Microbial Ecology, Volume 76, Issue 1, July 2018, pp. 215-225
DOI:10.1007/s00248-017-1118-4

1 **Distinctive soil archaeal communities in different variants of tropical equatorial forest**

2 Dorsaf Kerfahi[1], Binu M. Tripathi[2], J. W. Ferry Slik[3], Rahayu S. Sukri[3], Salwana Jaafar[3],
3 Jonathan M. Adams[4*]

4

5 [1]Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Biological Oceanography,
6 Seestrasse 15, D-18119 Rostock, Germany.

7 [2]Korea Polar Research Institute, 26 Songdomirae-ro, Yeonsu-gu, Incheon 21990, Republic of
8 Korea.

9 [3]Faculty of Science, Universiti Brunei Darussalam, Gadong, Brunei Darussalam.

10 [4]Division of Agrifood and Environment. Cranfield University, College Rd, Cranfield MK43
11 0AL, UK.

12

13 ***Corresponding author:** Jonathan M. Adams, Division of Agrifood and Environment.
14 Cranfield University, College Rd, Cranfield MK43 0AL, UK

15 E-mail:geograph.ecol@gmail.com and j.m.adams@cranfield.ac.uk

16

Published by Springer. This is the Author Accepted Manuscript issued with:
Creative Commons Attribution Non-Commercial License (CC:BY:NC 4.0).
The final published version (version of record) is available online at DOI:10.1007/s00248-017-1118-4.
Please refer to any applicable publisher terms of use.

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

41 **Introduction**

42 Tropical forests are known as one of the richest and most diverse environments on Earth [1,
43 2]. However, the composition and diversity of the microorganisms is poorly known compared
44 with macro-organisms [3]. Archaea represent one of the three main lineages of life on Earth
45 [4] and constitute a small but consistent percentage of soil microbial communities, usually
46 around 1-3% of the prokaryotic cells [5-8]. Thus, understanding their ecology and patterns in
47 the environment may be important in understanding ecosystem functioning.
48 Apart from their role in decay and methane production in waterlogged soils and wetlands,
49 archaea are also thought to play an important role in nitrogen cycling in soils in general, due
50 to the ability of many soil archaea to oxidize NH_3 into NO_2^-, the first step towards conversion
51 to NO_3^- which can be taken up more easily by plant roots [9]. In fact, the trophic mechanisms
52 of most archaea from aerobic soils are poorly known, because only a few have been cultured –
53 though of all those which have been cultured possess genes for ammonia oxidation [10, 11].
54 Amongst the diversity of aerobic soil archaea, which comprise several phyla, there have been
55 some hints of other trophic modes including heterotrophy of organic molecules, but the
56 importance of these remains unknown [11-13].

57 From previous studies, it is clear that soil archaeal communities can vary in predictable ways
58 along broad environmental gradients [10, 11, 14-16], and between different habitats [17]. Our
59 previous study [8] sampled archaea in both primary forest and agricultural environments on a
60 broad scale across Malaysia, finding that soil pH played a major role in community
61 composition. However, it is still unclear how strongly the composition, structure, and
62 diversity of the archaeal communities can differ on a local scale between common *terra firma*
63 tropical forest and distinctive and rare tropical forest variants such as white sand forests [18-
64 20]. Understanding this would give an added perspective on how specialized archaea are with
65 respect to habitat in the tropics. Given that the ecosystem of white sand forests is widely

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

70 We were also interested in understanding what influence conversion to secondary forest may
71 have on archaeal communities, as an indicator of their sensitivity to disturbance – an issue
72 that may be of conservation importance for understanding the effects of forest clearance.
73 Forest disturbance through logging or clearance, followed by regeneration of secondary
74 forest, is a major influence on tropical forests. The differences between secondary and
75 primary forests have been studied for a range of organisms, but never before for archaea [27-
76 31].

77 It is also unclear generally whether soil archaeal communities are governed by deterministic
78 or stochastic processes [11]. The role of stochasticity is a subject which has focused on a
79 range of different groups of organisms [32-35], but never apparently archaea. This could be of
80 relevance to understanding whether archaeal communities are strongly niche structured by
81 competition, and to understanding heterogeneity in ammonia oxidation potential in soils [36-
82 38].

83 In this study, we focused on different variants of rainforest in the global biodiversity hotspot
84 of Brunei Darussalam, Northwest Borneo. We investigated the variation in archaeal
85 community composition and diversity across dipterocarp primary forest (DP) – the major
86 *terra firma* forest type in SE Asia -, dipterocarp secondary forest (DS), inland heath white
87 sand forest (IH) and kerangas white sand forest (KS). This provides an opportunity to study
88 the composition and diversity of archaeal communities in different forest habitat types but
89 under same climatic conditions. We used 16S rRNA gene amplicon sequencing using Illumina
90 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

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

122

123 **Materials and methods**

124 **Study site**

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

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

144

145 Soil sampling and DNA extraction

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

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

175

176 **Soil Properties Analysis**

177 Geographical co-ordinates were measured using a GPS at each sampling quadrat during field
178 sampling. Soil pH, organic matter content (OM), total nitrogen and available phosphorus
179 concentrations were measured at Universiti Brunei Darussalam using the standard methods
180 [52]. Total nitrogen content was determined by Kjeldahl method. Soil available phosphorus
181 was extracted using Bray's reagent (0.025 M hydrochloric acid and 0.03 M ammonium
182 fluoride), and the phosphorus concentration in the extracts was then determined using a UV-
183 spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Soil organic matter content was
184 determined after incineration in a muffle furnace at 550°C for 2 hours, according to the
185 methodology described by [52].

186

187 **Sequence processing**

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

191 archaeal 16S rRNA gene sequences were aligned against EzTaxon-aligned database.
192 Sequences were denoised using the '*pre.cluster*' command in mothur, which applies a pseudo-
193 single linkage algorithm with the goal of removing sequences that are likely due to
194 sequencing errors [55]. Putative chimeric sequences were detected and removed via the
195 Chimera Uchime algorithm contained within mother [56]. The high quality archaeal 16Sr
196 RNA gene sequences were taxonomically classified against the EzTaxon-e database [57],
197 using the naïve Bayesian classifier implemented in mothur (at ≥80% bootstrap cutoff with
198 1000 iterations). All the 16S rRNA sequence data are available in MG-RAST server [58]
199 under project ID 80453 (http://metagenomics.anl.gov/linkin.cgi?project=mgp80453).
200

201 **Statistical analysis**

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

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

217 among the different forest types. We used permutational multivariate analysis of variance
218 (PerMANOVA, ‘adonis’ function in vegan R package) to test the effect of forest type on
219 Bray–Curtis distance matrices with 9999 random permutations. We performed regression
220 analysis using linear functions in SigmaPlot to test whether archaeal alpha-diversity (Shannon
221 index, OTUs richness, etc) was correlated with soil parameters across the different forest
222 types. To assess the differences in beta-diversity among different forest types, we used the
223 ‘betadisper’ function of ‘vegan’ R package using 999 permutations to determine the
224 significance of this test. Post-hoc Tukey tests were used for pairwise comparisons among
225 different forest types.
226 To test the nested structure of the archaeal communities across different forest types, we
227 performed nestedness using BINMATNEST with default input parameters [60]. We tested the
228 nestedness significance using default input parameters and null model 3 which calculates the
229 p-value for total row and column following [61]. We evaluated the correlation between OTUs
230 richness and packed matrix order (in which the nestedness is categorized from high to low) of
231 each sample using Spearman’s rho test [62]. To analyze the phylogenetic community
232 assembly processes, we measured the beta-NTI (beta nearest related taxon index) using
233 ‘phylocom comstruct’ command in Phylocom [63]. Beta-NTI<-2 or beta-NTI$>+2$ indicates
234 deterministic assembly process and $-2<$beta-NTI$<+2$ indicates stochastic assembly process of
235 the community.
236

237 **Results**

238 We obtained 92708 good quality archaeal 16S rRNA gene sequences in total from 28 samples
239 (8 samples were removed due to very low reads), which were classified into 966 operational
240 taxonomic units (OTUs) at 97% similarity level. Thaumarchaeota was the most abundant
241 archaeal phylum representing 90% of all detected archaeal sequences followed by
242 Euryarchaeota (8% of all archaeal sequences), and Crenarchaeota (1% of all archaeal

243 sequences) (Fig. 1; Table S1). Statistical analysis showed no difference in the relative
244 abundance of Thaumarchaeota across different tropical forest soils ($X^2(3)=1.55$, $P=0.66$). The
245 phyla Euryarchaeota was significantly greater in dipterocarp forests compared to the other
246 forest types with $X^2(3)=7.14$, $P=0.06$. Crenarchaeota (ABUNDANCE?) was significantly
247 different between different forest types with $X^2(3)=10.73$, $P=0.01$. Crenarchaeota was absent
248 in kerangas forest soils and present in very low percentages (0.02% of all archaeal sequences)
249 in inland heath forest. However, Crenarchaeota abundance as greater in dipeterocarp forests
250 with secondary forest having the highest abundance (5% of all archaeal sequences) (Fig. 1).
251 The majority of sequences belonged to FFSB_c of Thaumarchaeota (87% of total archaeal
252 reads), whose abundance was not statistically different amongst different forest types
253 ($F_{(3,24)}=0.59$, $P=0.62$; Fig. 2 and Table 1). However, the second abundant group belonged to
254 Euryarchaeota, the Thermoplasmata representing around 9% of all archaeal reads was found
255 to be significantly less abundant in secondary forest compared to the other forests. Groups
256 1a_c and MCG_c were absent in kerangas forest whereas, Methanobacteria was absent in
257 inland heath forest (Fig. 2; Table 1).
258 Archaeal 16S rRNA gene OTUs richness and diversity indices (Shannon index) marginally
259 differed across the four different forest types, with kerangas forest having overall lower
260 diversity compared to the other forest soils (OTUs richness: $X^2(3)=7.78$, $P=0.05$; Shannon
261 index: $F_{(3,24)}=2.97$, $P=0.05$; Fig. S2). Regression analysis results showed that among measured
262 soil parameters, organic matter and sand were negatively correlated to both OTU richness and
263 Shannon index of the total archaeal community. Shannon index was positively correlated with
264 pH, whereas negatively correlated with soil moisture (Fig. 3). All the measured soil
265 parameters showed significant variation among different forest types except for total nitrogen,
266 available phosphorous and silt concentrations (Table 2), with dipterocarp primary and
267 secondary forests having distinct soil properties from the other forests (Fig. S3).
268 We performed an NMDS based on the Bray-Curtis similarity matrix to assess differences in

269 archaeal community composition. Although the NMDS plot showed an overlap of archaea,
270 the community composition varied significantly across the soils in different forest types
271 (Global R=0.64, P=0.001; Fig. 4). The PerMANOVA analyses indicated that forest type
272 explained 37% variation in archaeal community composition (P<0.001, 9999 permutations).
273 Using the 'envfit' function, the vectors of environmental variables were fitted onto ordination
274 space to investigate the effect of soil properties on archaeal community structure. The
275 environmental fitting analysis indicated that of the measured soil properties, soil pH, organic
276 matter content, gravimetric water content, sand content and temperature were acting as strong
277 structuring factors of the archaeal assemblages in the different tropical forest types (Fig. 4).
278 The beta-diversity calculated using 'betadisper' command in R differed significantly across
279 different forests ($F_{(3,24)}$=2.95, P=0.04), with DP forest having the lowest beta-diversity
280 compared to the other forest types (Fig. 5).
281 Nestedness analysis showed that the archaeal communities followed a nested structure
282 (P<0.0001) across different forest types. We generated a packed matrix order of all samples,
283 in which the nestedness of each sample was categorized from high to low, and the lower ones
284 are nested in the higher ones (Table S2). The samples from dipterocarp forests had the highest
285 rank of nestedness compared to the other forest types. Thus, the OTUs composition of IH and
286 KS forests could be a subset of the archaeal community in the dipterocarp forest.
287 The beta-NTI analysis of variation in archaeal community assembly processes between
288 different forests suggested that overall, stochastic processes were dominant across the various
289 forest sites. However, DS forest showed some effects of deterministic assembly process due to
290 the higher variation of beta-NTI among DS soil samples (Fig. 6).
291

292 **Discussion**

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

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

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

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

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

325

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

334 In contrast to alpha diversity, archaeal beta-diversity was greatest in the two white sand
335 forests. It is unclear what characteristics of the environment might bring about this greater
336 spatial heterogeneity in the community – possibly the existence of extensive bare patches of
337 soil between vegetated areas, which is normal within the white sand forest. Different archaeal
338 species might be adapted to the physical and chemical characteristics of each of these types of
339 microsites. It is of interest that the two white sand forest types also have greater beta-diversity
340 in the bacterial community, although fungal beta-diversity was lower – possibly due to fewer
341 tree species being present [3]. One possible reason for greater beta-diversity is a greater role
342 of stochasticity in the archaeal community composition – whereby lottery/dispersal limitation
343 effects will be more significant where NH_3 supply occurs as isolated unpredictable bursts
344 [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.

Acknowledgments

This work was supported by a grant from the National Research Foundation (NRF) funded by

419 the Korean Government, Ministry of Education, Science and Technology (MEST) (NRF-
420 0409-20150076). We thank the Brunei Forestry Department and the Biodiversity Research
421 and Innovation Centre for entry and export permits respectively, and Universiti Brunei
422 Darussalam for permission to conduct research.

423 **References**

424 1. Corlett RT (2014) The ecology of tropical East Asia. Oxford University Press (UK)
425 2. Whitmore T (1984) Tropical rain forests of the Par East. Oxford: Oxford University
426 Press
427 3. Tripathi BM, Song W, Slik J, Sukri RS, Jaafar S, Dong K, Adams JM (2016)
428 Distinctive Tropical Forest Variants Have Unique Soil Microbial Communities, But
429 Not Always Low Microbial Diversity. Front Microbiol 7.
430 4. Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms:
431 proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci 87:
432 4576-4579.
433 5. Bates ST, Berg-Lyons D, Caporaso JG, Walters WA, Knight R, Fierer N (2011)
434 Examining the global distribution of dominant archaeal populations in soil. The ISME
435 J 5: 908.
436 6. Bengtson P, Sterngren AE, Rousk J (2012) Archaeal abundance across a pH gradient in
437 an arable soil and its relationship to bacterial and fungal growth rates. Appl Environ
438 Microbiol 78: 5906-5911.
439 7. Cao P, Zhang L-M, Shen J-P, Zheng Y-M, Di HJ, He J-Z (2012) Distribution and
440 diversity of archaeal communities in selected Chinese soils. FEMS Microbiol Ecol 80:
441 146-158.
442 8. Tripathi BM, Kim M, Lai-Hoe A, Shukor NA, Rahim RA, Go R, Adams JM (2013)
443 pH dominates variation in tropical soil archaeal diversity and community structure.
444 FEMS Microbiol Ecol 86: 303-311.
445 9. Prosser JI, Nicol GW (2008) Relative contributions of archaea and bacteria to aerobic
446 ammonia oxidation in the environment. Environ Microbiol 10: 2931-2941.
447 10. Singh D, Takahashi K, Park J, Adams JM (2016) Similarities and contrasts in the
448 archaeal community of two Japanese mountains: Mt. Norikura compared to Mt. Fuji.
449 Microb Ecol 71: 428-441.
450 11. Tripathi BM, Kim M, Tateno R, Kim W, Wang J, Lai-Hoe A, Shukor NAA, Rahim
451 RA, Go R, Adams JM (2015) Soil pH and biome are both key determinants of soil
452 archaeal community structure. Soil Biol Biochem 88: 1-8.
453 12. He J-Z, Hu H-W, Zhang L-M (2012) Current insights into the autotrophic
454 thaumarchaeal ammonia oxidation in acidic soils. Soil Biol Biochem 55: 146-154.
455 13. Zhang L-M, Hu H-W, Shen J-P, He J-Z (2012) Ammonia-oxidizing archaea have more
456 important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly
457 acidic soils. The ISME J 6: 1032.
458 14. Angel R, Soares MIM, Ungar ED, Gillor O (2010) Biogeography of soil archaea and
459 bacteria along a steep precipitation gradient. The ISME J 4: 553.
460 15. Nicol GW, Leininger S, Schleper C, Prosser JI (2008) The influence of soil pH on the
461 diversity, abundance and transcriptional activity of ammonia oxidizing archaea and
462 bacteria. Environ Microbiol 10: 2966-2978.
463 16. Singh D, Takahashi K, Adams JM (2012) Elevational patterns in archaeal diversity on
464 Mt. Fuji. PLoS One 7: e44494.
465 17. Shi Y, Adams JM, Ni Y, Yang T, Jing X, Chen L, He J-S, Chu H (2016) The
466 biogeography of soil archaeal communities on the eastern Tibetan Plateau. Sci Rep 6.
467 18. Katagiri S, Yamakura T, Lee SH (1991) Properties of soils in kerangas forest on
468 sandstone at Bako National Park, Sarawak, East Malaysia.
469 19. Proctor J (1999) Heath forests and acid soils. Botanical Journal of Scotland 51: 1-14.
470 20. Moran JA, Barker MG, Moran AJ, Becker P, Ross SM (2000) A comparison of the soil
471 water, nutrient status, and litterfall characteristics of tropical heath and mixed-
472 dipterocarp forest sites in Brunei. Biotropica 32: 2-13.

473 21. Din H, Metali F, Sukri RS (2015) Tree diversity and community composition of the
474 Tutong white sands, Brunei Darussalam: a rare tropical heath forest ecosystem. Int J
475 Ecol 2015.
476 22. Könneke M, Bernhard AE, de La Torre JR, Walker CB (2005) Isolation of an
477 autotrophic ammonia-oxidizing marine archaeon. Nature 437: 543.
478 23. Treusch AH, Leininger S, Kletzin A, Schuster SC, Klenk HP, Schleper C (2005) Novel
479 genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated
480 mesophilic crenarchaeota in nitrogen cycling. Environ Microbiol 7: 1985-1995.
481 24. Lipp JS, Morono Y, Inagaki F, Hinrichs K-U (2008) Significant contribution of
482 Archaea to extant biomass in marine subsurface sediments. Nature 454: 991.
483 25. Thauer RK (2011) Anaerobic oxidation of methane with sulfate: on the reversibility of
484 the reactions that are catalyzed by enzymes also involved in methanogenesis from CO
485 2. Curr Opin Microbiol 14: 292-299.
486 26. Thauer RK, Kaster A-K, Seedorf H, Buckel W, Hedderich R (2008) Methanogenic
487 archaea: ecologically relevant differences in energy conservation. Nat Rev Microbiol
488 6: 579-591.
489 27. Lee-Cruz L, Edwards DP, Tripathi BM, Adams JM (2013) Impact of logging and
490 forest conversion to oil palm plantations on soil bacterial communities in Borneo.
491 Appl Environ Microbiol 79: 7290-7297.
492 28. Kerfahi D, Tripathi BM, Lee J, Edwards DP, Adams JM (2014) The impact of
493 selective-logging and forest clearance for oil palm on fungal communities in Borneo.
494 PloS one 9: e111525.
495 29. Tripathi BM, Edwards DP, Mendes LW, Kim M, Dong K, Kim H, Adams JM (2016)
496 The impact of tropical forest logging and oil palm agriculture on the soil microbiome.
497 Mol Ecol 25: 2244-2257.
498 30. Edwards DP, Larsen TH, Docherty TD, Ansell FA, Hsu WW, Derhé MA, Hamer KC,
499 Wilcove DS (2011) Degraded lands worth protecting: the biological importance of
500 Southeast Asia's repeatedly logged forests. P Roy Soc London B: Biol Sci 278: 82-90.
501 31. Gibson L, Lee TM, Koh LP, Brook BW, Gardner TA, Barlow J, Peres CA, Bradshaw
502 CJ, Laurance WF, Lovejoy TE (2011) Primary forests are irreplaceable for sustaining
503 tropical biodiversity. Nature 478: 378.
504 32. Wang J, Shen J, Wu Y, Tu C, Soininen J, Stegen JC, He J, Liu X, Zhang L, Zhang E
505 (2013) Phylogenetic beta diversity in bacterial assemblages across ecosystems:
506 deterministic versus stochastic processes. The ISME J 7: 1310.
507 33. Stegen JC, Freestone AL, Crist TO, Anderson MJ, Chase JM, Comita LS, Cornell HV,
508 Davies KF, Harrison SP, Hurlbert AH (2013) Stochastic and deterministic drivers of
509 spatial and temporal turnover in breeding bird communities. Glob Ecol Biogeogr 22:
510 202-212.
511 34. Stegen JC, Lin X, Konopka AE, Fredrickson JK (2012) Stochastic and deterministic
512 assembly processes in subsurface microbial communities. The ISME J 6: 1653.
513 35. Hu W, Zhang Q, Tian T, Li D, Cheng G, Mu J, Wu Q, Niu F, Stegen JC, An L (2015)
514 Relative roles of deterministic and stochastic processes in driving the vertical
515 distribution of bacterial communities in a permafrost core from the Qinghai-Tibet
516 Plateau, China. PloS one 10: e0145747.
517 36. Wu Y, Xiang Y, Wang J, Zhong J, He J, Wu QL (2010) Heterogeneity of archaeal and
518 bacterial ammonia-oxidizing communities in Lake Taihu, China. Environ Microbiol
519 Rep 2: 569-576.
520 37. Alves RJE, Wanek W, Zappe A, Richter A, Svenning MM, Schleper C, Urich T (2013)
521 Nitrification rates in Arctic soils are associated with functionally distinct populations
522 of ammonia-oxidizing archaea. The ISME J 7: 1620.
523 38. Liu S, Shen L, Lou L, Tian G, Zheng P, Hu B (2013) Spatial distribution and factors

524 shaping the niche segregation of ammonia-oxidizing microorganisms in the Qiantang
525 River, China. Appl Environ Microbiol 79: 4065-4071.
526 39. Miyamoto K, Suzuki E, Kohyama T, Seino T, Mirmanto E, Simbolon H (2003)
527 Habitat differentiation among tree species with small-scale variation of humus depth
528 and topography in a tropical heath forest of Central Kalimantan, Indonesia. J Trop
529 Ecol 19: 43-54.
530 40. Proctor J, Anderson J, Chai P, Vallack H (1983) Ecological studies in four contrasting
531 lowland rain forests in Gunung Mulu National Park, Sarawak: I. Forest environment,
532 structure and floristics. J Ecol: 237-260.
533 41. Adeney JM, Christensen NL, Vicentini A, Cohn-Haft M (2016) White-sand
534 Ecosystems in Amazonia. Biotropica 48: 7-23.
535 42. Whitmore T, Flenley J, Harris D (1982) The tropics as the norm in biogeography?
536 Geograph J 148: 8-18.
537 43. Huston MA, Huston MA (1994) Biological diversity: the coexistence of species.
538 Cambridge University Press
539 44. McGrath DA, Smith CK, Gholz HL, de Assis Oliveira F (2001) Effects of land-use
540 change on soil nutrient dynamics in Amazonia. Ecosystems 4: 625-645.
541 45. Murty D, Kirschbaum MU, Mcmurtrie RE, Mcgilvray H (2002) Does conversion of
542 forest to agricultural land change soil carbon and nitrogen? A review of the literature.
543 Glob Change Biol 8: 105-123.
544 46. Davies SJ, Becker P (1996) Floristic composition and stand structure of mixed
545 dipterocarp and heath forests in Brunei Darussalam. J Trop For Sci: 542-569.
546 47. Becker P (1992) Seasonality of rainfall and drought in Brunei Darussalam. Brunei
547 Museum Journal 7: 99-109.
548 48. David A, Sidup S (1996) Brunei Metereological Services, Updated 2008, Climate of
549 Brunei Darussalam.
550 49. Fierer N, Schimel JP, Holden PA (2003) Variations in microbial community
551 composition through two soil depth profiles. Soil Biol Biochem 35: 167-176.
552 50. Fontaine S, Barot S, Barré P, Bdioui N, Mary B, Rumpel C (2007) Stability of organic
553 carbon in deep soil layers controlled by fresh carbon supply. Nature 450: 277.
554 51. Comeau AM, Li WK, Tremblay J-É, Carmack EC, Lovejoy C (2011) Arctic Ocean
555 microbial community structure before and after the 2007 record sea ice minimum.
556 PLoS One 6: e27492.
557 52. Allen SE, Grimshaw HM, Parkinson JA, Quarmby C (1974) Chemical analysis of
558 ecological materials. Blackwell Scientific Publications.
559 53. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski
560 RA, Oakley BB, Parks DH, Robinson CJ (2009) Introducing mothur: open-source,
561 platform-independent, community-supported software for describing and comparing
562 microbial communities. Appl Environ Microbiol 75: 7537-7541.
563 54. Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD (2012)
564 PANDAseq: paired-end assembler for illumina sequences. BMC bioinformatics 13:
565 31.
566 55. Huse SM, Welch DM, Morrison HG, Sogin ML (2010) Ironing out the wrinkles in the
567 rare biosphere through improved OTU clustering. Environ Microbiol 12: 1889-1898.
568 56. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves
569 sensitivity and speed of chimera detection. Bioinformatics 27: 2194-2200.
570 57. Kim O-S, Cho Y-J, Lee K, Yoon S-H, Kim M, Na H, Park S-C, Jeon YS, Lee J-H, Yi
571 H (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database
572 with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62: 716-
573 721.
574 58. Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, Paczian T,

575 Rodriguez A, Stevens R, Wilke A (2008) The metagenomics RAST server–a public
576 resource for the automatic phylogenetic and functional analysis of metagenomes.
577 BMC bioinformatics 9: 386.
578 59. Oksanen J, Kindt R, Legendre P, O'Hara B, Stevens MHH, Oksanen MJ, Suggests M
579 (2007) The vegan package. Community ecology package 10: 631-637.
580 60. Rodríguez-Gironés MA, Santamaría L (2006) A new algorithm to calculate the
581 nestedness temperature of presence–absence matrices. J Biogeogr 33: 924-935.
582 61. Geel M, Ceustermans A, Hemelrijck W, Lievens B, Honnay O (2015) Decrease in
583 diversity and changes in community composition of arbuscular mycorrhizal fungi in
584 roots of apple trees with increasing orchard management intensity across a regional
585 scale. Mol Ecol 24: 941-952.
586 62. McDonald JH (2009) Handbook of biological statistics. Sparky House Publishing
587 Baltimore, MD
588 63. Webb CO, Ackerly DD, Kembel SW (2008) Phylocom: software for the analysis of
589 phylogenetic community structure and trait evolution. Bioinformatics 24: 2098-2100.
590 64. Lauber CL, Strickland MS, Bradford MA, Fierer N (2008) The influence of soil
591 properties on the structure of bacterial and fungal communities across land-use types.
592 Soil Biol Biochem 40: 2407-2415.
593 65. Tripathi BM, Kim M, Singh D, Lee-Cruz L, Lai-Hoe A, Ainuddin A, Go R, Rahim
594 RA, Husni M, Chun J (2012) Tropical soil bacterial communities in Malaysia: pH
595 dominates in the equatorial tropics too. Microb Ecol 64: 474-484.
596 66. Cornejo FH, Varela A, Wright SJ (1994) Tropical forest litter decomposition under
597 seasonal drought: nutrient release, fungi and bacteria. Oikos: 183-190.
598 67. Dong K, Kim W-S, Tripathi BM, Adams J (2015) Generalized soil Thaumarchaeota
599 community in weathering rock and Saprolite. Microb Ecol 69: 356-360.
600 68. Siles JA, Margesin R (2016) Abundance and diversity of bacterial, archaeal, and
601 fungal communities along an altitudinal gradient in alpine forest soils: what are the
602 driving factors? Microb Ecol 72: 207-220.
603 69. Stahl DA, de la Torre JR (2012) Physiology and diversity of ammonia-oxidizing
604 archaea. Annu Rev Microbiol 66: 83-101.
605 70. Tupinambá DD, Cantão ME, Costa OYA, Bergmann JC, Kruger RH, Kyaw CM,
606 Barreto CC, Quirino BF (2016) Archaeal community changes associated with
607 cultivation of Amazon Forest soil with oil palm. Archaea 2016.
608 71. Zhong W, Cai Z (2007) Long-term effects of inorganic fertilizers on microbial
609 biomass and community functional diversity in a paddy soil derived from quaternary
610 red clay. Appl Soil Ecol 36: 84-91.
611 72. Auguet J-C, Casamayor EO (2013) Partitioning of Thaumarchaeota populations along
612 environmental gradients in high mountain lakes. FEMS Microbiol Ecol 84: 154-164.
613 73. Pedneault E, Galand PE, Potvin M, Tremblay J-É, Lovejoy C (2014) Archaeal amoA
614 and ureC genes and their transcriptional activity in the Arctic Ocean. Sci Rep 4.
615 74. Nicol GW, Campbell CD, Chapman SJ, Prosser JI (2007) Afforestation of moorland
616 leads to changes in crenarchaeal community structure. FEMS Microbiol Ecol 60: 51-
617 59.
618 75. Nicol GW, Tscherko D, Embley TM, Prosser JI (2005) Primary succession of soil
619 Crenarchaeota across a receding glacier foreland. Environ Microbiol 7: 337-347.
620 76. Fierer N (2017) Embracing the unknown: disentangling the complexities of the soil
621 microbiome. Nat Rev Microbiol.
622 77. Chase JM (2010) Stochastic community assembly causes higher biodiversity in more
623 productive environments. science 328: 1388-1391.
624 78. Rodrigues JL, Pellizari VH, Mueller R, Baek K, Jesus EdC, Paula FS, Mirza B,
625 Hamaoui GS, Tsai SM, Feigl B (2013) Conversion of the Amazon rainforest to

626 agriculture results in biotic homogenization of soil bacterial communities. Proc Natl
627 Acad Sci 110: 988-993.
628 79. Carvalho TS, Jesus EdC, Barlow J, Gardner TA, Soares IC, Tiedje JM, Moreira FMdS
629 (2016) Land use intensification in the humid tropics increased both alpha and beta
630 diversity of soil bacteria. Ecology 97: 2760-2771.
631 80. Fine PV, García-Villacorta R, Pitman NC, Mesones I, Kembel SW (2010) A Floristic
632 Study of the White-Sand Forests of Peru1. Annals of the Missouri Botanical Garden
633 97: 283-305.
634 81. Phillips O, Miller JS (2002) Global patterns of plant diversity: Alwyn H. Gentry's
635 forest transect data set. Missouri Botanical Press
636 82. Edwards DP, Hodgson JA, Hamer KC, Mitchell SL, Ahmad AH, Cornell SJ, Wilcove
637 DS (2010) Wildlife-friendly oil palm plantations fail to protect biodiversity effectively.
638 Conser Lett 3: 236-242.
639 83. Acosta-Martínez V, Dowd S, Sun Y, Allen V (2008) Tag-encoded pyrosequencing
640 analysis of bacterial diversity in a single soil type as affected by management and land
641 use. Soil Biol Biochem 40: 2762-2770.
642 84. Shange RS, Ankumah RO, Ibekwe AM, Zabawa R, Dowd SE (2012) Distinct soil
643 bacterial communities revealed under a diversely managed agroecosystem. PLoS One
644 7: e40338.

645

646 **Table legends**

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

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

651

652 **Figure legends**

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

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

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

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

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

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

667

668 **Supplementary Online Material**

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

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

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

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

677

 Click here to download Figure Fig. 1.tif

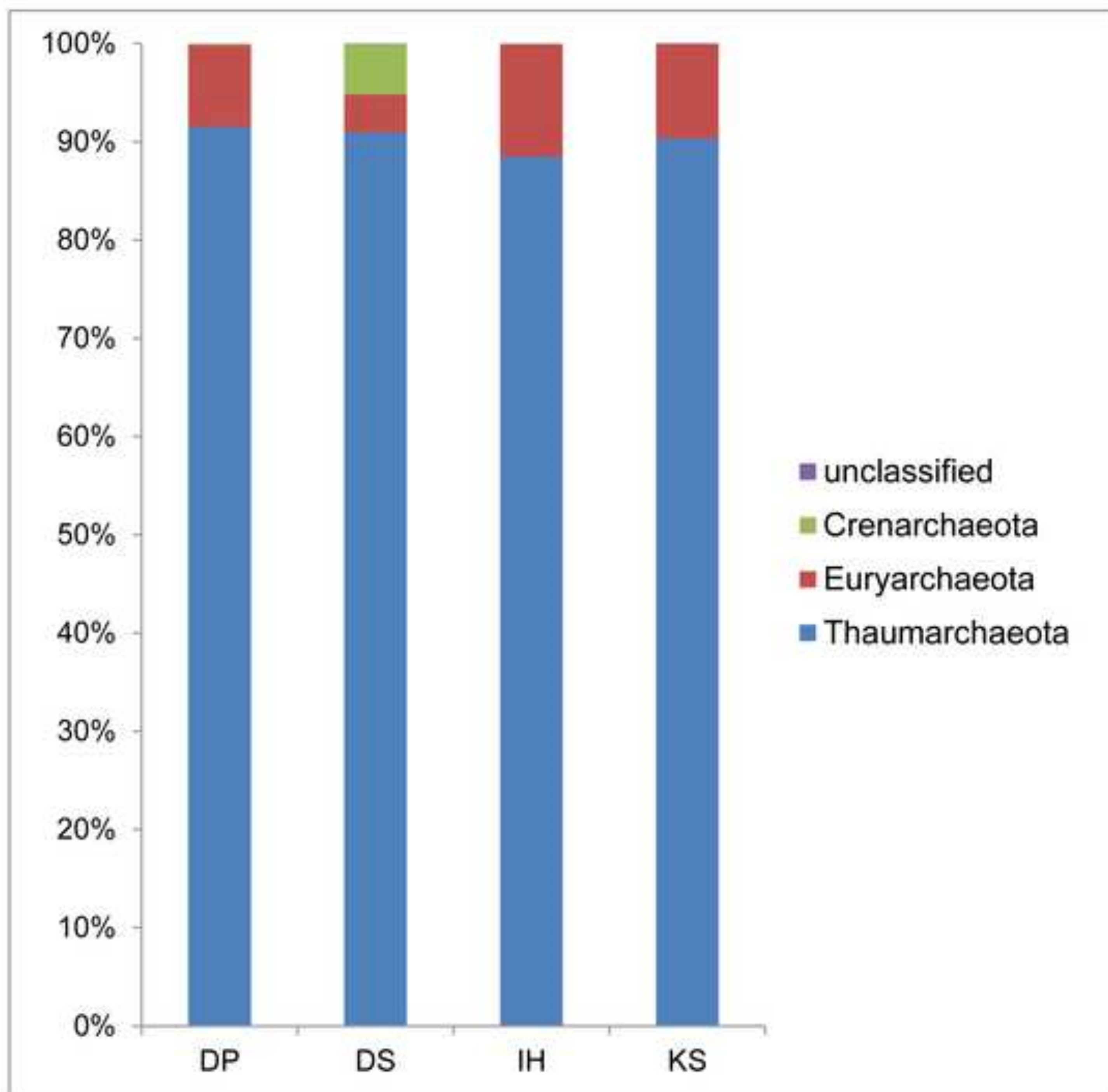


Figure 2 Click here to download Figure Fig. 2.tif

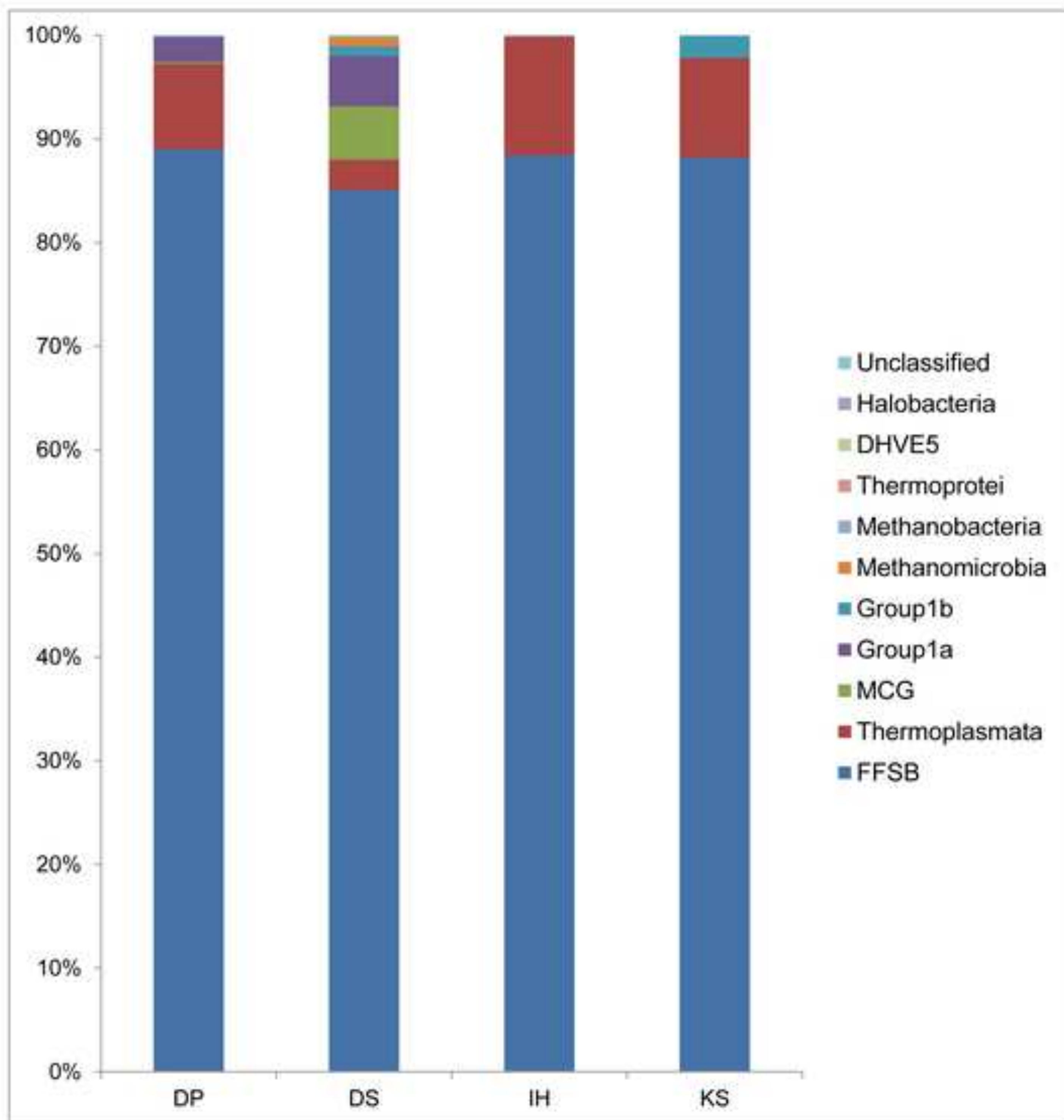


Figure 3

Click here to download Figure Fig. 3.tif

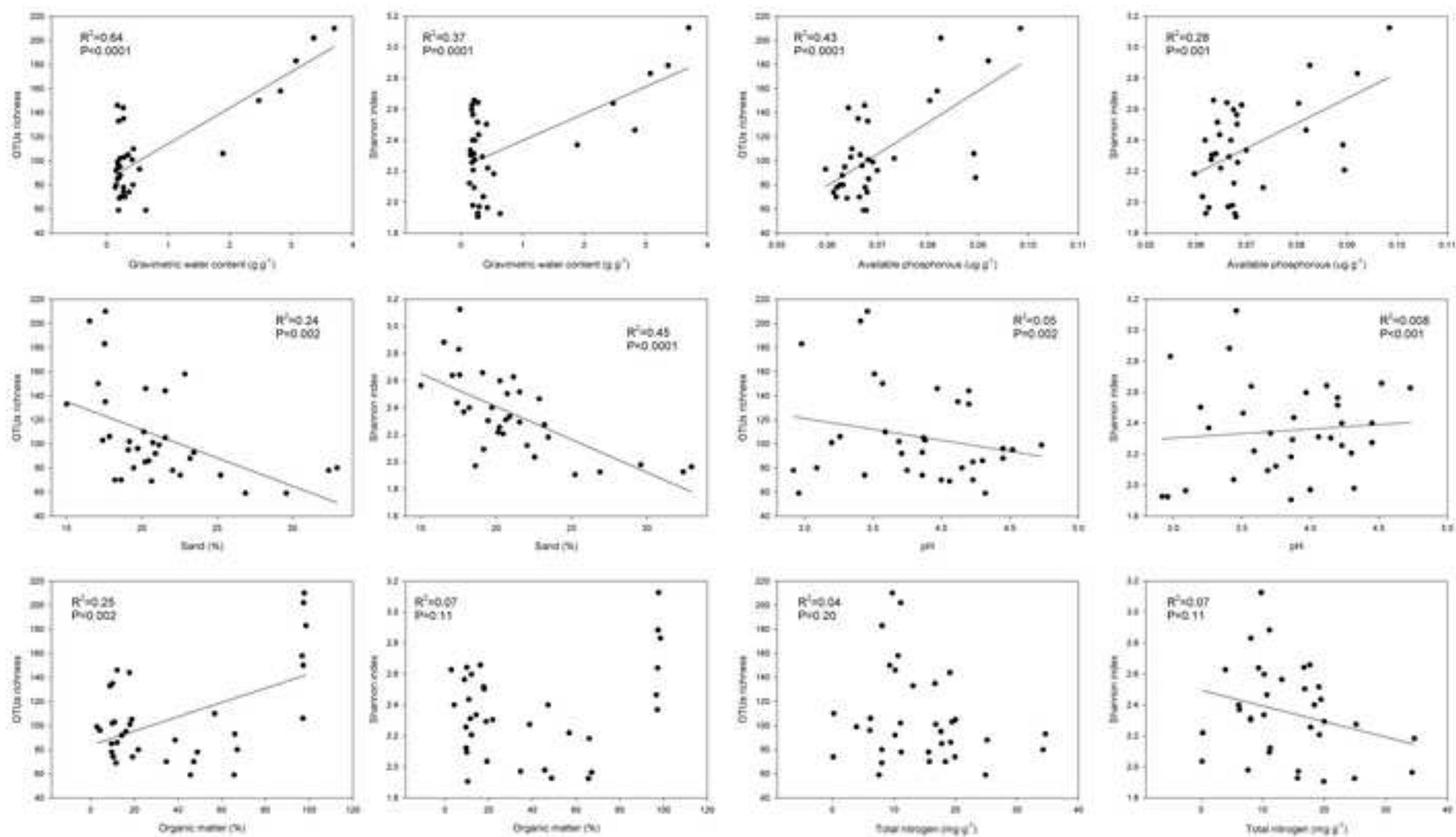


Figure 4

Click here to download Figure Fig. 4.tif

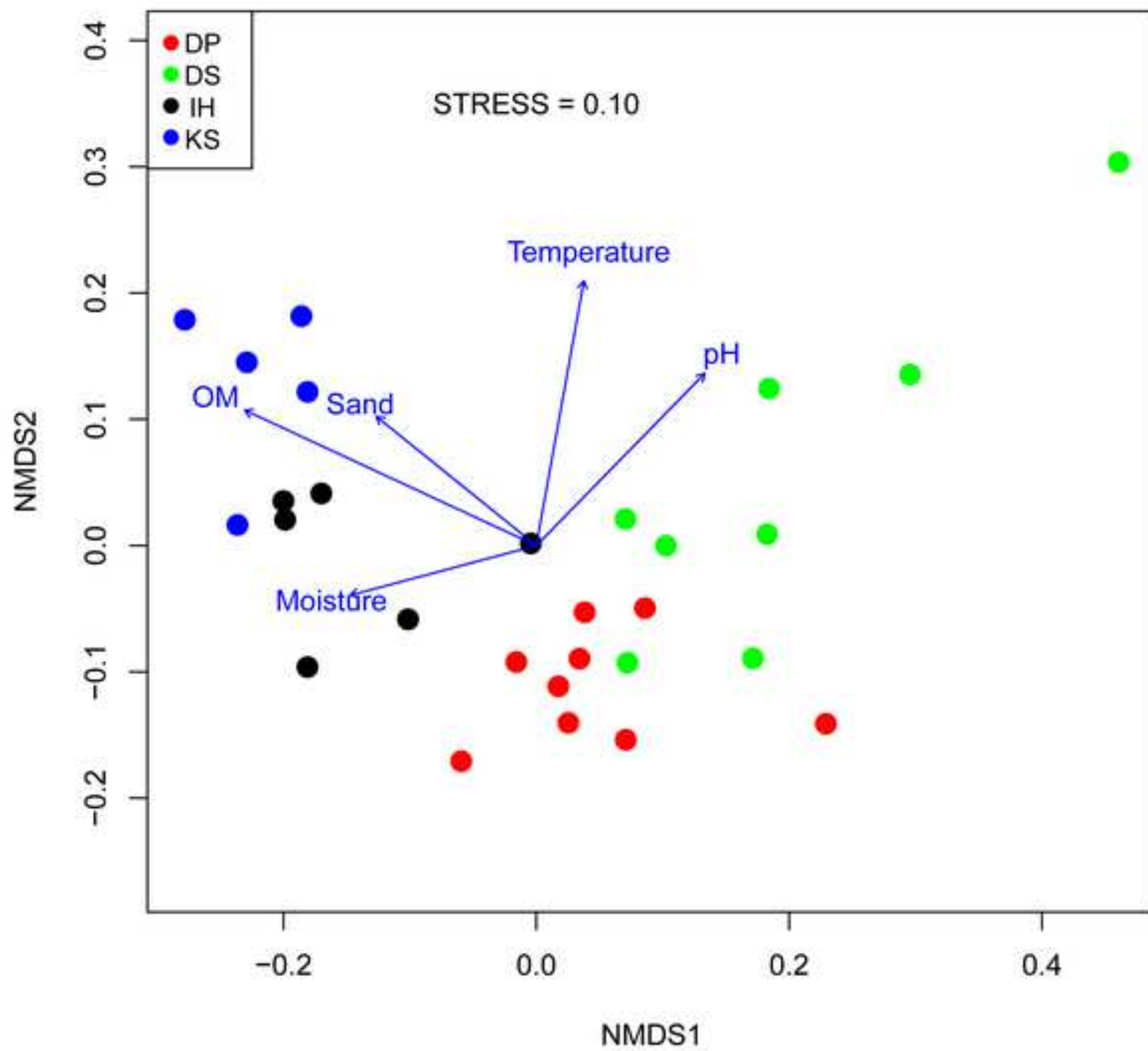


Figure 5 Click here to download Figure Fig. 5.tif

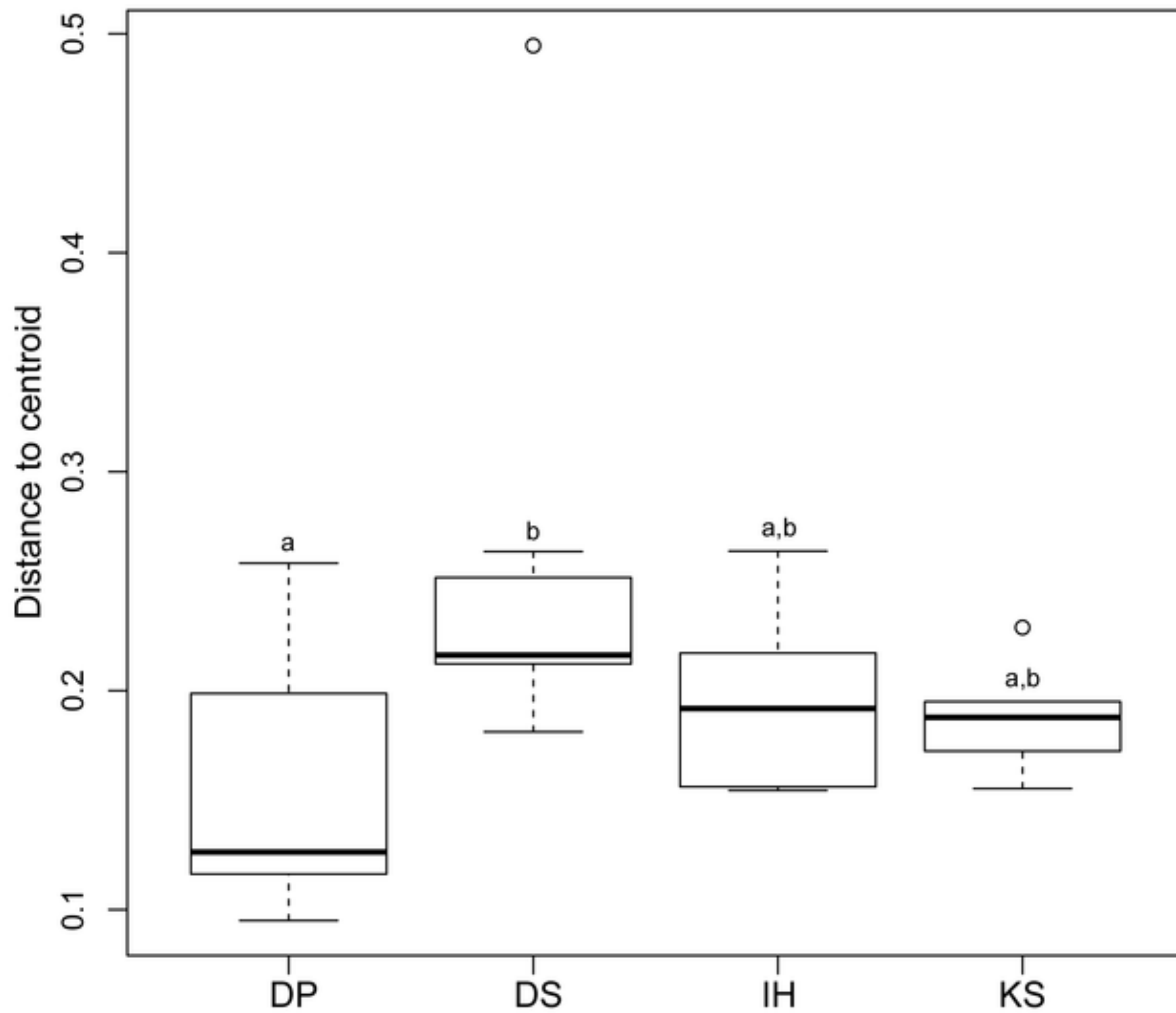
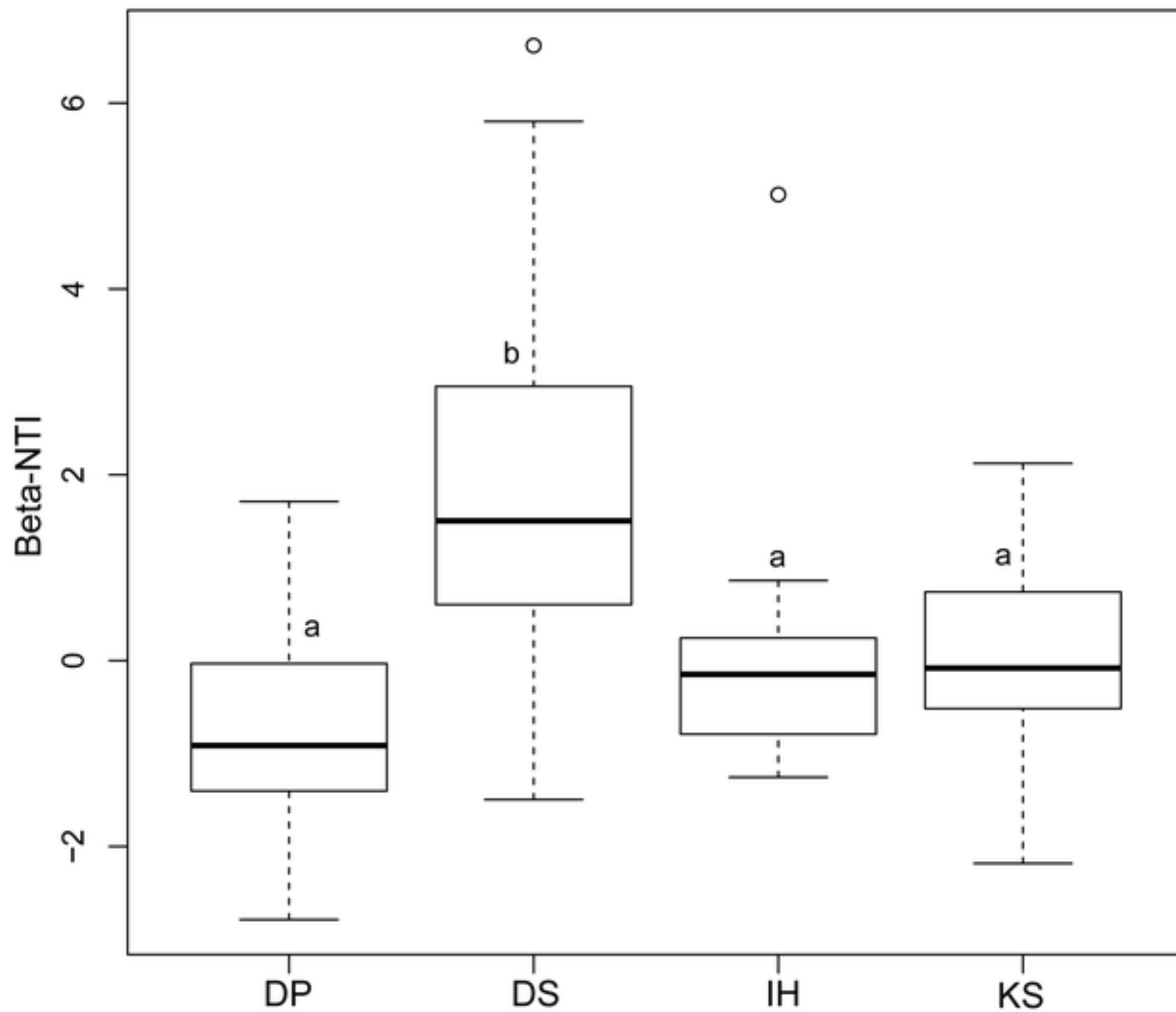


Figure 6

Click here to download Figure Fig. 6.tif



Table

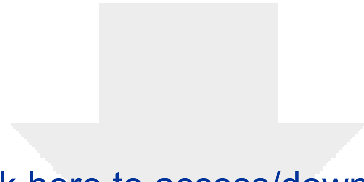
Table 1 Comparison of relative abundance of the archaeal sub-phyla among different tropical forest types in Brunei.

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

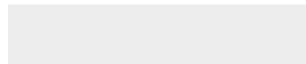
*Effect of forest type on relative abundance of archaeal taxa evaluated by Kruskal–Wallis test or ANOVA (*)

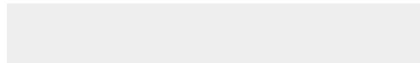
Table 2 Comparison of the variation of soil parameters across different tropical forest types in Brunei evaluated by Kruskal–Wallis test or ANOVA (*).

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 (^{o}C)	<0.001	23.34	3



Click here to access/download
Supplementary Material
Supplementary Materials.docx





Authors Click here to download Authors' Response to Reviewers' Comments Response to Reviewerscomments.docx

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.