Effect of short term variation in temperature and water content on the bacterial community in a tropical soil

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ABSTRACT

The community dynamics of environmental bacteria is dependent on micro-climatic factors such as temperature and water potentials. A shift in any one of these factors can have a major impact on the richness and composition of the microbes in the soil. However, microbial responses towards these climate stressors are poorly characterised. Here, we undertook a microcosm study to assess the effect of temperature and water content on the bacterial community structure in a tropical soil over a four week period. The microcosms were incubated at three different temperatures (25 °C, 30 °C, and 35 °C) and maintained under two different water levels (2 and 5 mL). Using a combination of molecular assessment tools and numerical inference, we showed that short-term variation in both temperature and water content induced significant changes to the soil bacterial community composition. The greatest difference in community structure between treatments was observed in the Week 2 microcosms, but in the Week 4 microcosms, the community structure between treatments became more similar. Compared to temperature, water content exerted a greater effect on the bacterial diversity. *Firmicutes* was the most abundant phylum in all the analysed samples, and its relative abundance increased with elevation in temperature and water content. Out of six functional genes analysed, the nitrogen fixation gene (nifH) and denitrification gene (nosZ) showed significant correlations to the bacterial community structures.

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1. Introduction

Bacteria are the dominant members of soil biota and they play crucial roles in the biogeochemical cycling of elements in the environment, thus contributing to the functioning of wider related ecosystems (Aislabie and Deslippe, 2013; Dominati et al., 2010; Nannipieri et al., 2003). It has been estimated that each gram of soil contained more than 10^8 bacterial cells (Torsvik et al., 1998). A high level of diversity is considered a vital aspect of ecosystem health as it begets ecosystem stability by acting as a genetic and functional reservoir that increases community resilience toward perturbations (Bissett et al., 2007; DeAngelis et al., 2013). Therefore, the loss of community richness provides a proxy indicator of deterioration in ecosystem health and functions (Allison and Martiny, 2008; Griffiths and Philippot, 2013).

The complexity of bacterial community in soil is dependent on underlying environmental stressors such as temperature and water potential (Schimel et al., 2007). Shifts in any of these modulators which are usually controlled by both short and long term local climatic gradients have the potential to change the structure and composition of soil bacteria (Bond-Lamberty et al., 2016; Brockett et al., 2012; Deslippe et al., 2012; Evans and Wallenstein, 2014; Manzoni et al., 2012; Rinnan et al., 2009; Yergeau et al., 2012). Separately, the degree to which microbial community structure can be perturbed by future environmental fluctuations is influenced by the contemporary environmental conditions (Evans and Wallenstein, 2011; Fierer et al., 2003; Waldrop and Firestone, 2006). For instance, bacterial communities that experienced frequent disturbances such as water stress (Fierer et al., 2003) or redox fluctuations (DeAngelis et al., 2010) were more resistant to perturbation than those subjected to narrow climatic conditions (Cavaleri et al., 2015). Understanding the short-term effects of variations in temperature and watering on tropical soil can therefore facilitate prediction for long-term environmental responses.

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Tropical ecosystems are generally regarded to house the highest biological diversity on earth (Gibson et al., 2011; Mandic-Mulec et al., 2015). The tropics are characterised by hot and humid climate throughout the year (Teygeler et al., 2001) with relatively low seasonal fluctuations in temperature and rainfall (Bonebrake and Mastrandrea, 2010). In Malaysia, the inter-annual variation of temperature and rainfall intensity was largely influenced by El Niño and La Nina events (Juneng and Tangang, 2005; Tangang, 2001; Tangang and Juneng, 2004; Tangang et al., 2007). Severe drought and excessive rain can cause significant alteration of soil parameters (Hamza and Anderson, 2005), leading to community shift of environmentally sensitive members of the community (Evans and Burke, 2013). For example, intense rainfall would increase the amount of water flow into soil. Waterlogged soil is generally low in oxygen, a condition which preferentially selects for taxa such as *Syntrophobacterales* (phylum *Proteobacteria)* (Castro et al., 2010). Drought, on the other hand, was found to reduce soil water potential, resource availability and exerted physical and physiological stresses on the bacterial communities (Schimel et al., 2007), such that *Actinobacteria,* a Gram-positive bacterial phylum, was favoured (Bouskill et al., 2012).

Similarly, seasonal warming was reported to increase the relative proportion of Gram-positive bacteria (Allison and Martiny, 2008). Such shifts in the community would trigger alterations in functional gene expressions which would in turn affect biogeo-chemical cycling and ecosystem feedback in future (Bell et al., 2009; Chodak et al., 2015; Singh et al., 2007). While Xiong et al. (2014) found that warming treatment increased the average ratio of *Alphaproteobacteria*-to-*Acidobacteria* with concomitant increase in the rates of soil CO_2 efflux, other studies reported that variability in these factors was not accompanied by changes in the bacterial communities and functions (Rinnan et al., 2009; Stres et al., 2008). It remains unclear how resilient would tropical soil system be to external perturbations and whether the soil system harbours populations that could adapt to temperature and water variations (Leibler and Kussell, 2010).

While studies on the long-term effects of warming and/or altered precipitation on bacterial community composition had been documented (DeAngelis et al., 2015; Kuffner et al., 2012; Liu et al., 2014; Pold et al., 2015), relatively little is known about short-term effects of these variables on the soil bacterial communities. Nevertheless, from the limited independent studies employing different assessment approaches, strong community shifts (e.g. increase in *Actinobacteria* in warmed and wetted soil) were detected within 4 weeks to 1 year (Curiel Yuste et al., 2014 (454 Pyrosequencing); Riah-Anglet et al., 2015 (Q-PCR); Waring and Hawkes 2015 (454 Pyrosequencing); Wu et al., 2015 (MiSeq Sequencing); Xiong et al., 2014 (454 Pyrosequencing). These studies focused on a single factor (e.g. effect of temperature or water only). Thus, short-term multi-factorial studies are warranted to understand the dynamic interaction between temperature and moisture.

In nature, temperature and the amount and availability of water are correlated with other environmental parameters such as ionic-potential, UV irradiation, evaporation and soil topography as effectors of the soil biotic community. Thus, it is hard to design experiments in the field to test the effect of each parameter singly. For example, it is difficult to control the level of heating and moisture in the field even with the utilisation of open top chambers (Aronson and McNulty, 2009). Therefore, in this study, we used a microcosm model under controlled laboratory conditions to elucidate the direct effect of temperature and water content on the bacterial community attributes (e.g. richness, abundance, and composition) in a tropical soil.

We hypothesized that (1) increase in temperature and water content would induce community shifts as species sensitive to environmental variations would decrease in abundance while tolerant species would increase in proportion and that (2) changes may affect the soil abiotic factors and functional gene abundance. We employed T-RFLP and statistical analyses to test hypothesis (1). For hypothesis (2), we monitored the soil abiotic parameters including pH, electrical conductivity/salinity, water content, nitrate, nitrite, and phosphate in the microcosms. Functional genes abundance was determined by quantitative PCR. All these parameters were correlated with the bacterial community for each microcosm.

2. Materials and methods

2.1. Sampling sites and sample collection

Tropical soil samples were collected from Rimba Ilmu (3.1311° N, 101.6580° E), the botanical garden in the University of Malaya, Kuala Lumpur, Malaysia, during the dry season. Surface soil samples were collected to a depth of 10 cm. Eilers et al. (2012) reported that bacterial diversity was typically highest in the top soil. The soil was fine-texture and dark brown in colour. The collected soil was sieved through a 2-mm mesh sieve on the same day and stored at 4 °C for less than 24 h before the start of the soil microcosm experiment.

2.2. Soil incubation

Microcosms are ecosystem models in which natural environments are mimicked and manipulated, and they have proven to be useful tools in studying parameters that shape bacterial communities (Kennedy et al., 2004; Kim et al., 2013). In the present study, microcosms incubated at lower water content were used to simulate soil ecosystem under short-term drought while micro-cosms incubated with high water input were used to simulate short-term intense rainfall (IPCC, 2007; Stres et al., 2008). Soil microcosms were established in sterile falcon tubes (3 cm diameter, 50 mL capacity) each filled with approximately 50 g (± 0.5 g) of sieved soil. Each microcosm headspace was 3 cm and partially filled with a sterilized rolled cotton wool plug to trap dust and allow aeration during incubation. The bottom part of each tube was filled with sterilized glass beads, up to 2 cm in height, before the soil was added. This was to prevent the soil at the bottom of the tube from being potentially submerged in water.

Malaysia is hot and humid throughout the year with annual mean air temperature ranging from 23 °C to 32 °C (Dominic et al., 2015). Soil temperature in Malaysia generally ranged from 26.9 °C to 30.2 °C (Sanusi et al., 2013). According to the Malaysian Meteorological Department, the annual average temperatures are projected to rise in the coming decades (Yau and Hasbi, 2013). Based on these data, we incubated our soil samples at three different temperatures (25 °C, 30 °C, and 35 °C), using tempera-ture-controlled orbital shaker incubators (YIHDER, Taiwan), to simulate current and predicted ranges of annual mean air temperature in Malaysia. Air humidity was maintained by placing a beaker of water inside each incubator. The amount of water lost from the beakers was in the range of 4.8 mL to 5.1 mL after 4 weeks, suggesting relatively similar humidity in all three incubators.

The climate in Malaysia can be divided into dry and wet seasons, with average daily rainfall measured as 10–25 mm (1–16 mL) (Althuwaynee et al., 2014). Based on this data, the water treatments conducted in this study were within the range of the rainfalls reported. As 1 mL of water weighs 1 g (Berdanier and Zempleni, 2008), adding more than 6 mL of water for four weeks may result in waterlogging because each microcosm contained only 50 g of soil samples. Thus, in this study, two different water levels were administered: 2 mL or 5 mL sterile water was added to