

**Title:** Soil bacterial community structure is insensitive to rising mean annual temperature in tropical montane wet forests.

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**Abstract:**

Soil bacteria play a key role in regulating terrestrial biogeochemical cycling and greenhouse gas fluxes across the soil-atmosphere continuum. Despite their importance to ecosystem functioning, we lack a general understanding of how bacterial communities respond to climate change, especially in relatively understudied ecosystems like tropical montane wet forests. We used a well-studied and highly constrained 5.2 °C mean annual temperature (MAT) gradient in tropical montane wet forests on the Island of Hawaii to test the hypothesis that long-term, whole-ecosystem warming increases the diversity and alters the composition of soil bacterial

communities. Across this MAT gradient, dominant vegetation, substrate type and age, soil moisture, and disturbance history are held constant, allowing us to effectively isolate the influence of rising MAT on soil bacterial community structure. Contrary to our hypothesis, we found that the richness, evenness and phylogenetic diversity of the soil bacterial community remained remarkably stable with MAT, and that MAT did not predict variation in bacterial community composition despite a substantial increase in belowground soil carbon fluxes across the gradient. Our results suggest that other factors that are constant across this gradient – such as soil pH, water availability and plant composition – may be more important than warming in influencing soil bacterial community composition and diversity, at least within the temperature range studied here (~13-18°C MAT). Ours is the first study to demonstrate stability of soil bacterial community structure with rising MAT and increased carbon flux in a tropical wet forest ecosystem. Moreover, our results add to growing evidence that the diversity and composition of bacterial communities dominated by Proteobacteria and Acidobacteria in low-pH forest soils may be insensitive to the direct effect of climate warming.

## **Introduction:**

Climate warming may have both direct and indirect effects on soil bacterial communities, but the magnitude and direction of these effects remain poorly resolved (Allison and Martiny 2008, Shade et al. 2012, Cregger et al. 2014). In particular, there are surprisingly few studies on how climate warming affects soil bacterial community composition and diversity in tropical forest ecosystems. This represents a significant knowledge gap given that tropical forests play a very large role in global carbon cycling (Bonan 2008, Beer et al. 2010, Pan et al. 2011), and that soil bacterial diversity and composition are closely linked to biogeochemical cycling and greenhouse gas fluxes (Heemsbergen et al. 2004, Carney et al. 2007, Singh et al. 2014). Moreover,

incorporation of microbial community dynamics improves the predictive capacity of soil carbon models (Allison et al. 2010, Li et al. 2014). Understanding how climate warming affects soil bacterial community composition and diversity in carbon-dense tropical wet forest ecosystems is, therefore, a high research priority given the potentially large and rapid feedback effects on both ecosystem functioning and the global climate system (Allison and Martiny 2008).

The temperature sensitivity of bacterial communities in tropical forest soils remains poorly resolved in part because there are currently no large-scale warming experiments in tropical forest ecosystems (Cavaleri et al. 2015). It is tempting to extrapolate results from warming experiments in other forest ecosystems to the tropics, especially since there is broad overlap in bacterial community composition at the phylum level between low-pH temperate and tropical forest soils (Lauber et al. 2009). However, forest warming experiments typically do not warm the canopy (Kuffner et al. 2012, Cregger et al. 2014, DeAngelis et al. 2015), which can increase gross photosynthesis and overall carbon input belowground (Lu et al. 2013). Increasing carbon availability to bacteria can greatly affect their community composition and diversity (Carney et al. 2007, Allison and Martiny 2008, Landa et al. 2013), especially in tropical forest soils (Nemergut et al. 2010). By not incorporating the canopy, warming experiments to date in temperate forests may not accurately reflect the integrated effect of whole-ecosystem warming on forest soil bacterial communities.

Elevation gradients provide an alternative to experimentally manipulating the ambient temperature of an entire forest stand, and can be a more tractable approach to examine how biota respond to climate change in tropical forest ecosystems (Malhi et al. 2010). Isolating the effect of temperature can be difficult, however, because a number of environmental variables typically co-vary with temperature across elevation gradients. These potentially confounding factors include

moisture availability, soil pH and plant species composition - all of which can have strong individual impacts on the diversity and composition of soil bacterial communities (Lauber et al. 2009, Cregger et al. 2014, Schlatter et al. 2015, Urbanová et al. 2015). High levels of local plant diversity and high turnover of plant species composition can be especially problematic along tropical forest elevation gradients (Rapp et al. 2012), and can complicate interpretation of how soil bacterial community composition and diversity vary in response to temperature.

Here we examine trends in soil bacterial community composition and diversity across a well-studied, highly constrained 5.2 °C mean annual temperature (MAT) gradient spanning 800 m elevation in native-dominated tropical montane wet forests on Hawaii Island. Unlike other elevation gradients in the tropics, dominant plant species are constant across the Hawaii MAT gradient, as are other potentially confounding variables including soil water balance, geologic substrate and soil type (Litton et al. 2011, Iwashita et al. 2013). Previous work demonstrated that rates of ecosystem carbon fluxes increase substantially with rising MAT across this gradient, including soil CO<sub>2</sub> efflux (Litton et al. 2011), leaf litter decomposition (Bothwell et al. 2014), litterfall and total belowground carbon flux (Giardina et al. 2014), while total ecosystem carbon storage remains constant (Selmants et al. 2014). Based on these findings, and on evidence that carbon availability shapes bacterial community structure (Nemergut et al. 2010, Landa et al. 2013), we hypothesized that rising mean annual temperature and the concurrent increase in belowground carbon availability would significantly increase diversity and alter the composition of soil bacterial communities across this MAT gradient.

## **Methods:**

The Hawaii MAT gradient consists of nine permanent 20 x 20 m plots along the eastern slope of Mauna Kea volcano within the Hawaii Experimental Tropical Forest and the Hakalau Forest

National Wildlife Refuge on the Island of Hawaii (Litton et al. 2011). The nine plots range in elevation from 800 to 1600 m, which corresponds to a 5.2° C difference in MAT (13 – 18.2° C; Table 1). All plots are in mature, closed-canopy tropical montane wet forest dominated by *Metrosideros polymorpha* with very similar geology and soil type. Soils are well-drained Acrudoxic Hydudands developed from ~20,000 year old volcanic ash (Litton et al. 2011, Giardina et al. 2014) deposited on a single Pleistocene-age Mauna Kea lava flow dominated by hawaiite and mugearite (Wolfe and Morris 1996, Litton et al. 2011). Mean annual rainfall is not constant across the MAT gradient, but both rainfall and potential evapotranspiration decline consistently with increasing elevation. As a result, soil water content is nearly constant across the MAT gradient (Table 1) because declining rainfall with elevation is balanced by reduced evapotranspiration driven by lower air temperatures.

Within each of the nine MAT plots, we collected eight soil samples (Oa and A horizon material) in March of 2010 to a depth of 10 cm after removing undecomposed and partially decomposed litter (Oi and Oe horizon material). We extracted DNA from soil samples with the Powersoil DNA Isolation Kit (Mo-Bio Laboratories, Carlsbad, CA, USA) and submitted DNA samples to the Research and Testing Laboratory (RTL, Lubbock, TX, USA) for tag-pyrosequencing. The V1-V3 region of the 16S rRNA gene was amplified with primers Gray28F (5' GAGTTTGATCNTGGCTCAG) and Gray519R (5' GTNTTACNGCGGCKGCTG) by RTL. Reads were generated in the forward direction from 28F with Roche 454 Titanium chemistry sequencing. Quality trimming, denoising and chimera removal were performed by RTL. We assigned sequences to operational taxonomic units (OTUs) using QIIME version 1.7.0 (Caporaso et al. 2010). To cluster reads into operational taxonomic units (OTUs), we used the open-reference OTU picking algorithm using the UCLUST tool (Edgar 2010) at 97% sequence

identity against the 13-5 release of the Greengenes database (DeSantis et al. 2006). OTUs represented by a single sequence were removed from the analysis. We rarefied samples to 2200 sequences because of unequal numbers of sequences among soil samples. The rarefaction process resulted in two soil samples with less than 2200 sequence reads, each from a different MAT plot, which were excluded from further analysis. We repeated the rarefaction process 10 times for each sample, with subsequent statistical analyses based on within-sample means of the 10 random iterations.

We quantified OTU richness, phylogenetic diversity and community similarity metrics in QIIME version 1.7.0 (Caporaso et al. 2010). We used the Chao1 richness estimator (Chao 1984) to estimate OTU richness per sample and Faith's (1992) phylogenetic diversity index (Faith's PD) to estimate phylogenetic diversity. To compare OTU composition across MAT plots, we constructed both taxonomic and phylogenetic community similarity matrices. We used the Bray-Curtis distance index to construct a taxonomic similarity matrix and the weighted UniFrac distance metric (Lozupone and Knight 2005) to construct a phylogenetic similarity matrix.

All statistical analyses were conducted using R version 3.0.2 with an  $\alpha$ -level of 0.10 for all tests due to small sample sizes ( $n = 9$ ). We used ordinary least-squares regression to determine whether OTU richness, phylogenetic diversity or the relative abundance of individual phyla varied significantly as a function of MAT. To visualize similarities in community composition across the MAT gradient, we used taxonomic (Bray-Curtis) and phylogenetic (weighted UniFrac) similarity matrices to construct non-metric multidimensional scaling (NMDS) ordinations using the vegan package (version 2.0-10) in R. We quantified whether bacterial community composition varied as a function of MAT, total belowground carbon flux (Giardina et al. 2014) and soil pH with permutational multivariate analyses of variance (PERMANOVA)

using taxonomic (Bray-Curtis) and phylogenetic (weighted UniFrac) measures of community similarity. We used the ‘adonis’ function (Anderson 2001) in the vegan R package to conduct PERMANOVA tests. We estimated bacterial OTU evenness in each MAT plot using Pielou’s J (Pielou 1966) and used ordinary least squares regression to determine whether OTU evenness varied as a function of MAT.

## **Results:**

In contrast to our hypothesis, bacterial diversity did not vary with temperature across this well-constrained MAT gradient in tropical montane wet forests. The observed number of bacterial OTUs averaged 571 ( $\pm 93$ ; 95% CI) but did not vary with MAT ( $R^2 = 0.03$ ,  $P = 0.64$ ; Fig. 1). Likewise, Chao1 estimated OTU richness did not vary with MAT ( $R^2 = 0.06$ ,  $P = 0.53$ ), averaging 1106 ( $\pm 174$ ; 95% CI) across the MAT gradient. Finally, there was no relationship between MAT and phylogenetic diversity (adj.  $R^2 = 0.07$ ,  $P = 0.48$ ), which averaged 45 ( $\pm 7$ ; 95% CI) across the MAT gradient (Fig. 1).

Similar to trends for diversity, we found no support for our hypothesis that temperature drives bacterial community composition in tropical montane wet forest soils. Soils across the MAT gradient were dominated by the phyla Proteobacteria ( $44\% \pm 4\%$  of sequences per MAT plot) and Acidobacteria ( $43\% \pm 4\%$  of sequences per MAT plot; Fig. 2). Previous studies have also found these phyla to dominate soil bacterial communities in low-pH wet tropical forest soils (Lauber et al. 2009, Nemergut et al. 2010, Fierer et al. 2011, Rodrigues et al. 2013, Lee-Cruz et al. 2013). None of the bacterial phyla varied significantly as a function of MAT (adj.  $R^2 < 0.015$ ,  $P > 0.17$  for all phyla). Approximately 87% ( $\pm 5\%$ ) of sequences within phylum Proteobacteria were in the class Alphaproteobacteria, and most Alphaproteobacteria were either in the genus Rhodoplanes (family Hyphomicrobiaceae;  $18\% \pm 3\%$  of sequences per MAT plot) or the family

Rhodospirillaceae ( $10\% \pm 3\%$  of sequences per MAT plot). Bacteria in phylum Acidobacteria were dominated by taxa within the family Koribacteraceae, a group of acidophilic heterotrophs, which averaged  $23\% (\pm 5\%)$  of sequences per MAT plot.

At the OTU-level, evenness estimated by Pielou's J did not vary as a function of MAT (adj.  $R^2 = 0.16$ ,  $P = 0.16$ ; mean Pielou's J =  $0.56 \pm 0.02$  across the MAT gradient). There was also a large amount of overlap in community composition among MAT plots (Fig. 3). Temperature was not a significant predictor of variance in either taxonomic similarity or phylogenetic similarity between communities across the MAT gradient (Table 2). Likewise, total belowground carbon flux (TBCF) and soil pH were not significant predictors of either taxonomic or phylogenetic similarity between soil bacterial communities across the MAT gradient (Table 2). Within-plot heterogeneity in bacterial composition was lower than between-plot heterogeneity based on lower mean phylogenetic distance in soil samples within MAT plots than in soil samples between MAT plots ( $t = -2.74$ ,  $P < 0.01$ ).

## **Discussion:**

Quantifying the sensitivity of soil bacterial communities to rising temperature is critical to understanding how tropical forest ecosystems will respond to a changing climate. We examined how long-term, whole-ecosystem warming influences the diversity and composition of soil bacterial communities across a highly constrained MAT gradient in closed-canopy Hawaiian tropical montane wet forests where other potentially confounding factors, including dominant vegetation, soil chemistry and soil moisture, were constant. Previous work along this gradient showed that increasing MAT drove sharp increases in above and belowground carbon process rates (Litton et al. 2011, Bothwell et al. 2014, Giardina et al. 2014). Increasing carbon fluxes with temperature, along with evidence from other studies indicating that bacterial diversity and



community composition are sensitive to increasing carbon inputs (Carney et al. 2007, Nemergut et al. 2010, Landa et al. 2013), led us to hypothesize that rising MAT and the concurrent increase in belowground carbon availability would drive substantial shifts in the diversity and composition of soil bacteria.

In contrast to our hypothesis, we found that bacterial community structure was insensitive to large increases in MAT and belowground carbon availability in Hawaiian tropical montane wet forests, suggesting that other environmental factors held constant across this gradient may be more important drivers of soil bacterial community structure. In the western hemisphere, soil pH is more closely related to microbial diversity than trends in climate across latitudinal gradients (Lauber et al. 2009), and in manipulative global change experiments, variation in soil moisture appears to have a much stronger effect on bacterial communities than variation in temperature (Castro et al. 2010, Zhang et al. 2013, Cregger et al. 2014). Soil pH and soil moisture did not vary appreciably across this MAT gradient (Table 1), which could explain the insensitivity of bacterial community structure to our 5.2° C increase in MAT. Dominant plant species also exert a strong control over the structure of soil bacterial communities (Lynch et al. 2012, Urbanová et al. 2015), especially in forests where dominant canopy tree species are responsible for the majority of ecosystem carbon input. Along the gradient studied here, all nine plots are dominated by *Metrosideros polymorpha* in the upper canopy with the mid-canopy dominated by a combination of the tree *Cheirodendron trigynum* and the tree fern *Cibotium menziesii* (Selmants et al. 2014). Taken together, our results suggest that, in the absence of changes to other variables that influence bacterial communities, even a 5.2° C increase in MAT is insufficient to alter soil bacterial community structure in these tropical montane wet forests.

There is substantial evidence from field and laboratory experiments that increasing carbon availability alters soil bacterial composition and diversity (Nemergut et al. 2010, Leff et al. 2012, Berthrong et al. 2013, Landa et al. 2013, 2014). However, and in contrast to prior microbial research, we found that soil bacterial composition and diversity are remarkably insensitive to even large gradients in MAT and belowground carbon flux. We offer three hypotheses, none of which are mutually exclusive, to explain why soil bacterial communities did not respond to the temperature-driven increase in belowground carbon input across this MAT gradient. First, increased carbon availability could have increased bacterial abundance without altering community composition or diversity – the forest ecosystem equivalent to the aphorism ‘a rising tide lifts all boats’. The chemical composition of leaf litter, fine root detritus and root exudates was likely similar across the gradient despite the overall increase in carbon input because plant species composition remained largely constant. Second, the overall metabolic activity of the soil bacterial community could have increased in response to the combined effects of rising MAT and increased belowground carbon input, but without changing bacterial abundance, composition or diversity. Finally, and related to the second hypothesis, increasing MAT and belowground carbon input may have increased the active fraction of the soil bacterial community. Dormancy is a widespread strategy among soil bacteria, allowing avoidance of unfavorable environmental conditions while maintaining high levels of diversity (Jones and Lennon 2010, Shade et al. 2012). Our analysis captured any bacterial cell with DNA without discriminating between active bacteria and the ~90% of bacterial cells in soil that are estimated to be metabolically inactive at any given time (Lennon and Jones 2011). It is possible that rising MAT and increased belowground carbon availability might increase the active fraction of existing soil bacterial communities rather than altering community composition or diversity. However, the relatively

aseasonal nature of the MAT gradient and year-round favorable moisture, temperature and carbon conditions suggest that release from dormancy is likely a minor factor.

The current study, conducted along a highly-constrained 5.2° C MAT gradient in tropical montane wet forests, adds to the growing body of evidence from environmental gradients and manipulative experiments that temperature alone may not be a primary factor structuring soil bacterial communities (Lauber et al. 2009, Fierer et al. 2009, 2011, Cregger et al. 2014, DeAngelis et al. 2015). These results further suggest that climate warming in the absence of changes in soil pH, soil water balance or plant community composition is unlikely to lead to drastic alterations of soil bacterial diversity and community composition, at least within the MAT range studied here, which may partially explain why soil carbon storage remains stable across the Hawaiian MAT gradient despite substantial increases in belowground carbon flux (Bothwell et al. 2014, Selmants et al. 2014, Giardina et al. 2014). In addition, these results lend support to Fierer et al.'s (2011) suggestion that broad hypotheses linking biodiversity to temperature-driven variation in metabolism may not apply to soil bacteria.

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Table 1. Site characteristics across a 5.2 C mean annual temperature gradient in closed-canopy tropical montane wet forests on the Island of Hawaii.

Elevation (m)	Air temp. (°C) <sup>a</sup>	Rainfall (mm y <sup>-1</sup> ) <sup>b</sup>	Soil moisture (m <sup>3</sup> m <sup>-3</sup> ) <sup>a</sup>	Soil pH <sup>a</sup>	Soil temp. (°C) <sup>a</sup>	Potential evapotranspiration (mm y <sup>-1</sup> ) <sup>c</sup>	Solar radiation (W m <sup>-2</sup> y <sup>-1</sup> ) <sup>c</sup>
800	18.2	4570	0.55	4.1	18.0	2298	201.1
934	17.3	4292	0.55	4.2	17.3	2232	200.9
1024	16.7	3975	0.57	3.7	16.3	2214	202.4
1116	16.1	3734	0.48	3.8	15.9	2127	204.9
1116	16.1	3433	0.51	3.6	15.6	2137	210.1
1204	15.5	3181	0.40	3.7	15.5	2211	214.5
1274	15.1	3101	0.51	3.9	14.9	2234	216.2
1468	13.8	4119	0.55	4.2	13.6	1888	202.6
1600	13.0	3282	0.57	4.1	12.6	1961	213.1

<sup>a</sup>data from Litton et al. (2011), <sup>b</sup>data from Giambelluca et al. (2012), <sup>c</sup>data from Giambelluca et al. (2014)

Table 2. Variation in taxonomic and phylogenetic community composition of soil bacteria as a function of temperature, total belowground carbon flux and soil pH across a 5.2 °C MAT gradient in closed-canopy tropical montane wet forests on the Island of Hawaii. Data were analyzed using permutational multivariate analyses of variance (PERMANOVA) on Bray-Curtis (taxonomic) and weighted UniFrac (phylogenetic) distance matrices.

<b>Factor</b>	<u>weighted UniFrac</u>		<u>Bray-Curtis</u>	
	<b>R<sup>2</sup></b>	<b>P-value</b>	<b>R<sup>2</sup></b>	<b>P-value</b>
Mean annual temperature	0.003	0.97	0.002	0.99
Total belowground carbon flux	0.011	0.54	0.005	0.82
Soil pH	0.004	0.89	0.005	0.82
Residuals	0.982		0.988	
Total	1.00		1.00	

**Figure legends:**

**Fig. 1.** Observed OTU richness and phylogenetic diversity (Faith's PD) of soil bacteria across a 5.2 °C mean annual temperature (MAT) gradient in closed canopy tropical montane wet forests on the Island of Hawaii. Blue circles are within-plot means and error bars represent 95% confidence intervals; n = 8 per MAT plot.

**Fig 2.** Relative abundance of soil bacteria by phylum across a 5.2° C mean annual temperature gradient in closed canopy tropical montane wet forests on the Island of Hawaii.

**Fig. 3.** Non-metric multidimensional scaling (NMDS) ordination of soil bacterial communities across a 5.2° C mean annual temperature (MAT) gradient in closed canopy tropical montane wet forests on the Island of Hawaii based on weighted UniFrac distances. Circles represent the centroid of replicate soil sample NMDS axis scores within MAT plots (n = 8 replicates per MAT plot) and grey error bars represent 95% confidence intervals. NMDS stress = 0.14.