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Distinctive soil archaeal communities in different variants of tropical equatorial forest

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Abstract

Little is known of how soil archaeal community composition and diversity differ between local variants of tropical rainforests. We hypothesized that, 1) as with plants, animals, fungi, and bacteria, the soil archaeal community would differ between different variants of tropical forest, 2) that spatially rarer forest variants would have a less diverse archaeal community than common ones, 3) that a history of forest disturbance would decrease archaeal alpha and beta diversity, and 4) that archaeal distributions within the forest would be governed more by deterministic than stochastic factors. We sampled soil across several different forest types within Brunei, Northwest Borneo. Soil DNA was extracted and the 16S rRNA gene of archaea was sequenced using Illumina MiSeq. We found that: 1) as hypothesized there are distinct archaeal communities for each forest type, and community composition significantly correlates with soil parameters including pH, organic matter and available phosphorous. 2) as hypothesized, the 'rare' white sand forest variants kerangas and inland heath had lower archaeal diversity. A nestedness analysis showed that archaeal community in inland heath and kerangas was mainly a less diverse subset of that in dipterocarp forests. However, primary dipterocarp forest had the lowest beta-diversity among the other tropical forest types. 3) Also, as predicted, forest disturbance resulted in lower archaeal alpha diversity – but increased beta diversity in contrast with our predictions. 4) contrary to our predictions, the BetaNTI of the various primary forest types indicated community assembly was mainly stochastic. The possible effects of these habitat and disturbance-related effects on N cycling should be investigated.

Keywords: archaeal composition, archaeal diversity, kerangas,Tropical forests, inland heath forest, white sand forest

Introduction

Tropical forests are known as one of the richest and most diverse environments on Earth [1, 2]. However, the composition and diversity of the microorganisms is poorly known compared with macro-organisms [3]. Archaea represent one of the three main lineages of life on Earth [4] and constitute a small but consistent percentage of soil microbial communities, usually around 1-3% of the prokaryotic cells [5-8]. Thus, understanding their ecology and patterns in the environment may be important in understanding ecosystem functioning.

Apart from their role in decay and methane production in waterlogged soils and wetlands, archaea are also thought to play an important role in nitrogen cycling in soils in general, due to the ability of many soil archaea to oxidize NH_3 into NO_2^-, the first step towards conversion to NO_3^- which can be taken up more easily by plant roots [9]. In fact, the trophic mechanisms of most archaea from aerobic soils are poorly known, because only a few have been cultured – though of all those which have been cultured possess genes for ammonia oxidation [10, 11]. Amongst the diversity of aerobic soil archaea, which comprise several phyla, there have been some hints of other trophic modes including heterotrophy of organic molecules, but the importance of these remains unknown [11-13].

From previous studies, it is clear that soil archaeal communities can vary in predictable ways along broad environmental gradients [10, 11, 14-16], and between different habitats [17]. Our previous study [8] sampled archaea in both primary forest and agricultural environments on a broad scale across Malaysia, finding that soil pH played a major role in community composition. However, it is still unclear how strongly the composition, structure, and diversity of the archaeal communities can differ on a local scale between common *terra firma* tropical forest and distinctive and rare tropical forest variants such as white sand forests [18-20]. Understanding this would give an added perspective on how specialized archaea are with respect to habitat in the tropics. Given that the ecosystem of white sand forests is widely

considered to be strongly limited by nitrogen supply (in addition to other nutrients) [19, 21] and that soil archaea play an important part in ammonia oxidation [22-26], understanding the archaeal ecology of white sand forests and how it differs may ultimately have a role in understanding this peculiar ecosystem.

We were also interested in understanding what influence conversion to secondary forest may have on archaeal communities, as an indicator of their sensitivity to disturbance – an issue that may be of conservation importance for understanding the effects of forest clearance. Forest disturbance through logging or clearance, followed by regeneration of secondary forest, is a major influence on tropical forests. The differences between secondary and primary forests have been studied for a range of organisms, but never before for archaea [27-31].

It is also unclear generally whether soil archaeal communities are governed by deterministic or stochastic processes [11]. The role of stochasticity is a subject which has focused on a range of different groups of organisms [32-35], but never apparently archaea. This could be of relevance to understanding whether archaeal communities are strongly niche structured by competition, and to understanding heterogeneity in ammonia oxidation potential in soils [36-38].

In this study, we focused on different variants of rainforest in the global biodiversity hotspot of Brunei Darussalam, Northwest Borneo. We investigated the variation in archaeal community composition and diversity across dipterocarp primary forest (DP) – the major *terra firma* forest type in SE Asia -, dipterocarp secondary forest (DS), inland heath white sand forest (IH) and kerangas white sand forest (KS). This provides an opportunity to study the composition and diversity of archaeal communities in different forest habitat types but under same climatic conditions. We used 16S rRNA gene amplicon sequencing using Illumina MiSeq platform to test the following hypotheses and expectations:

1. We expected that like plants, animals, fungi, and bacteria, the soil archaeal community composition would differ on a habitat basis between different variants of tropical forest, and that archaeal community would be structured by soil edaphic and habitat characteristics. Past ecological studies have shown clearly that the flora, fauna, soil fungal and soil bacterial communities of white sand forests are distinct from *terra firma* forest [3, 9, 21].

2. We hypothesized that rare and environmentally distinctive forest variants (in this instance, white sand forests) would have lower alpha and beta diversity of archaea than to normal *terra firma* tropical forests. In ecology in general, it has been noted that habitats which are both extreme and rare tend to have a lower diversity of animals and plants [39-42]. This is thought to be partly because they offer too great an evolutionary barrier for many of the lineages existing in surrounding habitats to adapt to [43]. In addition, these rare habitats also tend to be relatively ephemeral on a geological timescale, so that any lineages which do successfully adapt to them will tend to go extinct. This combination of factors is thought to keep diversity lower in these environments.

3. Given that soil archaea are generally thought to be slow growing - existing on a limited range of low energy substrates at low concentrations - we hypothesized that the soil archaeal community would be very sensitive to large scale disturbance of soils and vegetation, which would alter many aspects of the physical environment [44, 45]. We expected that in previously cleared and regenerating secondary forest areas, both the alpha and beta diversity of soil archaea would be lower, compared to the unlogged *terra firma* forests from which they were derived.

4. We hypothesized that, if soil archaea are generally slow growing and have low population turnover, their populations would be mostly structured by deterministic

processes [11], with species composition and abundance determined by competition for available niches. We expected this to be especially so in the white sand forests, which are regarded as strongly N limited and presumably have low rates of ammonia input. However, we anticipated that in areas of secondary forest, the slow responsiveness of archaeal populations to disturbance would lead to a greater role of stochastic processes.

Materials and methods

Study site

Soil sampling took place in four different low land tropical rainforest types in Brunei Darussalam, Northwest Borneo. We collected soil samples from mixed dipterocarp primary forest (DP), recently logged (last 2 years) mixed dipterocarp secondary forest (DS), and the two types of white sand forest: inland heath forest (IH), and kerangas forest (KS) [3]. The mixed dipterocarp primary forest is dominated by large tree species belonging to the family of Dipterocarpaceae and the forest structure is complex and multi-layered. The age of the trees of the DP forest is around 60 years [46]. The DS forest contains many of the same plant species as the DP forest, but differs by the dominance of pioneer tree species such as *Macaranga*, *Vitex*, and *Dillenia* species. The DS forest has a more open structure, consisting of a complex mosaic of isolated trees from the original forest, shrubs and weed trees, regenerating tree seedlings, and largely bare patches of soil (vehicle ruts and piles of loose bulldozed soil) and broken parts of branches and roots exposed to full sunlight, with contrasting plant compositions and micro-climates. Both kerangas (KS) and inland heath (IH) forests differ considerably from dipterocarp forests in plant species composition and structure, having a low and uniform single-layered canopy with dense undergrowth full of shrubs, herbs, pitcher plants, etc. The main difference between the two heath forest types sampled in the

present study is that inland heath forest has low drainage capacity compared to kerangas forest, which means that the kerangas forest is being more susceptible to drought, while the inland heath soil can sometimes be flooded for part of the year.

Soil sampling and DNA extraction

Soil samples were collected in June 2014, during a period characterized by climate conditions in which afternoon rain storms occurred about every other day. Brunei has a seasonal climate, with two drier periods occurring in February/March and July/August [47]. The mean annual rainfall is above 2300 mm [48]. Three clusters of samples were taken in each forest type within a 3 km transect (Figure1). Within each cluster, three quadrats (10 m x 10 m in size) were collected at least 30 m apart along a smaller scale linear transect (Fig. S1). Each individual sample consisted of five pooled samples (each approximately 50 g from the four corners and one center point of the quadrat). The top 10 cm of soil was collected in a sterile sampling bag after removing the litter layer. In tropical forest soils, as with most soils, the highest microbial density and activity are detected near the surface [49, 50]. Thus, this is a more accurate representation of microbial communities inhabiting soils. The sampling quadrats were randomly located with respect to tree roots. When a sample point fell directly onto a large root or woody stem, it would be moved several centimeters to the side of the root/stem. Within each quadrat, some samples might be closer to tree roots than others, but the five subsamples per quadrat were mixed into one composite sample - which in effect integrates spatial heterogeneity. After gently removing surface moss, leaves, and stones, the five subsamples from within each quadrat were combined into one plastic bag. The collected soil samples were homogenized by sieving (2 mm sieve), and stored at -20˚C until DNA extraction. A total of 36 samples were collected from four different forest types (nine replicates from each forest type).

The soil DNA was extracted from 0.3 g of each sample of soil, using the Power Soil DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA) following the protocol described by the manufacturer. The DNA samples were sent to the Dalhousie University, Canada, for sequencing using Illumina MiSeq platform (www.cgeb-imr.ca). PCR primers targeted the archaeal 16S rRNA gene within V6-V8 region (~ 440-450 bp) and were adapted from existing rRNA primers and designed *de novo* to ensure amplicon sizes appropriate for 454- Roche™ chemistry. Forward primers included Roche's A adaptor and MIDs (''multiplex identifiers'') in the form of: 5'-[A-adaptor]+[MID1 to 10]+[specific F primer]-3'; reverse primers included Roche's B adaptor in the form of: 5'-[B-adaptor]+[specific R primer]-3' [51].

Soil Properties Analysis

Geographical co-ordinates were measured using a GPS at each sampling quadrat during field sampling. Soil pH, organic matter content (OM), total nitrogen and available phosphorus concentrations were measured at Universiti Brunei Darussalam using the standard methods [52]. Total nitrogen content was determined by Kjeldahl method. Soil available phosphorus was extracted using Bray's reagent (0.025 M hydrochloric acid and 0.03 M ammonium fluoride), and the phosphorus concentration in the extracts was then determined using a UV-spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Soil organic matter content was determined after incineration in a muffle furnace at 550˚C for 2 hours, according to the methodology described by [52].

Sequence processing

The sequenced data generated from MiSeq sequencing platform was processed using the mothur pipeline [53]. Two paired sequences were assembled using Pandaseq [54] with an assembly quality score of 0.9, which is the most stringent option to reduce errors. The

archaeal 16S rRNA gene sequences were aligned against EzTaxon-aligned database. Sequences were denoised using the '*pre.cluster*' command in mothur, which applies a pseudo-single linkage algorithm with the goal of removing sequences that are likely due to sequencing errors [55]. Putative chimeric sequences were detected and removed via the Chimera Uchime algorithm contained within mother [56]. The high quality archaeal 16Sr RNA gene sequences were taxonomically classified against the EzTaxon-e database [57], using the naïve Bayesian classifier implemented in mothur (at ≥80% bootstrap cutoff with 1000 iterations). All the 16S rRNA sequence data are available in MG-RAST server [58] under project ID 80453 (http://metagenomics.anl.gov/linkin.cgi?project=mgp80453).

Statistical analysis

To correct the differences in the number of reads, which can bias diversity estimates, a subset of 3311 sequences was randomly selected of 16S rRNA gene from each sample of the different rainforest types. To determine if the relative abundance of the most abundant archaeal taxa and diversity indices differ between tropical forest types, we used analysis of variance (ANOVA) or Kruskal–Wallis tests for normal and non-normal data, respectively. Furthermore, parametric (Tukey's HSD test) or non-parametric (pairwise Wilcox test) post-hoc tests were used in case of significant results of ANOVA or Kruskal– Wallis tests, respectively.

The Bray-Curtis distance matrix was built using the 'vegdist' function in the vegan package of R [59]. We performed a nonmetric multi-dimensional scaling (NMDS) plot using the 'metaMDS' function in the vegan package of R. We used the Bray-Curtis distance matrix to assess patterns in archaeal species composition. To evaluate whether archaeal community composition was structured in relation to any of the environmental variables measured (i.e. pH, total N, organic matter, available phosphorous), we used the 'envfit' function in package Vegan in R. Then, we performed an analysis of similarity (ANOSIM) to test the difference

among the different forest types. We used permutational multivariate analysis of variance (PerMANOVA, 'adonis' function in vegan R package) to test the effect of forest type on Bray–Curtis distance matrices with 9999 random permutations. We performed regression analysis using linear functions in SigmaPlot to test whether archaeal alpha-diversity (Shannon index, OTUs richness, etc) was correlated with soil parameters across the different forest types. To assess the differences in beta-diversity among different forest types, we used the 'betadisper' function of 'vegan' R package using 999 permutations to determine the significance of this test. Post-hoc Tukey tests were used for pairwise comparisons among different forest types.

To test the nested structure of the archaeal communities across different forest types, we performed nestedness using BINMATNEST with default input parameters [60]. We tested the nestedness significance using default input parameters and null model 3 which calculates the p-value for total row and column following [61]. We evaluated the correlation between OTUs richness and packed matrix order (in which the nestedness is categorized from high to low) of each sample using Spearman's rho test [62]. To analyze the phylogenetic community assembly processes, we measured the beta-NTI (beta nearest related taxon index) using 'phylocom comstruct' command in Phylocom [63]. Beta-NTI<-2 or beta-NTI>+2 indicates deterministic assembly process and -2<beta-NTI<+2 indicates stochastic assembly process of the community.

Results

We obtained 92708 good quality archaeal 16S rRNA gene sequences in total from 28 samples (8 samples were removed due to very low reads), which were classified into 966 operational taxonomic units (OTUs) at 97% similarity level. Thaumarchaeota was the most abundant archaeal phylum representing 90% of all detected archaeal sequences followed by Euryarchaeota (8% of all archaeal sequences), and Crenarchaeota (1% of all archaeal

sequences) (Fig. 1; Table S1). Statistical analysis showed no difference in the relative abundance of Thaumarchaeota across different tropical forest soils ($X^2(3)=1.55$, P=0.66). The phyla Euryarchaeota was significantly greater in dipterocarp forests compared to the other forest types with $X^2(3)=7.14$, P=0.06. Crenarchaeota (ABUNDANCE?) was significantly different between different forest types with $X^2(3)=10.73$, P=0.01. Crenarchaeota was absent in kerangas forest soils and present in very low percentages (0.02% of all archaeal sequences) in inland heath forest. However, Crenarchaeota abundance as greater in dipeterocarp forests with secondary forest having the highest abundance (5% of all archaeal sequences) (Fig. 1). The majority of sequences belonged to FFSB_c of Thaumarchaeota (87% of total archaeal reads), whose abundance was not statistically different amongst different forest types ($F_{(3,24)}=0.59$, P=0.62; Fig. 2 and Table 1). However, the second abundant group belonged to Euryarchaeota, the Thermoplasmata representing around 9% of all archaeal reads was found to be significantly less abundant in secondary forest compared to the other forests. Groups 1a_c and MCG_c were absent in kerangas forest whereas, Methanobacteria was absent in inland heath forest (Fig. 2; Table 1).

Archaeal 16S rRNA gene OTUs richness and diversity indices (Shannon index) marginally differed across the four different forest types, with kerangas forest having overall lower diversity compared to the other forest soils (OTUs richness: $X^2(3)=7.78$, P=0.05; Shannon index: $F_{(3,24)}=2.97$, P=0.05; Fig. S2). Regression analysis results showed that among measured soil parameters, organic matter and sand were negatively correlated to both OTU richness and Shannon index of the total archaeal community. Shannon index was positively correlated with pH, whereas negatively correlated with soil moisture (Fig. 3). All the measured soil parameters showed significant variation among different forest types except for total nitrogen, available phosphorous and silt concentrations (Table 2), with dipterocarp primary and secondary forests having distinct soil properties from the other forests (Fig. S3).

We performed an NMDS based on the Bray-Curtis similarity matrix to assess differences in

archaeal community composition. Although the NMDS plot showed an overlap of archaea, the community composition varied significantly across the soils in different forest types (Global R=0.64, P=0.001; Fig. 4). The PerMANOVA analyses indicated that forest type explained 37% variation in archaeal community composition (P<0.001, 9999 permutations). Using the 'envfit' function, the vectors of environmental variables were fitted onto ordination space to investigate the effect of soil properties on archaeal community structure. The environmental fitting analysis indicated that of the measured soil properties, soil pH, organic matter content, gravimetric water content, sand content and temperature were acting as strong structuring factors of the archaeal assemblages in the different tropical forest types (Fig. 4). The beta-diversity calculated using 'betadisper' command in R differed significantly across different forests ($F_{(3,24)}$=2.95, P=0.04), with DP forest having the lowest beta-diversity compared to the other forest types (Fig. 5).

Nestedness analysis showed that the archaeal communities followed a nested structure (P<0.0001) across different forest types. We generated a packed matrix order of all samples, in which the nestedness of each sample was categorized from high to low, and the lower ones are nested in the higher ones (Table S2). The samples from dipterocarp forests had the highest rank of nestedness compared to the other forest types. Thus, the OTUs composition of IH and KS forests could be a subset of the archaeal community in the dipterocarp forest.

The beta-NTI analysis of variation in archaeal community assembly processes between different forests suggested that overall, stochastic processes were dominant across the various forest sites. However, DS forest showed some effects of deterministic assembly process due to the higher variation of beta-NTI among DS soil samples (Fig. 6).

Discussion

Hypothesis 1. Archaeal community composition and diversity will differ on a habitat basis between different variants of tropical forest. As we had hypothesized, different forest types

had distinctive archaeal communities. Samples from each forest type clustered separately. Similar results were earlier found for bacteria and fungi at the same study sites [3]. The habitat-related difference in archaeal community composition might be due to the significant difference of soil environmental characteristics including pH, organic matter, soil moisture, etc. Previous studies on tropical rainforests showed that logging and land use change alter soil chemical and physical proprieties [44, 45]. Soil biochemistry plays a major role in determining the composition of microbial communities [64-66], therefore they can be affected by changes in soil physicochemical characteristics [27, 28, 64, 65].

The most abundant phylum detected in our tropical soil samples was Thaumarchaeota (representing about 90% of total archaeal reads). This finding is consistent with previous studies which have found that Thaumarchaeota dominate many different environments including most aquatic and terrestrial habitats [67-69]. Previous studies had already reported Thaumarchaeota as the predominant archaeal phyla in the soils of tropical forests [70, 71]. Thaumarchaeota is a mesophilic group, and most of its taxa whose genomes have been analyzed are apparently ammonia-oxidizing. Thus, members of this phylum have a major role in the nitrogen cycle [17, 72, 73]. Phylum Crenarchaeota were much more abundant in our secondary forest site than in the other forest soils. Crenarchaeota have been reported to dominate soils with higher pH [74, 75], and indeed the secondary forest soils had slightly higher pH (average pH 4.27) than other forest soils.

The presence of methanogens in the secondary forest areas (Fig. 2) seems odd for soils which did not have high-water content. These secondary forest areas are not normally flooded, and we suggest that the presence of methanogens relates to the considerable compaction of soils that is associated with forest clearance using heavy machinery. In clayey soils such as these, water pools readily over vehicle tracks and other compacted areas, forming small leaf-filled puddles and this has presumably gone anaerobic in parts. It is interesting to consider whether

this makes any contribution to methane fluxes from cleared/regenerating forest areas. Another possibility is that some of these methanogens can be found in secondary forest due to the presence of microhabitats (e.g. aggregates) in aerated soils that have contained low concentrations of oxygen [76] – again likely a product of soil compaction by heavy logging vehicles in this case.

Hypothesis 2. We hypothesized that rare and environmentally distinctive forest variants (in this instance, white sand forests) would have lower alpha and beta diversity of archaea than the normal terra firma tropical forests. The results of our study suggest that indeed, rarer habitat types (the white sand forest 'inland heath' and 'kerangas') have lower alpha-diversity compared to the more common dipterocarp primary and secondary forest types. This finding contrasts with bacteria and fungi in the same sites, where Tripathi et al [3] found higher alpha-diversity of bacteria in white sand forest than primary forest, and the same fungal alpha-diversity in all forest types.

In contrast to alpha diversity, archaeal beta-diversity was greatest in the two white sand forests. It is unclear what characteristics of the environment might bring about this greater spatial heterogeneity in the community – possibly the existence of extensive bare patches of soil between vegetated areas, which is normal within the white sand forest. Different archaeal species might be adapted to the physical and chemical characteristics of each of these types of microsites. It is of interest that the two white sand forest types also have greater beta-diversity in the bacterial community, although fungal beta-diversity was lower – possibly due to fewer tree species being present [3]. One possible reason for greater beta-diversity is a greater role of stochasticity in the archaeal community composition – whereby lottery/dispersal limitation effects will be more significant where NH_3 supply occurs as isolated unpredictable bursts [77]. It would be interesting to study through further field observations whether this pattern

345 does actually hold true in terms of NH_3 supply and archaeal population levels.

346

347 *Hypothesis 3. We hypothesized that the soil archaeal community would be very sensitive to*
348 *history of forest clearance, and both alpha and beta-diversity of soil archaea would be lower*
349 *in secondary forest.* Our results showed that forest logging had an effect on both soil archaeal
350 alpha and beta-diversity, with secondary dipterocarp forest having lower alpha diversity, but
351 higher beta-diversity of archaea. Thus, our hypothesis is only partially supported. Previous
352 studies have reported similar uncoupling – a decrease in alpha-diversity along with an
353 increase in beta-diversity due to forest logging – for the fungal community in Sabah where
354 primary forest was once and twice-logged, or converted to oil palm agriculture [28], or for
355 bacterial community where Amazonian primary rainforest was converted to pasture [78].
356 However, our results contrast somewhat with a study in Sabah on the impact of tropical forest
357 logging and conversion to oil palm plantations on soil metagenome of [29], where both alpha
358 and beta-diversity of amplicon and shotgun metagenomes were not influenced by logging and
359 land use change. Another study on bacterial community in Amazon rainforest showed that the
360 land use intensification resulted in an increase of both alpha and beta-diversity of soil bacteria
361 [79].

362 The ecological reasons behind the increase of archaeal beta-diversity after logging may be due
363 to the changes in soil environmental proprieties – such as increased spatial variation in
364 temperature and light supply, soil water content and pH – produced by logging and bulldozing
365 the forest [27]. The area we sampled had been logged and bulldozed within the previous two
366 years, and the canopy cover was very open (<10% coverage by trees over 20 m height),
367 mainly covered by weeds and fast-growing shrubs, with around 20% bare soil exposed to
368 sunlight – much of this in the form of compacted vehicle ruts or loose bulldozed piles of soil.
369 Broken branches and uprooted stumps covered parts of the area, often bulldozed into large

370 piles. This extreme heterogeneity contrasts with the uniform dampness and shade of the
371 undisturbed forest, the unbroken leaf litter layer and the gentle microtopography.

372

373 *Hypothesis 4. We hypothesized that archaeal populations would be mostly structured by*
374 *deterministic processes, but in areas of secondary forest the slow responsiveness to*
375 *disturbance would lead to a greater role of stochastic processes.* In contrast to our prediction,
376 stochastic processes dominated the archaeal community assembly in all the different forest
377 types, with a lesser role of deterministic processes. Paradoxically, that the apparently
378 'disturbed' environment of secondary forest is actually more deterministic in terms of
379 archaeal community. One possible explanation is that due to increased decay of dead material
380 (e,g. roots of dead cleared trees) in the secondary forest, there is an increase in NH_3 supply to
381 the soil, resulting in rapid population increase, high population densities and competition
382 within archaeal communities. This would produce a more deterministic pattern. On the other
383 hand, more NH_3-poor primary forest types, archaeal populations may be effectively inactive
384 and at lower and fluctuating population densities more of the time, such that competition and
385 niche structuring are less important. When isolated and short lived bursts of NH_3 do become
386 available, for example from decay of a dead organism, the increase in archaeal populations
387 may depend more on founder effects and dispersal limitation – hence the greater stochasticity
388 in community structure. It is important to point out, however, that our own data did not
389 support the expected pattern of decreased N abundance in white sand forests, although other
390 studies have supported this [80, 81]. It is possible that in fact lower available P levels, which
391 we observed, could also limit archaeal abundances and their ability to respond to NH_3 supply.
392 In this particular study, practical limitations on analyzing soils in Brunei prevented us from
393 analyzing NH_3 or NO_3 content of our soils – although in any case such nutrients are highly
394 labile and fluctuate markedly over time, such that instantaneous measurements would

probably not be representative. It would also be interesting to compare whether the pattern of predominant stochasticity we observed here in archaeal populations holds true for archaea generally in other environments.

Conclusions

This study has yielded some examples of ways in which soil archaea appear to fit a 'conventional' pattern that holds true for larger organisms. It is clear that – like plants and animals - soil archaea do show strong habitat differentiation within rainforest environments, and it appears that their diversity is lower in the rare and physiologically extreme white sand forest environment. Paradoxically, however, archaea do not show the same trend in terms of beta-diversity as they do in alpha-diversity – a pattern that might relate to heterogeneity in population activity in relation to patchy and short-lived nutrient availability in the nutrient-poor white sand forest.

Also, as expected, secondary dipterocarp forest have lower alpha diversity of archaea than primary forest. This suggests that the undisturbed, stable environment of primary forest favors diversity, just as it does for larger organisms [31, 82], though this is apparently not always the case for fungi and bacteria [27, 83, 84].

The strong role of stochasticity in archaeal ecology - in all the habitats we studied - deserves further consideration in other studies. This contrasts with the deterministic pattern seen for bacteria in the same samples, and would be interesting to consider why and how a stochastic pattern predominates for archaea.

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Table legends

Table 1 Comparison of relative abundance of the archaeal sub-phyla among different tropical forest types in Brunei.

Table 2 Comparison of the variation of soil parameters across different tropical forest types in Brunei evaluated by Kruskal–Wallis test or ANOVA (*).

Figure legends

Fig. 1 Relative abundance of archaeal taxa across different tropical forest types in Brunei at the phyla level.

Fig. 2 Relative abundance of archaeal taxa across different tropical forest types in Brunei at the class level.

Fig. 3 Relationship between soil parameters and diversity of total archaea (OTUs richness and Shannon index) across different tropical forest types in Brunei.

Fig. 4 NMDS ordination of total archaea community composition among tropical forest in Brunei, based on Bray-Curtis distance in relation to edaphic parameters.

Fig. 5 Community beta-diversity of archaeal communities across different tropical forest types in Brunei. Tukey pairwise comparisons are shown; different letters denote significant differences between groups at P values less than 0.05.

Fig. 6 Variation of beta-NTI of archaeal communities across different tropical forest types in Brunei. Tukey pairwise comparisons are shown; different letters denote significant differences between groups at P values less than 0.05.

Supplementary Online Material

Table S1 A packed matrix order categorizing nestedness of each sample from high to low across different tropical forest types in Brunei Forest type and OTUs richness are listed.

Fig. S1 Soil sample locations and sampling scheme of different forest types in Brunei.

Fig. S2 The alpha diversity indices (OTUs richness and Shannon index) of archaeal community in different tropical forest types in Brunei.

Fig. S3 Variation of measured soil parameters in different tropical forest types in Brunei. Tukey pairwise comparisons are shown; different letters denote significant differences between groups at P values less than 0.05.

Figure 1

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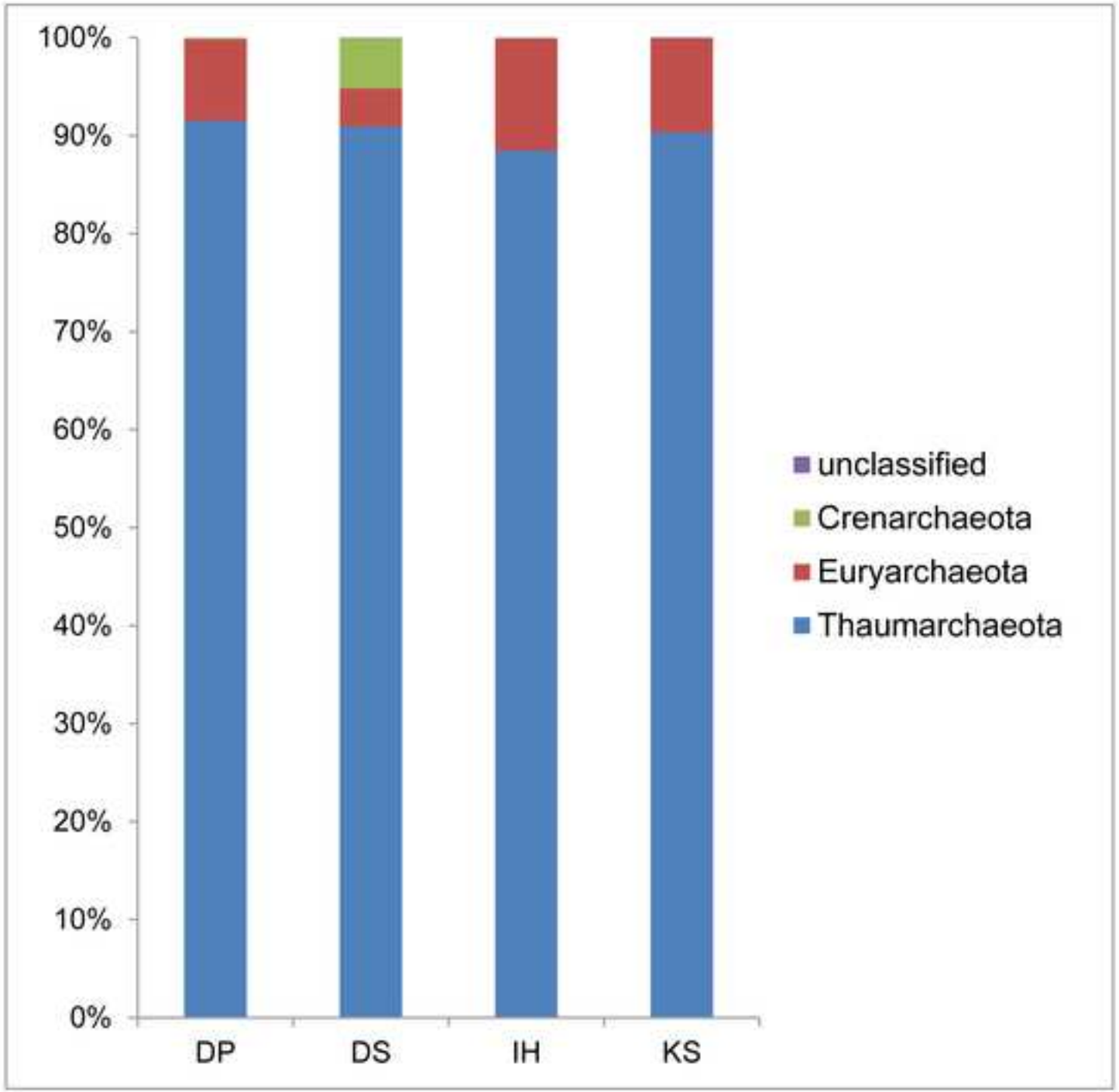


Figure 2

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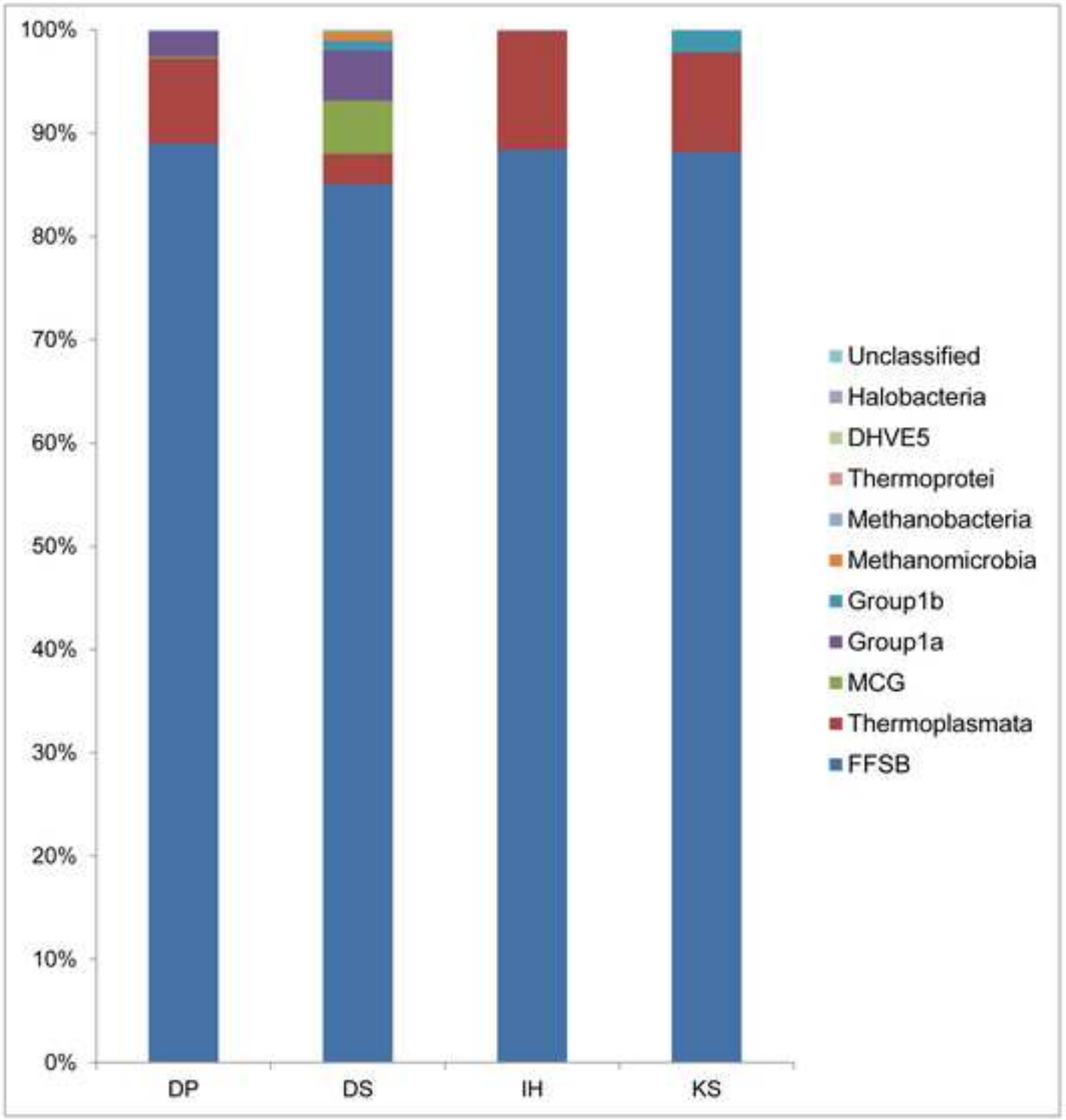


Figure 3

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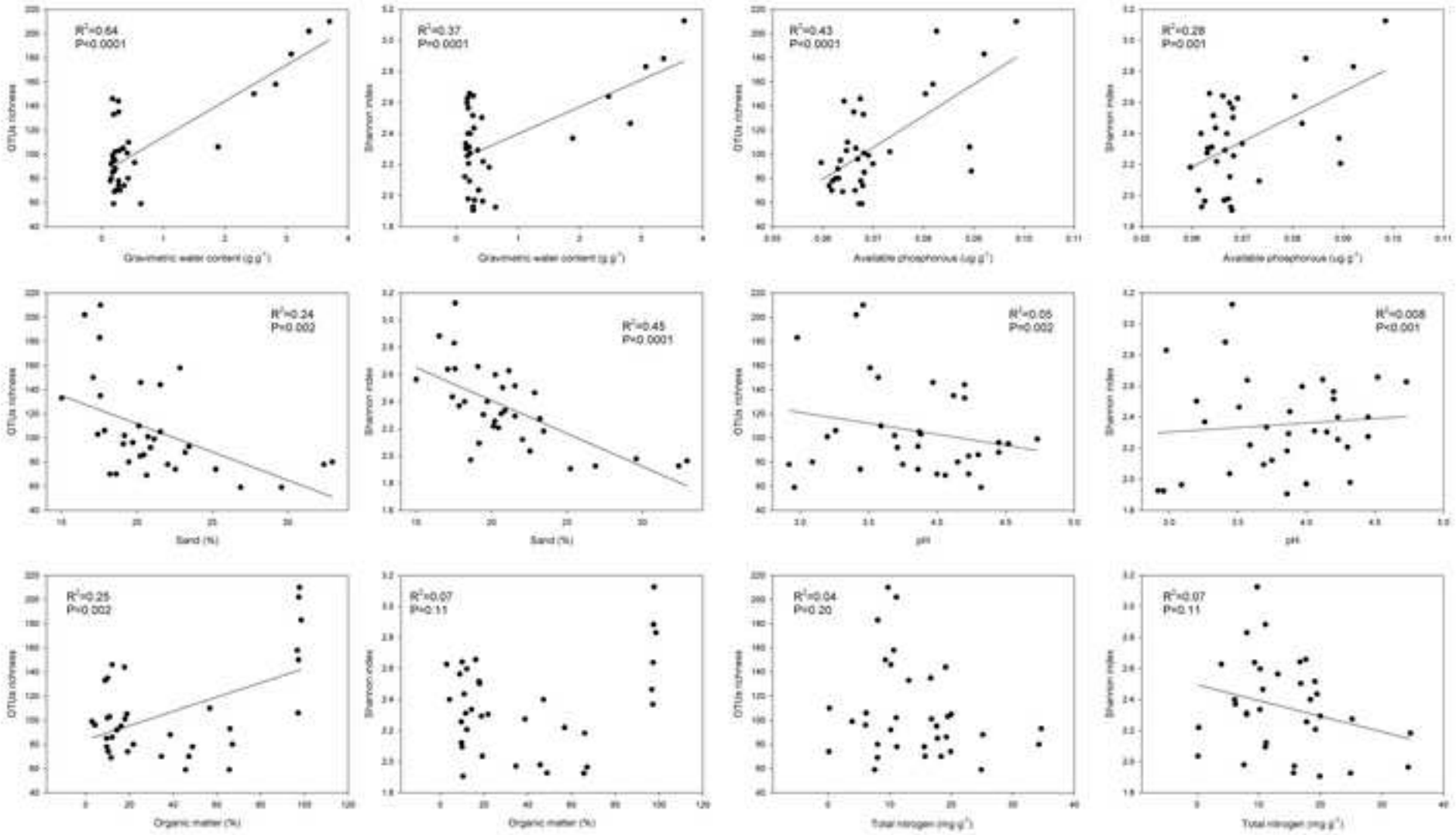


Figure 4

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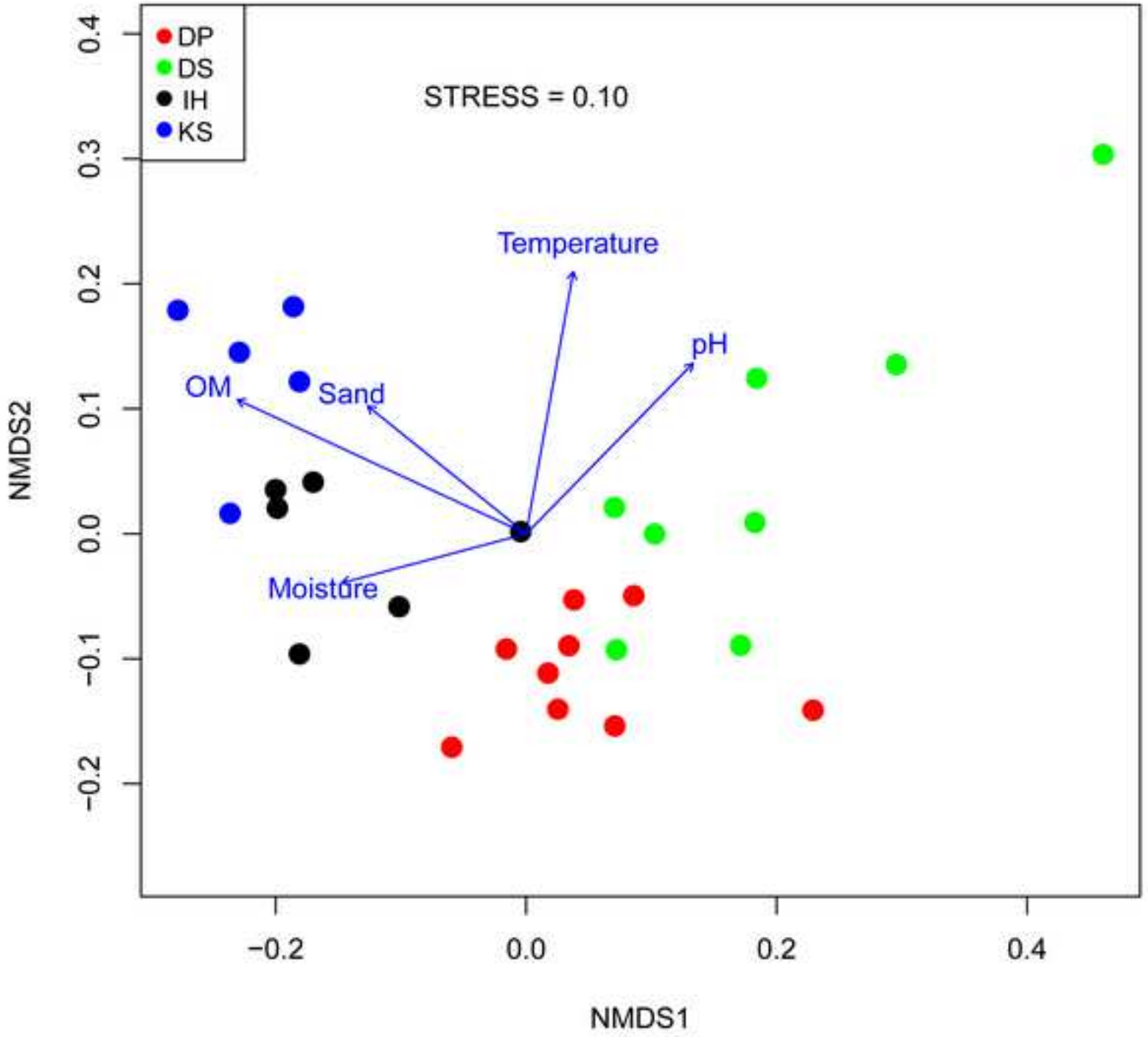


Figure 5

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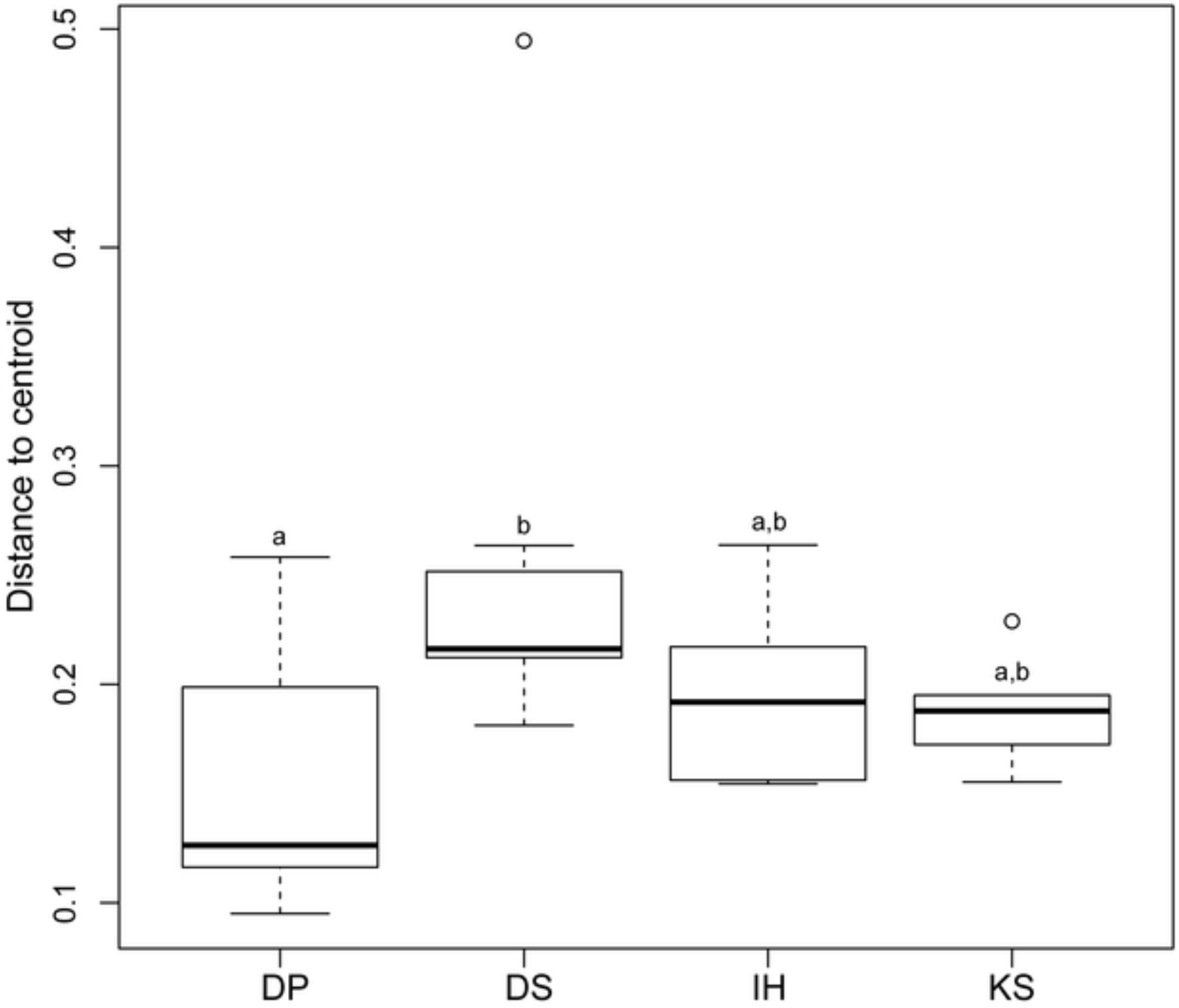


Figure 6

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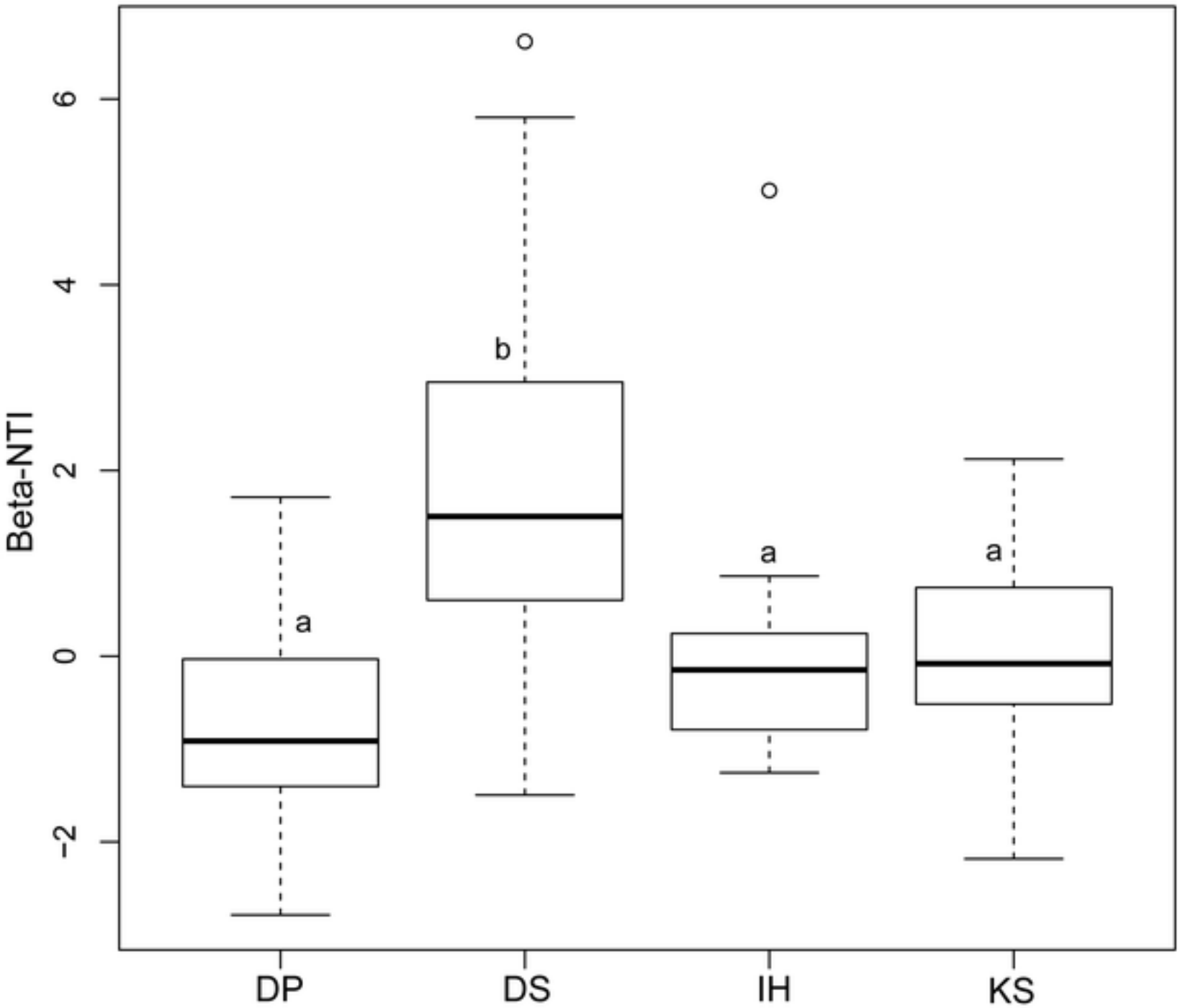


Table 1 Comparison of relative abundance of the archaeal sub-phyla among different tropical forest types in Brunei.

Sub-phylum	**P value**	**X^2 or F***	**DF**
FFSB_c	0.62	0.59*	3,24
Thermoplasmata	0.01	10.06	3
MCG_c	0.01	10.73	3
Group1a_c	<0.001	20.29	3
Methanomicrobia	0.05	7.43	3
Group1b_c	0.01	10.48	3
Methanobacteria	0.33	3.42	3

*Effect of forest type on relative abundance of archaeal taxa evaluated by Kruskal–Wallis test or ANOVA (*)

Table 2 Comparison of the variation of soil parameters across different tropical forest types in Brunei evaluated by Kruskal–Wallis test or ANOVA (*).

Soil parameters	**P value**	**X^2 or F***	**DF**
pH	<0.001	37.1*	3,24
Gravimetric water content (g g^{-1})	<0.001	17.33	3
Total nitrogen (mg g^{-1})	0.37	1.07*	3,24
Available phosphorous (ug g^{-1})	0.52	7.72	3
Organic matter (%)	<0.001	25.64	3
Sand (%)	0.04	8.28	3
Clay (%)	0.001	14.83	3
Silt (%)	0.4	2.83	3
Temperature (ºC)	<0.001	23.34	3

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Reviewers' comments:
Ref.: Ms. No. MECO-D-17-00286
Distinctive soil archaeal communities in different variants of tropical equatorial forest
Microbial Ecology

Reviewer #1:

This paper compared archaeal community structures in different types of tropical forests by employing MiSeq analysis. Considering the lack of information about archaea in tropical forests, this paper has a merit. And the methods are well-established ones with proper statistical analysis. However, the conclusion and discussion are rather weak while the authors proposed interesting hypotheses. Overall, the paper appears to be quite descriptive with a lot of speculation. The paper would be considered as a short communication after the following issues have been addressed. Or, the authors could run complementary experiments where how different factors (pH, ammonium or others that the authors speculated as key controlling variables) may influence archaeal community even in the short-term.

1. My main concern is the spatio-temporal variations of archaea in soil ecosystems. Top 10 cm is a place where microbial activities and diversity would be higher. However, the paper lacks information about depth profile and influences of roots (and their exudates). As such, it does not reflect 'effects of different forest types', but just compared, probably the effects of leaf litter, shading and/or surface water content.

Response: **We have now better explained the sampling method. The 0-10cm layer is generally agreed to be the focus of microbial activity in soils, where most of the microbial biomass and most of the biogeochemical transformation of dead material is taking place. L153-165:**

"In tropical forest soils, as with most soils, the highest microbial density and activity are detected near the surface (Fierer et al 2003, Fontaine et al 2007). Thus, this is a more accurate representation of microbial communities inhabiting soils. The sampling quadrats were randomly located with respect to tree roots. When a sample point fell directly onto a large root or woody stem, in which case it would be moved several centimeters to the side of the root/stem. Within each quadrat, some samples would likely be closer to tree roots than others, but the five subsamples per quadrat were mixed into one composite sample - which in effect integrates spatial heterogeneity. After gently removing the surface moss, leaves, and stones, the five subsamples from within each quadrat were combined into one plastic bag. The collected soil samples were homogenized by sieving (2 mm sieve), and stored at -20˚C until DNA extraction. A total of 36 samples were collected from four different forest types (nine replicates from each forest type)."

2. L286-289: Can the authors speculate further why methanogens are present in the secondary forest areas? Are the areas flooded during rainy seasons, and the results would be the 'legacy' microbes that are dormant? Any comparative data or literature from tropical soils?

Response: **In fact these secondary forest areas are not normally flooded, and we suggest that the presence of methanogens relates to the considerable compaction of soils that is associated with forest clearance using heavy machinery. In clayey soils such as these, water pools readily over vehicle tracks and other compacted areas, forming small leaf-filled puddles and this has presumably gone anaerobic in parts. It is interesting to consider whether this makes any contribution to methane fluxes from cleared/regenerating forest areas. Another possibility is that some of these methanogens can be found in secondary forest due to the presence of**

microhabitats (e.g. aggregates) in aerated soils that have contained low concentrations of oxygen (Fierer 2017) – again likely a product of soil compaction by heavy vehicles in this case. L314-324.

3. L298-309: It would be interesting to compare the data with similar set of data (where bacteria, fungi, and archaea are all determined) from other biomes or ecosystems. Would there be general patterns where bacteria (or fungi) diversity differ from archaea? Or, is it a unique pattern in tropical forest only?

Response: **There has been little systematic study of archaea across habitats anywhere in the world, and relatively little clear comparison with extreme soil variants. In an earlier broad scale study (Tripathi et al 2015), we found that more extreme pH soils in both tropical and temperate environments had distinct communities of archaea – with parallel patterns of habitat gradient differentiation seen in both bacteria and fungi. However, unlike bacteria, archaeal diversity was not lower in the more extreme habitats. In the case of our earlier temperate-tropical study, there was no real equivalent (in terms of being an extreme environment) to the white sand forests studied here.**

"Tripathi, B. M., Kim, M., Tateno, R., Kim, W., Wang, J., Lai-Hoe, A., ... & Adams, J. M. (2015). Soil pH and biome are both key determinants of soil archaeal community structure. Soil Biology and Biochemistry, 88, 1-8."

4. L326-329: Again too sketchy and patchy speculation. Logging could supply extra carbon source and aeration in the short-term, but may reduce overall carbon supply and water content in the soils in the longer-term. The authors need to discuss this issue in depth with more references from temperate forests, considering the limitation of information on tropical forests.

Response: **In fact we did not suggest that carbon sources or aeration necessarily increase, just that the haphazard physical disturbance would tend to create patchiness in the environment. It is a matter of quite straightforward observation that secondary tropical forest after logging and bulldozing has piles of debris in some areas, bare areas in others, shaded areas interspersed with areas open to sunlight, dips and puddles interspersed with piles of loose soil. The secondary forest area we sampled had been logged and bulldozed within the previous 2 years before sampling, and was still in a highly disturbed state with much bare soil and debris – so there had been little chance for the spatial heterogeneity to be 'blurred out' as the reviewer suggests. We are at a loss to justify this rigorously from the literature, as published studies on recently disturbed forest sites take it as a given that readers with an ecological background will understand what such places are like: chaotic, jumbled, and very patchy. It is best that we describe it verbally ourselves (please see the passage below).**

We edited these lines to:
"The ecological reasons behind the increase of archaeal beta-diversity after logging may be due to the changes in soil environmental proprieties – such as increased spatial variation in temperature and light supply, soil water content and pH – produced by logging and bulldozing the forest (Lee-Cruz et al. 2013). The area we sampled had been logged and bulldozed within the previous two years, and the canopy cover was very open (<10% coverage by trees over 20 m height), mainly covered by weeds and fast-growing shrubs, with around 20% bare soil exposed to sunlight – much of this in the form of compacted vehicle ruts or loose bulldozed piles of soil. Broken branches and uprooted stumps covered parts of the area, often bulldozed into large piles. This extreme heterogeneity contrasts with the uniform dampness and shade of the undisturbed forest, the unbroken leaf litter layer and the gentle microtopography." L362-371.

Reviewer #2:

The manuscript" Distinctive soil archaeal communities in different variants of tropical equatorial forest" describes the Archaea present in four different soil samples from Brunei.

The manuscript is written with four hypotheses, which the authors tested. I don't like this way of writing, because the focus is on each of the hypotheses rather than the overall story. A manuscript should always tell a story rather than list hypotheses and the outcome of testing them. Therefore, I would recommend to re-write the introduction and the discussion with focus on the overall story.

Response: We have now extensively rewritten the introduction as suggested.

The number of sequences in general is very low, while the number of OTU's is quite high. Normally scientists can get higher numbers of sequences per Illumina run.

Response: We obtained 121822 sequences in total, but used quite a low cutoff because some samples had lower numbers of reads and we wanted to keep a high number of replicates. However, the number of reads used (3311 reads per sample) should be adequate for general purposes of comparing communities.

Specific comments:
Line 48 - 49: NO2- and NO3- rather than NO2 and NO3.

Response: We have now corrected NO_2 and NO_3 to NO_2^- and NO_3^- accordingly. L50-51.

Line 154 - 157: It would be good to add some more information about the sequencing. Which primers were used to amplify the DNA and what approach was used for sequencing?

Response: Now we have added more information about the sequencing process. L168-174.

Line 171 - 178: What were the quality cut-offs for the sequences, how long were the sequences? Please add some more information about the initial quality control after sequencing.

Response: We have now added more lines to clearly describe the steps of sequences analysis and the quality control. For sequence quality control, we used pandaseq for merging paired ends with stringent quality control criteria. Sequences were then denoised using the 'pre.cluster' command in mothur, which applies a pseudo-single linkage algorithm with the goal of removing sequences that are likely due to sequencing errors. Putative chimeric sequences were detected and removed via the Chimera Uchime algorithm in Mothur. L188-199.

The sequences median length is 446 base pair.

Line 214 - 216: The number of sequences is rather low for 36 samples. This means that you only got around 3000 sequences per sample, which is for NGS data really low. I would normally expect 5-10 x higher number of sequences.

Response: We agree with the reviewer. However, the number of sequences is low because a lot of sequences belonged to bacteria and/or other taxa because of the universal primer pair used for amplification (http://cgeb-imr.ca/protocols.html**). Nevertheless, 3000 reads should still be adequate for general comparisons of communities, and it is capable of showing clear differences here.**

Line 217 - 230: The Archaea were distributed into phyla, but no information is provided on any more detailed phylogenetic affiliation. Thaumarchaeota are not just AOA. There are other Archaea in that phylum and it would be good to give the readers a little more information on the groups that are dominant in the different phyla, because it has a huge impact on the metabolic capacities of the soils.

Response: Now, we have provided more detailed phylogenetic information of archaeal taxa. L251-257.

Line 241: Is the Bray-curtis similarity index really the best index to describe the changes in the communities? Bray-Curtis just measures presence-absence rather than abundance and/or phylogenetic affiliation. I would suggest to use indices that include information about the abundance of certain strains as well as about the phylogenetic relationship!

Response: We do not agree with reviewer on this point, that Bray-Curtis just measures presence and absence. Bray-Curtis also takes abundance into account (Baselga et al 2013, Wolda 1981). ***"Baselga, A. (2013). Separating the two components of abundance-based dissimilarity: balanced changes in abundance vs. abundance gradients. Methods in Ecology and Evolution, 4(6), 552-557.***

Wolda, H. (1981). Similarity indices, sample size and diversity. Oecologia, 50(3), 296-302."

In the discussion, you often mention alpha and beta diversity per sample. While alpha diversity is determined per sample and tells you the diversity in a sample, beta diversity compares different samples by calculating the distances/dissimilarities between two samples. Those data are afterwards presented in 2D plots like NMSD or PCoA. In Figure 5 you present the "average distance to the centroid of the different soils" and use those data to interpret beta diversity. The distances of all samples are pretty similar, while when looking at Figure 4 comparing the different soils, more differences could be detected. Beta diversity should be used comparative for all samples.

Response: We agree with the reviewer that beta diversity compares different samples by calculating the distances/dissimilarities between two samples. To avoid this mistake, we removed using the term beta diversity per sample through the manuscript. We also removed the use of "distance to centroid". L278.

Figure 4: The archaeal community in general is not extremely different, when looking at Figure 4, IH and KS are even overlapping.

Response: We agree that the archaeal community is not extremely different. However, it is clear that the archaeal community is clustered separately by each forest type. The statistics confirm this difference (Global R=0.64, P=0.001). Now we have clarified the sentence. L268-271.



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Distinctive soil archaeal communities in different variants of tropical equatorial forest

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