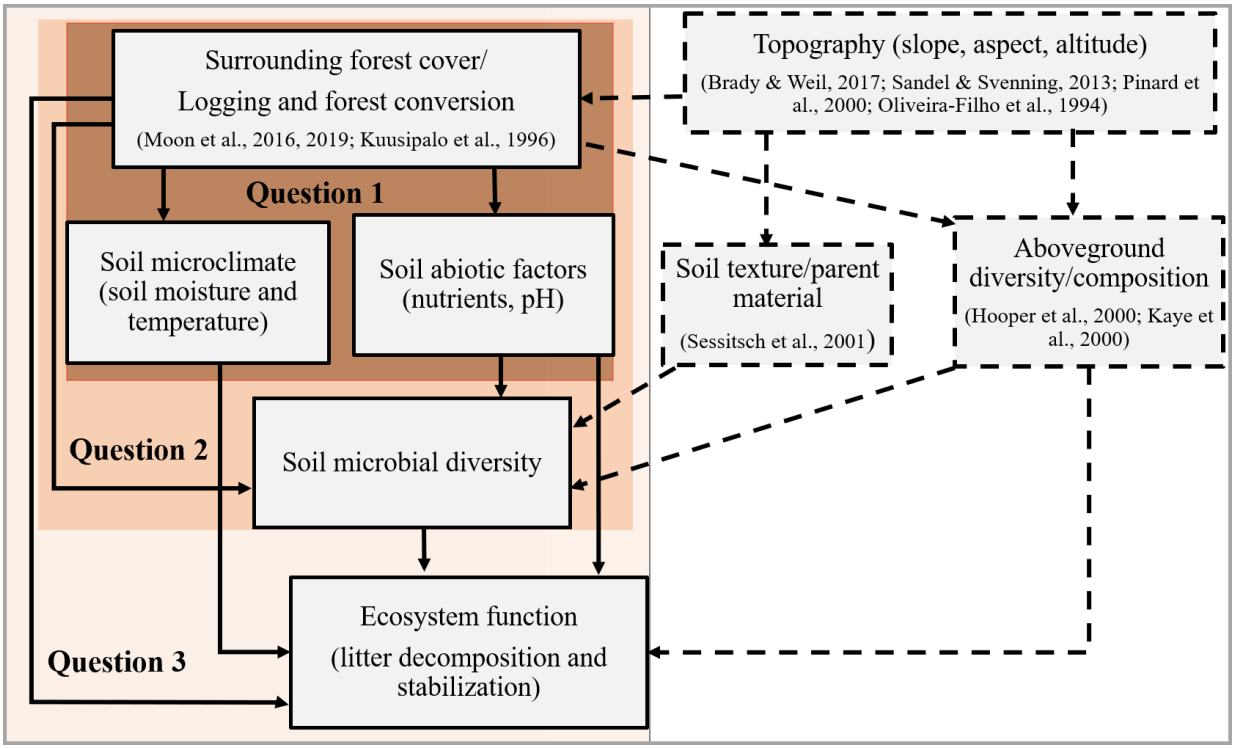

Figure 1. Conceptual diagram of the hierarchical approach and proposed pathways by which anthropogenic activities such as logging and forest conversion affect soil microclimate, soil structure (abiotic (texture, pH, and nutrients) and biotic (bacterial and fungal diversity)), and ecosystem functions (e.g., litter decomposition and stabilization). Arrows indicate directionality and dashed lines indicate factors/effects not measured in this study. Shaded boxes represent the focus area of each question.

2. Materials and Methods

2.1 Study Area

We conducted this study in the Tambunan District of central Sabah, Malaysia (5.71750, 116.40055) (**Figure 2**). The study sites ranged in elevation from 870 to 1150 m asl. This region receives ~1968 mm of rainfall each year and has a mean annual temperature of 24.3 ℃. We chose to study five sites along a land use (i.e., old growth tropical forests, rubber (*Hevea brasiliensis*) plantation, agriculture) and a recovery time gradient (time since the last disturbance ranged from 4–150 years) (**Table 1**). Two of the sites, Mahua Falls Forest (MaF) and Malungung Forest (MuF), were within the federally protected Crocker Range Park area covering about 139,919 ha. The MaF and MuF sites were heavily logged ~100 and ~70 years prior, respectively. The remaining three sites are located in nearby privately-owned lands within the Tambunan Valley. The oldest site (Angelo's Forest, AnF) had not been clear-cut/logged for over 150 years. The rubber site (Rub) is an abandoned rubber plantation, cleared, terraced, and planted 41 years ago. The rubber trees were untapped at the time of the sampling. The agricultural site (Agri) was previously cultivated for chili, cleared again in 2014, and left abandoned for four years prior to sampling.

2.2 Site Land Cover Classification and Analysis

We classified land cover within a 100-m radius buffer of the transect center using a maximum likelihood supervised classification. The buffer represented the area of influence surrounding each study site (Brown and Vivas, 2005). Maximum likelihood supervised classification uses pixel color and pattern recognition aided by user-provided training samples to classify satellite imagery into land cover classes (Parece et al., 2014). Our land cover

classification system was modified from Churches et al. (2014), and included the following land uses: forest, herbaceous/shrub, bare/non-vegetated, water, and no data. High-resolution SENTINEL-2 satellite imagery was obtained from the Copernicus Open Access Hub (https://scihub.copernicus.eu/, last accessed 17 March 2019). SENTINEL-2 products were chosen because of their high spatial resolution (10 m) and availability in our study area. Land cover classification was completed using ArcMap (version 10.6.1, ESRI, Inc., © 1995–2018). Once all pixels within the 100-m radius buffer were classified using the maximum likelihood classification, the number of pixels classified as forest versus pixels classified as non-forest (i.e., herbaceous/shrub, bare/non-vegetated, water, no data) were used to calculate percent forest cover.

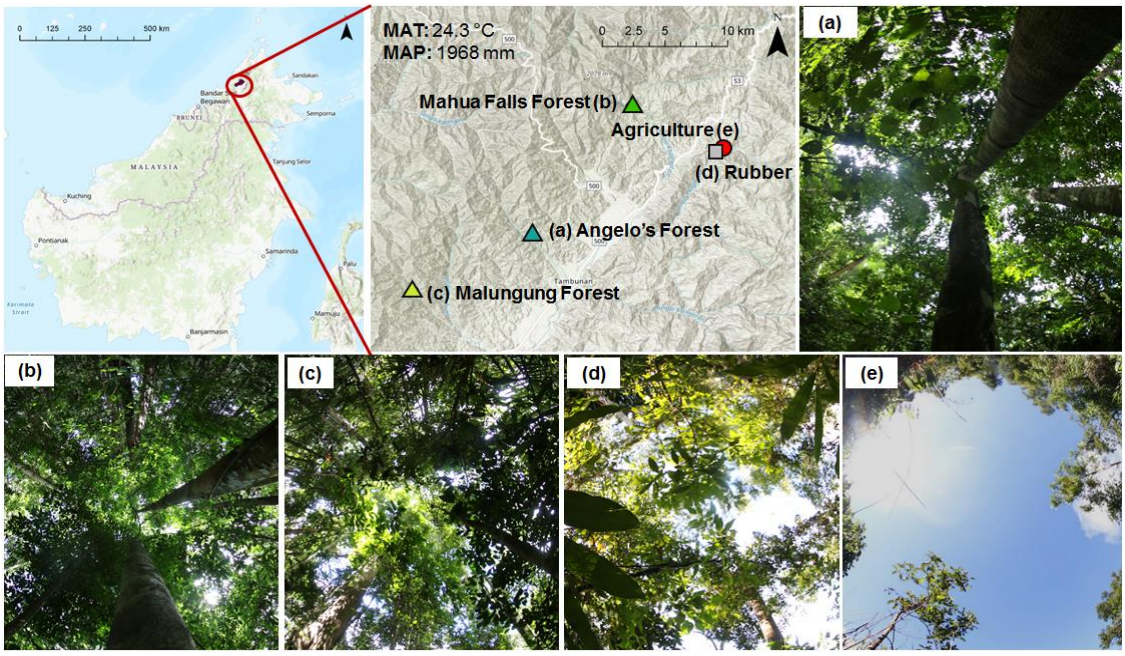

Figure 2. Map of the study area showing the location of the five study sites across a gradient of land use types: Angelo's Forest (AnF, a), Mahua Falls Forest (MaF, b), Malungung Forest (MuF, c), Abandoned Rubber Plantation (Rub, d), and Agriculture (Agri, e), in the state of Sabah, Malaysian Borneo. Sites are displayed using different colors and symbols. The average elevation of these sites is 934 m, mean annual air temperature (MAT) is 24.3 °C, and mean annual precipitation (MAP) is 1968 mm. Canopy photos (a – e) were taken using a Canon EOS 7D Mark II Digital SLR camera with an Altura Ultra Wide Angle Aspherical Fisheye Lens (8 mm f/3.0).

2.3 Field Sampling

Soils in this region are characterized as orthic acrisols, having low base saturation (< 50%) and high clay content in B Horizon (FAO 2009; Chesworth, 2008). We collected soil samples at 5-m intervals along a 45-m transect positioned along a slope gradient, to sample maximum within-site variability, at each of the five sites in June 2018 (**Supplementary Figure S1, Table 1**). Ten composite samples were created along each transect using 2 – 5 soil cores (i.e., 6.35-cm diameter x 5-cm depth) collected within 1 m of each plot center (**Supplementary Figure S1**). Due to extremely rocky conditions at AnF, we were unable to utilize the 6.35-cm diameter soil auger and used a 2.54-cm diameter corer for soil sampling instead. We removed large roots, rocks and undecayed litter from the samples and our samples. The soil samples include both organic and mineral soil. We homogenized each sample by vigorously shaking soil in a plastic sample bag, which broke large soil aggregates and mixed the multiple cores collected for each sample. We then immediately preserved a 1 g subsample in RNALater stabilization solution (ThermoFisher Scientific), a total of 10 samples per site. The preserved samples were shipped to the Molecular Research Laboratory (MR DNA, Shallowater, TX, USA) for microbial analysis. Due to relatively low seasonal fluctuations in temperature and precipitation in this region, we assume that microbial diversity would stay fairly constant during the growing season. Average near-surface air temperature measured across the study sites was 20.54 ± 0.05 and 20.45 ± 0.03 ℃ in June and July, respectively. Average relative humidity was 90.85 ± 0.19 and 93.01 ± 0.11 % in June and July, respectively. While we did not collect precipitation data, precipitation stayed constant during June and July, with moderate to heavy rain nearly every afternoon. In the remaining samples, we removed rocks, litter, roots and other large, undecayed organic material. We then air-dried, ground (using a pestle and mortar), and sieved (2 mm) the soil to remove non-

soil fractions and shipped the soil samples to the Texas Tech University's soil laboratory (contact person: Dr. David Weindorf) for analysis of soil physical and chemical properties (i.e., elemental characterization, pH, texture, total C and N, % soil organic matter). We measured surface soil (0 – 5 cm) temperature (digital soil thermometer probe, HANNA Instruments) in the field and collected additional soil samples for gravimetric soil water content. Soil samples collected for gravimetric water content measurement were stored in air-tight containers at ambient temperature prior to analysis.

Table 1. Study site description including geographical location (decimal degrees), altitude (m +/- SE), slope (% +/- SE), dominant vegetative cover, time since disturbance (TSD, years), and current/historical land use.

Site Name	Latitude (decimal degrees)	Longitude (decimal degrees)	Altitude (m) ± SE	Slope (%) ± SE	Slope Aspect	Vegetation	TSD (Years)	Land Use (Current/Historical)
Angelo's Forest (AnF)	5.713	116.335	900 ± 5	44 ± 19	WNW	Mixed Dipterocarp forest	>150	Not clear cut for >150 years; Currently forested.
Mahua Forest (MaF)	5.797	116.406	1140 ± 2	78 ± 0	NNE	Mixed Dipterocarp forest	100	Clear cut ~100 yrs ago; Selective logging until approximately 1983. Currently forested.
Malungung Forest (MuF)	5.661	116.251	862 ± 2	26 ± 4	WNW	Mixed Dipterocarp forest	70	Clear cut ~70 yrs ago; Selective logging until 1993. Currently forested.
Rubber (Rub)	5.765	116.469	879 ± 2	26 ± 4	SSE	Rubber trees, roadside fern	41	Terraced and planted with rubber trees in 1977. Trees currently untapped and abandoned.
Agriculture (Agri)	5.766	116.470	891 ± 1	56 ± 11	SSW	Bamboo; mostly cleared	4	Previously cultivated for chili. Cleared in 2014. Currently used for agriculture.

*Land use history based on personal accounts from private landowners and local land managers.

2.4 Soil Microbial Diversity

We received final operational taxonomic units (OTUs) that were assigned based on 97% similarity and followed the proprietary MR DNA analysis pipeline (Dowd et al. 2008) from the

Molecular Research Laboratory (MR DNA, Shallowater, TX, USA). See appendix S8 for detailed information on molecular sample processing as provided by the lab. We calculated Chao1 Bacterial and Fungal diversity at the OTU level for each soil sample in R (R Core Team, 2017) using the package "SpadeR" (Chao et al., 2016).

2.5 Soil Physicochemical Analysis

We measured soil pH using the saturated paste method, characterized soil elements (aluminum (Al), sulphur (S), potassium (K), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), lead (Pb)) using portable x-ray fluorescence (pXRF), determined soil organic matter (SOM, %) concentration using the loss-on-ignition method, estimated total C and N using the Dumas method, and determined soil texture (i.e., % sand, silt, clay) using a Hydrometer particle size analysis (152H Hydrometer). Due to insufficient sample size, we could only estimate soil texture of 28 soil samples out of 50 (**Supplementary Figure S2**). We measured gravimetric water content using the oven-dry method (i.e., 60 ℃ until sample weight remained constant).

2.6 Litter Decomposition and Stabilization

Inherent differences in litter quality across sites pose a complication when studying changes in microbial decomposition rates following disturbance (Keuskamp et al., 2013). The Tea Bag Index (TBI) method employs a standardized litter (tea) bag technique to compare plant litter decomposition and stabilization across sites without the influence of litter quality; this method focuses on identifying environmental factors that influence decomposition rates across sampling locations (Keuskamp et al., 2013). We buried 10 pairs of pre-weighed teabags (i.e.,

nonwoven tetrahedron-shaped polypropylene tea bags, Lipton, Unilever), one each green tea (fast decomposing litter) and rooibos tea (slow decomposing litter), at 8-cm below surface. After 72 to 77 days, we retrieved the teabags and manually removed debris and roots (*Tea Bag Index: Scientific Protocol*, 2016). We used the initial and final oven-dried (70 ℃ for 48 hours) masses to calculate decomposition rate (k, day^{-1}) and stabilization factor (S, unitless).

2.7 Statistical Analyses

First, we checked the correlation between different measured variables by generating a correlation matrix in R using the package "corrplot" (Wei and Simko, 2017) (**Supplementary Figure S3**). We then used SigmaPlot 14.0 (Systat Software, Inc., San Jose California USA) to display and fit the statistical models to the bivariate relationships between time since last major disturbance and different soil properties. All statistical models were checked for the significance and alpha value, and only significant ($p < 0.05$) models are displayed (**Figure 3**).

We used the "randomForest" package (Liaw and Wiener, 2002) in R to rank the importance of predictors in explaining bacterial diversity, fungal diversity, litter decomposition rate, and litter stabilization (**Figure 4**). Random forest is an ensemble machine learning technique that creates a series of uncorrelated regression trees from random subsamples of the dataset. These trees decide as a committee which variables are the most predictive, and rank the variables in order of their ability to increase the mean square error when removed from the model (Breiman, 2001). Before running the random forest analysis, we removed highly correlated variables ($|r| > 0.70$), retaining the most ecologically relevant and/or proximal predictor variables (Dormann et al., 2013). Through this process Fe, C, Mn, S, K, C:N, and Zn were removed from the random forest analysis. Although time since disturbance and pH were

highly correlated (|r| = 0.82), we chose to leave both variables in the analysis because of their unique ecological contributions to the model. To ensure that the importance given to each predictive variable would not be biased by the presence of the two correlated variables, we ran the random forests analyses again removing each of the two correlated variables one at a time and then simultaneously. We found reductions in the overall variance explained by the model, however the top predictive variables for each model were the same – excluding the variable(s) removed. Therefore, we concluded that the correlation of the variables had little effect on our model and chose to include them both in the final analyses. One extreme fungal diversity outlier was also removed from the random forest and bivariate analyses, but is displayed in figures and noted with an asterisk. We built 200 trees (ntrees = 200) for each run and sampled 3 – 4 variables (Mtry = 3 – 4, depending on the number of predictor variables) with replacement for each. We then took the variables with the highest importance and used bivariate analysis to further explore dominant trends in the data (**Figures 5, 6, Supplementary Figures S4, S5**). Models of bivariate plots were fitted using SigmaPlot 14.0 (Systat Software, Inc., San Jose, California, USA).

3. Results

3.1 Effect of Time Since Disturbance on Soil Physicochemical Properties

Average soil pH at our sites ranged from 3.7 – 5.4, with the highest pH at AnF and the lowest at Rub (**Table 2**). Daytime soil temperature had a fairly narrow range, 19.9 – 21.5 ℃, across sites. Soil water content ranged from 0.4 – 1.01 g/g, with water content over 100% at MaF corresponding with relatively high %SOM. Percent SOM varied across the sites (i.e., 3.4 – 10.8%), with the greatest SOM concentration at MaF and the lowest at Agri. The C:N ratio ranged from 8.0 – 13.0. Surface soil textures were characterized based on the USDA

classification system as sandy clay (AnF, MaF), clay loam (MuF), and clay (Rub, Agri). (**Supplementary Figure S2**).

We found a strong link between the time since disturbance and soil physicochemical properties **(Figure 3, Supplementary Figure S6**). Our results show a linear increase in pH and total N with the time since disturbance; and a peaked response of sulfur, total C, SOM and % sand to the time since disturbance with the peak at ~100 years (**Figure 3**). Time since disturbance was not significantly related to daytime soil temperature or gravimetric water content, however daytime soil temperature had a linear negative relationship with surrounding forest cover ($p < 0.001$, $R^2adj = 0.26$, $n = 50$) (**Supplementary Figure S7**).

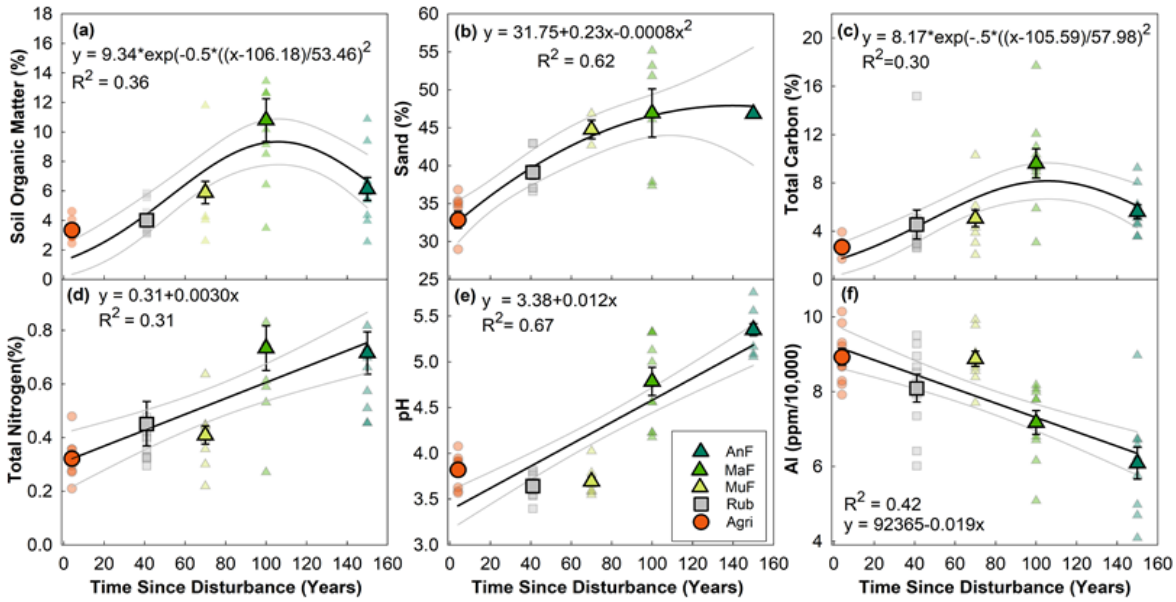

Figure 3. Relationship between the time since last major disturbance (years) and soil properties across a gradient of land use types: Angelos Forest (AnF), Mahua Falls Forest (MaF), Malungung Forest (MuF), Abandoned Rubber Plantation (Rub), and Agriculture (Agri), displayed using different colors and symbols. Smaller transparent symbols represent the observed data, while larger solid symbols represent the site means. Bars represent the standard error of the mean, black solid lines represent model means and solid gray lines represent 95% confidence intervals. All statistical models were run on observed data and significant at $p < 0.05$, except the relationship between % sand and time since disturbance ($p < 0.1$). Sample size for all variables is 10, except for % sand (i.e., AnF: n = 1, MaF: n = 6, MuF: n = 3, Rub: n = 8, Agri: n = 10) due to the logistical difficulties of collecting enough soil for the analysis.

Table 2. Soil properties from the five study sites, including pH in water, gravimetric water content (g/g), daytime soil temperature (°C), soil organic matter (%), and carbon to nitrogen ratios (C:N). Sites are arranged from longest to shortest time since disturbance.

Site Name	pH	Soil Water Content (g/g)	Soil Temp (°C)	Soil Organic Matter (%)	C:N
Angelo's Forest (AnF)	5.35 ± 0.07	0.47 ± 0.04	21.09 ± 0.06	6.13 ± 0.78	8.04 ± 0.57
Mahua Forest (MaF)	4.79 ± 0.15	1.01 ± 0.16	19.92 ± 0.09	10.80 ± 1.45	12.97 ± 0.50
Malungung Forest (MuF)	3.70 ± 0.04	0.40 ± 0.11	21.25 ± 0.11	5.89 ± 0.76	11.94 ± 0.67
Rubber (Rub)	3.65 ± 0.04	0.54 ± 0.03	21.54 ± 0.03	4.03 ± 0.31	9.44 ± 0.49
Agriculture (Agri)	3.82 ± 0.05	0.51 ± 0.08	20.96 ± 0.08	3.36 ± 0.20	8.32 ± 0.12

3.2 Factors Affecting Soil Microbial Diversity

We obtained a total of 3,439,724 fungal and 2,920,915 bacterial high-quality sequences among the 50 samples, averaging 68,794 and 58,418 reads per sample, respectively. These were assigned to 15,980 fungal and 25,106 bacterial OTUs at 97% similarity. Our results show that random forest analysis explained 67.08% of the total variability in Chao1 bacterial OTU diversity (hereafter, bacterial diversity) and 18.69% of the variability in Chao1 fungal OTU diversity (hereafter, fungal diversity) (**Figure 4**).

3.2.1 Bacterial Diversity

Bacterial diversity shows a peaked response with soil pH with peak at ~ 5 ($p < 0.001$, $R^2adj = 0.63$); a saturating response with time since disturbance ($p \leq 0.01$, $R^2adj = 0.56$); and a linear increase with the surrounding forest cover ($p < 0.001$, $R^2adj = 0.43$) (**Figure 5**). Daytime soil temperature and SOM are weaker predictors of bacterial diversity (**Figure 4a**). Bacterial

diversity shows a saturating response with SOM ($p < 0.001$, $R^2adj = 0.29$, $n = 50$) and a linear decline with increasing daytime soil temperature ($p < 0.001$, $R^2adj = 0.21$, $n = 50$) (**Supplementary Figure S4**).

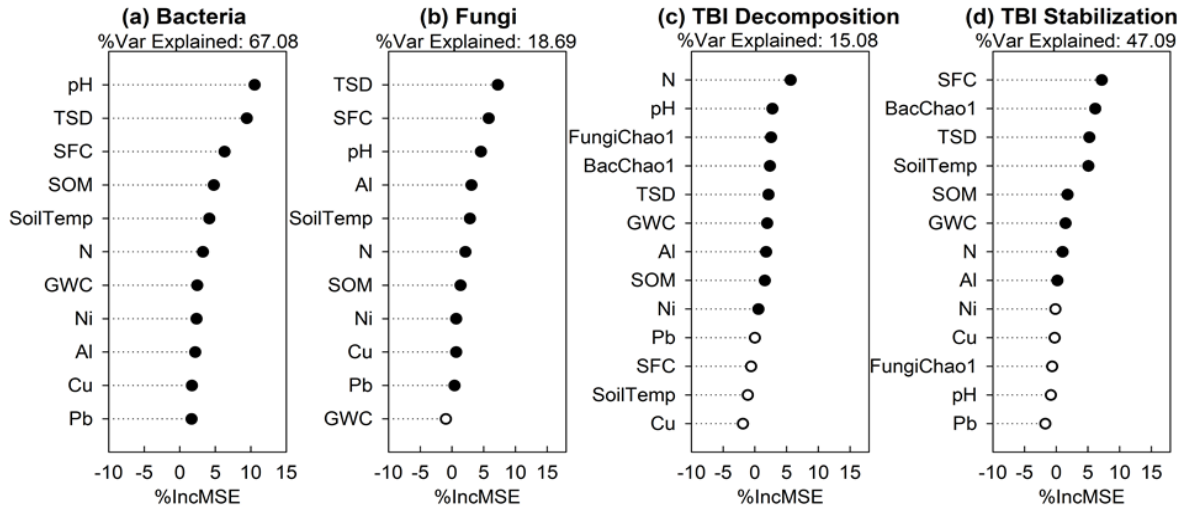

Figure 4. Importance of each predictor for Chao1 bacterial OTU diversity (a) Chao1 fungal OTU diversity (b) TBI decomposition rate (c) and TBI stabilization factor (d) using random forest analyses. Predictors included: TSD (time since disturbance, years), SFC (surrounding forest cover, %), soil pH, SOM (soil organic matter, %), Soil Temp (daytime soil temperature, °C), N (total soil nitrogen, %), GWC (gravimetric water content, g/g), Ni (ppm), Al (ppm), Cu (ppm), and Pb (ppm). The x-axis displays the mean importance of each predictor, represented by the increase in mean square error (%IncMSE) following predictor permutations. The y-axis displays the predictors in order of their mean variable importance. Unfilled symbols indicate a negative importance value, meaning variable has no predictive power. For each analysis, ntree (number of trees grown) was 200, and mtry (number of predictors sampled at each node) was as follows: mtry = 3 (a,b) and mtry = 4 (c,d).

3.2.2 Fungal Diversity

Fungal diversity increases with time since disturbance with a saturating response ($p < 0.001$, $R^2adj = 0.34$) (**Figure 5d**). Surrounding forest cover is the second most important predictor of fungal diversity, with a positive linear relationship ($p < 0.001$, $R^2adj = 0.21$, **Figures 4b, 5e**). Among soil properties, soil pH is the best predictor of fungal diversity (**Figures. 4b, 5f**), and shows a peaked response similar to bacterial diversity, followed by soil Al concentration,

and daytime soil temperature (**Figure 4, Supplementary Figure S4**). Soil Al concentration displays a weak inverse relationship with fungal diversity ($p \leq 0.01$, $R^2adj = 0.09$, $n = 50$). Fungal diversity showed a slight decrease with increasing daytime soil temperature, however the relationship between the two is not significant ($p > 0.1$, $n = 49$) (**Figure S4**).

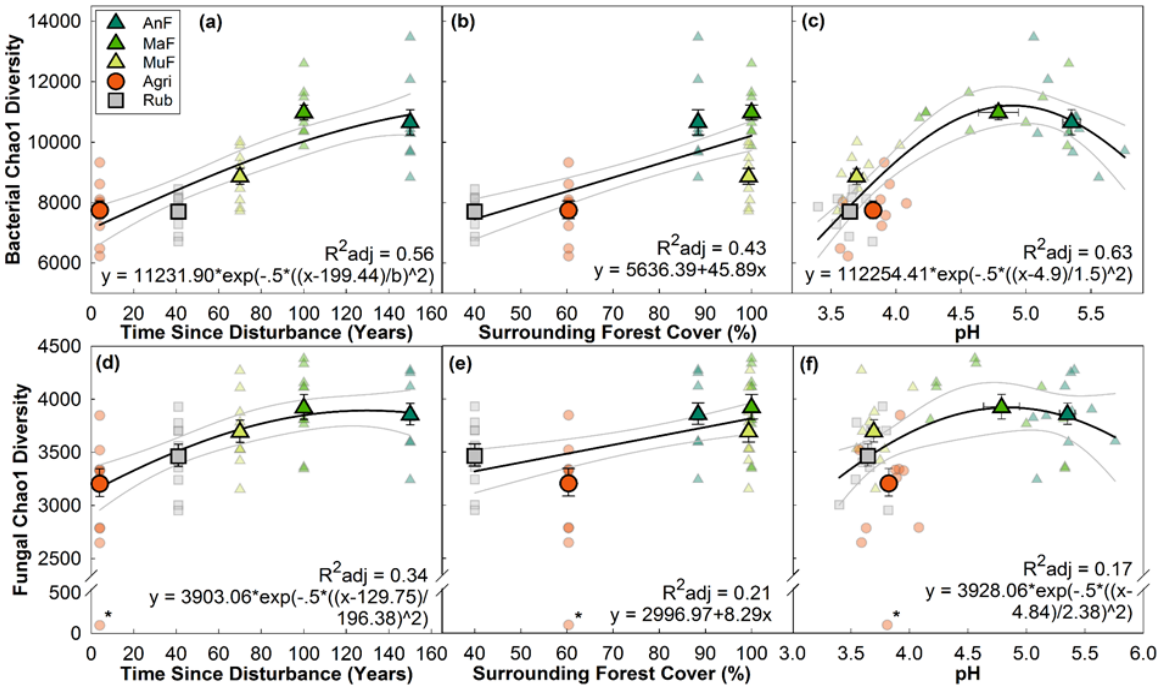

Figure 5. Relationships between best predictors (from random tree analysis) of Chao1 microbial OTU diversity (bacterial (a,b,c) and fungal (d,e,f)) across a gradient of land use types: Angelos Forest (AnF), Mahua Falls Forest (MaF), Malungung Forest (MuF), Abandoned Rubber Plantation (Rub), and Agriculture (Agri), displayed using different colors and symbols. Smaller transparent symbols represent the observed data, while larger solid symbols represent the site means. Bars represent the standard error of the mean, solid black lines represent model means and gray lines represent 95% confidence intervals. All statistical models were run on observed data and significant at $p < 0.001$, except the relationship between Chao1 bacterial OTU diversity and time since disturbance ($p \leq 0.01$). All sites had a sample size of 10, except for Agri, where $n = 9$ for (d), (e), and (f). One extreme outlier indicated with asterisk on (d), (e), and (f) was not included in the regression analyses.

3.3 Factors Affecting Litter Decomposition Rate and Stabilization

3.3.1 Comparison with Global Tea Bag Index Data

Our results match the global pattern (Keuskamp et al. 2013) of the relationship between litter decomposition rate and litter stabilization factor (**Figure 6**). The litter decompose faster in the two old growth forests (AnF = 0.032 and MaF = 0.036 day^{-1}), and the litter decomposition rates are comparable to the other lowland tropical forests from global data (**Figure 6**). All three naturally regenerating forests show relatively high litter stabilization factors (AnF = 0.239, MaF = 0.245, and MuF = 0.242) similar to stabilization factors for mixed and birch forests (**Figure 6**). Interestingly, the younger natural forest (MuF) closely matched the agriculture (Agri) site with intermediate litter decomposition rates (MuF = 0.020, Agri = 0.016 day^{-1}) and relatively high litter stabilization (MuF = 0.242, Agri = 0.201). Contrastingly, rubber plantation had a low litter stabilization factor (0.070) similar to the lowland tropical forest, coupled with an intermediate decomposition rate (0.024 day^{-1}), placing it near the geothermal wet grassland ecosystem within the global dataset.

3.3.2 Factors Affecting Litter Decomposition Rate

Overall random forest analysis explained 15.08% overall variance in the litter decomposition rate. The most important predictor of litter decomposition rate is soil N (**Figure 4c**) followed by soil pH and soil microbial diversity. Decomposition increases linearly with increasing soil N ($p < 0.05$, $R^2adj = 0.22$, $n = 41$) (**Supplementary Figure S5a**) and shows a peaked response with soil pH with peak around 5 ($p < 0.05$, $R^2adj = 0.13$, $n = 41$) (**Supplementary Figure S5b**). The litter decomposition rate increased with increasing fungal (p

≤ 0.05, **Figure 7a**) and bacterial ($p < 0.001$, **Figure 7b**) diversity. Random forest analysis found time since disturbance to have little effect on litter decomposition.

3.3.3 Factors Affecting Litter Stabilization (*S*)

Random forest analysis explained 47.09% of the overall variance in litter stabilization (**Figure 4d**). Percent onsite forest cover explained the most variance in litter stabilization, followed by bacterial diversity, time since disturbance, and daytime soil temperature (**Figure 4d**). Litter stabilization increased significantly with surrounding forest cover with a saturating response ($p < 0.005$, $R^2adj = 0.63$); where the average litter stabilization at the three oldest forested sites is consistent at 0.24 (unitless). Litter stabilization increases linearly with increasing fungal diversity ($p < 0.001$, **Figure 7c**) and shows a saturating response with bacterial diversity ($p < 0.001$, **Figure 7d**). Litter stabilization shows a peaked relationship with daytime soil temperature, with the peak between 20 – 21 ℃ ($p < 0.001$, $R^2adj = 0.22$). While random forest analysis identified time since disturbance as the second most important predictor of litter stabilization, bivariate analysis revealed no significant relationship between the two (**Figure S5**).

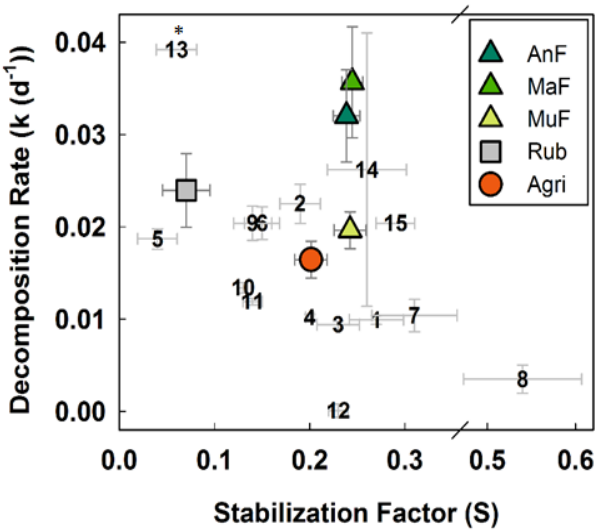

Figure 6. Relationship between litter stabilization factor (*S*, unitless) and decomposition rate (*k*, day^{-1}) compared with global data set from Keuskamp et al. (2013). Filled symbols represent site means: Angelos Forest (AnF), Mahua Falls Forest (MaF), Malungung Forest (MuF), Abandoned Rubber Plantation (Rub), and Agriculture (Agri), while numbered symbols represent a range of sites: mangrove (1, 2); oceanic raised bog (3, 4); grassland (5, 6); semi-arid desert (7, 8); forest (9); wet forest (10); pasture (11); floating fen (12); lowland tropical forest (13); mixed forest (14); birch forest (15). Bars represent the standard error of mean. *Vertical error bar absent due to overdispersion. Global data was created using TBI 1.0 (woven tea bags), while tropical montane data (this paper) was created using TBI 2.0 (non-woven tea bags).

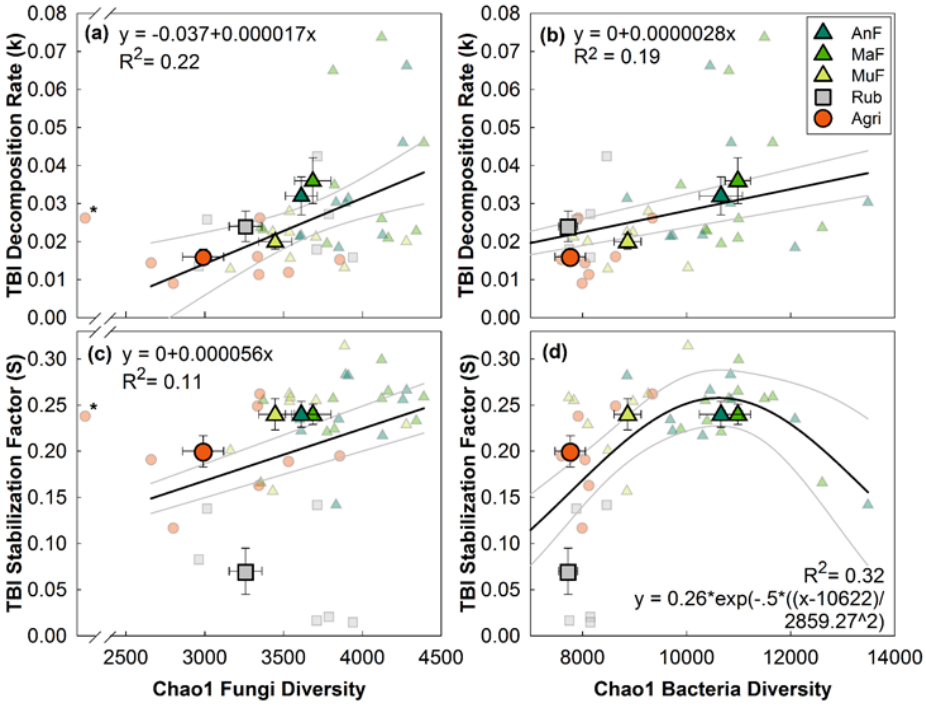

Figure 7. Relationship between Chao1 microbial © diversity (fungal (a,c) and bacterial (b,d)) and litter decomposition rate (*k*, day^{-1}) and stabilization factor (*S*, unitless) across a gradient of land use types: Angelo's Forest (AnF), Mahua Falls Forest (MaF), Malungung Forest (MuF), Abandoned Rubber Plantation (Rub), and Agriculture (Agri), displayed using different colors and symbols. Smaller transparent symbols represent the observed data, while larger solid symbols represent the site means. Bars represent the standard error of the mean, solid black line represents mean model and solid gray lines represent 95% confidence intervals. All statistical models were run on observed data and significant at $p < 0.0001$, except the relationship between Chao1 fungal © diversity and decomposition rate, ($p \leq 0.05$). Sample sizes varied for each analysis plot due to logistics. Sample sizes were: AnF (n = 9), MaF (n = 10), MuF (n = 8), Rub (n = 6), and Agri (n = 8); except for plots (a) and (c) where Agri (n=7). One extreme outlier indicated with asterisk on (a) and (c) was not included in the regression analyses.

4. Discussion

4.1 Effect of Time Since Disturbance on Soil Physicochemical Properties

Logging and forest conversion can have lasting impacts on soil physicochemical conditions (Waldrop et al., 2000). We compare soil properties at five sites across a land use disturbance gradient, with sites ranging from 4 to over 150 years of post-disturbance

regeneration. As expected, the number of years since disturbance significantly affected soil physicochemical properties (**Figure 3**). Soil pH significantly changed along the disturbance gradient, increasing from 3.7 at the youngest site (Agri) to 5.4 at the oldest site (AnF) **(Figure 3)**. This pH range is similar to the range reported by Tripathi et al. (2016a) across a forest disturbance gradient in Sabah, Borneo, and is consistent with the previous studies that reported lower soil pH following the conversion of tropical forests to plantations (Alele et al., 2014; Waldrop et al., 2000; Templer et al., 2005). Lower pH levels measured in recently recovered sites could be attributed to recent harvesting. For example, plants are capable of altering the pH of the rhizosphere and bulk soil through ion uptake, and removal of soil cations such as Ca^{2+} and Mg^{2+} can lead to soil acidification when plant material is removed from a site during harvest (Rengel, 2003). Previous logging and cultivation of the sites may have resulted in a decrease in soil pH, with soil pH gradually increasing over time in older forests with organic matter decomposition (Allen, 1985).

We found that soil C, N, S, and SOM generally increased with time since disturbance, with soil C, S, and SOM beginning to decline at ~100 years post disturbance (**Figures 3, Supplementary Figure S6**). Our results are consistent with prior work that reported higher concentrations of total soil C, soil N (Dinesh et al., 2003), and SOM (Templer et al., 2005) in forested sites compared to both agricultural and plantation sites, mainly attributed to a decrease in quantity and quality of plant residues returned to the soil. Though there is no information on how S changes along a time since disturbance gradient, soil S concentrations can be tightly coupled with total soil C content and SOM levels (Kirkby et al., 2011). Our results reflect this nutrient coupling, with the concentration of soil S increasing proportionally with soil C along the disturbance gradient. Previous studies have observed significant losses of soil nutrients due to

leaching (Brouwer and Riezebos, 1998), biomass removal (Martinelli et al., 2000; Olander et al., 2005), and altered nutrient allocation (Swinfield et al., 2020) associated with logging. The increased soil C pools and nutrients along the time since disturbance gradient in our results are possibly due to the soil nutrient stocks rebuilding to pre-disturbance levels.

While SOM and soil C increased linearly until ~100 years, we did see a slight decline at our oldest site, AnF (**Figure 3**). Given that rates of organic matter accumulation typically slow over time (Silver et al., 2000), the saturating relationships we observed were not surprising. Percent SOM has been found to return to pre-disturbance levels within 40–50 years of succession in tropical forests (Brown and Lugo, 1990), however, other studies have observed this to occur in less than a decade (Templer et al., 2005). The length of time required to accumulate organic matter in the soil is variable and can be affected by vegetation (i.e., quantity and quality of litter inputs), abiotic and biotic soil properties (i.e., soil texture and microbial activity), and land use history (Post and Kwon, 2000). Furthermore, inherent differences among our forested sites in terms of soil texture, the oldest site (AnF) had rocky terrain, could have resulted in the high variability and lower amounts of SOM as compared to the other old growth forested site (MaF). Similar to soil C and SOM, the C:N ratio peaked at ~100 years and subsequently declined at the oldest site, due to the combined effects of total soil C decreasing and total soil N increasing (**Figure 3**). Nitrogen fixing organisms, particularly microbial plant symbionts, are considered an important source of new N in tropical soils (Gehring et al., 2005). Some late-successional plant species have higher leaf N content, with correspondingly high soil N content, as compared with early-successional forests (Yan et al., 2006). Higher N content in litter could result in enhanced decomposition rates, driving soil C and organic matter levels down.

Soil texture also changed significantly along the time since disturbance gradient; % sand increased with time with a saturating response around 100 years post-disturbance. Fine particles can be lost from soil following disturbance events such as tillage due to erosion and runoff, leaving a higher percentage of larger particles such as sand (Brady and Weil, 2017). Our results contradict these findings and could be a result of variations in soil parent material, however our study did not assess soil parent material. Because our study did not specifically focus on the differences in parent material and natural erosion patterns, it is difficult to determine the cause for the observed increase in % sand over time. It is also possible that the selection of fertile, low sand content soils for agriculture and rubber plantation development led to the observed patterns.

While we found no significant effects of time since disturbance on soil microclimate, we found a decrease in daytime soil temperature with increasing forest cover (**Figure S7**). Removal of the forest canopy during logging events increases daytime soil temperature (Yashiro et al. 2008), and reforestation can have significant surface cooling effects (Novick and Katul, 2020). Tropical secondary forests in Borneo can maintain a similar near-surface microclimate to unlogged forests through thermal buffering (Jucker et al., 2018), however rubber monocultures have shown to be warmer and drier than naturally forested areas (Meijide et al., 2018). While our sites did vary in terms of their forest cover, all of our sites had ample cover to support thermal buffering. However, we did find a significant relationship between surrounding forest cover and daytime soil temperature.

4.2 Factors Affecting Soil Microbial Diversity

Logging alters biodiversity and community composition of plants and animals through the introduction of edge-tolerant species and the decline of forest-interior specialists (Hill and

Curran, 2003; Struebig et al., 2013; Wells et al., 2007). We found that both bacterial and fungal diversity increased significantly with time since disturbance until ~100 years, which is consistent with prior studies in subtropical (Zhu et al., 2010) and Norway spruce (Wallander et al., 2010) ecosystems that found increasing microbial diversity with stand age. This increase in microbial diversity over time may reflect spatial heterogeneity in accumulation of SOM (Grayston et al., 1998; Klein et al., 1995) or increased variability in litter quality and quantity as a result of changes in plant diversity (Zhu et al., 2010) as forests mature.

4.2.1 Bacteria

We found that the surrounding forest cover, a proxy of current surrounding land use, is a significant predictor of bacterial diversity. Human-dominated landscapes can affect the ecological communities and processes of adjacent areas through the introduction of pollutants, physical damage of the site (Brown and Vivas, 2005), and through biotic homogenization (Devictor et al. 2008; Lôbo et al. 2011). The lowest bacterial diversity is found in the landscapes dominated by human activities (Rub and Agri). These sites appear to be frequented by humans and/or animals based on the existence of social trails, evidence of grazing (Agri), amount of exposed soil, and the proximity to inhabited areas. The three naturally regenerated forested sites (MuF, MaF, and AnF) were relatively undisturbed and surrounded by a large tropical forest buffer. While it is impossible for us to pinpoint the exact mechanism through which surrounding forest cover is related to bacterial diversity, previous research suggests that anthropogenic disturbance such as conversion to pasture or plantation can induce biotic homogenization of the soil bacterial community, leading to the loss of some more endemic taxa and to the broader dispersal of the existing taxa within a site (Alele et al., 2014; Rodrigues et al., 2013). This loss in

bacterial diversity is likely to be better quantified by the Chao1 diversity index, used in this study, as it is weighted by rare taxa.

Our results show that soil pH is the strongest overall predictor of bacterial diversity which is consistent with the previous work on bacterial diversity in the tropics (Tripathi et al., 2012; Tripathi et al., 2016b), temperate forests, grasslands (Bahram et al., 2018; Fierer and Jackson, 2006) and alpine meadows (Shen et al. 2019). Bacterial diversity shows a peaked response with soil pH with peak around 5, which is consistent in pattern with the prior work in European forests and grasslands (Kaiser et al., 2016) and Malaysian tropical forests (Tripathi et al., 2012), but differs in the peak from one of the studies (Tripathi et al. 2012)., where diversity peaked at near-neutral pH. Bacteria are known to experience physiological constraints at extreme pH and optimum soil pH varies among bacterial taxa (Tripathi et al., 2012). However, the intracellular pH of bacteria is often close to neutral (Krulwich et al., 2011; Slonczewski et al., 1981), and a more extreme external pH may place stress on bacterial cells (Lauber et al., 2009). Moreover, an increase in abundance of a particular taxa can lead to a decrease in overall bacterial diversity (Wood et al., 2017). The current understanding of bacterial diversity response to pH suggests that changes in bacterial diversity may be the result of pH-driven changes in availability of various soil nutrients (Lauber et al., 2009; Tripathi et al., 2012). Controlled experiments are needed to clarify the relationship between pH and bacterial diversity through both indirect and direct pathways.

Soil bacterial communities rely on the decomposition of organic matter for energy (Trivedi et al., 2013) and as such, SOM has previously been identified as one of the main drivers of soil bacterial diversity on a global scale (Delgado-Baquerizo et al., 2016; Tian et al., 2018).

However, our results show a weak correlation between bacterial diversity and SOM with a saturating response after about 8% SOM.

Previous studies in the tropics have also made links between a decline in bacterial diversity with increasing soil temperatures (Tripathi et al., 2016b) and increasing mean annual air temperatures (Nottingham et al., 2018). A study from a geothermal field (soil temperatures ranging from 50 – 90 ℃) in China also found bacterial diversity to be lower in warmer soils (Li et al., 2015). While average daytime soil temperature across our sites shows only ~2 ℃ difference, this was sufficient to produce a negative effect on bacterial diversity at greater temperature (**Figure S4**). These results are consistent with prior work (Li et al., 2015) that suggest that higher soil temperatures can lead to lower microbial diversity.

4.2.2 Fungi

Our results demonstrate a strong positive relationship between time since disturbance and fungal diversity with a saturating response after ~ 100 years (**Figure 5**). Soil disturbance and homogenization from tillage and logging have shown to have a negative effect on fungal communities (Brinkmann et al., 2019; Doran, 1980; van Groenigen et al., 2010). The most recently disturbed sites (Agri and Rub) show lower variation along the transect for a number of soil parameters including pH, SOM, and total C content. These findings suggest that the sites incurred resource homogenization during forest clearing, cultivation, and continued anthropogenic disturbance. Nearby roads and structures, social trails, and grazing animals that frequented the area also contributed to the anthropogenic impact on these sites and potentially to shifts in fungal diversity.

Many fungal species (e.g., namely ectomycorrhizal fungi), are plant-symbionts and often exhibit strong host tree preference (Tedersoo et al., 2008). Thus, the alteration of tree species composition and abundance can also affect fungal community structure and diversity (Gao et al., 2013; Urbanová et al., 2015). We found that the current surrounding forest cover (i.e., cover by a mature tree canopy) is a significant predictor of fungal diversity, with younger sites (Agri and Rub) exhibiting the lowest levels of fungal diversity, and mature forests exhibiting higher fungal diversity (**Figure 5**). Kerfahi et al. (2014) found lower β-diversity of fungal species in oil palm plantations and lower ectomycorrhizal fungi abundance in logged areas and oil palm plantations compared to tropical forests in Borneo. These shifts in abundance were partially attributed to a decrease in host roots, as Dipterocarp species were absent from the oil palm plantations (Kerfahi et al., 2014). Future studies in this region, relating plant community cover, composition and root structure to the relative abundance of fungal taxa would aid in establishing these linkages.

We show that soil pH and soil Al concentrations, which were negatively correlated with each other ($|r| = 0.66$), are the strongest soil physicochemical predictors of fungal diversity (**Figure 4**), similar to a previous study (Zheng et al., 2019) that reported increasing fungal diversity with increasing soil pH. Though soil pH has frequently been identified as a strong driver of bacterial community composition and diversity (Fierer and Jackson, 2006; Lauber et al., 2009; Tripathi et al., 2012; Tripathi et al., 2016b), the effect of pH on fungal communities is found to be much weaker (Lauber et al., 2008; Rousk et al., 2010). Unlike bacteria which appear to be sensitive to suboptimal pH levels, fungi show less specificity to soil pH (Rousk et al., 2010). Soil pH may be more strongly related to significant shifts in fungal community composition (Rousk et al., 2010; Zheng et al., 2019). The shifts in relative abundance of

dominant fungal taxa may (Dumbrell et al., 2010; Kyaschenko et al., 2017) or may not be associated with shifts in fungal diversity.

Our results (**Figure 4**) also suggest a weak negative relationship between daytime soil temperature and fungal diversity. Previous studies have shown mixed effects of soil temperature on fungal diversity, including some which found little to no effect (Cao et al., 2020; Looby and Treseder, 2018; Tripathi et al., 2016b). For example, Tripathi et al. (2016b) found no relationship between soil temperature and fungal diversity in a tropical rainforest in Borneo, while Looby and Treseder (2018) found higher fungal diversity in warmer soils of a tropical montane forest in Costa Rica. Further, Cao et al. (2020) found ectomycorrhizal fungi diversity was significantly lower in experimentally warmed plots compared to control plots in a subtropical forest in China. It is likely that these differences can be explained by variation in their mechanistic underpinnings; fungal diversity can be directly affected by variation in microbial metabolic activity and growth rates (Brown et al., 2016), and indirectly affected by changes in ecosystem productivity and increased plant diversity (Brown et al., 2016). Analysis of the daytime soil temperature at our study sites over a longer time period and the incorporation of climatic variables such as mean annual temperature could further our understanding of the effects of temperature on fungal diversity in this system.

Overall, random forest analysis explained 18.69% of variation in fungal diversity. The lack of strong direct ties between fungal diversity and soil physicochemical properties could be tied to the fungal scale of operation; fungal hyphae give it the ability to access a larger volume of soil, making fungi more able to survive in a variety of soil conditions, while bacteria are more heavily impacted by local substrate and nutrient availability (Zheng et al., 2019). It is also likely that soil properties that were not included in our analysis would have improved the predictive

power. For example, previous studies have found correlations between ectomycorrhizal fungi diversity and soil P (Tripathi et al., 2016b), and soil texture (Tripathi et al., 2016b; Wakelin et al., 2008); we chose not to include these measures in our analysis due to an insufficient sample size (see Methods, Soil Physicochemical Analysis), however bivariate analysis of the limited soil texture data revealed positive linear relationships between % sand and bacterial and fungal diversity (although relationship between sand and fungal diversity was non-significant). Further, plant community composition and diversity can be important predictors of fungal diversity due to their symbiotic relationships (Gao et al., 2013; Peay et al., 2010). Including these variables in future analysis (e.g., soil P, soil texture properties, plant composition and diversity) may improve our understanding of these agroforestry systems.

4.3 Controls on Litter Decomposition and Stabilization

4.3.1 Litter Decomposition Rate

Our results show that litter decomposition rate fell within the range of previously measured litter decomposition rates from a global dataset assembled by Keuskamp et al. (2013). While time since disturbance isn't a strong predictor of decomposition rates, microbial diversity and some soil physicochemical properties are strongly correlated with litter decomposition. Our analysis suggests that soil N is the most important predictor of litter decomposition (**Figure 4**). Because microbially mediated litter decomposition is generally thought to be nutrient limited (Berg et al., 2007; Crews et al., 1995), it is likely that increases in nutrient levels in these ecosystems increased litter decomposition rates. It is generally accepted that tropical forests are P-limited as opposed to N-limited like many temperate forests (Vitousek, 1984). However, some tropical montane (higher elevation) forest studies show N and P co-limitation (Camenzind et al.,

2018; Tanner et al., 1998). These limitations can be caused by a variety of factors, including differences in litter, precipitation, and temperature (Tanner et al., 1998). A meta-analysis (Knorr et al., 2005a) found that N addition often increased decomposition of high-quality litter, but slowed decomposition of low-quality litter. This pattern is also consistent with the prior studies of N addition in tropical soils (Cusack et al., 2010).

Soil microbial communities are one of the main drivers of soil C cycling through processes such as litter decomposition and stabilization (Gleixner, 2013; Schimel and Schaeffer, 2012). We found that litter decomposition rate increased linearly with increasing fungal and bacterial diversity (**Figure 7**) suggesting a broader metric such as diversity can be used as an indicator of functional shifts in these tropical montane ecosystems. These findings also have significant implications on current debate about diversity versus functional redundancy (Giller et al., 1996) by suggesting that reduced microbial diversity results in reduced functional capabilities of the soil. Functional redundancy among the high diversity of soil microbial taxa (Jurburg and Salles, 2015; Griffiths et al., 2000) confounds the diversity-function relationship, and differences in microbial community composition (Balser and Firestone, 2005; Nielsen et al., 2011; You et al., 2014) or simply the removal of keystone species may have a greater effect on ecosystem function than overall microbial diversity (Nielsen et al., 2011). Previous studies have found mixed results, with some showing little to no effect of microbial diversity on litter decomposition (Degens, 1998; Griffiths et al., 2000) and others finding positive relationships between the two (Baumann et al., 2013; Maron et al., 2018; Meyer et al., 1998; Wagg et al., 2014). Diversity-function studies often use pre-sterilized soil inoculated with a microbial slurry of varying dilutions, or chloroform fumigation (Griffiths et al., 2000), radiation (Philippot et al., 2013), or heavy metal treatments (Meyer et al., 1998) to artificially lower microbial diversity. These

controlled studies can create environments with unnaturally low diversity (Griffiths et al., 2000; Nielsen et al., 2011), or environments with an over-dominance of a single species due to stochastic recolonization following the artificial disturbance (Dumbrell et al., 2010; Lekberg et al., 2012; Nielsen et al., 2011). Given the strong diversity-function links found in this study, we suggest further research to investigate shifts in bacterial and fungal diversity and their relationships to other functional shifts in natural environments.

Climate is regarded as another important driver of litter decomposition (Berg et al., 1993; Keuskamp et al., 2013; Trofymow et al., 2002). Several studies, each spanning numerous ecoclimatic regions, have found significant increase in decomposition rates with precipitation (Berg et al., 1993; González and Seastedt, 2001; Trofymow et al., 2002;). The effects of precipitation on decomposition in tropical rainforests is underrepresented in literature, however Keuskamp et al. (2013) found that precipitation was the most important driver of litter decomposition rates in the tropical ecosystems. In addition, Wieder et al. (2009) found that simulated rain throughfall reductions significantly suppressed litter decomposition rates in a tropical rainforest. While we did not include climate drivers as a factor in our study, we did find a slight increase in decomposition rates with gravimetric water content. We attribute the lack of climatic effects on litter decomposition rate across our sites to the narrow climatic range of sites that were chosen intentionally to study the effect of disturbance gradient.

4.3.2 Litter Stabilization

During litter decomposition, some labile plant tissues are microbially and biochemically transformed into recalcitrant, stable compounds. The diversion of labile plant tissues, which would otherwise decompose, into stabilized organic matter builds upon humus pools, thereby

sequestering more C in the soil (Prescott, 2010). Based on the observation that decomposition rates obtained from different types of litter often converge after several years, Prescott (2010) suggested that the amount of litter converted into humus (stabilized) is more important than decomposition rate. Litter decomposition and stabilization are not necessarily inversely related. A few litterbag studies, including this study, have found decomposition and stabilization to be decoupled, with both decomposition rate and stabilization factor increasing in parallel in response to a given treatment (Sarneel et al., 2020; Sarneel and Veen, 2017). For example, our results show that both litter decomposition and stabilization increased with microbial diversity (**Figure 7**), but there was no relationship between litter decomposition and stabilization (**Figure 6**).

Mayer et al. (2004) suggested that organic matter is stabilized through two pathways: 'organic recalcitrance', which refers to the difficulty of breaking down certain biomolecules, and 'biotic exclusion', which refers to the protection of organic matter through anoxic conditions or other physical barriers. Previous studies have found that both the presence/absence of woody vegetation (Sarneel et al., 2020) and vegetation type (Kaye et al., 2000) can affect C turnover. Litter stabilization increased significantly with increasing surrounding forest cover, reaching a maximum at ~ 90% forest cover in this study, possibly due the formation of a specialized microbial community with distinct stabilizing capabilities (Keiser et al., 2011), the addition of a more preferential microbial energy source through root exudates (Sparling et al., 1982), the increased input of recalcitrant root tissue, the physical protection of organic matter by the formation of soil aggregates (Prescott, 2010), and reduced erosion and leaching (Ratta and Lal, 1998; Ribolzi et al., 2017). It is possible that increased forest cover and live root mass protected the litter bags from decomposition and led to higher stabilization. The three forested sites (AnF,

MaF, and MuF) had the highest stabilization factor followed by converted sites (Agri, and Rub). The youngest site (Agri) had relatively low tree cover, but had a dense herbaceous understory that could have provided some physical protection to the soil, an effect that has been previously documented in fallow land in tropical montane regions (Ribolzi et al., 2017). The abandoned rubber plantation (Rub) had the lowest litter stabilization, possibly due to lack of understory vegetation that led to a considerable amount of exposed soil both beneath the tree cover and in the surrounding area, and soil disturbance as suggested by prior studies in managed plantations (Ribolzi et al., 2017) and agriculture ecosystems (Balesdent et al., 2000, Reichert et al., 2001). Soil disturbances, such as tillage, disrupt soil aggregates that leads to increased organic matter degradation (Balesdent et al., 2000), and soil losses through erosion and runoff (Reichert et al., 2001) can exacerbate those losses.

Previous studies have found that the most stable C in soils is often of microbial origin and emphasized the importance of microbial transformation and partial degradation in organic matter stabilization (Gleixner, 2013; Grandy and Neff, 2008; Oades et al., 1988). We found increased litter stabilization with increasing microbial (bacterial and fungal) diversity, reaching a maximum at the mid-range of bacterial diversity. Contrary to our findings, Maron et al. (2018) reported a positive correlation between microbial diversity and the degradation of recalcitrant litter relative to labile plant material, resulting in lower stabilization. Few other studies have analyzed the effects of microbial diversity on litter stabilization, however some have linked changes in microbial community composition to C stabilization and turnover rates (Leifheit et al., 2015; You et al., 2014). Changes in microbial growth efficiency, or the ability of the microbial community to incorporate substrates into biomass and byproducts, associated with changes in microbial community composition can alter litter transformation rates (Six et al.,

2006). For example, Leifheit et al. (2015) suggested that the presence of arbuscular mycorrhizal fungi suppresses saprotrophic organisms through reducing available soil nutrients, subsequently slowing the decomposition of woody plant litter and increasing soil aggregation.

We found a peaked response of litter stabilization with daytime soil temperature and a peak stabilization between 20 - 21 ℃. Previous work has shown that litter stabilization decreases with increasing mean annual air temperature (Keuskamp et al., 2013; MacDonald et al., 2018; Sarneel et al., 2020) and soil temperature (Keuskamp et al., 2013), however the temperature ranges studied are frequently much wider than the range found among our sites. For example, Keuskamp et al. (2013) compared decomposition and stabilization of tea bags incubated at 10 and 25 ℃, while average daytime soil temperatures among our sites varied by only 3.5 ℃. Furthermore, Knorr et al. (2005b) found that recalcitrant C pools are more temperature sensitive than labile C pools. This could help to explain our finding that stabilization, but not decomposition was affected by daytime soil temperature.

5. Conclusion

We explored how current land use and time since disturbance affected soil physicochemical properties, soil microbial diversity, and soil functions in tropical montane forests of Malaysian Borneo. We found that soils of the older forest harbored significantly higher microbial diversity and stabilized greater amounts of litter than soils of the younger forests and converted sites. There is a big gap in our understanding of how the link between soil microbial diversity and function changes across disturbance gradients of land use. Considering the rapid rate of land use change in the tropics and microbial role in nutrient cycling, it is critical to investigate how land use disturbance affects soil structure and function in these systems. Our

results suggest that logging and forest conversion significantly affect soil microbial diversity and can have lasting effects on carbon cycling in tropical montane forests. Considering the potential global impacts of land use on the terrestrial C cycle in one of the most productive biomes on Earth (Esquivel-Muelbert et al., 2019), these results have wide implications for land management and biodiversity conservation.

6. Declaration of Conflict

Authors declare no conflict of interests.

7. Author Contribution

KN designed the study; MF collaborated with private landowners for land access and created land use history; RS, JBM, AR, JG, and KN conducted field work; DW conducted soil physicochemical analysis; RS analyzed the data with assistance from KN, JBM and JSSS; RS wrote the first draft of the manuscript with assistance from KN, JSSS, and JBM; all authors discussed and approved the paper.

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10. Supplementary Material

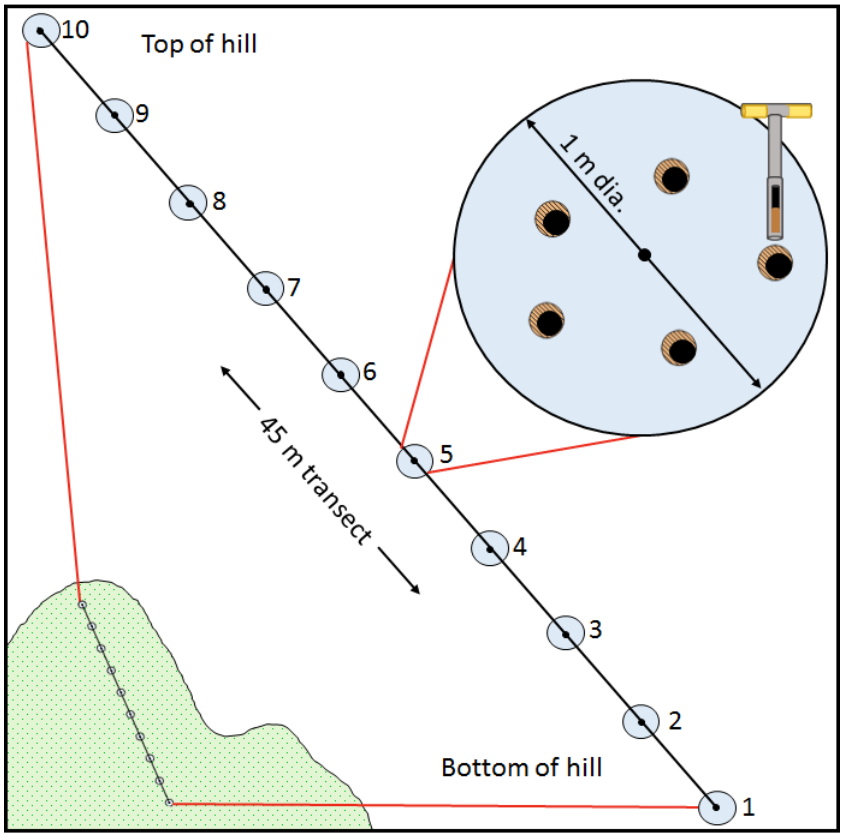

Figure S1. Diagram of soil sampling design displaying 10 plots along a 45-m transect at each of the study sites. Soil cores (n = 2 to 5) were collected within a 1-m radius circle of each plot's center to create 1 composite soil sample per plot and 10 samples per site. Transects were positioned along the slope gradient to maximize the variance in soil properties within each site.

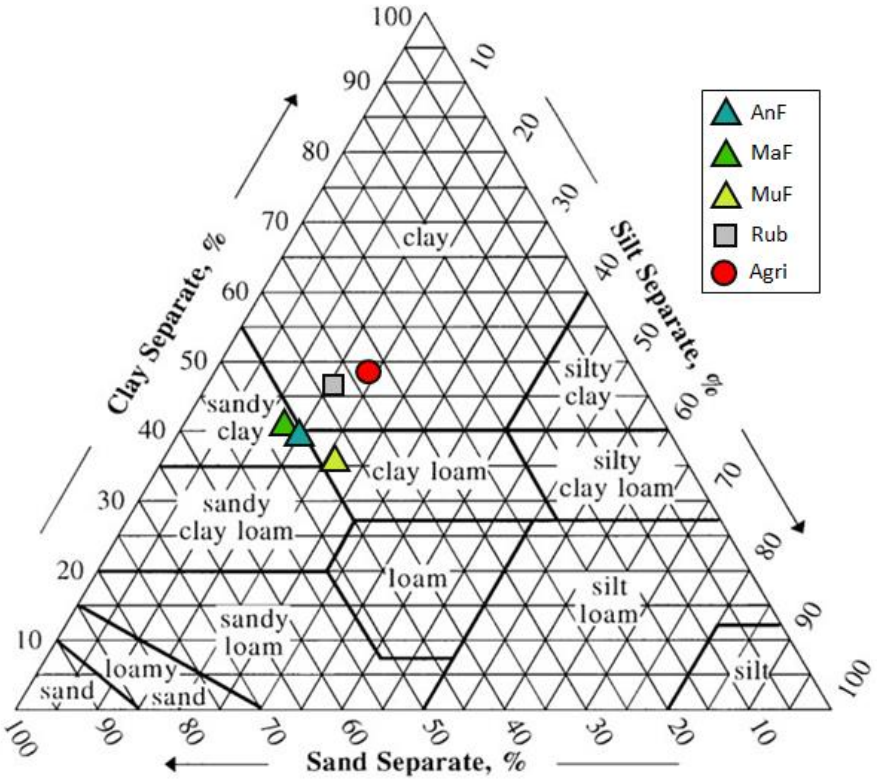

Figure S2. Modified USDA soil texture pyramid displaying the soil texture for the 5 study sites: Angelos Forest (AnF), Mahua Falls Forest (MaF), Malungung Forest (MuF), Abandoned Rubber Plantation (Rub), and Agriculture (Agri).

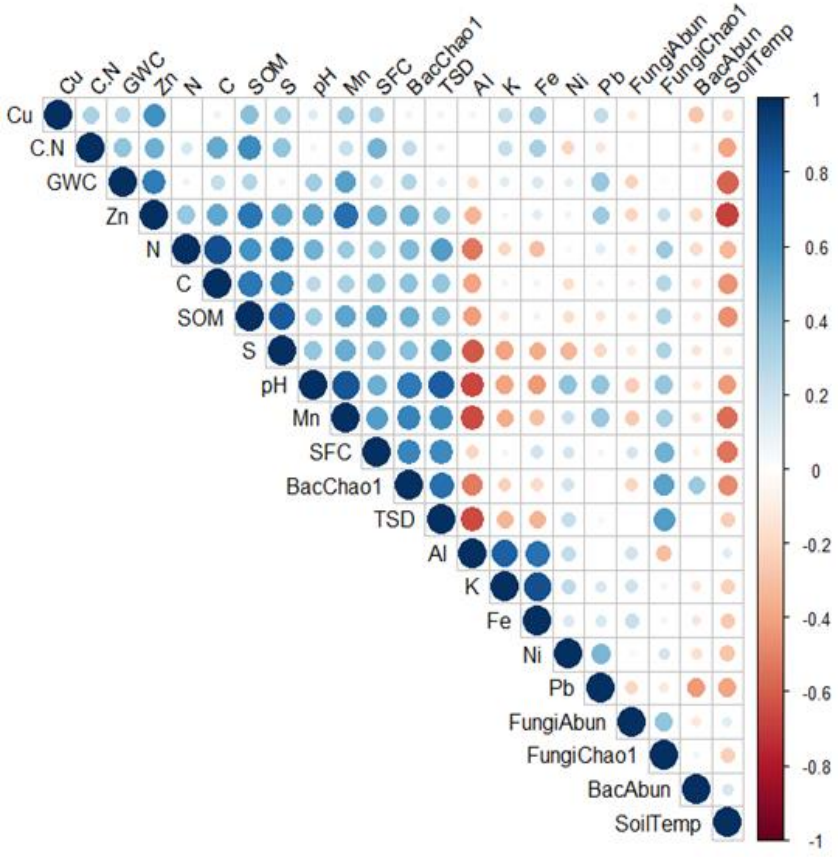

Figure S3. Correlation matrix of onsite forest cover and soil properties: Cu (copper, ppm); C.N (carbon and nitrogen ratio); GWC (gravimetric water content, g/g); Zn (zinc, ppm); N (total soil nitrogen, %); C (total soil carbon, %); SOM (soil organic matter, %); S (sulphur, ppm); pH; Mn (manganese, ppm); SFC (surrounding forest cover, %); BacChao1 (Chao1 bacterial OTU diversity index); TSD (time since disturbance, years); Al (aluminum, ppm); K (potassium, ppm); Fe (iron, ppm); Ni (nickel, ppm); Pb (lead, ppm); FungiAbun (fungal OTU abundance); FungiChao1 (Chao1 fungal OTU diversity index); BacAbun (bacterial OTU abundance); SoilTemp (soil temperature, ℃).

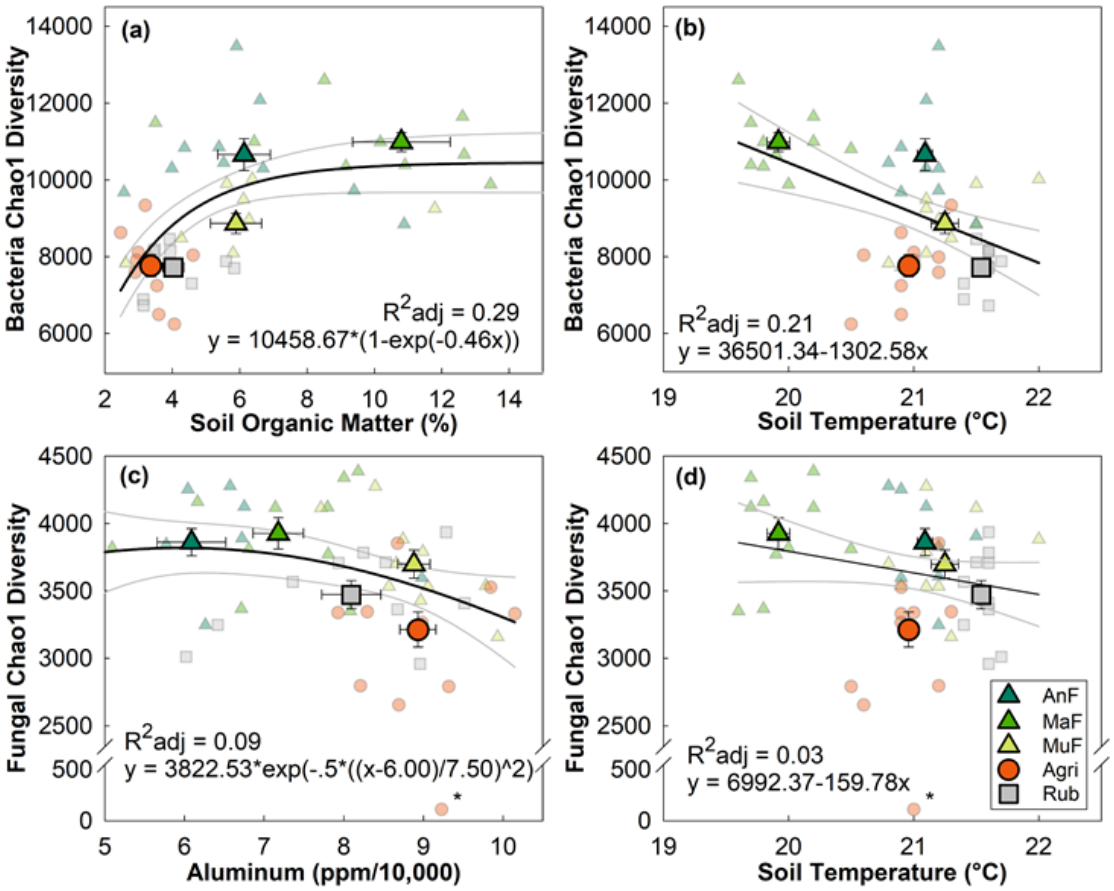

Figure S4. Relationship between additional soil properties identified by the random forest analysis as important predictors and Chao1 microbial OTU diversity (bacterial (a,b) and fungal (c,d)) across the gradient of land use types: Angelos Forest (AnF), Mahua Falls Forest (MaF), Malungung Forest (MuF), Abandoned Rubber Plantation (Rub), and Agriculture (Agri), displayed using different colors and symbols. Smaller transparent symbols represent the observed data, while larger solid symbols represent the site means. Bars represent the standard error of the mean, solid black line represents mean model and solid gray lines represent 95% confidence intervals. Statistical models were run on all observations and fitted lines are significant at $p < 0.001$ except (b) at $p < 0.05$. Sample size is 10 for each site, except for Agri where n = 9 for (b) and (d). One extreme outlier indicated with asterisk on (b) and (d) not included in regression analyses.

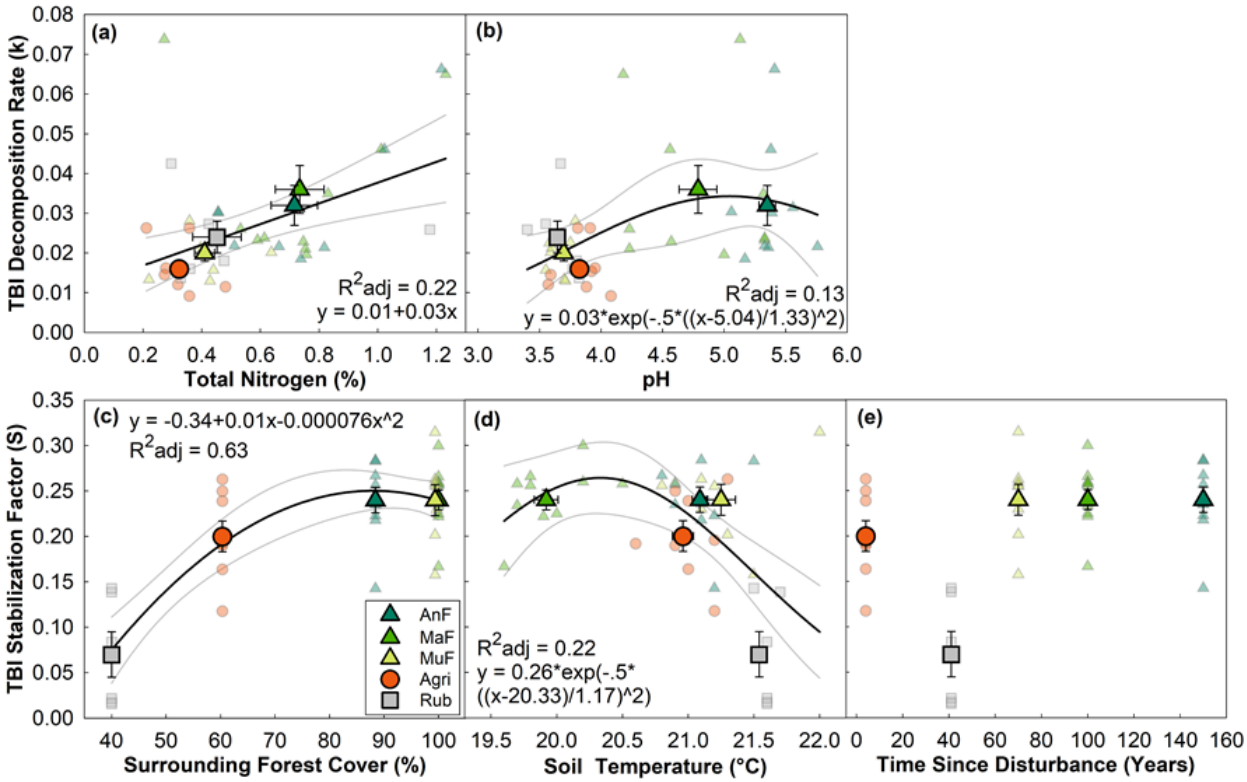

Figure S5. Relationship between additional soil properties identified by the random forest analysis as important predictors of litter decomposition rate (*k*, day^{-1}) (a, b) and stabilization factor (*S*, unitless) (c,d,e) across a gradient of land use types: Angelo's Forest (AnF), Mahua Falls Forest (MaF), Malungung Forest (MuF), Abandoned Rubber Plantation (Rub), and Agriculture (Agri), displayed by different colors and symbols. Smaller transparent symbols represent observed data points, while solid symbols represent site means. Bars represent the standard error of the mean, solid black lines represent model means and gray lines represent 95% confidence intervals. All statistical models were run on observed data and significant at $p < 0.05$. Sample sizes varied due to logistics: AnF (n = 9), MaF (n = 10), MuF (n = 8), Rub (n = 6), and Agri (n = 8).

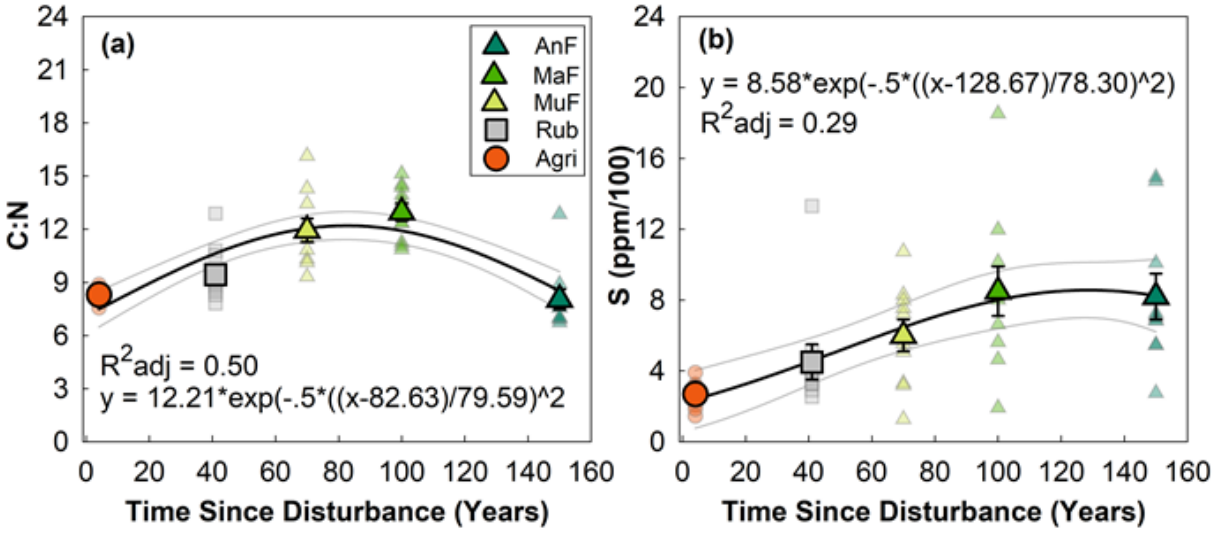

Figure S6. Relationship between time since disturbance and C:N (a) and soil S (sulphur, ppm/100) (b) across a gradient of land use types: Angelo's Forest (AnF), Mahua Falls Forest (MaF), Malungung Forest (MuF), Abandoned Rubber Plantation (Rub), and Agriculture (Agri). Smaller transparent symbols represent observed data points, while solid symbols represent site means. Bars represent standard error of the mean, solid black line represents model mean and gray lines represent 95% confidence intervals. Statistical model was run on observed data and significant at $p < 0.001$. Sample size is 10 for each site.

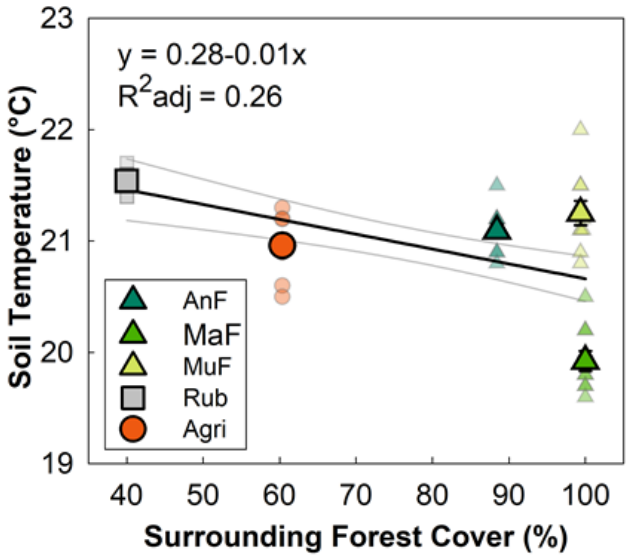

Figure S7. Relationship between surrounding forest cover (%) and soil temperature (°C) across a gradient of land use types: Angelo's Forest (AnF), Mahua Falls Forest (MaF), Malungung Forest (MuF), Abandoned Rubber Plantation (Rub), and Agriculture (Agri). Smaller transparent symbols represent observed data points, while solid symbols represent site means. Bars represent standard error of the mean, solid black line represents model mean and gray lines represent 95% confidence intervals. Statistical model was run on observed data and significant at $p < 0.001$. Sample size is 10 for each site.

S8. Soil Molecular Analyses

The 16s rRNA gene (bacteria) and ITS1 region (fungi) were amplified. Polymerase chain reaction (PCR) primers 515, ITS1 and ITS2, along with HotStarTaq Master Mix Kit (Qiagen, USA) were used in 30 PCR cycles with the following steps: 94 ℃ for 3 minutes followed by 30 cycles of 94 ℃ for 30 seconds; 53 ℃ for 40 seconds and 72 ℃ for 1 minute; and a final elongation step at 72 ℃ for 5 minutes. Following amplification, DNA fragments were sorted by molecular weight and concentration using 2% agarose gel. Sorted DNA samples were then purified using AmpureXP beads and checked on Agilent High Sensitivity (HS) chip on Bioanalyzer 2100 and quantified on fluorimeter by Qubit dsDNA HS Assay kit (Brand from MR DNA). The library was loaded onto the Illumina Platform for clustering and sequencing. Paired-End sequencing allows the template fragments to be sequenced in both the forward and reverse directions. Sequencing was carried out on an illumina MiSeq following the manufacturer's guidelines and data were analyzed using the proprietary MR DNA analysis pipeline (Dowd et al. 2008). Sequences with < 150 bp or with ambiguous base calls were removed. In brief, sequences were denoised, operational taxonomic units (OTUs) were assigned based on 97% similarity, and chimeras were removed. Archaea and mitochondrial or chloroplast OTUs were removed from the 16S data and non-fungi OTUs from ITS1 data. In total, 31,261 bacterial and 0 fungal chimeric were removed from further downstream analysis. Final OTUs were assigned using BLASTn; cloned sequences were searched against a database derived from RDP-II and NCBI (www.ncvi.nlm.nih.gov, http://rdp.cme.msu.edu).