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Debra Peters, Ph.D.  
Editor-in-Chief  
*Ecosphere*

Dear Dr. Peters,

We are pleased to submit a revised version of the manuscript 'Increases in mean annual temperature do not alter soil bacterial community structure in tropical montane wet forests', previously submitted to *Ecology* as manuscript no. 15-1142. Our manuscript represents an important advance in understanding how soil bacterial communities respond to a changing climate in tropical montane wet forests, an understudied ecosystem that is also a significant global carbon sink.

In response to the reviewers' comments, we have re-written large portions of the Introduction and Discussion sections and added new citations in support of our conclusions. This revised text more clearly emphasizes that our unique design allowed us to isolate the long-term, integrated effect of rising temperature on soil bacterial community structure in tropical montane forests by holding constant many potentially confounding factors (e.g., soil pH, dominant vegetation, soil moisture). As such, our study provides strong evidence that neither warming nor warming-related increases in belowground carbon flux influence soil bacterial community structure, and that prior evidence to the contrary was either weak or confounded by other factors.

We have fully addressed each of the two referees' comments in detail below. Our responses are in normal typeface and immediately follow each reviewer comment, which are listed in boldface type. Page and line numbers listed in our responses refer to the revised version of the manuscript.

Consistent with ESA's data policy, we have made our data and R code publicly available as supplementary material at:

[https://github.com/selmants/bacteria\\_MAT](https://github.com/selmants/bacteria_MAT)

We expect our paper to be of immediate interest to a wide audience of community and ecosystem ecologists, climate change modelers and microbiologists. We hope you find our revisions and responses to reviewers' comments sufficient to merit publication of our manuscript in *Ecosphere*.

Sincerely,

Paul C. Selmants, Ph.D.

Reviewer #1 (Comments to Author):

**This is a well-written manuscript that follows on from recent work describing carbon flux and carbon storage across the same thermal gradient in Hawaii. The physical and biogeochemical characteristics of these sites lend themselves well to understanding how microbial community composition varies (or does not vary) with temperature.**

**While there are certainly a number of positives associated with this manuscript, I fear it leaves too many unanswered questions to warrant publication. In particular it leaves a number of questions which could have easily been addressed by this study, and perhaps should have been. Some of these questions are raised by the authors in their discussion.**

**I don't believe the central hypothesis is sufficiently justified in the introduction. Why should temperature fundamentally alter community diversity? Given the capacity of enzymes and membrane transporters to acclimate to localized temperatures, what are the selective pressure that drive differentiation of the communities? The authors never really delve into the relevance of potential changes in community composition. We know microbial communities are characterized by functional redundancy, therefore, why would a community change matter? If the differences in carbon fluxes are driven by an enhancement in metabolic activity then what is the point of characterizing phylogenetic diversity? Given the previous work measuring carbon fluxes across this thermal gradient, it was a little surprising these connections were not made.**

Our results indicate that increasing temperature and carbon flux may enhance metabolic activity without altering community composition or diversity, yet the evidence for this was unclear prior to our study. It is true that enzymes and membrane transporters can acclimate to localized temperatures, but this acclimation has limits and these limits are not known for the vast majority of bacterial taxa because they have not been cultured. Given the results from prior studies that we cite in the Introduction and Discussion sections, we considered it reasonable to hypothesize a shift in bacterial community composition and diversity with a 5.2 °C increase in MAT and a ~50% increase in carbon flux belowground. The fact that our results do not support this hypothesis is a valuable contribution not only to the broad factors that shape soil bacterial communities, but also to the specific impacts of climate change on the structure of soil bacterial communities in tropical forest ecosystems. In response to this comment, we have re-written a portion of the Introduction (lines 85-89) and the Discussion (lines 215-270) to emphasize that our unique study design provides strong evidence that neither warming nor warming-related increases in belowground carbon flux have any influence on soil bacterial community structure, and that prior evidence to the contrary was either weak or confounded by other factors.

**I also don't believe the methodology is adequate to address the broader question at hand. While community composition across a thermal gradient is a primary question for the authors, there is a strong attempt to relate it to the carbon fluxes previously measured. This would require analysis at a finer phylogenetic resolution than reported here (which is mainly restricted to the phyla level). Functional variability at the genus and class level could far better explain the seeming lack of impact of the functional potential of these communities is therefore the relevant measurement rather than the phylogeny.**

Our analyses are not mainly restricted to the phylum level, as suggested by this reviewer. In fact, most of our analyses were conducted at the OTU level – including our analyses of OTU richness, phylogenetic diversity (which this reviewer appears to misunderstand as being conducted at the phylum level), and the two separate similarity matrices used to compare community composition across the MAT gradient. We specifically stated that community composition analyses were conducted at the OTU level on line 148 of this manuscript (line 126 in previous version). Phylogenetic diversity in particular is highly correlated with functional trait diversity, so any sizeable shift in phylogenetic diversity could indicate a change in functional capacity of the soil bacterial community. It is clear from this reviewer's comments that we did not explain phylogenetic diversity in sufficient detail - specifically how it is calculated, why it is relevant to this study, and its functional implications. Therefore, we have added text and citations to the Methods section (lines 144-148) to better explain the importance and functional implications of phylogenetic diversity. While we agree with this reviewer that a direct measure of the functional potential of the entire soil bacterial community within each MAT plot would be ideal, such an all-encompassing method does not currently exist for highly diverse soil bacterial communities.

**Added to this, the authors could have dispelled a great deal of their hypothesizing in the discussion had bacterial abundance or enzyme potential been measured. These are relatively simple measurements to make, and would have added a great deal to this story.**

We agree that measuring abundance would have strengthened our arguments in the Discussion section, but the focus of this study was on community composition and diversity. In response to this comment, we have re-written this portion of the Discussion section to emphasize the lack of variability in bacterial community composition and diversity in response to large changes in MAT and belowground carbon flux (lines 215-270).

**Finally, the authors mention a couple of times in the abstract and discussion that we currently don't understand how microbial communities will respond to climate changes. This is a fundamental question, and an important one given the potentially constrained adaptive capacity of tropical organisms (e.g., Deutsch et al., PNAS, 2008). However, I fail to see how this paper addresses that question. The authors are looking across a natural temperature gradient where organisms are presumably adapted to localized conditions. This is not a manipulation experiment where temperature is raised above the annual mean and the metabolic response of bacteria addressed.**

This reviewer is correct that we assessed soil bacterial community composition and diversity across a natural temperature gradient where these communities have adapted to localized conditions. This is exactly what makes our MAT gradient so valuable; manipulative experiments are necessarily short-term (1-3 years) and so may only capture the short-term oscillations that can often accompany a rapid change in temperature. Our design captures the long-term, integrated, whole-ecosystem impact of rising temperature on soil bacteria in tropical montane forests – an understudied ecosystem type where manipulative warming experiments would likely be confined to just the soil. In response to this comment, we have added text and citations to the Introduction that highlight the value of our highly constrained MAT gradient (lines 85-89).

Specific comments:

**Ln 77: What is the annual variance in the temperature? If it's high would that be a reason to suspect some overlap within a geographically isolated area?**

Within each MAT plot, the standard deviation of mean monthly temperature on an annual basis is  $\sim 1.2$  °C. This is quite low compared with the 5.2 °C range in mean annual temperature across the MAT gradient. We have added text and a citation to the Methods section stating that within-plot variation in air temperature is low compared to the range in air temperature across the MAT gradient (lines 109-110).

**Ln 94: How do the air temperatures relate to the soil temperatures at 10 cm?**

Mean annual soil temperature at 10 cm differs from MAT by only  $\sim 0.5$  °C on average across the gradient, and MAT explains almost all of the variance in soil temperature ( $T_{\text{soil}} = 1.02 \cdot \text{MAT} - 0.45$ ;  $R^2 = 0.99$ ;  $P < 0.01$ ). We have added text and a citation to the Methods section that explains the nearly 1:1 relationship between soil temperature and air temperature across the MAT gradient (lines 111-112).

**Ln 131: Why report the alpha value before reporting the test?**

The alpha level, which is the probability of falsely rejecting the null hypothesis (Type I error), should always be selected before conducting a statistical test. We used  $\alpha = 0.10$  for all statistical tests, and so reported the alpha level here rather than repeating it in the description of each individual test conducted. No changes were made to the manuscript based on this comment.

**Ln 150: 45 OTUs?**

No, not OTUs. Faith's phylogenetic diversity (PD) metric measures the length of evolutionary pathways (number of branches on a phylogenetic tree) that connect a given set of taxa. Larger PD values correspond to greater expected trait diversity within a community. As stated above, this reviewer's comments make clear that we did not sufficiently explain phylogenetic diversity or its relevance to our study. Therefore, we have added text and citations to the Methods section that better explains the concept of phylogenetic diversity and its relevance to our study (lines 144-148).

**Ln 153: If something isn't changing in situ then I don't see how it can be 'driving'?**

This reviewer is correct that MAT isn't changing in situ. However, MAT does vary dramatically across the well-constrained MAT gradient that we established, while most other major factors known to control soil bacterial community composition remain constant (e.g., plant community composition, soil pH, soil water availability). We believe this 'space-for-time' substitution design, which has a long and fruitful history in ecology, is an ideal natural laboratory to examine the long-term, integrated effect of temperature on soil bacterial community structure, as we have explained in detail throughout the Introduction and Methods section. In response to this comment we have replaced the word 'drives' with 'influences' to avoid confusion (line 181).

**Ln 177: This statement seems at odds with the data showing temperature has no effect, no? In addition, I don't see what the data here contributes to assessments of community sensitivity?**

We have rewritten a large portion of the discussion to emphasize that, by controlling for several other factors known to shape bacterial community structure, our results provide strong evidence that soil bacterial community composition and diversity are largely insensitive to rising MAT in these tropical montane wet forests (lines 215-270). Given that soil bacteria provide essential functions contributing to carbon and nutrient cycling, our data demonstrating that a 5.2 °C increase in mean annual temperature has no effect on soil bacterial community structure is a valuable contribution to ecosystem modelers seeking to incorporate microbial community dynamics into projections of ecosystem carbon storage with climate change.

**There is an argument to be made that the adaptive capacity of tropical forest soils is lower than elsewhere (see the work of Deutsch or Tewksbury), but can that argument be applied here to justify this statement?**

We believe an argument based on the work of Deutsch et al. (2008) would be highly speculative. Deutsch et al. had access to a large dataset of population growth curves for terrestrial insects at multiple temperatures, but population growth curves for the vast majority of bacterial taxa are unknown, at any temperature, because most have not been cultured. No changes were made to the manuscript in response to this comment.

**Ln 186: This was a little confusing to me at first. This manuscript presents no data on carbon (either bulk or fluxes). To get at this data readers have to go back to Giardina et al., manuscript of a few years ago. This manuscript should stand on its own, therefore, some of this published data should appear here in some manner should the authors wish to refer to it.**

We now include total belowground carbon flux values for each MAT plot from Giardina et al. (2014) in Table 1.

**Ln 193: What does 'more closely related' refer to here? Explains more of the variance? How much more relative to temperature?**

The relationship between pH and bacterial diversity is much stronger than the relationship between climate and bacterial diversity. Across a latitudinal gradient, pH explains about 50% of the variance in soil bacterial diversity and MAT explains virtually none - see Lauber et al. (2009). We have rewritten this entire paragraph and no longer use the phrase 'more closely related' (lines 215-234).

**Ln 219: This speaks to my earlier point. Simple measurements, such as quantifying the bacterial abundance would have lent strong support for this hypothesis. Without this data I'm afraid I feel this study is a little incomplete.**

We agree that measuring abundance would have helped to explain these trends. However, our study was designed to examine the response of soil bacterial community composition and diversity to rising MAT. We have re-written a large portion of the discussion to provide a detailed and complete assessment of the evidence against MAT being a strong controlling factor of soil bacterial community structure (lines 215-247).

Reviewer #2 (Comments to Author):

General Comments:

**This manuscript describes bacterial community diversity and composition across an 800 m elevation gradient which spans a 5 °C mean annual temperature gradient in tropical montane forest in Hawaii. The results presented convincingly demonstrate that across a highly-constrained temperature gradient with a consistent tropical montane forest vegetation cover, bacterial diversity is insensitive to warming.**

**The authors use the temperature gradient to infer effects of climate warming on bacterial diversity, addressing a pressing question in ecology which is currently unresolved, how will climate change affect the functioning of soil microbial communities and consequent feedbacks to the atmosphere. This is an important question as the soil microbial community has a critical role in carbon and nutrient cycling influencing greenhouse gas fluxes. Climate warming experiments have been used to examine this question, but not in tropical forests, and the authors correctly argue that the gradient approach has considerable advantages over warming experiments which exclude the forest canopy.**

**The scope of the paper is original and scientifically robust in its design and analysis. The paper is also very well-written, concise, easy to understand and clear in its conclusions. The manuscript contains a unique dataset with interesting insights for the journals readership on the influence of temperature on bacterial community composition and I recommend this paper for publication, providing the specific comments below can be addressed.**

Specific comments:

**A small number of gradient studies of this type have been conducted in temperate ecosystems around the world, but there has been very limited study in tropical forests with (to my knowledge) only one comparable study by Fierer et al 2011, Ecology. Gradient studies can be problematic due to confounding factors i.e. other factors changing with elevation in addition to temperature. The strength of this particular gradient is that temperature is the differentiating factor, with dominant vegetation, underlying substrate and soil moisture status relatively constant. The Fierer study spanned a larger elevation and temperature gradient (200-3400m) and a more diverse range of plant communities. The consistent vegetation along this gradient and narrower overall temperature range of the sites makes this study different from the Fierer et al 2011 study, providing different insights but this is not brought out in the manuscript. Given the similarity in the scope of the two papers the advance of this paper compared to the Fierer paper needs to be addressed in the introduction.**

We have added text to the Introduction that specifically compares our MAT gradient and study design to that described in the Fierer et al. 2011 paper (lines 78-89).

**The authors report that across the gradient, dominant vegetation and plant composition are constant and report the dominant canopy and mid-canopy species. Whilst dominant species are likely to have a strong influence on soil microbial composition, plant diversity is also likely to influence microbial diversity. If a more detailed assessment of plant diversity changes with elevation is available this would strengthen the argument that plant composition is constant (line 31). If plant diversity data is available this should be referred to in the manuscript.**

We did not quantify plant diversity directly, but plant species richness in these forests is quite low because of the extreme geographic isolation of the Hawaiian Islands. We do list dominant species in the canopy and mid-canopy in the Discussion section, and cite previous work demonstrating that these dominant species remain constant across the MAT gradient (lines 224-226).

**Line 136-138 - a short justification is needed of why these parameters were selected from the large range of potential climatic and edaphic factors which could have been explored.**

We have added text that justifies why these specific parameters were selected as predictor variables in our analysis (163-165).

#### **Discussion:**

**One area missing from the discussion is a comparison of the manuscript findings with other similar studies, in particular Fierer et al 2011, which also reported no trend in bacterial diversity with elevation. Whilst this is the only study in tropical forest, there are other elevation/temperature gradient studies of microbial diversity which should be referred to. E.g. Bryant et al 2008, Shen et al 2014, Singh et al 2012.**

We now include text in the Discussion section (lines 216-234) that compares our results with those from other elevation gradients, specifically Fierer et al (2011) Bryant et al (2008), Shen et al (2014), and Singh et al (2012).

**Line 209. Re-phrase "However, and in contrast to....."**

We have rephrased this sentence (line 239).

**Line 216. The sentence "The chemical composition of leaf litter....." It is not clear if this sentence is a justification for hypothesis 1 or an introduction to hypothesis 2.**

We have re-written this portion of the discussion section to more clearly describe why soil bacterial community structure remained constant despite evidence that their functional capacity

may have increased with warming and warming-related increases in belowground carbon flux (lines 235-270).

**Line 283 Incomplete reference**

The reference to this paper is now complete (lines 340-341).

**Line 348 paper title missing from reference**

We have added the paper title to this reference (lines 420-423).