Consequences of tropical forest conversion to oil palm on soil bacterial community and network structure

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Abstract

Tropical forest conversion to agriculture is a major global change process. Understanding of the ecological consequences of this conversion are limited by poor knowledge of how soil microorganisms respond. We analyzed the response of soil bacteria to conversion from primary rain forest to oil palm plantation and regenerating logged forest in Malaysia. Bacterial diversity increased by approximately 20% with conversion to oil palm because of higher pH due to liming by plantation managers. Phylogenetic clustering indicated that bacterial communities were determined by environmental filtering. Regenerating logged forests did not have significantly different soil chemistry, which did not correspond with significant differences in bacterial richness, diversity, or the relative abundances of particular taxa. However, there were significant differences in the structure of bacterial community networks between regenerating logged forests and primary forests, highlighting previously unobserved effects of these two land uses. Network analysis highlighted taxa that are potentially central to bacterial networks, but have low relative abundances, suggesting that these rare taxa could play an ecological role and therefore warrant further research.

Keywords: bacteria; microbial diversity; microbial networks; oil palm; rare microbes; tropical deforestation

1. Introduction

Tropical forests have long been under threat of conversion to other land uses—more than half of the original extent of rain forests has been converted (Asner et al., 2009). Since tropical forests are home to more than two-thirds of all terrestrial plant and animal species (Brooks et al., 2002; Dirzo and Raven, 2003; Gardner et al., 2009), this loss of tropical forest comes hand-in-hand with a loss in biodiversity. Yet this story of conversion and species loss may or may not translate to loss of the huge diversity of soil organisms found under foot.

Soil microorganisms, which make up the bulk of soil diversity, are widely recognized to be essential to the functioning of terrestrial ecosystems. Microbial activity is responsible for many biogeochemical redox reactions (Falkowski et al., 2008). Both negative and positive feedbacks between soil organisms and plant communities contribute to ecological structure and functioning in the tropics (Bagchi et al., 2010; Kiers et al., 2000; Mangan et al., 2010). Given the importance of soil microorganisms to biogeochemical cycling and plant-soil feedbacks, understanding if soil microbes are threatened by large-scale tropical land-use change is necessary to understand and predict broader functional consequences of land-use change.

An growing body of work has documented how soil microbial communities respond to human-induced environmental change (Thomas W Crowther et al., 2014; da C Jesus et al., 2009; de Carvalho et al., 2016; Fierer et al., 2012; Lee-Cruz et al., 2013; Leff et al., 2015; McGuire et al., 2015; Ramirez et al., 2012, 2010; Rodrigues et al., 2013; Tripathi et al., 2016; Wood et al., 2015). Several consistent patterns have emerged from this work. Changes in the bacterial community are largely governed by changes in soil chemical properties, mainly pH (Lauber et al., 2009; Rousk et al., 2010). Bacterial diversity decreases sharply with decreases in pH, partly due to an associated increase in the relative abundance of taxa such as *Acidobacteria*. By

contrast, fungi are less sensitive to changes in pH. Instead, the dominant control on fungi tends to be a combination of factors such as soil carbon, local soil moisture, and plant composition (Barberán et al., 2015; Fierer et al., 2003; Prescott and Grayston, 2013; Prober et al., 2015; Toberman et al., 2008). The response of soil microbial communities to land-use change is in part determined by the properties of the underlying soil, with the greatest difference between forest communities and grassland communities occurring on sandier soils (Thomas W Crowther et al., 2014). This constraint of soil type may be due to lower moisture and carbon holding capacity in sandier soils or inability of sandier soil to buffer against changes in pH, which are dominant controls of fungal and bacterial communities, respectively.

These now robust patterns rely on inference generated from the relative abundance of soil microbes, whether directly or through abundance-weighted diversity metrics. Most microbial taxa are, however, rare (Locey and Lennon, 2016)—i.e. low in relative abundance—and these abundance-weighted metrics may miss possible contributions of rare species. In plant communities, rare species can make important contributions to ecosystem structure and function (Jain et al., 2014; Lyons and Schwartz, 2001). Whether the same is true for microbes remains less well known, but evidence is mounting that loss of rare microbial taxa can play an important role in community structure (Shade et al., 2014) and ecosystem functioning, especially through modifying plant-soil feedbacks (Hol et al., 2015, 2010). Rare taxa, by virtue of being rare, may exhibit different life history strategies than abundant taxa (Murray et al., 2002) and therefore respond differently to land-use change. If this is the case, then understanding their responses may highlight different trends in the response of microbial communities to land-use change. Network analysis, which has been widely used to study the impacts of global change on plant and animal diversity (Ings et al., 2009), may help inform understanding of the ecological role of rare bacteria

by highlighting how rare taxa co-occur with well-studied taxa, which could indicate similar ecological roles between rare and well-studied taxa (Ma et al., 2016).

Based on the literature cited above showing that bacterial communities are strongly structured by abiotic conditions, we expected that bacterial community composition and diversity would follow land-use changes that modified soil chemical properties, particularly pH. Because McGuire et al (2015) found elevated pH under oil palm—but no differences between regenerating and primary forests—we expected that bacterial diversity and community composition would differ between oil palm and the native forest types, but not among the native forest types. For network composition, a chronosequence of abandoned agricultural land showed that fungal networks became more connected in older sites with a shift towards more fungal-dominated food webs (Morriën et al., 2017). Based on this we developed two competing hypotheses: (H1) bacterial network structure follows patterns observed in fungi and becomes more interconnected moving from disturbed to primary vegetation; (H2) because food webs shift to fungal dominance under primary vegetation, bacterial networks decrease in complexity as fungal communities increase in complexity.

To evaluate our expectations and the consequences of forest conversion on soil microbial composition, we compared bacterial communities from three sites in Malaysia: a primary lowland mixed dipterocarp forest, a regenerating dipterocarp forest that had been selectively logged 50 years ago, and a 25-year old oil palm plantation. Over the past few decades, palm oil, the commercial commodity extracted from the oil palm plant (*Elaeis guineensis;* Arecaceae) has been the most rapidly growing crop in the tropics. Indonesia and Malaysia alone account for more than 80% of all palm oil production and not coincidentally, this region of the world also experiences the highest proportional rate of deforestation (Carlson et al., 2012; Hansen et al.,

2013). Thus, oil palm plantations are highly relevant for evaluating the consequences of large-scale tropical deforestation on soil microbial communities.

2. Material and methods

2.1 Site description and field sampling

Soil samples were collected from lowland sites in peninsular Malaysia in the state of Negeri Sembilan, as previously described (McGuire et al., 2015). Briefly, we sampled from three land-use types: primary rain forest (primary forest), forest regenerating from logging 50 years prior (regenerating forest) and an oil palm plantation in active cultivation for 25 years (oil palm). The regenerating and primary forests area are located in the Pasoh Forest Reserve (2º5' N, 102º18' W, 80 m asl), with the Dipterocarpaceae family comprising nearly one-third of the basal area of canopy trees (Manokaran et al., 2004). The oil palm plantation was located less than 500 m from the Pasoh Forest Reserve. Climate in this region is aseasonal with mean annual precipitation of 1,788 mm and average minimum and maximum temperatures of 22.7 and 33.2 C, respectively. The dominant soil type in the lowland forest plots sampled is Ultisols (Adzmi et al., 2010).

Within each land-use type (primary forest, regenerating forest, and oil palm plantation), three replicate plots (20 x 20 m) were established and five soil samples were collected from each plot during a single sampling event. All sampling plots were at least 1 km away from each other, but selected on the same underlying soil type and slope position. The collected samples were divided into three sampling depths: 0-2 cm, 2-10 cm, and 10-20 cm. All plots were separated by at least 500 m. Sample replicates were composited by depth to one sample per depth, per plot and were placed in sterile plastic bags, sealed and frozen at -20 ºC on the day of collection. In the

laboratory, all soil samples were passed through a 2 mm sieve, homogenized, and stored frozen at -20ºC until laboratory analyses were performed.

2.2 Laboratory analyses

We amplified and sequenced a portion of the 16S rRNA gene to assess bacterial communities in a similar manner as described previously (Caporaso et al., 2012). Amplifications were performed on DNA isolates from the MoBio PowerSoil extraction kit (MoBio, Carlsbad, CA), which were the isolates used for prior analysis of soil fungi (McGuire et al., 2015). PCR amplification was performed with the primers 515f and 806r, which included sequencing adapters for the Illumina sequencing platform, and the reverse primer contained a 12-bp barcode unique to each sample. Amplicons combined and sequenced on an Illumina MiSeq instrument using a paired-end 151-bp sequencing kit. Raw amplicon sequences were demultiplexed and processed with the UPARSE pipeline (Edgar, 2013) as in Ramirez et al (Ramirez et al., 2014). Paired end sequence reads were merged prior to additional processing. Sequences were quality filtered using a "maxee" value of 0.5 and singletons were removed. Sequences were clustered into operational taxonomic units at a threshold of ≥97% sequence similarity. Merged, demultiplexed sequences were mapped against our *de novo* database of clustered OTUs to get counts of sequences per OUT and sample. Taxonomy was assigned to OTUs using the RDP classifier (Wang et al., 2007) with a confidence threshold of 0.5 and trained on the Greengenes database (McDonald et al., 2012). Samples were rarefied to 7,000 sequences per sample prior to downstream analysis. Previous analyses focused on analysis of fungal community dynamics contain data on microbial biomass, enzymatic activity, fungal diversity, fungal composition, and soil physical and chemical properties (D'Angelo et al., 2015; McGuire et al., 2015).

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164 *2.3 Analytic methods*

165 2.3.1 Diversity

166 We calculated several common ecological diversity metrics including species richness,
167 evenness, Shannon, and Faith's Phylogenetic Diversity (PD). Shannon is a diversity metric
168 where the relative abundance of species is weighted by evenness. Faith's PD sums the branch
169 lengths of a phylogeny for a given site and uses the resulting branch length sum as a metric of
170 phylogenetic diversity. Diversity metrics were calculated for each site using the *vegan* package
171 (Oksanen et al., 2016) of R (Core, 2016), except for phylogenetic diversity, which was calculated
172 in the *picante* package (Kembel et al., 2010).

173

174 2.3.2 Phylogenetic analysis

175 Assessing the extent of phylogenetic clustering in a community can be used to infer the
176 degree to which communities are likely structured by environmental filtering or competition
177 (Cavender-Bares et al., 2009; Webb and Ackerly, 2002). Using this approach, the observed
178 phylogenetic distribution of a community is compared to a null model or randomization
179 procedure to determine whether the observed phylogeny is more or less clustered than would be
180 expected at random. Clustering suggests environmental factors structure community assembly,
181 whereas overdispersion suggests that biological interactions, such as competition, are the
182 dominant force in community assembly. To apply this approach, we used a phylogeny generated
183 from sequence OTUs to create a community dissimilarity matrix in the *picante* package in R
184 (Kembel et al., 2010). We then calculated a relative abundance-weighted standardized effect size
185 for mean pairwise distance (MPD) and mean nearest neighbor distance (MNTD), which are

metrics of mean pairwise phylogenetic distance within the community (Webb et al., 2008). MPD calculates mean pairwise distance between all OTUs in each site. MNTD calculates the average distance separating each OTU in a site from its nearest phylogenetic relative. The standardized effect size of these metrics compares the observed phylogenetic distribution to an expected distribution under some null model or randomized scenario. To ensure robustness of our approach, we calculated MPD and MNTD using two null model scenarios, one that randomizes the tips of the phylogeny (Tip Randomization) and a second that randomizes that community abundances within samples, but holds richness constant (Richness Randomization).

2.3.3. Network analysis

To analyze network structure, we used data on the relative abundances of bacterial taxa by land-use types to create a taxonomic association network. This procedure suggests an association network by comparing observed taxonomic co-occurrences with a set of predicted co-occurrences from null models with the same richness and relative abundances as the observed community. Standardized effect-size scores are calculated for the observed vs. predicted data, significant associations are retained, and scores are converted to an association network. We generated association networks using the *netassoc* package (Blonder and Morueta-Holme, 2015).

We calculated a number of statistics to characterize the nature of networks under the three land-use categories. Modularity measures the compartmentalization of a network into sub-networks, or modules (Newman, 2006). High modularity scores indicate the presence of many connections among vertices within a module, but few connections to vertices of different modules. We calculated modularity using the *modularity* function in the *igraph* package (Csardi and Nepusz, 2006). Assortativity measures the tendency for similar vertices to be linked with

each other (Newman, 2002). We calculated assortativity using the *assortativity_degree* function in the *igraph* package. Transitivity represents the likelihood that neighboring vertices are linked, and then linked to other adjacent vertices à la transitivity property (Barrat et al., 2004). We calculated transitivity using the *transitivity* function in the *igraph* package. We also determine, for each land-use category, which taxa (vertices) had the highest number of paths connected to other vertices. This is also known as betweenness or network centrality (Freeman, 1978). We calculated this using the *vertex_connectivity* function in the *igraph* package.

To determine whether network statistics were significantly different among the three land-use categories, we generated 10,000 random networks with similar sizes and calculated the mean and standard deviation of the same statistics. We then calculated a z-score for each of the observed networks to determine how many standard deviations it fell away from the expected value given from the network randomization procedure. The randomly generated network was a regional network that had the same number of vertices and edges as the observed network that includes all sites, regardless of land-use category. Comparing to this regional network therefore highlights how environmental changes would affect the locally observed network.

2.3.4 Statistical analyses

We determined bacterial community similarity among land-use types and soil horizons using ANOSIM; non-metric multi-dimensional scaling (NMDS) was used to visualize clusters. We used linear models to determine the impact of land-use type on bacterial diversity. We first tested response variables for normality using the Shapiro-Wilk test. In cases of non-normality, response variables were transformed using a Box-Cox transformation. All differences were

considered significant at a 0.05 threshold and marginally significant at a 0.10 threshold (Hurlbert and Lombardi, 2009).

3. Results

The soil bacterial community did not differ by sampling depth (ANOSIM R = -0.05, P = 0.77). We therefore pooled samples across depths to increase sample size and statistical power. Because samples collected from the same site at different depths are not independent, we controlled for non-independence by clustering standard errors of all samples from the same site. We observed a significant difference in the soil bacterial community among land-use types (ANOSIM R = 0.59, P < 0.01). Specifically, oil palm soil bacterial communities clustered independently of regenerating and primary forest (Figure 1; Stress = 0.06). There was strong evidence that bacterial communities from all land-use types were phylogenetically clustered (as opposed to overdispersed) relative to a null model (MPD & MNTD < 0; P = 0.01; Table 1). There was no evidence for significant differences in the degree of phylogenetic clustering among land-use types.

3.1 Diversity

To explore the nature of the difference between bacterial communities, we assessed potential differences in several ecological diversity metrics. We observed significantly elevated diversity of soil bacteria in oil palm compared to regenerating and primary forests (Table 2). For instance, Shannon diversity of soil bacteria increased by approximately 20% under oil palm, compared to primary and regenerating forests ($p < 0.001$). This general pattern was robust to all diversity indices used, including species richness ($p < 0.001$), evenness ($p = 0.001$), and Faith's

phylogenetic diversity ($p < 0.001$). Statistical models that included only land-use type as predictor variables, explained between 50% and 77% of the variation in diversity metrics (Table 2).

3.2 Community Composition

We observed significant changes in the relative abundances of several key taxa among land-use types (Figure 2; $p < 0.05$ for all groups shown). The most significant changes were between oil palm and the two forest types, with little difference between regenerating and primary forests. Most taxa increased in relative abundance under oil palm, compared to regenerating and primary forest (Figure 2). A notable exception was *Acidobacteria*, which was the only taxonomic group to significantly decrease in relative abundance (by approximately 40%) under oil palm compared to regenerating and primary forest.

3.3 Network Structure

Networks of soil bacterial communities were more modular under oil palm and logging than was expected at random, given the taxa present in the regional species pool (Figure 3; Table 3a, b). Regenerating forests were around eight times more modular, and oil palm plantations approximately five times more modular, than primary forests, but oil palm was only 0.4 times less modular than regenerating forests. Similar taxa were 2.5 times less likely to be associated with each other under oil palm compared to primary forests, whereas similar taxa were three times more likely to be associated in regenerating forest soil compared to primary forest (Figure 3; Table 3a, b). All land-use types had similar values of transitivity—the likelihood that neighboring vertices are linked—but were all less than expected by random (Table 3a, b).

For all taxa, we calculated the number of edges connecting to other vertices, for each land-use type. We found that several taxa played central roles (high degree of connectivity) in certain land-use networks, but were not present or were unimportant in others (Figure 3; Table 4). For instance, *Acidobacteria* had 244 connections under oil palm, but not under regenerating or primary forest. *Actinobacteria* had 250 connections under primary forest, but none under regenerating forest and oil palm. *NKB19* was not present under oil palm and regenerating forest, but had the most connections under primary forest. *Planctomycetes* and *Gemmatimonadetes* were two of the most central taxa under regenerating forest soils, but neither had connections in either oil palm or primary forest. Some taxa were central across all land-use types, such as *GN02* and *Nitrospirae*.

The regenerating forest network had the fewest number of taxa co-occurring, but most relationships were high in magnitude—whether positive or negative (Figure 4). Oil palm had the most co-occurrence relationships, but was dominated by a few strong positive interactions (Figure 4). Primary forest had a mix of positive and negative interactions, both weak and strong (Figure 4). The correlation between the relative abundances of two taxa was a significant predictor of the co-occurrence strength between those two taxa ($P < 0.00$), but explained only 19% of the variance in co-occurrence scores.

4. Discussion

4.1 Effects of oil palm on the soil bacterial community

We expected that bacterial diversity would be greatest under oil palm because of greater pH. We found support for this hypothesis, which conforms with other work in tropical forests that find increases in measures of bacterial diversity after conversion of tropical forest (da C

Jesus et al., 2009; de Carvalho et al., 2016; Lee-Cruz et al., 2013; Rodrigues et al., 2013; Tripathi et al., 2016). Increases in bacterial diversity were associated with significant increases in evenness, suggesting a disrupted microbial community. In our study, conversion of primary forest to oil palm plantation was associated with an increase in pH—from 4.7±0.1 to 5.1±0.1(Brearley, 2015; McGuire et al., 2015). Higher pH is often associated with greater soil bacterial diversity, with the slope of this relationship greatest in low pH conditions (Lauber et al., 2009). Because of highly acidic tropical forest soils, oil palm plantation managers lime soils for improved production (Tripathi et al., 2012). Since pH is the dominant driver of soil bacterial communities across biomes (Lauber et al., 2009; Rousk et al., 2010; Tripathi et al., 2013, 2012), an increase in bacterial diversity accompanying liming conforms with expectations from the literature. Our observed decrease in the relative abundance of *Acidobacteria*—which tend to have higher relative abundances with low pH—in oil palm soils also supports our conclusion that changes in the bacterial community under oil palm cultivation were largely due to changes in pH. This claim is furthermore supported by our finding that the bacterial community is highly phylogenetically clustered, which is often used to infer that environmental filtering is the dominant driver of community assembly (Cavender-Bares et al., 2009). Other work has found evidence for abiotic stress leading to phylogenetic clustering of soil bacteria (Goberna et al., 2014).

4.2. Bacterial communities in regenerating vs. primary forest

We found little evidence of differences in bacterial diversity and community composition between regenerating and primary forest. Similar findings have been made in a similar system in Borneo (Lee-Cruz et al., 2013; Tripathi et al., 2016, 2012). Network analysis, however,

illuminated previously unnoticed differences in the structure of bacterial communities between primary and regenerating forest. We proposed two, competing hypotheses for differences in network structure: (H1) bacterial network structure would increase in complexity towards primary vegetation; (H2) bacterial network structure would become less complex as fungal networks became more complex under primary forest. We found that regenerating forest networks were eight times more modular than primary forest networks, providing evidence that the response of bacterial networks and fungal networks could be different, given that fungal networks have been shown to increase in interaction strength with primary vegetation (Morriën et al., 2017). Regenerating forest and oil palm networks were also significantly more modular than random. Modularity measures the compartmentalization of a network into sub-networks, or modules (Newman, 2006). A lower modularity value indicates that taxa within the network tend to co-occur more with a wider range of other taxa. Ecologically, modularity has been interpreted to indicate partitioning into groups of ecologically similar taxa and, thus, resistance of a network to disturbance and loss of individual species (Burgos et al., 2007; Ding et al., 2015). Based on this interpretation, high modularity in regenerating forest could indicate that these forests include more ecological types than in primary forest.

We also found that all land-use types had lower transitivity than random. In some cases, transitivity has been shown to be an indicator that network structure is dominated by keystone species—species whose removal can have a disproportionate effect on overall community structure (Berry and Widder, 2014). Our observed lower-than-random transitivity across all land-use types suggests that bacterial community structure is not highly sensitive to loss of particular taxonomic groupings. Because lower transitivity can be indicative of weaker interactions and couplings within the bacterial community, non-transitive network structure has been inferred as

346 indicative of co-existence (Narisawa et al., 2008). Our finding of lower-than-random transitivity
347 suggests fairly stable co-existence of bacterial types across land-use categories.
348 The taxa that played a key role in bacterial networks were also different between
349 regenerating and primary forests. In regenerating forests, the taxa with the most connections to
350 other taxa were *Planctomycetes*, *GN02*, *Gemmatimonadetes*, and *Nitrospirae*. By contrast, in
351 primary forest the most important taxa were *NKB19*, *ZB3*, *Actinobacteria*, and *Elusimicrobia*.
352 Thus, the network analysis highlights that land use can significantly alter the network structure
353 of the soil bacterial community, even if diversity indices do not show differences.
354 There are several important caveats to drawing ecological interpretations from network
355 topology. Because network structure shows a pattern, it is difficult to infer ecological process
356 based on assessment of the pattern alone (Bascompte, 2007). There are only a few examples of
357 systems in which it is well understood how network typology and form connects to function,
358 many of which tend to be at the cellular rather than ecological level (Ingolia, 2004; Price et al.,
359 2004). As more information becomes available on the ecological strategies of particular
360 microbial taxa—such as how they respond to abiotic conditions—making inference about
361 ecological dynamics from network structure will become more fruitful. Another limitation to co-
362 occurrence network analysis is that the nature of the interactions is vague. Much network
363 analysis in ecology focuses on well defined and quantified biotic interactions, such as food web
364 and mutualistic interactions (Ings et al., 2009). The nature of co-occurrence interactions could be
365 due to several factors, some being more ecologically meaningful than others. Methodologically,
366 network structure can be influenced by the method of network construction and null model
367 testing used (Connor et al., 2016; Weiss et al., 2016).

Yet, despite these limitations, network analysis may be a powerful tool to highlight potential ecological roles of understudied taxa. Because many microbial taxa are hard to culture, network analysis may highlight the ecological strategies of organisms that are difficult to observe directly. In our study, we observed that several of the taxa that play important network roles are underdescribed ecologically. For instance, taxa that strongly positively co-occur with a well-studied taxon may play similar ecological roles. This inference is supported by our finding that the overall bacterial community is phylogenetically clustered, meaning that environmental filtering is likely important to bacterial community structure. Co-occurring taxa, therefore, should be co-occurring because they have similar environmental response strategies. However, we observed that correlation in relative abundance only explains 19% of the variation in co-occurrence, which suggests that similar response of taxa's relative abundances to environmental conditions only explains a part of the nature of complex co-occurrence patterns.

4.3. Combining diversity, phylogenetic, and network analyses provides more insight

We found many rare taxa with high network centrality highlighting potentially important, but understudied, microbial taxa that are overlooked by analyses of diversity or relative abundance patterns. Specifically, taxa such as *GN02*, *NKB19*, *ZB3*, *NC10*, *AD3*, *Parvarchaeota*, *Armatimonadetes*, and *Fibrobacteres* all played important roles in network centrality, but had low relative abundances. Identifying understudied taxa has previously focused on identifying taxa with high relative abundances in novel systems, such as surprisingly high relative abundances of *Verrucomicrobia* in remnant patches of native prairie across the U.S. Midwest (Fierer et al., 2013). Our approach suggests that rare taxa that are low in relative abundance also warrant further research effort, as they may play an important role in bacterial communities and

potentially connect to broader ecosystem functioning—this potential importance of rare taxa has been shown for plant systems (Jain et al., 2014; Lyons and Schwartz, 2001) but not for microscopic taxa that are far less well known but lend themselves well to network analysis due to their high diversity.

Further integration of network approaches into microbial analyses requires understanding how patterns between the two approaches overlap. For instance, it will be particularly important to understand when and why relative abundances translate to network importance and when they do not. In our analysis the relative abundance of *Acidobacteria* was lowest in oil palm soils, but *Acidobacteria* had the greatest network centrality. Similarly, *Actinobacteria* relative abundance was lowest in primary forest soils, but had the highest network centrality score. These patterns between relative abundance and network centrality are seemingly idiosyncratic, so further research into the drivers of network influence in the soil bacterial community is needed. This work will likely require improved understanding of life history strategies of microbial taxa, which overlaps with the research needs to develop understanding of microbial functional traits (Aguilar-Trigueros et al., 2015; Thomas W. Crowther et al., 2014; Krause et al., 2014; Martiny et al., 2015; Wallenstein and Hall, 2011; Wieder et al., 2014).

4.4 Conclusion

Though soil fungal communities appear to be highly responsive to changes in vegetation and carbon loss (McGuire et al., 2015), changes in bacterial communities under deforestation may be principally driven by changes in environmental conditions associated with land-use change. This implies that diversity changes in bacterial communities may be more ephemeral

than changes to fungal communities, given that pH can change over shorter time periods than soil carbon and dominant vegetation structure.

Understanding the nature of the change in these bacterial communities has largely focused on shifts in relative abundances and diversity. Our finding that bacterial diversity increases under oil palm aligns with previous findings, but we also shed new light on the nature of bacterial community disassembly with land-use change. Network analysis highlights strong differences in network structure between regenerating and primary forests that do not appear in analyses of diversity or relative abundance patterns. Our analysis identified bacterial taxa that play central roles in network structure, but have low relative abundances. These taxa warrant further research effort to identify their functional roles in the ecosystem.

Our finding that the structure of bacterial networks differed between regenerating and primary forests also suggests that microbial community analysis needs to go beyond assessment of diversity and relative abundance patterns to unravel the nature of changes to bacterial communities under land-use change. Analytic tools that go beyond diversity analyses are widely applied in community ecology and our data suggests that greater application of these methods could strongly benefit inference in microbial ecology.

Author Contributions

K.L.M., H.D., C.B., S.M.G, C.M.G, M.R.G, A.M, and N.Y designed the field sampling, conducted field work, processed soils, and extracted DNA; B.L.T helped with study design; P.M and F.Q.B helped with study design and field sampling; J.A.G amplified and sequenced DNA; J.L and N.F processed sequence data; S.A.W analyzed data and wrote the first draft of the manuscript; all authors provided feedback on the final version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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Figure Headings

Figure 1. NMDS plot of soil bacterial communities under primary rainforest, regenerating rainforest, and oil palm plantation in peninsular Malaysia.

Figure 2. Bar plot of relative abundances of bacterial taxa among the three land-use categories. Taxa were included for which there was significant differences in relative abundances among at least two of the categories. For visualization, plots are broken up by taxa with high relative abundances (a) and low relative abundances (b).

Figure 3. Association network maps of soil bacterial communities under the three land-use categories: regenerating forest (a), oil palm (b), and primary forest (c). The size of vertices is proportional to the number of edges connecting each vertex.

Figure 4. Heat map of significant ($p < 0.05$) co-occurrence values among individual taxa under the three land-use categories: regenerating forest (a), oil palm (b), and primary forest (c). Red indicates negative co-occurrence scores and blue indicates positive co-occurrence scores. Correlation of species taxonomic relative abundances is a significant explanatory variable of co-occurrence scores, but only explains a small portion of the overall variation (d).

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