**Title:** Soil bacterial community structure remains stable with 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 to the atmosphere. Despite their importance to ecosystem functioning, we lack a general understanding of how bacterial communities will respond to climate change, especially in relatively understudied ecosystems like tropical montane wet forests. We used a well-studied 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 alters the diversity and composition of soil bacterial communities. This MAT gradient is highly constrained, with dominant vegetation, substrate type and age, soil moisture, and disturbance history all held nearly constant, allowing us to effectively isolate the effect of rising MAT on soil bacterial community structure. Contrary to our hypothesis, we found that the richness, phylogenetic diversity and evenness of bacteria remained remarkably stable across the MAT gradient, and that MAT explained < 10% of the variation in community composition, despite a substantial increase in carbon fluxes with warming across this gradient. Consistent with previous studies, our results suggest that other factors held constant across this gradient, such as soil pH, water availability and plant species composition, may be more important factors influencing soil bacterial communities than warming – at least within the temperature range studied here. Our results add to growing evidence that soil bacterial communities in low-pH forest soils dominated by Proteobacteria and Acidobacteria may be largely resistant to the direct effect of climate warming.

**Introduction:**

Climate warming may have both direct and indirect effects on soil bacterial community structure (Allison and Martiny 2008, Shade et al. 2012), but the magnitude and direction of these effects remain poorly resolved because bacterial communities in different ecosystems respond differently to rising temperature (Cregger et al. 2014). It is thus surprising that there are few published 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 are large terrestrial carbon sinks (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). Understanding how climate warming affects soil bacterial community structure in a variety of tropical 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). Moreover, incorporating microbial dynamics improves the predictive capacity of soil carbon models (Allison et al. 2010, Li et al. 2014), which further demonstrates the importance of examining the temperature sensitivity of soil bacterial communities in tropical forest ecosystems.

There are currently no large-scale warming experiments in tropical forest ecosystems (Cavaleri et al. 2015), but evidence from experiments in temperate forests suggests that warming may have little to no effect on soil bacterial community structure (Kuffner et al. 2012, Cregger et al. 2014, DeAngelis et al. 2015). These forest warming experiments do not incorporate canopy warming, however, which can increase gross photosynthesis and thus overall carbon input to ecosystems (Lu et al. 2013), and increased carbon availability can strongly affect soil bacterial diversity and community composition (Carney et al. 2007, Allison and Martiny 2008, Leff et al. 2011, Landa et al. 2013, 2014). Thus soil warming experiments may not accurately reflect the integrated effect of whole-ecosystem warming on forest soil bacterial communities.

Elevation gradients can also be a valuable approach to examine how biota respond to environmental change in tropical forest ecosystems (Malhi et al. 2010), but isolating the effect of temperature can be difficult. A number of environmental variables can co-vary with temperature across elevation gradients, including moisture availability, soil pH and plant species composition – all of which have strong individual impacts on soil bacterial community structure (Lauber et al. 2009, Cregger et al. 2014, Schlatter et al. 2015, Urbanová et al. 2015). These potentially confounding factors complicate interpretation of variation in soil bacterial community composition and diversity along elevation gradients. High levels of local diversity and high turnover of plant species composition are especially problematic in comparisons of tropical forest plots across elevation gradients (Rapp et al. 2012).

Here we examine trends in soil bacterial 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 held largely 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 demonstrates that rates of ecosystem carbon fluxes increase substantially with rising MAT across this gradient, including soil CO2 efflux, leaf litter decomposition, total belowground carbon flux and litterfall (Litton et al. 2011, Bothwell et al. 2014, Giardina et al. 2014), while total ecosystem carbon storage remains constant (Selmants et al. 2014). Based on these findings, we hypothesized that rising mean annual temperature and increasing carbon availability would significantly alter the diversity and 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, Bothwell et al. 2014, Selmants et al. 2014, Giardina et al. 2014). 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 similar soil type and geology. Soils are well-drained Acrudoxic Hydradands developed from ~ 20,000 year old volcanic ash deposited on top of 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 mineral soil samples (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). Individual soil samples were collected near the center of eight 5x5 m subplots within each MAT plot, ~20-cm from the soil CO2 efflux collar within that subplot (Litton et al. 2011). 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 according to their protocols. 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 UCLUST (Edgar 2010) at 97% sequence identity against the 13-5 release of the Greengenes database (DeSantis et al. 2006). OTUs containing 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 being excluded from further analysis, each from a different MAT plot. 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 estimated OTU richness, phylogenetic diversity and community similarity metrics using python scripts 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 phylogenetic diversity index (PD) (Faith 1992) to estimate phylogenetic diversity. To compare OTU composition across MAT plots, we constructed community similarity matrices based on both taxonomic similarity and phylogenetic similarity. We used the Bray-Curtis distance index to construct a taxonomic similarity matrix and both weighted and un-weighted UniFrac distance metrics (Lozupone and Knight 2005) to construct phylogenetic similarity matrices.

All statistical analyses were conducted using R version 3.0.2 with an α-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 and un-weighted UniFrac) similarity matrices to construct non-metric multidimensional scaling (NMDS) ordinations using the vegan package in R. We quantified whether bacterial community composition varied as a function of MAT, soil pH and total belowground carbon flux (Giardina et al. 2014) with permutational multivariate analyses of variance (PERMANOVA) using taxonomic (Bray-Curtis) and phylogenetic (weighted and un-weighted UniFrac) measures of community similarity. PERMANOVAs were conducted using the ‘adonis’ function (Anderson 2001) in the vegan R package. 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:**

We found no evidence that bacterial diversity varied with temperature across this well-constrained MAT gradient. The estimated number of bacterial OTUs averaged 1106 (± 174; 95% CI) across the MAT gradient but did not vary significantly with MAT (adj. R2 = 0.06, P = 0.54; Fig. 1). There was also no significant relationship between MAT and phylogenetic diversity (adj. R2 = 0.07, P = 0.48), which averaged 45 (± 7; 95% CI) across the MAT gradient (Fig. 2).

Similar to trends for diversity, we found little to no support for our hypothesis that temperature is a major driver of bacterial community composition in these tropical montane wet forest soils. Soils across the MAT gradient were dominated by the phyla Proteobacteria (44% ± 4 % of sequences per MAT plot) and Acidobacteria (43% ± 4% of sequences per MAT plot; Fig. 3), a pattern consistent with other 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 we identified varied significantly as a function of MAT (adj. R2 < 0.015, P > 0.17 for all phyla). Approximately 87% (± 5%) of sequences within phylum Proteobacteria were in the class Alphaproteobacteria, and most Alphaproteobacteria were either in the genus Rhodoplanes (family Hyphomicrobiaceae; 18% ± 3% of sequences per MAT plot) or the family Rhodospirillaceae (10% ± 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% (± 5%) of sequences per MAT plot across the gradient.

At the OTU-level, evenness estimated by Pielou’s J did not vary significantly as a function of MAT (adj. R2 = 0.16, P = 0.16; mean Pielou’s J = 0.56 ± 0.02 across the MAT gradient), and there was a large amount of overlap in community composition among MAT plots (Fig. 4). Although statistically significant, MAT explained only 9% of the variance in taxonomic similarity and ~ 3% of the variance in phylogenetic similarity between communities across the gradient (Table 1). Total belowground carbon flux (TBCF) was also a significant predictor of bacterial taxonomic and phylogenetic community similarity, but explained an even smaller proportion of the variance than did MAT, and soil pH was not a significant predictor of either taxonomic or phylogenetic similarity between soil bacterial communities across the MAT gradient (Table 1). The mean phylogenetic distance (weighted UniFrac) of bacterial communities in soil samples within each MAT plot was significantly lower than the mean phylogenetic distance of bacterial communities in soil samples between MAT plots (t = -2.74, P < 0.01), indicating that within-plot heterogeneity in bacterial composition was lower than between-plot heterogeneity.

**Discussion:**

Quantifying the temperature sensitivity of soil bacterial communities is a critical component to understanding how tropical forest ecosystems will respond to climate change. To test the hypothesis that long-term, whole-ecosystem warming will alter both the diversity and composition of soil bacterial communities, we used a highly constrained MAT gradient in closed-canopy Hawaiian tropical montane wet forests where most other potentially confounding factors, including dominant vegetation, soil chemistry and soil moisture, were held largely constant. Previous work along this gradient indicates that rising MAT drives sharp increases in several ecosystem processes (Litton et al. 2011, Bothwell et al. 2014, Giardina et al. 2014), many of which involve soil bacteria, leading us to hypothesize that rising MAT would drive substantial shifts in the diversity and composition of soil bacteria.

In contrast to our hypothesis, we found no evidence that temperature exerts a strong control over soil bacterial diversity and composition 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. Trends in soil pH are more closely related to microbial diversity than trends in climate across latitudinal gradients in the western hemisphere (Lauber et al. 2009), and variation in soil moisture appears to have a much stronger effect on bacterial communities than variation in temperature in manipulative global change experiments (Castro et al. 2010, Zhang et al. 2013, Cregger et al. 2014). Neither soil pH or soil moisture varied appreciably across this gradient (Table 1), which may partially explain the stability of bacterial community structure despite a 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 carbon inputs to the ecosystem. Along the gradient studied here, all nine plots were dominated by *Metrosideros polymorpha* in the upper canopy with the mid-canopy dominated by a combination of the woody tree species *Cheirodendron trigynum* and the tree fern *Cibotium menziesii* (Selmants et al. 2014). Taken together, our results suggest that even a 5.2° C increase in MAT is insufficient to alter soil bacterial community structure in these tropical montane wet forests if other factors such as soil chemistry, soil water availability and plant species composition remain relatively constant.

There is substantial evidence from field and laboratory experiments that increasing carbon inputs alters bacterial composition and diversity (Nemergut et al. 2010, Leff et al. 2011, Berthrong et al. 2013, Landa et al. 2013, 2014). Several lines of evidence from previous research along this gradient indicate that rising MAT has increased carbon inputs to the soil in these tropical montane wet forests (Litton et al. 2011, Bothwell et al. 2014, Giardina et al. 2014), yet our current results demonstrate that soil bacterial composition and diversity remain remarkably stable. We offer three hypotheses, none of which are mutually exclusive, to explain why soil bacteria did not respond to the temperature-driven increase in carbon inputs across this MAT gradient. First, increased carbon resource availability could have increased bacterial abundance without altering community structure – 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 is likely similar across the gradient despite the overall increase in carbon inputs because plant species composition remains largely constant, which lends credence to this hypothesis. Second, the overall metabolic activity of the soil bacterial community has increased in response to the combined effects of rising temperature and increased carbon inputs, with little to no change in either bacterial abundance or community structure. Finally and related to the second hypothesis, increasing MAT and carbon inputs 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, and did not discriminate between active and inactive organisms. An estimated 90% of bacterial cells in soil are metabolically inactive at any given time (Lennon and Jones 2011) suggesting that rising temperature and increased carbon availability might lead to an increase in the active fraction of existing soil bacterial communities rather than altering community structure.

This study, conducted along a well-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). Our results 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 a number of carbon fluxes (Selmants et al. 2014, Giardina et al. 2014). In addition, our results lend further 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.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| MAT Plot | Elevation (m) | Air temperature (°C)a | Rainfall (mm y-1)b | Soil volumetric water content (m3 m-3)a | Soil pHa | Soil temperature (°C)a | Potential evapotranspiration (mm y-1)c | Solar radiation (W m-2 y-1)c |
| 1 | 800 | 18.2 | 4570 | 0.55 | 4.1 | 18.0 | 2298 | 201.1 |
| 2 | 934 | 17.3 | 4292 | 0.55 | 4.2 | 17.3 | 2232 | 200.9 |
| 3 | 1024 | 16.7 | 3975 | 0.57 | 3.7 | 16.3 | 2214 | 202.4 |
| 4 | 1116 | 16.1 | 3734 | 0.48 | 3.8 | 15.9 | 2127 | 204.9 |
| 5 | 1116 | 16.1 | 3433 | 0.51 | 3.6 | 15.6 | 2137 | 210.1 |
| 6 | 1204 | 15.5 | 3181 | 0.40 | 3.7 | 15.5 | 2211 | 214.5 |
| 7 | 1274 | 15.1 | 3101 | 0.51 | 3.9 | 14.9 | 2234 | 216.2 |
| 8 | 1468 | 13.8 | 4119 | 0.55 | 4.2 | 13.6 | 1888 | 202.6 |
| 9 | 1600 | 13.0 | 3282 | 0.57 | 4.1 | 12.6 | 1961 | 213.1 |

Table 2. Variation in bacterial community composition as a function of temperature, soil pH and total belowground carbon flux 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 both taxonomic (Bray-Curtis) and phylogenetic (weighted and unweighted UniFrac) distance matrices.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | UniFrac (weighted) | | UniFrac (unweighted) | | Bray-Curtis | |
| **Factor** | **R2** | **P-value** | **R2** | **P-value** | **R2** | **P-value** |
| Mean annual temperature | 0.03 | 0.071 | 0.05 | 0.010 | 0.09 | 0.002 |
| Soil pH | 0.01 | 0.559 | 0.02 | 0.165 | 0.02 | 0.213 |
| Total belowground C flux | 0.03 | 0.096 | 0.03 | 0.034 | 0.04 | 0.045 |
| Residuals | 0.97 |  | 0.90 |  | 0.85 |  |
| Total | 1.00 |  | 1.00 |  | 1.00 |  |