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Title

Identifying the microbial taxa which consistently respond to soil warming across time and space

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Abstract

Soil microbial communities are the key drivers of many terrestrial biogeochemical processes. However, we currently lack a generalizable understanding of how these soil communities will change in response to predicted increases in global temperatures and which microbial lineages will be most impacted. Here, using high-throughput marker gene sequencing of soils collected from 18 sites throughout North America included in a 100-day lab incubation experiment, we identified a core group of abundant and nearly ubiquitous soil microbes that shift in relative abundance with elevated soil temperatures. We then validated and narrowed our list of temperature sensitive microbes by comparing the results from this laboratory experiment with data compiled from 210 soils representing multiple, independent global field studies sampled across spatial gradients with a wide range in mean annual temperatures. Our results reveal predictable and consistent responses to temperature for a core group of 189 ubiquitous soil bacterial and archaeal taxa, with these taxa exhibiting similar temperature responses across a broad range of soil types. These microbial 'bioindicators' are useful for understanding how soil microbial communities respond to warming and to discriminate between the direct and indirect effects of soil warming on microbial communities. Those taxa that were found to be sensitive to temperature represented a wide range of lineages and the direction of the temperature responses were not predictable from phylogeny alone, indicating that temperature responses are difficult to predict from simply describing soil microbial communities at broad taxonomic or phylogenetic levels of resolution. Together these results lay the foundation for a more predictive understanding of how soil microbial communities respond to soil warming and how warming may ultimately lead to changes in soil biogeochemical processes.

Introduction

Climate change is warming soils in many regions worldwide, likely altering biogeochemical process rates (Butler et al., 2012; Grimm et al., 2013). Soil microbial communities mediate many of these processes (Li et al., 2014; Treseder et al., 2012; Wieder et al., 2015), including those that are central to carbon and nitrogen cycling at local to global scales. Yet how warming affects the structure and functioning of soil microbial communities is unclear. This knowledge gap persists because the enormous diversity of soil microbial communities hinders our ability to discern the organismal responses that determine the collective physiology, abundance and functional

diversity of the microbial biomass, which in turn shape biogeochemical cycling (Amend et al., 2016). A taxon-specific focus for plants and animals has contributed to the development of trait-based approaches that have yielded detailed insights into how and why plant and animal communities may shift in response to warming (Corlett and Westcott, 2013; Garcia-Robledo et al., 2016). Similar insights into microbial communities require comparable work with soil microbial taxa. Identifying those microbial taxa that specifically respond to temperature will enable determination of temperature-response traits from among the multitude of other traits that dictate the responses of highly-diverse soil communities to other environmental factors. The first challenge, however, is to identify that subset of microbial taxa, from the hundreds or thousands that can be found in any given soil sample, that consistently change in abundance in response to changes in soil temperature.

There are several reasons why we still have a limited understanding of how soil microbial communities will respond to changes in temperature. First, most of the bacterial taxa found in soil remain understudied (Ramirez et al., 2014), with their specific responses to temperature unknown. Although there has been extensive research on thermophilic and psychrophilic bacteria (e.g. Aanniz et al., 2015; Bottos et al., 2008; Siddiqui et al., 2013), these taxa are not likely to be abundant in most soils and the temperature optima that define thermophiles and psychrophiles (40 - 70°C and -20 to 10°C, respectively) are either outside or overlap minimally with the range of temperatures experienced by many soils. Thus, knowing what taxa are thermophilic or psychrophilic is unlikely to be useful for predicting how microbial communities in most soils will respond to anticipated mean increases in global surface temperatures of 2-4°C by the year 2100 (IPCC, 2013).

Second, despite the myriad of studies looking at microbial community responses to experimental warming, both in the field (e.g. DeAngelis et al., 2015; Castro et al., 2010; Frey et al., 2008) and in the lab (e.g. Waldrop and Firestone, 2004; Andrews et al., 2000), it remains hard to predict the specific taxonomic shifts in microbial communities that are likely to occur across a diversity of soil types. Most studies are typically site or soil-type specific, making it difficult to infer if results from one study are relevant to the broad range of soils found across the world. Likewise, despite previous work documenting how soil microbial communities change across climatic

gradients, including elevational (Whitaker et al., 2014) and latitudinal gradients (Wu et al., 2009), multiple soil biotic and abiotic characteristics often co-vary with temperature across these gradients. For example, changes in temperature regimes often coincide with changes in soil moisture or the amounts and types of organic carbon inputs (Reichstein & Beer, 2008). These correlations make it challenging to disentangle whether shifts in the relative abundances of specific microbial taxa are driven by temperature itself or by corresponding changes in other factors that can also shape soil microbial communities.

The primary goal of this study was to identify soil bacterial and archaeal taxa that consistently respond to temperature differences, focusing on those microbial taxa that occur across a wide range of soil and ecosystem types. We refer to such taxa as 'microbial bioindicators', and in this study focus on microbes that are particularly sensitive to changes in temperature and are ubiquitous enough in soil that their relative abundances can be ultimately used to help understand and predict the community and ecosystem consequences of changes in soil temperature regimes. There has been some suggestion that a few keystone species may be particularly important in understanding microbial responses to changing climate (DeAngelis et al., 2015), but these analyses have been limited to one study site. Just as microbial bioindicators have previously been used to assess environmental conditions such as soil toxin levels (Shen et al., 2016) or wetland health (Sims et al., 2013), if we can identify microbial indicators of temperature we can then identify when soil communities are affected directly or indirectly by changes in temperature itself, versus the numerous other biotic and abiotic factors that may similarly change across environmental gradients.

We secondarily sought to determine if there was a phylogenetic signal to the microbial temperature responses. If there is a strong phylogenetic signal it should be easier to predict temperature responses of individual soil microbial taxa, because it would suggest that those traits associated with temperature preferences are sufficiently conserved that they can be inferred from phylogenetic information alone. There appears to be potential for using a phylogenetic, trait-based framework to predict shifts in community composition for traits with a strong phylogenetic signal such as pH preferences (Martiny et al., 2015). Likewise, a strong phylogenetic signal for temperature response in fungi was found in a recent warming experiment in boreal forest soils,

with variance in warming response best explained at the taxonomic rank level of Order (Treseder et al., 2016). In *Cyanobacteria* and *Actinobacteria*, temperature preference appears to be shallowly conserved at the species level (Martiny et al., 2015). However, we do not have a sufficient amount of pre-existing data to infer whether temperature preferences are predictable from phylogeny alone across the broad diversity of bacteria and archaea common in soil.

Here we use both a lab incubation study and field studies to identify bacterial and archaeal taxa that are consistently sensitive to changes in soil temperature. For the lab incubation study, we collected grassland and forest soils from 11 Long Term Ecological Research (LTER) areas across North America and compared changes in bacterial and archaeal community composition after a 100-day incubation at different temperatures. We then identified a subset of taxa that changed in relative abundance in response to differences in soil incubation temperature.

To test the validity of our incubation-based predictions of temperature preferences, we quantified how the temperature-responsive taxa identified from the lab-based study changed in response to natural gradients in mean annual temperature across three different sets of soils collected from field sites across the globe (210 soils in total). Only those taxa that consistently 'preferred' warmer or colder temperatures in both laboratory and field studies were considered for downstream analyses to identify temperature 'bioindicators' and to quantify the phylogenetic signal in microbial temperature preferences.

Materials and Methods.

Lab-based soil incubation study

Our aim was to compare microbial community responses to changes in temperature across a wide range of distinct soils to see if there are microbial taxa that exhibit consistent responses to changes in temperature regime, i.e. taxa that consistently increase or decrease in relative abundance across a wide range of soils. We collected samples from 11 Long Term Ecological Research (LTER) areas (Supplementary Table 1), during Spring 2012. At each LTER, mineral soils (0-5 cm in depth) from two distinct vegetation types were sampled, both forest and grassland soils, to yield soil samples from 18 unique sites included in this lab experiment (for 4

of the LTER areas only a forest or grassland soil sample was obtained, thus we obtained samples from 18 unique sites). These soils are not meant to be representative of each LTER site, but rather to capture a range of ecosystem types (including wet tropical forest, tundra, prairies, and boreal forest). As such, the sites spanned a broad range in climatic conditions, with mean annual temperatures (MAT) ranging from -3.2°C to 22.8°C and mean annual precipitation ranging from 262 to 1,898 $mm \cdot y^{-1}$. For additional site information, see Supplementary Table 1. We pre-incubated 60 g dry weight of each of the 18 sites at 20°C for 14 days, after passing each through a 2 mm sieve. Then we transferred a 30 g sub-sample of each soil to either high (28°C) or low (12°C) temperature incubators to test how microbial communities from diverse soils responded to the two different temperature conditions, yielding 36 soil sub-samples. Soil sub-samples were then incubated for 100 days. Water was added weekly to each soil during the incubation period to keep all soils between 50-70% water holding capacity throughout the incubation, which is considered optimal for microbial activity in lab incubated soils (Paul et al. 2001).

After the incubation period, DNA was extracted and a portion of the 16S rRNA gene was PCR amplified and sequenced as described in Leff et al. (2015). Briefly, a moistened sterile swab was inserted into each sample and DNA was extracted from the swab using the PowerSoil DNA extraction kit (MoBio Laboratories Inc.). We included multiple negative controls per extraction plate to check for possible contamination. After DNA extraction, a targeted portion of the 16S rRNA gene was PCR amplified in triplicate reactions using the 515f/806r primer set that included Illumina sequencing adapters and 12-bp barcode to permit multiplexed sequencing. This primer pair amplifies the V4-V5 region of the 16S rRNA gene for Archaea and Bacteria with relatively few biases (Caporaso et al., 2012). After normalizing amplicon concentrations, all amplicons were pooled and sequenced (2 x 150 bp paired-end chemistry) on the Illumina MiSeq platform at the University of Colorado Next Generation Sequencing Facility.

Assessment of microbial community composition

Raw sequence data from the lab incubation samples were processed following an approach described previously (Leff et al., 2015), using a custom Python script ('prep_fastq_for_uparse_paired.py', at: https://github.com/leffj/helper-code-for-uparse) to de-multiplex sequences before processing in the UPARSE pipeline (Edgar 2013). After trimming all

sequences to the same length (100 bp), sequences were quality filtered to a "maxee" value of 0.5 (maximum per sequence expected error frequency value) and clustered into phylotypes (sequences that shared ≥97% sequence similarity) with USEARCH (Version 7). Additionally, all sequences were dereplicated and singleton phylotypes (phylotypes represented by only a single read) were removed. The raw reads were mapped to the clustered *de novo* database at 97% similarity generating phylotype counts. Taxonomy was assigned to each phylotype using the RDP classifier (Wang et al., 2007) against the Greengenes database (McDonald et al., 2012) with a confidence threshold of 0.5. Finally, chloroplast and mitochondrial reads were removed prior to downstream analyses. To control for differences in sequence coverage, all samples from the lab study were rarefied to 10,000 sequences per sample (using the R package 'mctoolsr', at: https://github.com/leffj/mctoolsr). A total of 33 soil samples were included in the final analyses, with three of the 36 samples that were incubated discarded due to insufficient sequence coverage.

Quantifying microbial community shifts with temperature in the lab

We tested for shifts in community composition across soils and temperature treatments using permutational multivariate ANOVA (PERMANOVA; Supplementary Table 5) with the "Adonis" function in the vegan package in R (Version 3.2.2). Bray-Curtis dissimilarity matrices were used to quantify differences in community composition, as calculated using the R package mctoolsr (Version 0.3.2). When testing for shifts in composition with temperature, incubation temperature and site were used as the predictor variables with 999 permutations.

To identify phylotypes that were responsive to temperature in the lab, we first restricted our analyses to only include phylotypes that were found in soils from at least six of the 18 sites and had percent abundances exceeding 0.05%. We did this to ensure that rare taxa were not included, as our goal was to identify abundant phylotypes and to restrict our analyses only to those phylotypes that were reasonably ubiquitous and found in multiple soils. To measure the temperature response of each individual phylotype that met these criteria, the percentage change in relative abundances from 12°C to 28°C were averaged across all sites for each phylotype (visualized in Fig. 1a for six selected phylotypes). Zeroes were transformed to 0.001 in

calculations (similar to Amend et al., 2016). We then tested to see if those phylotypes identified as being responsive to temperature in the lab also shifted with temperature in natural field soils.

Field data selection and compilation

To test if temperature-responsive phylotypes from the lab experiment exhibited directionally similar responses across natural gradients in soil temperature regimes in the field, we collated previously-published microbial community data from three, independent field studies that encompassed a diverse array of mineral soils (Supplementary Table 3). The field studies included: 1) soils from both forested and herbaceous-dominated plots collected from the same 11 Long-Term Ecological Research (LTER) areas across the United States but never lab incubated ("US LTER areas", Crowther et al., 2014a); 2) grassland soils collected from across the globe ("global grasslands", Leff et al., 2015); and 3) soils collected from a 3400 m elevation gradient in the Peruvian Andes ("Peru elevation gradient", Nottingham et al., 2016). The US LTER areas dataset included 64 soil samples from 18 sites (the same 11 US LTER areas, with forest and grassland samples from most LTER areas), and samples were collected once yearly from each site in 2010-2012. The 2012 soils were identical to those used for the lab experiment, except DNA was extracted prior to the lab incubations. To ensure that we identified phylotypes that would be found even more broadly than the LTER dataset, we included the other two field studies, which markedly broadened the geographic breadth of our meta-analysis. The global grasslands' dataset included 105 grassland soil samples from 19 sites on three continents, with mean annual temperatures ranging from 0.3°C to 17.2°C. The Peru elevation gradient dataset included 41 samples from 12 sites along an elevation gradient in the Peruvian Andes, with mean annual temperatures ranging from 6.5°C to 26.4°C. The global grasslands dataset was filtered to discard samples that received a nutrient treatment, and the Peru elevation dataset was filtered to only include samples collected from surface mineral soil horizons. Together, these studies represent globally diverse soils (210 field soil samples in total included in downstream analyses) that span four continents and a broad range of mean annual temperatures.

All 210 of the soil samples from these three datasets were analyzed using the exact same 16S rRNA gene sequencing approach and bioinformatics pipeline described above. Sequencing depth was normalized to 4,000 sequences per sample for US LTER areas, 100,000 for global

grasslands, and 4,000 for Peru elevation gradient. The resulting datasets were filtered to include only phylotypes that were also found in the lab study and represented at least 0.05% of the sequence reads per individual soil. These criteria were used to restrict our analyses only to those phylotypes that were reasonably abundant in any one sample and shared across broad geographic gradients. By setting these abundance and ubiquity criteria our objective was to capture phylotypes that would be useful as bioindicators, because taxa that are rare and/or restricted to only a few soil types would not be broadly useful as bioindicators. As in the lab incubation, phylotypes were discarded if found in fewer than at least 25% of the sites in each field dataset (at least 6 of 18 sites for US LTER samples, 6 of 19 for global grasslands, and 3 of 12 for Peru). Phylotype tables with corresponding metadata and representative sequences for bioindicator taxa are available via Figshare (doi: https://dx.doi.org/10.6084/m9.figshare.3468734.v4).

Statistical analyses comparing results of lab versus field studies

Our objective in comparing the lab incubation results to the field data were to assess if each of the temperature-responsive phylotypes identified from the lab study – and which met the minimum abundance and occurrence data in field soils to be included in downstream analyses – also responded across field climatic gradients in a direction that was predicted by our lab incubation data. In other words, if a phylotype increased (or decreased) in abundance in the lab incubation, we determined whether it also increased (or decreased) in relative abundance across field sites that vary with respect to their mean annual temperatures (MATs). To do this, we first correlated MAT and mean relative abundance of each phylotype across soils for every field dataset, using Spearman's rank correlation (example data from three OTUs represented in Fig. 1b-d with loess smoothing). Spearman's rank correlation is a non-parametric method to describe the strength of the relationship between two variables (e.g. to what extent relative abundance and MAT covary for each phylotype). Spearman correlation coefficient (ρ) values indicate the strength and direction of the association.

Our next goal was to test if our candidate lab responsive phylotypes predicted temperature response to the same corresponding phylotypes in the field better than we would expect if the field responses to temperature were random (not associated with response in lab). To do this we asked whether the number of taxa that had the same directional response, versus the number of

taxa that had differing responses, was greater than we would expect by random chance for each field dataset compared to the lab. Note, we only considered direction and not magnitude of response here. For each field dataset, we assigned each phylotype to one of four possible responses: increase in both (+ lab, + field), decrease in both (- lab, - field), or different responses where the possibilities were (+ lab, - field) or (- lab, + field). To compare observed differences in the number of taxa in each of the four categories versus expected differences under the null model of random response to temperature (e.g. no temperature effect), we ran Pearson's chi-square tests (χ^2) with Yates' continuity correction (Supplementary Table 7), with α set to 0.05 for a significance cutoff.

After confirming a significant directional response to temperature, we assessed the overall association of the relationship between lab predictions and field observations of temperature response. We used Spearman's rank correlations (R 'stats' Version 3.2) again, this time to assess the magnitude and direction of the relationship between lab responses (measured as average % change in relative abundance) and field responses (ρ values). The null hypothesis was that there would be no association ($\rho = 0$) between lab and field responses of phylotypes for each of three datasets.

Finally, we identified which phylotypes were consistently responding to temperature and termed those phylotypes 'bioindicator taxa', with these bioindicators classified as either preferring warmer or colder soil temperatures. To do this, we filtered the list of phylotypes further, to only include those phylotypes that responded the same way in all datasets (i.e. the three field datasets and lab study), as our objective was to identify temperature-responsive taxa that are found in a wide range of soil types. We note that the magnitude of temperature response was not necessarily the same (e.g. a phylotype would still be considered consistently 'warm-responsive' if it increased in relative abundance with increased temperatures in the lab and across field sites even if the magnitude of this increase was variable).

We investigated the percent of bacterial sequences recovered from each soil in the field datasets that were classified as bioindicators to determine what percentage of the bacterial community in these soils were represented by the taxa identified as being temperature-responsive. To test how

the proportion of warm-responsive relative to cold-responsive taxa shifted with MAT, we fit linear regressions to the percentage composition of soils across the MAT gradient for each field dataset for both warm and cold responsive taxa.

Phylogenetic analyses

To test for phylogenetic signal in temperature responses within each of the four major phyla containing warm and cold responsive taxa (Acidobacteria, Planctomycetes, Proteobacteria, and Verrucomicrobia), representative sequences from each of the phylotypes were aligned using the SILVA Incremental Aligner (SINA, v1.2.11) along with full length 16S rRNA gene sequences of nearest neighbors identified from the SILVA database. First, sequences were aligned to pre-aligned sequences from the SILVA core set aligned database (Pruesse et al., 2007) with climate sensitive archaea as the outgroup taxa for this alignment. Next, a maximum likelihood tree was built using RAxML version 7.3.9 (Stamatakis et al., 2006) with a GTRGAMMA model with 100 bootstrapped replicates. Lastly, we estimated the phylogenetic depth of temperature responses for major phyla using Blomberg's K, calculated with the R package 'Picante', version 1.6.2 with the function "multiPhylosignal". The K values indicate how well the species trait is correlated to the phylogeny as expected with a Brownian motion based metric of the strength of phylogenetic signal (Blomberg et al., 2003), where higher K values indicate better correlation. To test for significant phylogenetic signal, p values for each K value were calculated based on the variance of the phylogenetically independent contrasts relative to a null model that reshuffles trait values randomly 999 times.

Results and Discussion

Effect of lab incubation temperature on soil bacterial and archaeal communities

Microbial diversity and community composition were highly variable across the soils used for the lab experiment that were collected from 18 forest and grassland sites across North America (R^2 = 0.78; P < 0.001). Abundant phyla (mean relative abundance across samples > 5%) included *Proteobacteria*, *Acidobacteria*, *Verrucomicrobia*, *Actinobacteria*, *Bacteroidetes*, *Planctomycetes*, and *Chloroflexi*, and their relative abundances differed substantially across sites (Supplementary Table 4). For example, *Crenarchaeota* varied in relative abundance by as much as 33-fold across samples. Likewise, the relative abundances of two bacterial phyla, *Chloroflexi*

and *Verrucomicrobia,* varied by up to 7-fold across sites. Despite this considerable variability in overall prokaryotic community composition, we were still able to identify a subset of taxa that responded in a consistent manner to temperature conditions. Incubation temperature explained 3.9% of the variation of microbial community composition for those soils incubated at the two different temperatures in the lab (R^2 = 0.039; P < 0.001; Supplementary Table 5), with the same taxa generally responsible for the community changes observed between the incubation temperatures across diverse soils (Supplementary Table 6).

We identified a total of 1,639 unique phylotypes that responded to lab incubation temperature (response measured by percentage change in relative abundance between the low and high temperature treatments). Of the 1,639 phylotypes, 1,046 decreased in relative abundance with elevated temperature and 593 increased. A total of 16,494 unique phylotypes were not included in downstream analyses as they were found at less than six sites or at less than 0.05% abundance in individual soils.

We recognize that those phylotypes which changed significantly in abundance with lab incubation temperature are not necessarily responding just to changes in incubation temperature, as other soil variables also change when soils are incubated for extended periods of time under different temperature regimes. We began this study with controlled lab incubations because our intent was to minimize such changes in other factors by, for example, holding moisture levels constant. However, other factors beside temperature will change with differences in incubation temperature. For example, we would expect that microbial respiration rates are initially elevated at higher temperatures in lab incubations (Kirschbaum, 2004; Streit et al., 2014), and thus pools of available carbon would be expected to decline more rapidly in soils incubated at higher temperature. Thus, the availability of labile carbon was likely lower in the soils incubated at 28°C relative to those held at 12°C. As such, taxa that changed appreciably in relative abundance between the two temperatures are responding ultimately to changes in soil temperature, but their direct response may be to temperature and/or another proximal cause. However, we stress that the phylotypes identified through their temporal responses in the lab incubations were only a candidate set of temperature-responsive taxa. Thus, the next step in our approach was to pare

down this candidate subset to identify taxa most likely responding directly to temperature, as opposed to other soil factors.

Identification of temperature-responsive taxa across global soils

We compared our lab results to abundance patterns in field-collected soils representing multiple independent climatic gradients, imposed by latitude and/or elevation. Responses to temperature in the lab and across the three field gradients were used to identify 'warm-responsive' or 'cold-responsive' bioindicator taxa across a broad range of soils collected from across the globe. We first restricted the analyses to taxa that were shared between the field samples and the lab incubation samples, and then correlated the relative abundances of taxa across mean annual temperatures (MATs) to assess temperature sensitivity. There were 1,219 phylotypes in the US LTER areas data that overlapped with the incubation study, 1,499 phylotypes in the global grasslands data, and 778 phylotypes in the Peru elevation study.

We found that our candidate temperature-responsive taxa identified from the lab incubation were able to predict the direction of response (e.g. increase or decrease with temperature) better than expected under a null model of random response for each comparative field dataset (Pearson's χ^2 with Yate's correction for continuity, Supplementary Table 7; $p < 0.0001$ for global grasslands and Peru Elevation gradient and $p = 0.042$ for US LTER areas). Further, temperature responses observed in the lab (from 12°C to 28°C) and in the field (Fig. 2a) were positively correlated, as expected if field responses were predictable from the observed lab responses to changes in temperature. The identified phylotypes from the three field studies were positively correlated with the lab study phylotypes (global grasslands data, $\rho = 0.17$ and $p < 0.0001$, for Peru elevation gradient $\rho = 0.31$ and $p<0.0001$, and for US LTER areas, $\rho = 0.11$ and $p < 0.0001$). Together these results demonstrate that there are a core group of microbial taxa that consistently respond to temperature effects, and that these responses are detectable in both lab and field studies.

We found 659 phylotypes that overlapped across all studies that met the criteria of having relative abundance of at least 0.05% per soil and being present in at least 25% of sites (Fig. 2) for each field dataset and the lab incubation. Of these, 189 phylotypes consistently responded to temperature in the lab and in all field datasets (Fig. 2b, see Supplementary Table 2 for a

399 complete list), and we term these phylotypes 'bioindicator' taxa. Again, we note that these taxa
400 were selected based on consistency in direction of response, however the magnitude of response
401 for taxa may differ across the lab incubation and field studies. Of the 189 temperature-
402 responsive phylotypes, 51 preferred warm temperatures, increasing in relative abundance with
403 elevated temperature and 138 phylotypes preferred cooler temperatures, decreasing in relative
404 abundance with elevated temperature. These temperature-responsive phylotypes span a broad
405 range of soil phyla, including *Acidobacteria*, *Proteobacteria*, *Planctomycetes*, *Bacteroidetes*,
406 *Verrucomicrobia*, *Actinobacteria*, *Armatimonadetes*, *Chloroflexi*, *Elusimicrobia*,
407 *Gemmatimonadetes*, *Chlamydiae*, *AD3*, *Crenarchaeota*, *Cyanobacteria*, *Firmicutes*, and *WPS-2*.
408 Within *Acidobacteria*, 15 temperature-responsive phylotypes are within the family
409 *Koribacteraceae* (13 of which decrease with elevated temperatures). The other dominant families
410 are *Acidobacteriaceae*, which contain seven temperature-responsive taxa (six being cold-
411 responsive) and *Soilbacteres*, which contain six temperature-responsive phylotypes (five cold-
412 responsive). Unfortunately, we know relatively little about the ecology or metabolism of either
413 *Acidobacteria* family in part because most soil *Acidobacteria* have not been cultured or
414 described, despite their ubiquity in soils (Lee et al., 2007). However recent genomic and
415 metagenomic analyses have suggested members of *Acidobacteria* are likely to play a critical role
416 in rhizosphere carbon dynamics (Lee et al., 2007; Ward et al., 2009). *Proteobacteria* also
417 contained many temperature-responsive phylotypes (Fig. 4). While *Betaproteobacteria*,
418 *Deltaproteobacteria*, and *Gammaproteobacteria* contained mostly cold-responsive phylotypes,
419 *Alphaproteobacteria* were comprised of more warm-responsive phylotypes (12 of 22), including
420 those within the *Rhizobiales* group.
421
422 Some of the taxa identified as temperature-responsive were consistent with those taxa described
423 in previous studies as being sensitive to elevated temperature. For example, DeAngelis et al.
424 (2015) identified microbial indicator taxa from a 20-year warming study at Harvard Forest and
425 also found that those taxa which responded to elevated temperature were not necessarily
426 predictable from broader taxonomic identity. While our comparisons of which taxa respond are
427 qualitative, some similar taxonomic responses to temperature were observed. Within the phyla
428 *Proteobacteria*, we similarly identified that *Rhizobiales* taxa increased with elevated
429 temperatures and *Rhodospirilales* decreased. DeAngelis et al. (2015) cautioned that those taxa

that changed in abundance in response to elevated temperature may be responding to other soil factors that also change with temperature (e.g. soil moisture). Our combined lab (where moisture was held constant) and field approach to identify consistent controlled temporal and observational spatial responses to temperature, allow us to suggest that these taxa are in fact most likely responding directly to the soil warming.

Our list of temperature sensitive phylotypes is relatively short, in part reflecting the fact that while many rare taxa are also likely to be temperature sensitive, they were not the focus of our study. Our analyses also do not capture those taxa that have a site-specific temperature response as we restricted our analyses to those phylotypes exhibiting consistent temperature responses across soil types. However, the 189 warm- and cold-responsive taxa we identified could be used in future studies as taxa indicative of soil warming across a wide range of soil types. For example, these 'indicator taxa' may be useful to researchers that want to disentangle the multiple, correlated effects of changing climate on soil conditions to know which taxa are likely responding directly to temperature, versus indirectly responding to changes in soil moisture, soil N availability, soil carbon availability, or vegetation, that often accompany changes in soil temperature regimes. Moreover, with recent studies using microbial bioindicator species to inform reconstructions of paleo-environmental conditions (Schirrmeister et al., 2002; Zhang et al., 2008), it may also be possible to use temperature-responsive microbial bioindicators to reconstruct historical changes in soil temperature from microbial DNA preserved in soil or sediments. Specifically, we found that sites did vary in their abundances of cold to warm-responsive taxa, and as expected, these differences were correlated with the MAT of a site, with warmer field sites having higher relative abundances of warm-responsive taxa (Fig. 3) and lower relative abundances of cold-responsive taxa (and vice-versa). We found significant correlations between the percentage composition of warm- or cold-responsive taxa and field site temperature (Fig. 3, right panel; for global grasslands, R^2= 0.29, for Peru elevation gradient R^2= 0.92, and for US LTER areas R^2= 0.44 and for all, $p < 0.001$), emphasizing the utility of the bioindicators we identified for inferring soil temperature conditions.

While the list of temperature-responsive phylotypes is short, these 189 taxa collectively represent a large fraction of total soil bacterial communities (Fig. 3). Of the global field datasets we used

in this study, warm- and cold-responsive taxa make up on average 22% of the total community (sequence reads) across samples from the Peru elevation gradient, 20% from the US LTER areas, and 12% from global grasslands. Thus, although these taxa represent a small fraction of the taxonomic richness (18,133 total taxa detected across the lab dataset), they are sufficiently abundant to render them useful as bioindicators and to suggest that they would routinely be identified in soils from other sites or experimental manipulations. Perhaps most importantly, the huge microbial diversity in soils makes it challenging to link organismal, community and ecosystem responses to understand how global changes will affect the structure and functioning of these communities. These bioindicator taxa we have identified could serve as 'model' organisms – either in culture-dependent studies and/or *in situ* targeted -omic studies – to understand the ecological and biogeochemical consequences of soil microbial temperature responses. For example, shifts in cold- and warm-responsive taxa may be driven by trade-offs between tolerance of cooler temperatures and competitive dominance at warmer temperatures (Crowther et al. 2014b). Further, an organism-specific focus could help to discern whether and to what extent microbial physiology (e.g. growth efficiencies) respond directly to temperature, given the potential sensitivity of soil carbon stocks to such variables (e.g. Allison et al. 2010, Hagerty et al. 2014). That is, by knowing which of the thousands of taxa within any one soil to focus on, we can begin to understand the mechanisms underpinning how warming affects the structure and function of soil microbial communities.

Taxonomy and phylogeny are poor predictors of temperature response

Taxonomy generally did not correlate with temperature response across bacterial and archaeal phyla. Most major phyla including *Proteobacteria*, *Acidobacteria*, *Planctomycetes*, and *Verrucomicrobia* contained both cold- and warm-responsive phylotypes, suggesting that phylum identity alone is not useful for predicting which specific taxon will likely be responsive to temperature change (Fig. 4). However, there were a few interesting exceptions. All *Chloroflexi* and most *Actinobacteria* identified as being temperature sensitive were warm-responsive. This finding is consistent with our lab observations, as we observed an 82% increase in the relative abundance of *Actinobacteria* with elevated temperatures ($p<0.01$, Supplementary Table 6) and a 44% increase in *Chloroflexi* (although $p = 0.22$) on average, across sites. Within the *Bacteroidetes* phylum, we only recovered temperature-sensitive phylotypes that were cold

responsive, and the relative abundances of this phylum as a whole also consistently decreased in the lab incubation with elevated temperature (-44%, $p<0.05$). We want to emphasize, however, that although many members of these highlighted phyla were identified as being sensitive to temperature, there are many lineages within these phyla that either did not consistently respond to temperature or were not sufficiently abundant or ubiquitous to be included in our analyses. As such, phylum-level identity alone was not generally a useful predictor of the temperature sensitivity of individual phylotypes.

We next investigated the strength of the relationship between phylogeny and temperature response to see if temperature responses could be predicted at finer levels of phylogenetic resolution. We found that the phylogenetic depth of temperature response varies, but is not deeply conserved for most bacterial phyla. For major phyla where both cold and warm-responsive taxa were identified, we assessed the level of trait conservatism for temperature response and found only one of four phyla to have a significant phylogenetic signal. *Acidobacteria* had the strongest phylogenetic signal ($K = 0.23$ and $p = 0.001$; Fig. 5), however a $K<1$ indicates that within *Acidobacteria* closely related species were actually less similar for the given trait than expected under the Brownian motion model of trait evolution. For all other taxa including *Proteobacteria* ($K = 0.15$, $p = 0.15$), *Verrucomicrobia* ($K = 0.02$, $p = 0.08$), and *Planctomycetes* ($K = 0.02$), there was no significant phylogenetic signal at the set cutoff level of 0.05. This adds another line of evidence suggesting that merely knowing what taxonomic group or phylogenetic lineage a phylotype belongs to will not be very useful for predicting its temperature response in either field or lab studies.

If phylogenetic information alone is not a particularly useful predictor of temperature responses, then we must shift our approach to understanding and predicting microbial community responses to elevated soil temperatures. Indeed, most studies investigating climate change effects on soil microbial communities (e.g. Castro et al., 2010, Deslippe et al., 2012) only discuss shifts in the abundances of microbial taxa at broad taxonomic levels (typically phylum or class levels). Yet, our data suggest that interpreting changes in relative abundance with elevated temperatures at broad taxonomic levels likely obscures our ability to describe and understand the temperature responses of soil microbial communities.

523

524 ***Caveats and next steps***

525 The 189 microbial bioindicators identified in this study are not meant to be representative of all
526 temperature sensitive taxa. Indeed, while these temperature sensitive taxa were reasonably
527 abundant in most soil samples, we did not specifically examine those phylotypes that may have
528 appeared to respond to temperature at an individual site or field gradient (Fig. 2). On average, at
529 least half of the phylotypes in the top 20 temperature sensitive taxa for a given field study were
530 not found across all datasets. This was not surprising as relatively few microbial taxa are
531 ubiquitous across all soil types (Ramirez et al., 2014). Another challenge is that some of the
532 bioindicator taxa we identified as being responsive to temperature are not taxonomically well-
533 resolved (e.g. no taxonomic assignment at the genus or species level; Supplementary Table 2).
534 This is a common challenge in studies of soil microbes as many taxa are not well characterized
535 (Ramirez et al., 2014) and thus the specific identities and ecological attributes of many soil
536 microbes remain unknown.

537

538 A clear next step is to determine what gene categories or genomic attributes could be used to
539 predict the temperature responses of individual taxa or lineages. If such genes could be
540 identified, it would be possible to predict the temperature sensitivities of individual taxa by
541 analyzing the ever-expanding database of bacterial and archaeal genomes that are publicly
542 available. To test the feasibility of doing so, we compared the 189 temperature sensitive 16S
543 rRNA sequences against the current version of the largest publicly-available microbial genome
544 database, Integrated Microbial Genomes (IMG; Markowitz et al., 2012), using the basic local
545 alignment search tool (BLAST). However, only 25 of the 189 matched known genomes with ≥
546 97% similarity. Likewise, only 22 phylotypes had 16S rRNA gene sequences that matched those
547 of cultivated strains deposited in the Ribosomal Database Project (RDP). Measuring the
548 temperature optima of isolated strains representative of bioindicator phylotypes would be a
549 useful next step to confirm temperature growth optima and specific responses to soil
550 temperature. However, doing so will require a substantial amount of effort, given that so few soil
551 taxa have been cultivated and isolated under laboratory conditions (Pham and Kim, 2012). Such
552 efforts are essential for building a more mechanistic understanding of soil microbial responses to
553 temperature change.

Our analyses represent one of the first attempts to evaluate whether there are consistent patterns to the temperature responses of microbes across a broad range of soil types using both laboratory and field data. There have been extensive efforts to understand how 'macrobial' taxa (e.g. trees, mammals) are likely to respond to warming. By building a similar understanding for soil microbial communities, we hope to facilitate the same kinds of efforts that are possible for macrobes in terms of understanding and projecting community and functional responses to climate change. The 189 temperature sensitive bacterial and archaeal taxa can be used as indicators of soil temperature conditions, information that can be leveraged to identify when soil communities are likely directly responding to soil warming. Likewise, these bioindicators could provide the means to determine why some soil microbes are more sensitive to temperature than others and what physiological attributes are correlated with temperature sensitivity. More generally, this work begins to establish a broader understanding of how soil microbial communities across the globe may respond to changes in temperature and the biogeochemical consequences of the resulting community shifts.

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Figure Captions

Figure 1. Examples of six temperature-sensitive phylotypes and their shifts in relative abundance with temperature in both the lab and field studies. (a) Mean log percent change in relative abundances (from 12°C to 28°C) of six phylotypes by site in the lab incubation. Confidence limits from basic nonparametric bootstrap in R (base) to highlight the 95% confidence limits for the mean (marked by black circles) without assuming normality. (b-d) Relative abundances of the same six taxa (fitted with loess smoothing in this figure to illustrate general trend) across gradients in mean annual temperature for each of three field studies (Peru elevation gradient, Global grasslands, US LTER areas). Red and blue colors indicate warm-responsive and cold-responsive taxa respectively.

Figure 2. Phylotype responses to elevated temperatures in the lab compared to the field studies. (**Top row**) Points show phylotypes that were shared between the lab-incubated samples and each field gradient. The percent change in relative abundances in the lab (from 12°C to 28°C) and field estimates (via Spearman rank correlations). Spearman correlation coefficient (ρ) values in the bottom right hand corner indicate the strength and direction of correlation (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). (**Bottom row**) Phylotypes that were shared across all lab-incubated samples and across all three field gradients (n = 689 phylotypes). Blue and red points highlight phylotypes that consistently decreased or increased in relative abundance with elevated temperature across the lab study and *all* field studies.

Figure 3. Bioindicator taxa identified as being temperature sensitive make up a substantial portion of soil communities. **(Left panels)** Percent of total community composition of warm-responsive (red) and cold-responsive taxa (blue) phylotypes for each field site, by sample. Soils are ordered from left to right by increasing MAT. The average percent composition for global

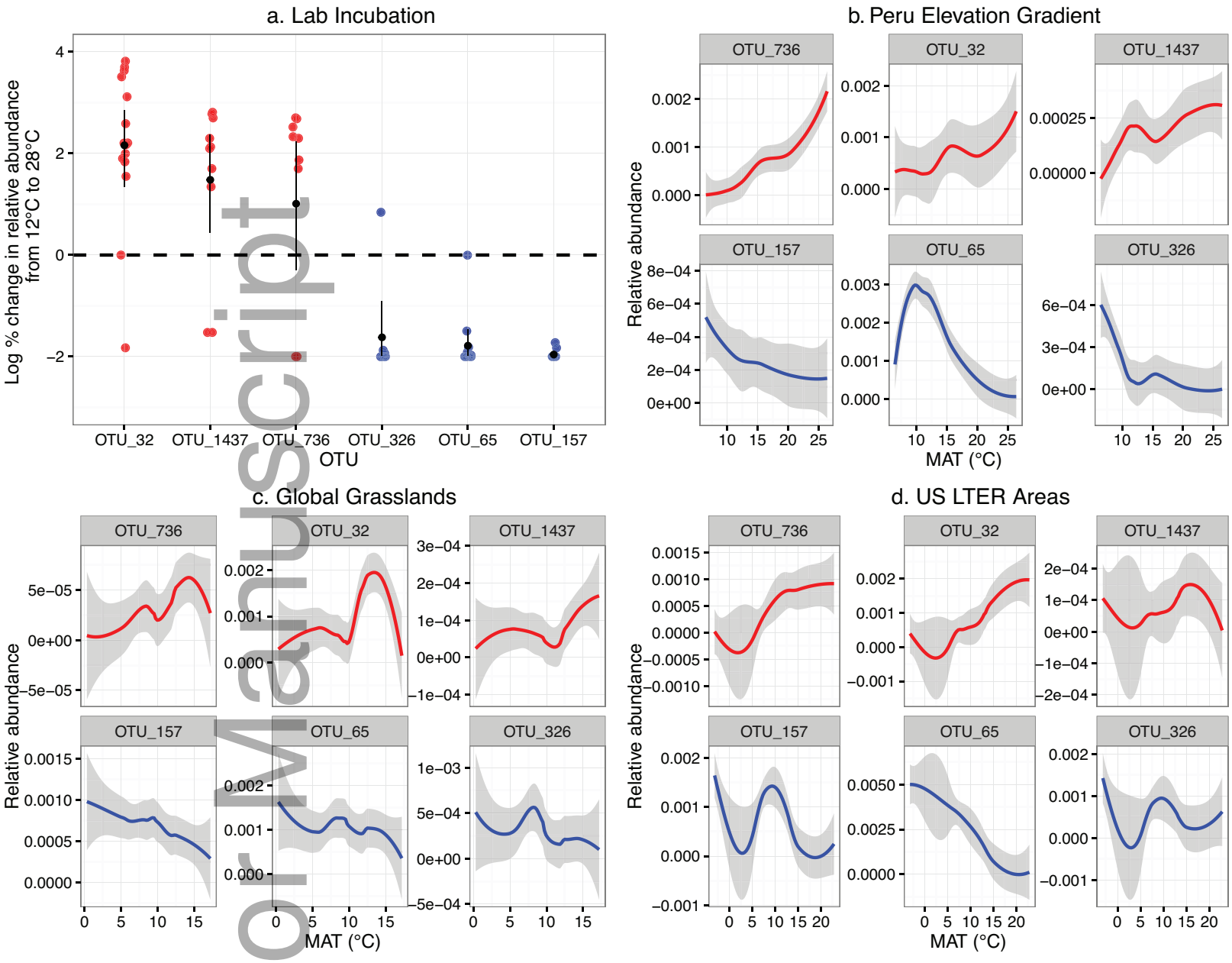
768 grasslands = 11.8%, for Peru elevation gradient = 21.9%, and for US LTER areas = 19.8%.
769 **(Right panels)** Percent community composition of sites are correlated with MAT. (*) denotes
770 significant correlation between percent of community composition made up of cold- responsive
771 versus warm- responsive phylotypes and R^2 indicates the coefficient of determination (fitted with
772 linear regressions). The gray shaded regions represent ± 10% confidence intervals.

773

774 **Figure 4.** Phylotypes identified as being temperature sensitive binned by phyla for both warm-
775 responsive (red) and cold- responsive taxa (blue).

776

777 **Figure 5.** Phylogenetic distribution of temperature-sensitive bacterial phylotypes across the four
778 most abundant bacterial phyla. Blue clade markers signify cold-responsive taxa and red signify
779 warm- responsive taxa. Trees were built using RAxML. For a small portion of phylotypes,
780 taxonomic assignment did not match tree topology; no manual corrections were made.



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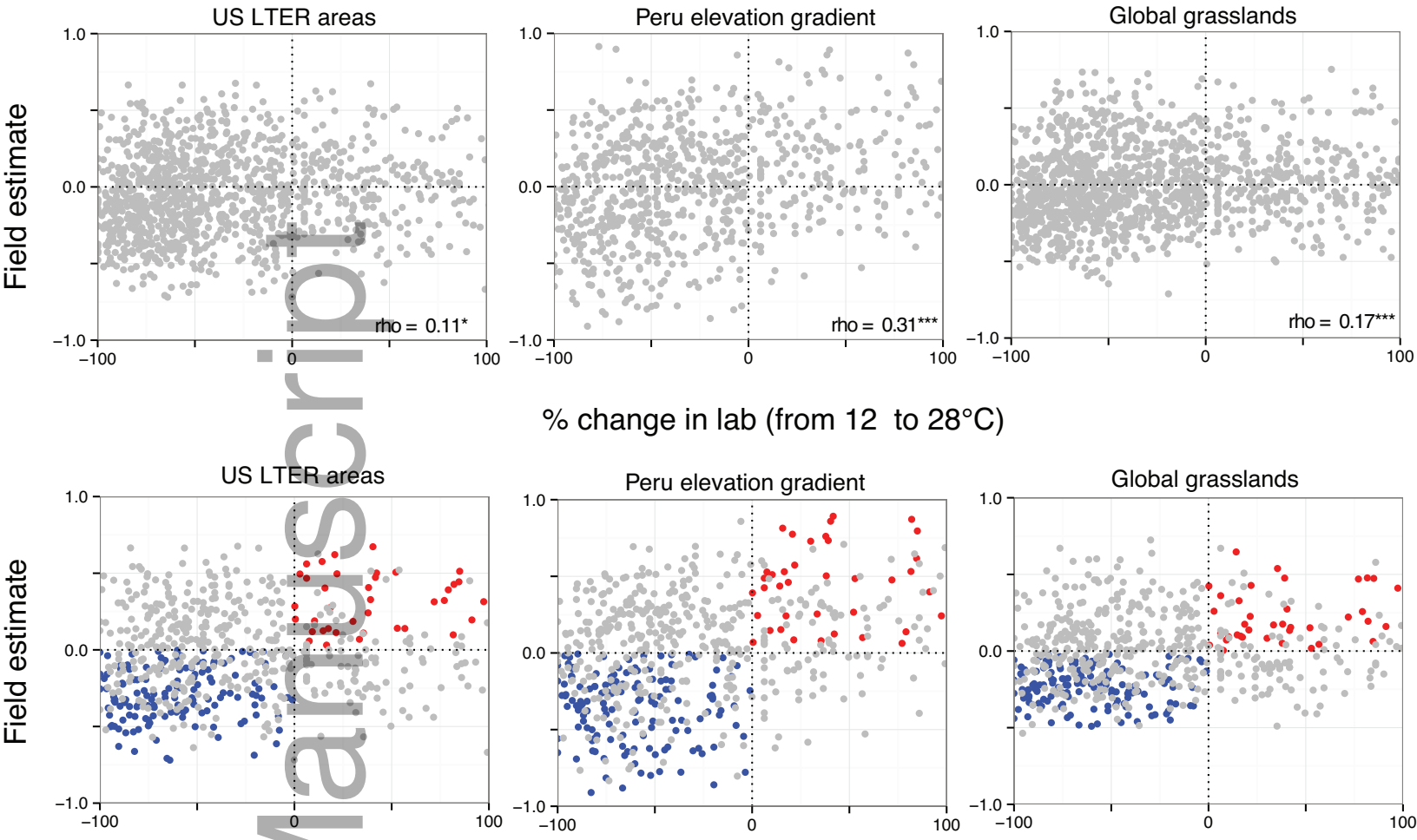

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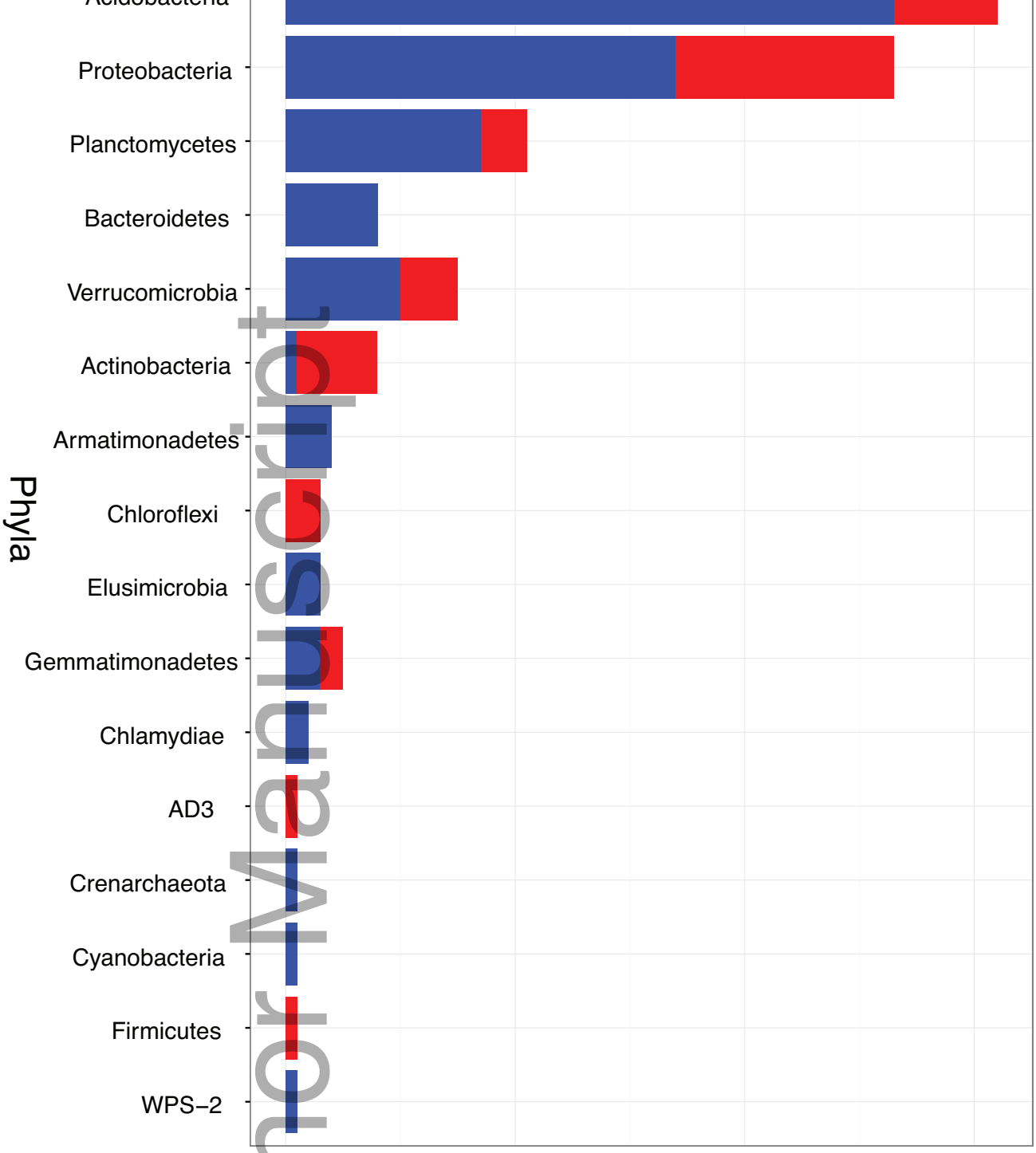

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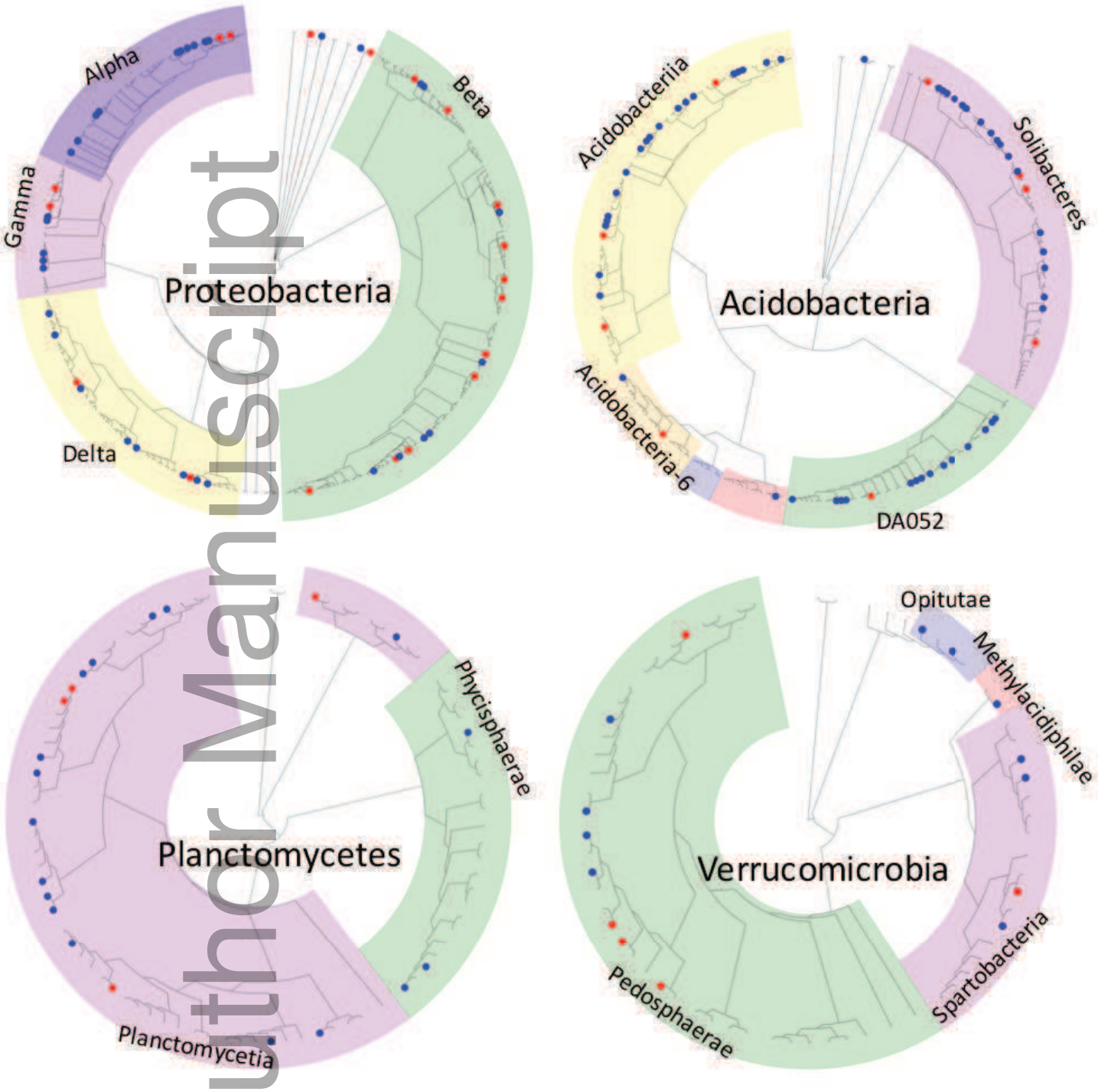

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