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Effects of Elevated Temperature on the Tropical Soil Bacterial Diversity

(Kesan Peningkatan Suhu terhadap Kepelbagaian Bakteria Tanah Tropika)

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ABSTRACT

Bacteria are important biological components of soil that play pivotal roles in improving soil quality and maintaining a balanced ecosystem. However, global climate change may have severe impacts on biodiversity and ecosystems including species loss and extinction of plants and animals, including microbes. Thus, it is crucial to determine how elevated temperature may alter soil bacterial diversity and composition. In this study, an in vitro *simulated temperature rise experiment was carried out on soils from three sampling sites, referring to S1, S2, and S3 around Sabah, Malaysia. Soils were incubated at 25 °C (control) and 27 °C (simulated warming) with constant parameters in a growth chamber up to 16 months. Total DNA was extracted from microbes in the soil and used for PCR amplification targeting the V3-V4 region of the 16S rRNA gene. These amplicons were sequenced using the MiSeq platform (Illumina, USA). Raw DNA sequences were trimmed, merged, and aligned against the 16S rRNA sequences in the NCBI 16S database. The results showed that the analyzed soils were mainly dominated by* Proteobacteria, Actinobacteria, Acidobacteria, *and* Verrucomicrobia. *After 16 months of simulated warming, a net decrease of* Proteobacteria, Acidobacteria, *and* Planctomycetes, *and an increase of* Actinobacteria *and* Chloroflexi *were observed for all three soil samples, indicating that these phyla were highly affected by a temperature rise. At the genus level,* Gaiella *and* Nocardioides *exhibited a net increase while* Bradyrhizobium, Mycobacterium, Tepidisphaera, *and* Paludibaculum *demonstrated net decrease after 16 months of simulated warming. Knowledge on the changes of soil bacterial diversity patterns as a result of temperature elevation will contribute to select the best intervention strategy to overcome global warming issue in the future.*

Keywords: 16S metagenomic sequencing; growth chamber; soil bacteria; temperature

ABSTRAK

Bakteria merupakan komponen biologi penting yang memainkan peranan dalam meningkatkan kualiti tanah dan mengekalkan keseimbangan ekosistem. Akan tetapi, perubahan iklim global mungkin akan memberi kesan buruk terhadap kepelbagaian bio dan ekosistem termasuk kehilangan spesies serta kepupusan haiwan, tumbuhan dan mikrob. Oleh itu, adalah penting untuk menentukan bagaimana peningkatan suhu akan menyebabkan perubahan kepelbagaian dan komposisi bakteria dalam tanah. Dalam kajian ini, uji kaji simulasi peningkatan suhu secara in vitro *telah dijalankan ke atas tanah yang diperoleh dari tiga tapak pensampelan di sekitar Sabah, Malaysia. Tanah tersebut dieram pada suhu 25 °C (kawalan) dan 27* °C (*simulasi) dengan parameter yang sama dalam kebuk pertumbuhan selama 16 bulan. DNA keseluruhan telah diekstrak daripada mikrob dalam tanah dan digunakan untuk amplifikasi PCR menyasarkan kawasan V3-V4 pada gen 16 SrRNA. Amplikon tersebut dijujuk dengan menggunakan platform Miseq (Illumina, USA). Data penjujukan telah dipangkas, digabungkan dan disejajarkan dengan jujukan 16 SrRNA pada pangkalan data 16S NCBI. Hasil kajian menunjukkan bahawa sampel tanah didominasi oleh* Proteobacteria, Actinobacteria, Acidobacteria *dan* Verrucomicrobia. *Selepas simulasi pemanasan selama 16 bulan, pengurangan bersih bagi* Proteobacteria, Acidobacteria *dan* Planctomycetes *serta peningkatan bagi* Actinobacteria *dan* Chloroflexi *dapat diperhatikan untuk ketiga-tiga sampel tanah. Pada peringkat genus,* Gaiella *dan* Nocardioides *menunjukkan peningkatan bersih manakala* Bradyrhizobium, Mycobacterium, Tepidisphaera *dan* Paludibaculum *menunjukkan penurunan bersih selepas 16 bulan. Pengetahuan corak perubahan kepelbagaian bakteria dalam tanah akibat peningkatan suhu persekitaran akan dapat membantu dalam strategi intervensi ke atas tanah bagi menangani isu pemanasan global pada masa hadapan.*

Kata kunci: Bakteria tanah; kebuk pertumbuhan; penjujukan metagenomik 16S; suhu

Introduction

Population growth, industrial development, and burning of fossil fuels around the world contribute significantly towards elevated CO_2 concentration that eventually leads to an increase in environmental temperature (NOAA/ESRL 2010). According to the 2019 Global Climate Report from NOAA, 2019 recorded the second warmest year in the 140-year record, with an increase of 1.15 ºC since the global record began in 1880 (NOAA 2020). IPCC (2018) affirmed that an increase of even 1.15 ºC in global temperature has severe impacts on biodiversity and ecosystems. This climatic variation showed to be one of the contributing factors to the decrease of microbial richness of terrestrial microbes (Classen et al. 2015; Rinnan et al. 2007; Schuur et al. 2008; Zhang et al. 2005). There is also increasing concern on the impact of elevated temperature in the surging of infectious disease. Colwell (1996) provided evidence that warming may directly increase the population of *Vibrio cholera* while Baker-Austin et al. (2010) reported that an increase of 1 °C in sea surface temperature to be associated with the two-fold increase of reported *Vibrio* cases in the Baltic Sea.

Soil microbes are an important biological component of soil function that plays a fundamental role in biogeochemical cycling, improving soil quality, shaping the habitats, and maintaining the ecosystem (Hayat et al. 2010). Although soil microbes remain as one of the most abundant and diverse organisms with important ecological functions, their responses towards elevated temperature have not been fully explored primarily due to their complex and diverse taxonomic groups (Sheik et al. 2011). Furthermore, only about 0.1% of microbes are culturable and hence, we have limited knowledge about their niches and functions (Schloss & Handelsman 2005; Torsvik et al. 2002; Zhang et al. 2013). Fortunately, due to the advancement of next-generation sequencing technology, metagenomic studies became more approachable, enabling in-depth analysis of soil bacterial communities. It is unclear how will bacterial diversity be affected when the global temperature rises in the tropical, especially bacterial from a terrestrial environment. Therefore, this study aims to investigate the effects of elevated environmental temperature on the diversity and composition of soil bacterial communities in the tropics using an *in vitro* simulated warming method in a growth chamber.

Materials and Methods

Experimental design and soil collection

In this study, 25 °C was selected as the control temperature by calculating the mean temperature for a year between August 2015 and July 2016 from the data obtained from Malaysian Meteorological Department, Kota Kinabalu, Sabah. Meanwhile, 27 °C which was 2 °C higher than the mean temperature was selected as the temperature for simulated warming. Higher temperature differences than 2 °C will be surreal and may fail to predict the effects of climate change towards soil bacteria. Other parameters such as humidity, day length, water, and photosynthetic active radiation (PAR) were also determined from the mean value from the meteorological data.

Based on the experimental design of Yergeau et al. (2012), five replicates of each sample were incubated at 25 °C (control) and 27 °C (simulated warming) in two different growth chambers. Other parameters such as humidity (81.2%), day length (12 h), water (100 mL) and photosynthetic active radiation (240 µmol. m^{-2} s^{-1}) were constants for both conditions. *In vitro* simulated warming using growth chamber was selected to manipulate the temperature in this study as it is cost-effective, using only growth chamber and more stable since the temperature and other parameters are fixed, indicating that the changes of the bacterial communities are mainly due to elevated temperature either directly or indirectly (Mateos-Rivera et al. 2016; Yergeau et al. 2012).

Soil from three sampling sites, S1 at Ranau (05°56.701' N 116°138.568' E), S2 at Kota Belud (06°01.168' N 116°29.890'E) and S3 at Kundasang (06°00.243' N 116°32.577' E) were collected in October 2015 for an *in vitro* simulated temperature rise experiment for 16 months. Five replicates of 600 g for each sample were incubated at 25 °C (control) and 27 °C (simulated), respectively, in growth chambers with constant parameters. Approximately 70 g of soil with five replicates at each location was retrieved from the growth chamber every 4 months for a period of 16 months.

Total DNA extractions

Total bacterial genomic DNA from the Sabah soils incubated in growth chambers was extracted using the PowerLyzer PowerSoil DNA Isolation Kit (Mo Bio Laboratories) and vortexed at maximum speed for 20 min as a bead-beating option. DNA extractions were performed separately for each of the five replicates taken per samples. Equal volumes of these five extractions were pooled to create the mixed total DNA to be used for downstream analysis. The integrity of genomic DNA extracted was verified by agarose gel electrophoresis and quantified using the NanoVue nano spectrophotometer (GE Healthcare, Buckinghamshire, UK). Extracted DNA was diluted to 5 ng/µl prior to library preparation.

Soil analysis

Soil analysis was carried out to determine total nitrogen, total organic carbon and total phosphorus content of the soil samples for the 0- and 16-month intervals. About 25 g of sieved soil for each soil sample were sent to Borneo Samudera Sdn. Bhd. for soil analysis.

16S rRNA GENE SEQUENCING

PCR amplification of the V3-V4 region of the 16S rRNA gene was carried out using the primer pair GC S-D-Bact-0341-b-S-17 (5'-CCT ACG GGN GGC WGC AG-3') and S-D-Bact-0785-a-A-21 (5'-GAC TAC HVG GGT ATC TAA TCC-3') (Klindworth et al. 2013). PCR amplification was performed in a 25 µL mixture containing 12.5 ng template DNA, 0.2 µM forward and reverse primers and 1X KAPA HiFi HotStart ReadyMix (Kapa Biosystems Inc., Wilmington, MA). The amplification began with an initial denaturation of 95 °C for 3 min and followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s and extension at 72 °C for 30 s before ending with a final elongation step at 72 °C for 5 min. The expected size of the PCR product was approximately 550 bp. The 16S rDNA amplicons obtained were subjected to Illumina MiSeq sequencing using a paired-end 250 bp sequence read to run with the MiSeq Reagent Kit v2 (Illumina® Inc., San Diego, California). The raw sequencing reads obtained for each soil sample were subjected to quality filtering using ConDeTri v2.2 (Smeds & Künstner 2011) and assembled into single contigs using PEAR 0.9.10 (Zhang et al. 2014). Processed reads were subjected to taxonomic classification using NCBI's 16S Microbial database and the results were visualized using MEGAN5 software (Huson et al. 2011). Two-way analysis of variance (ANOVA) was used to determine the interactive effects between temperature, incubation period and relative abundance of soil bacteria. The results between control and simulated community were compared to study the changes in soil bacterial diversity and composition at elevated temperatures.

RESULTS

TOTAL DNA EXTRACTION

Total DNA of the soil from three sampling sites, S1, S2, and S3 were extracted directly from the soil without enrichment and culturing steps yielded between 15.50 and 45.00 ng/µL (data not shown). The genomic DNA had $A_{260/280}$ and $A_{260/230}$ ratios range between 1.82-2.02 and 1.66-2.02, respectively. Extracted DNA was diluted to 5 ng/uL before the PCR amplification to minimize and eliminate the inhibitory effect of humic acid and other contaminants that co-purified with the genomic DNA.

SOIL ANALYSIS

Total nitrogen, organic carbon, and phosphorus content of the soil samples for the 0- and 16-month intervals for both control and simulated communities were analyzed (Table 1). In general, total organic carbon, nitrogen, and phosphorus for all soil samples were comparable between 0- and 16-month intervals. S1 and S2 demonstrated a slight increase in total organic carbon and phosphorus after treatment but readings in S3 decreases. As for the analysis of total nitrogen, there was a slight decrease in S1 and S3 while it increases in S2.

TABLE 1. Measurements of total nitrogen, total organic carbon and total phosphorus on soil samples collected from the Samples 1, 2, and 3 for 0-month and 16-month interval of simulated warming

Site	Soil sample	Time interval	Total organic carbon (%)	Total nitrogen (%)	Total phosphorus (ppm)
S1	Control	0-month	1.10	0.27	238
		16-month	1.36	0.24	224
	Simulated	0-month	1.10	0.27	231
		16-month	1.27	0.24	209
S2	Control	0-month	1.56	0.23	432
		16-month	2.07	0.30	360
	Simulated	0-month	1.56	0.23	432
		16-month	1.85	0.30	386
S3	Control	0-month	1.15	0.27	253
		16-month	0.71	0.15	307
	Simulated	0-month	1.15	0.27	253
		16-month	0.75	0.14	263

ANALYSES OF 16S METAGENOMIC SEQUENCES: CLASSIFICATION AT PHYLUM LEVEL

In general, S1 was dominated by Proteobacteria, Actinobacteria, Verrucomicrobia, and Acidobacteria. Proteobacteria emerged as the most dominant phyla at 0th until 8th-month sampling for both control and simulated communities with relative abundance more than 27% and followed by Verrucomicrobia (>17%) and Actinobacteria (>15%). The dominance of Proteobacteria was replaced by Actinobacteria at 12th and 16th-month sampling, emerging as the most prevalent phylum at both control and simulated environment covering more than 25% of the relative abundance. Figure 1 illustrates the major bacterial phyla classified for Sample 1 at control (25 °C) and simulated (27 °C) environment after 16 months of incubation.

After 16 months of simulated warming, a significant decrease of Proteobacteria (p=0.050), Verrucomicrobia (p=0.010), Acidobacteria (p=0.000), and Planctomycetes (p=0.010) was observed except for Firmicutes (p=0.070); while Actinobacteria (p=0.031) and Chloroflexi (p=0.014) showed a significant increase for both control and simulated communities in S1. Control communities experienced the same changes with the simulated communities at a different rate, and the differences in rate was calculated to give the net change of the relative abundance for each of the phyla after simulated warming. Most of the phyla did not show a linear change but fluctuated during the 16 months of simulated warming except Proteobacteria, Planctomycetes, and Chloroflexi that demonstrated continuous decrease and increase, respectively. Firmicutes experienced the highest net decrease of 154.16% while Chloroflexi demonstrated the highest net increase with 75.96% after 16 months of incubation.

Like S1, S2 was dominated by Proteobacteria, Actinobacteria, Verrucomicrobia, and Acidobacteria for most of the sampling time. Other less abundant phyla included Planctomycetes, Firmicutes, Chloroflexi, and Bacteroidetes that were not found in S1. Proteobacteria (>27%) emerged as the most dominant phylum over the 16 months of incubation for both control and simulated communities and followed by Actinobacteria (>10%) and Verrucomicrobia (>6%). Planctomycetes, Chloroflexi, Bacteroidetes, and Firmicutes remained as the less abundant phyla for both conditions over the 16th-month sampling with relative abundance less than 5.25%. Figure 2 illustrates the major bacterial phyla classified for Sample 2 at control (25 °C) and simulated (27 °C) environment after 16 months of incubation.

Even though Proteobacteria in S2 managed to maintain their position as the most dominant phylum, the relative abundance decreases over the 16 months and results in a net decrease of 32.83% when compared with 0th-month sampling. On the other hand, Actinobacteria (p=0.008) Firmicutes (p=0.060), Chloroflexi (p=0.026), and Bacteroidetes (p=0.020) experienced a net increase over the 16 months with Firmicutes enjoying the highest net increase with 117.91% and Bacteroidetes with a smallest net increase of 6.12%. Following the trend of Proteobacteria, Planctomycetes (p=0.027), Acidobacteria (p=0.055), and Verrucomicrobia (p=0.018) in S2 exhibited a net decrease in descending order with 29.73, 26.94 and 9.85%.

S3 was dominated by Proteobacteria, Actinobacteria, and Acidobacteria for most of the sampling time. Proteobacteria (>29%) emerged as the most dominant phylum over the 16 months of incubation for both control and simulated communities and followed by Actinobacteria (>20%) and Acidobacteria (>8%). Planctomycetes, Chloroflexi, Verucomicrobia, Bacteroidetes, and Firmicutes remained as the less abundant phyla for both conditions over the 16th-month sampling with relative abundance less than 6.84%. Figure 3 illustrates the major bacterial phyla classified for Sample 3 at control (25 °C) and simulated (27 °C) environment after 16 months of incubation.

Proteobacteria (p<0.05) showed a net decrease for all the samples after 16 months of incubation indicating that

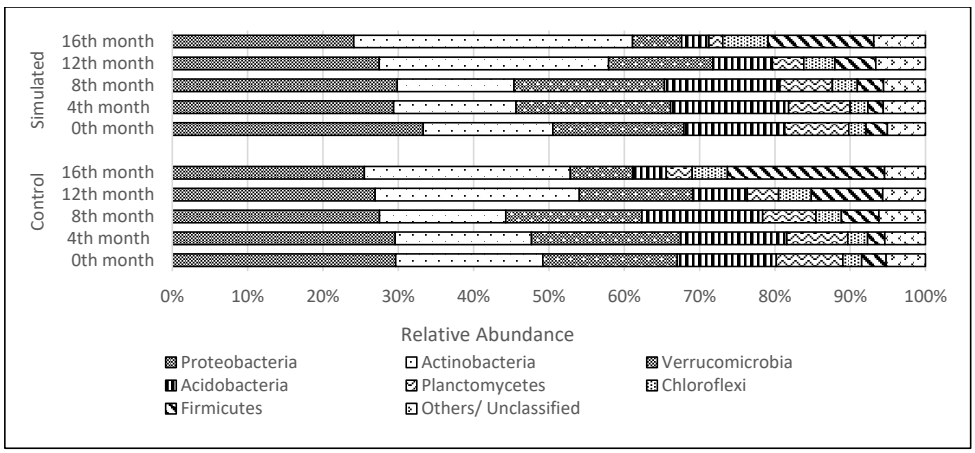

FIGURE 1. Major bacterial phyla classified for Sample 1 (control: 25 °C; simulated: 27 °C) at 0th, 4th, 8th, 12th and 16th-month sampling

Proteobacteria are highly affected by increased temperature. Proteobacteria in S3 were affected most with a net decrease of 38.30% followed by S2 with 32.83% and S1 with 13.60%. Besides Proteobacteria, Acidobacteria, and Planctomycetes also demonstrated a net decrease across the samples after 16 months of incubation. Acidobacteria experienced the highest decrease in S2 with 26.94% while Planctomycetes was also most affected at S2 with 29.73% of net decrease. On the other hand, the net increase for all samples was observed for Actinobacteria and Chloroflexi. Actinobacteria experienced the highest increase of 96% at S3 and followed by S1 with 74.75% and S2 with 57.61%. The details of the net change in relative abundance in Samples 1, 2, and 3 after 16 months of simulated warming are tabulated in Table 2.

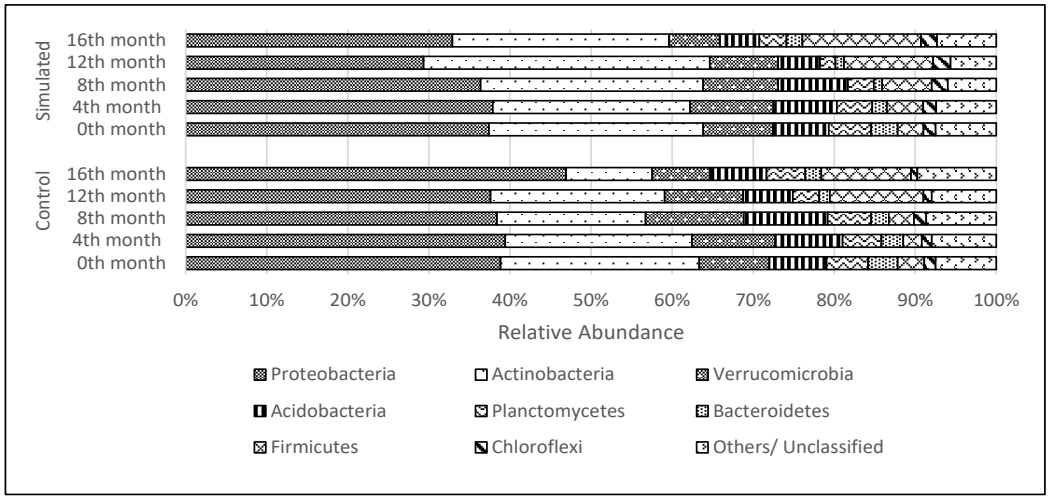

FIGURE 2. Major bacterial phyla classified for Sample 2 (control: 25 °C; simulated: 27 °C) at 0th, 4th, 8th, 12th and 16th-month sampling

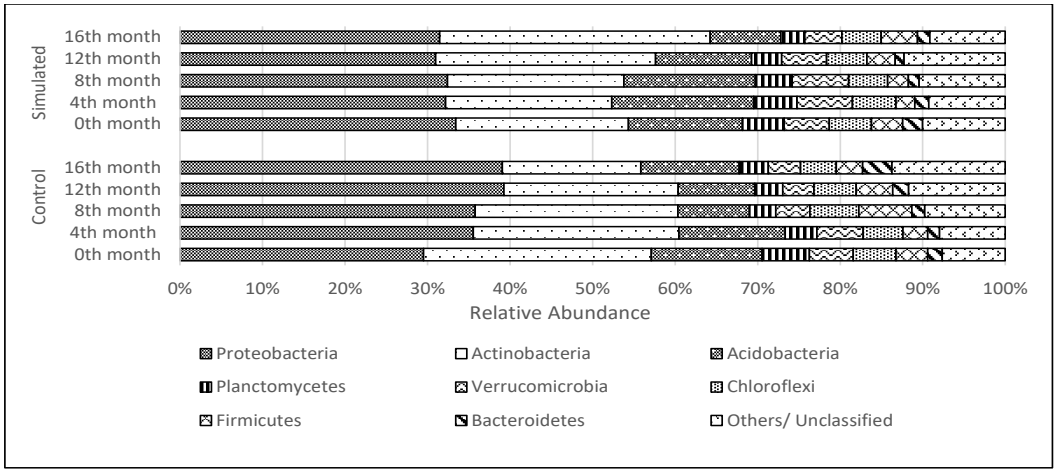

FIGURE 3. Major bacterial phyla classified for Sample 3 (control: 25 ºC; simulated: 27 ºC) at 0th, 4th, 8th, 12th and 16th-month sampling

TABLE 2. Net change in relative abundance (%) of major bacterial phyla in Sample 1, Sample 2 and Sample 3 after 16 months of simulated warming

Phyla	Net change in relative abundance		
	S1	S2	S3
Proteobacteria	-13.60	-32.83	-38.30
Actinobacteria	74.75	57.61	96.00
Verrucomicrobia	-9.06	-9.85	8.83
Acidobacteria	-6.75	-26.94	-26.75
Planctomycetes	-17.10	-29.73	-3.91
Chloroflexi	75.96	75.47	9.00
Firmicutes	-154.16	117.91	34.34
Bacteroidetes	-	6.12	-142.79

CLASSIFICATION AT GENUS LEVEL

The major bacterial genera of soils from S1, S2, and S3 collected from the growth chamber once every 4 months of simulated warming were identified. In general, S1 was dominated by *Chthoniobacter, Gaiella,* and *Paludibaculum* for most of the sampling times. *Chthoniobacter* (>11%) emerged as the most dominant genus at 0th until 12th-month sampling for both control and simulated communities and followed by *Gaiella* (>4%) and *Paludibaculum* (>3%) with a slight difference between the two genera. The dominance of *Chthoniobacter* was replaced by *Gaiella* at 16th-month sampling, emerging as the most prevalent genus at both conditions. *Chthoniobacter* decreased over the 16 months of incubation while *Gaiella* increased steadily and finally replaced *Chthoniobacter* as the most dominant genus with 8.39 and 10.08% at the control and simulated environment, respectively. Other bacterial genera that were found in S1 were *Rhodoplanes, Bradyrhizobium, Limisphaera, Mycobacterium, Acidibacter, Bacillus, Rhodomicrobium, Actinomicrobium*, and *Tepidisphaera*.

After 16 months of incubation, *Chthoniobacter* experienced a net decrease of 10.39% while *Gaiella* had a net increase of 78.57%, further illustrating the change of dominance due to a higher incubation temperature. While *Chthoniobacter* began with a high abundance, *Sphingomonas* had only 0.40 and 0.28% at 0th-month sampling. However, *Sphingomonas* increased rapidly throughout the 16 months of incubation and finally secured its position as one of the most dominant genera after *Gaiella* at the last sampling with a relative abundance of more than 7%. *Bacillus*, on the other hand, began with a low relative abundance of 1.24 and 1.26% but ended up as one of the dominant genera with 2.86 and 3.66% at the control and simulated, respectively.

All genera demonstrated net decrease except for *Gaiella, Sphingomonas,* and *Actinomadura*. Control communities experienced the same changes with the simulated communities at different rates and the differences in rates were calculated to give the net change of relative abundance for each of the genera after simulated warming. As expected, *Sphingomonas* experienced the highest net increase of 1884.64% that enabled *Sphingomonas* to rise as one of the dominant genera from abundance less than 0.5% initially. Likewise, *Gaiella* and *Actinomadura* also experienced a net increase of 78.57 and 199.86%, pushing them to become the 1st and 4th most dominant genus at 16th-month of incubation.

Similar to S1, S2 was generally dominated by *Gaiella, Chthoniobacter, and Bradyrhizobium* for most of the sampling time. However, in S2, *Gaiella* (>6.5%) emerged as the most dominant genus at most sampling time for both control and simulated communities instead of *Chthoniobacter*. Other bacterial genera that were found in S2 were *Nocardioides, Rhodoplanes, Limisphaera, Paludibaculum, Streptomyces, Mycobacterium, Bacillus, Sphingomonas, Acidibacter, Flavobacterium, Algisphaera,* and *Actinomadura*. It was noted that *Nocardioides*,

Streptomyces, Flavobacterium, and *Algisphaera* were found in S2 with relative abundance higher than 0.5% but were absent in S1. On the other hand, *Tepidisphaera* and *Rhodomicrobium* were only found in S1 but absent in S2.

The dominance of *Gaiella* was replaced by *Sphingomonas* (>9%) at 12th-month sampling at the control and 16th month at simulated, emerging as the most prevalent genus at both conditions. *Gaiella* dominated more than 6.5% of the total reads at the beginning while *Sphingomonas* only covered 1.07% for both conditions. However, at 12th-month sampling, *Sphingomonas* replaced *Gaiella* as the most dominant genus with a relative abundance of 9.42% while *Gaiella* only covered 4.94%. Similarly, *Bradyrhizobium* began as one of the most dominant genera but slowly being replaced by other genera and only managed to secure as the 8th and 7th most dominant at the control and simulated communities with the relative abundance of 2.60 and 1.76%. *Bacillus*, on the other hand, began with a low relative abundance of 1.26 and 1.14% but ended up as one of the dominant genera with 3.66 and 3.61% at the control and simulated, respectively.

The major bacterial genera found in S3 were *Gaiella, Chthoniobacter, Bradyrhizobium,* and *Sphingomonas*. Unlike S1 and S2, *Sphingomonas* began as one of the dominant genera with a relative abundance of 2.78 and 3.60% instead of the minor genus which covered less than 1% of the total reads. Other bacterial genera with the relative abundance of more than 0.5% that was found in S3 were *Paludibaculum, Nocardioides, Vicinamibacter, Limisphaera, Streptomyces, Tepidisphaera, Mycobacterium, Acidibacter, Rhodoplanes,* and *Brivitalea*. It was noted that *Vicinamibacter* and *Brivitalea* were only found in S3 with relative abundance higher than 0.5% but were absent in S1 and S2.

In the 0th-month, *Gaiella* emerged as the most dominant genus at both conditions with 3.37 and 4.21% of total reads. However, at the next sampling, *Sphingomonas* had successfully replaced *Gaiella* as the most dominant genus with 3.63 and 4.87% at the control and simulated environment. *Sphingomonas* managed to maintain its position until the 16th-month sampling with a relative abundance of 11.98 and 10.43%. Although *Gaiella* was replaced by *Sphingomonas*, *Gaiella* still secured their position as the second most prevalent genus until the last sampling with 3.32 and 5.49% while *Chthoniobacter* maintained their spot after *Gaiella*. *Bradyrhizobium* began as one of the dominant genera but slowly being replaced by other genera and only managed to secure as the 6th and 7th most dominant at the control and simulated communities; respectively with the relative abundance of 1.55 and 1.19% after 16 months of incubation. Following *Bradyrhizobium's* trend, *Limisphaera* had a decent relative abundance of 1.41 and 1.33% at the 0th-month and eventually decreased over time and only had less than 0.37% after 16 months.

DISCUSSION

The soils from all three sampling sites were dominated by Proteobacteria, Actinobacteria, Verrucomicrobia, and Acidobacteria. This corroborated with the results of Kerfahi et al. (2016) and Kim et al. (2014) which reported soil samples from West Malaysia were mainly dominated by Acidobacteria, Proteobacteria, and Actinobacteria, the most commonly found bacterial phyla in tropical soils. Although both East and West Malaysia were dominated by similar phyla, different relative abundance was recorded where Acidobacteria (36%) was identified as the most prevalent phylum in West Malaysia (Kim et al. 2014) while only 7-13% were found in Sabah's soil. Such contrast of relative abundance between soil in West and East Malaysia are probably due to different characteristics of the soil environment or the geographical locations of the sampling site.

After 16 months of simulated warming, a net decrease of Proteobacteria, Acidobacteria, and Planctomycetes were observed across all three soil samples indicating that they were highly affected by increased temperature. The results agree with previous studies by Mateos-Rivera et al. (2016), Pearce et al. (2013), and Schuette et al. (2010) that stated Proteobacteria decreases with higher incubation temperatures. Skidmore et al. (2005) suggested that Proteobacteria decreases with increasing temperature, as some of the members are adapted to live in lower temperature habitats with low organic matter content. The Proteobacteria are a major phylum, which includes a wide variety of metabolically diverse group. They are known to be able to degrade a wide range of toxic compounds, function as nitrogen fixers such as Rhizobium and Mesorhizobium and play a key role in the carbon cycle (Aislable & Deslippe 2013). Given the function of Proteobacteria as a metabolically diverse group, they contributed significantly to agriculture (Lugtenberg & Kamilova 2009; Verma et al. 2010). Proteobacteria exhibited properties to promote plant growth by assisting in nutrient acquisition and protects against disease through the production of inhibitory substances or increase the natural resistance of the plant (Lugtenberg & Kamilova 2009; Verma et al. 2010). Thus, exposure of soil at a higher temperature at a long duration can lead to disruption of soil function and agriculture activity as a lower abundance of Proteobacteria has important implications for plant productivity and soil health (Trivedi et al. 2016).

Besides Proteobacteria, Acidobacteria, and Planctomycetes also demonstrate a net decrease across the samples after 16 months of incubation. Acidobacteria is known to be able to perform nitrate and nitrite reduction (Ward et al. 2009), a very important stage in nitrogen cycles. Higher temperatures lead to a decrease of Acidobacteria and can indirectly affect nitrogen cycles in the soil resulting in poor soil function. Although all

three samples in this study showed that Acidobacteria and Planctomycetes decreased with increased temperature, several reports indicated otherwise (Mateos-Rivera et al. 2016; Zumsteg et al. 2013). This suggested that there could be other factor(s) influencing the relative abundance of Acidobacteria and Planctomycetes when temperature increases, for example soil type or sampling location considering that previous studies that used varying soil produced a different result. Additionally, the geographical locations between this, a tropical region, and the previous studies, in Antarctica were different in weather conditions.

On the other hand, Actinobacteria, Chloroflexi, and Bacteroidetes (in S2) experienced a net increase across all soil samples consistent with the results reported by Mateos-Rivera et al. (2016), Pearce et al. (2013) and Schuette et al. (2010) implying that Actinobacteria and Chloroflexi favored the increase of temperature compared to other phyla. According to Heuer et al. (1997) and Mateos-Rivera et al. (2016), members of Actinobacteria are known to able to catabolize organic carbon, justifying the reason they are present at a higher concentration at longer incubation time as the organic carbon in soil increases when the incubation time increases. However, due to poor understanding of Chloroflexi, the conclusion on why this phylum increased with increasing temperature cannot be made. Further characterization of bacteria from this phylum is needed before a conclusion is possible.

Analyses of sequencing results at genus level showed that S1, S2, and S3 were dominated by similar bacterial genera; Gaiella, Chthoniobacter, Bradyrhizobium as well as Paludibaculum in S1, consistent with the results reported by Jeanbille et al. (2016), Novello et al. (2017), and Wang et al. (2015). Gaiella was Gram-negative bacteria with non-motile rod-shaped cells, the dominant genus of Actinobacteria. They are strictly aerobic, oxidase, and catalase-positive, describe for the first time by Albuquerque et al. (2011), resulting in the little information available for this genus. Despite the abundance of Gaiella in soils from various geographic regions, the conclusion of the genus's dominance cannot be made due to poor understanding of the genus. Further characterization of bacteria from Gaiella is needed before a deduction is possible. Besides Gaiella, the dominance of Chthoniobacter and Bradyrhizobium in most of the soil samples were observed at 0^{th}-month sampling and their abundance in soils can be attributed to their involvement in carbon cycling and production of secondary metabolites (Jenkins et al. 2009).

After 16 months of incubation, Gaiella and Nocardiodies demonstrated a net increase for all the samples indicating that they were highly affected by temperature rise. Both genera belonged to the phylum Actinobacteria which gives them attributes as oligotrophic K-strategies, which able to grow under apparent optimal conditions (Ward et al. 2009). Their ability to grow in less than optimal conditions, for example at higher temperatures gives them leverage against other genera. However, due to the limited information on Gaiella and its functions, it is impossible to make inference how the growth of Gaiella will affect the soil function and ecosystem. The response of Bacillus towards simulated warming differed between soil samples. Growth of Bacillus in S1 was impeded by simulated warming but was promoted in S2 resulting in a net increase of 26.19% after 16 months of incubation. This implied that under a suitable condition, temperature rise promoted the growth of Bacillus, a genus that consists of many beneficial bacteria to the plant growth (Ryu et al. 2003; Shao et al. 2015) as well as other Bacilli (Carlin et al. 2010; Thwaite et al. 2012).

In conclusion, Proteobacteria were highly affected by temperature rise, showing a net decrease for all the samples. It can be concluded that a higher temperature reduces the abundance of Proteobacteria regardless of soil type and sample location. Given the function of Proteobacteria as a metabolically diverse group, exposure of soil at a higher temperature for a long duration can lead to disruption of soil function and affects agriculture activity. The findings of this study suggest that efforts should be made to consistently isolate and conserve beneficial bacterial species from the genus Bradyrhizobium spp., Gaiella spp. and certain Bacillus spp. that was greatly impeded by the elevated temperature. This will ensure that these vulnerable beneficial bacterial species are not lost in the future due to global warming.

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