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# Contrasting patterns and drivers of soil bacterial and fungal diversity across a mountain gradient

**Running title:** microbial elevational diversity patterns

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**Originality-Significance Statement**

This paper is the first study in examining soil microbial diversity and community compositions on Mt. Kilimanjaro, which is a substantial contribution by filling the knowledge gap regarding this rarely/hardly touched, but important region. Our results showed the contrasting patterns and drivers of soil bacterial and fungal diversity across a broad elevation gradient of a range of 3400 m on Mt. Kilimanjaro. The diversity patterns and drivers of those diversity patterns differ among taxonomic groups (phyla/classes) within bacterial or fungal communities. Our study demonstrated that bacterial and fungal diversity and community composition responded differently to climate and edaphic properties along an extensive mountain gradient and suggest that the elevational diversity patterns across microbial groups are determined by distinct environmental variables. These novel findings will add important knowledge for regional-scale species distributions, as well as microbial responses to climate change in montane ecosystems.

**Summary**

Microbial elevational diversity patterns have been extensively studied, but their shaping mechanisms remain to be explored. Here, we examined soil bacterial and fungal diversity and community compositions across a 3.4 km elevational gradient (consists of 5 elevations) on Mt. Kilimanjaro located in East Africa. Bacteria and fungi had different diversity patterns across this extensive mountain gradient – bacterial diversity had a U shaped pattern while fungal diversity monotonically decreased. Random forest analysis revealed that pH (12.61% importance) was the most important factor affecting bacterial diversity, whereas mean annual temperature (9.84% importance) had the largest impact on fungal diversity, which was consistent with results obtained from mixed-effects model. Meanwhile, the diversity patterns and drivers of those diversity patterns differ among taxonomic groups (phyla/classes) within bacterial or fungal communities. Taken together, our study demonstrated that bacterial and fungal diversity and community composition responded differently to climate and edaphic properties along an extensive mountain gradient, and suggest that the elevational diversity patterns across microbial groups are determined by distinct environmental variables. These findings enhanced our understanding of the formation and maintenance of microbial diversity along elevation, as well as microbial responses to climate change in montane ecosystems.

## Introduction

Ecologists have been searching for generalizable patterns describing biodiversity for 100's of years and observations along elevational gradients have been key in shaping debates about how biodiversity responds to climate (Rahbek, 1995; Lomolino, 2001; Martiny *et al*., 2006). In fact, elevational species diversity patterns for plants and animals have been studied for two centuries, and related hypotheses (e.g., climate, mid-domain effect) have been proposed to explain general patterns along gradients (e.g., decreasing, unimodal) (Rahbek, 2005; McCain, 2009). In spite of their high abundance and vital roles in ecosystem functioning, until recently, microorganisms have been left out of in these analyses because their abundance and composition was difficult to observe and measure (but see, Bryant *et al*., 2008; Singh *et al*., 2012; Shen *et al*., 2014; Peay *et al*., 2017; Hendershot *et al*., 2017; Nottingham *et al*., 2018).

Microbial responses to elevational gradients remain mixed. Some studies found that soil bacteria showed decreasing diversity patterns (Bryant *et al*., 2008; Wang *et al*., 2015; Nottingham *et al*., 2018; Shen *et al*., 2019). For example, one of the earliest studies reported that the diversity of soil *Acidobacteria* monotonically decreased with the increasing elevation (Bryant *et al*., 2008). Some studies found hump-shaped or U-shaped diversity patterns for soil bacteria (Singh *et al*., 2012; Li *et al*., 2016; Peay *et al*., 2017). There were also studies showing that soil bacterial diversity did not vary with elevation (Fierer *et al*., 2011; Shen *et al*., 2013; Singh *et al*., 2014). Nonetheless, the lack of a discernable trend in microbial diversity with elevation across studies may be the result of compounding effects of multiple environmental factors or undetected

factors that are relevant to microbes, as well as the possibility that microbial diversity may not vary on a spatial scale that corresponds in any way to elevational gradients.

There are likely different elevational diversity patterns across microbial groups at multiple taxonomic levels. For example, one study on the Kohala Volcano of Hawai'i found contrasting diversity patterns with a hump-shaped diversity-elevation trend for bacteria and an increased diversity-elevation trend for fungi (Peay *et al*., 2017). While within bacterial communities, one study on Fuji Mountain found a decreasing diversity pattern for *Acidobacteria* and a hump-shaped diversity pattern for *Proteobacteria*, while actinobacterial diversity did not show any pattern with elevation (Singh *et al*., 2012). Given phyla or classes possess unique phylogenetic and ecological traits (e.g., r-strategy and k-strategy), an in-depth analysis of specific phyla or classes may help to inform hypotheses about why variance in diversity patterns emerges (Fierer *et al*., 2007).

There are two main scale-dependent categories of environmental factors that likely impact soil microbial diversity patterns along elevational gradients: (1) regional-scale factors such as climate, area, parent material, historical impacts (evolutionary constraints), plant productivity (Gaston, 2000); and (2) local-scale abiotic factors including pH and nutrients availability, and biotic interactions (e.g. mutualism and competition). Some studies suggest regional-scale climate factors (particular temperature) could strongly affect soil microbial diversity and community composition along elevational gradients (Singh *et al*., 2014; Ding *et al*., 2015; Nottingham *et al*., 2018), whereas others suggest the elevational microbial diversity

patterns are determined by local-scale variation in soil pH (Shen *et al*., 2013; Geml *et al*., 2014; Wang *et al*., 2015; Peay *et al*., 2017). However, the relative importance of these hierarchical environmental factors in shaping soil biodiversity along elevation gradient remains less evaluated.

Theories have been proposed to explain the relationship between environmental factors and species diversity. For example, the mid-domain effect, assuming that spatial boundaries cause more overlap of species' ranges towards the centre of an area, has been widely used to explain the unimodal diversity pattern with elevation (Colwell *et al*., 2004; McCain, 2004; Miyamoto *et al*., 2014). Temperature has been shown positively correlate with species richness for macroorganisms (Hawkins *et al*., 2003; Evans *et al*., 2005) and microorganisms (Furhman *et al*., 2008; Zhou *et al*., 2016) in large-scale diversity patterns. The positive temperature-diversity relationship was commonly explained by the metabolic theory of ecology, which predicts changes of organisms' diversity along temperature gradients via biochemical kinetics of metabolism (Brown *et al*., 2004). The metabolic theory predictions have been specifically applied to microorganisms in horizontal and elevational investigations (Zhou *et al*., 2016; Nottingham *et al*., 2018). Species diversity often increases with increasing available energy, which is termed species-energy theory (Wright, 1983). This theory was widely accepted for plants and animals (Hawkins *et al*., 2003; Phillips *et al*., 2010). However, its applicability to soil communities has been less explored, although there is some evidence it holds for fungal functional guilds in wood and leaf environments (Schmit *et al*., 2005; Yang *et al*., 2016).

Mt. Kilimanjaro, the highest free-standing mountain on earth, has a distinct vertical distribution of vegetations that extends from tropical to frozen zones. This extensive gradient mirrors the latitudinal vegetation gradient in the northern hemisphere and thus provides us with an excellent laboratory to study microbial distribution patterns at a regional scale (Hemp, 2006). Previous studies showed that the species richness of plants and animals significantly decreased with increasing elevation on Mt. Kilimanjaro (Hemp, 2006; Peters *et al*., 2016). Here, we measured soil bacterial and fungal communities along an elevation gradient (3.4 km elevation gradient, consists of 5 elevations) on Mt. Kilimanjaro. We used this gradient to test the following hypotheses: (1) Soil bacteria and fungi will have unique elevational diversity patterns, but different environmental drivers will be correlated with those patterns. (2) Fungal diversity will be positively related to plant productivity, as predicted by species-energy theory and recent findings which showed a significant relationship between them (Hiiesalu *et al*., 2017; Yang *et al*., 2017). (3) The composition of bacterial and fungal communities differed with elevation. Based on recent findings of global topsoil microbial studies by Bahram and colleagues (2018), bacterial community composition will be related to local soil conditions such as pH, whereas fungal community composition will be related to larger scale processes, such as climate and plant productivity.

**Results**

*Contrasting patterns with elevation: U-shaped for bacterial diversity, decreasing for*

*fungal diversity*

For richness, evenness and Shannon diversity metrics, the whole bacterial community showed a significant ($r^2 = 0.721$, $p < 0.001$) U-shaped pattern, whereas the whole fungal community significantly ($r^2 = 0.404$, $p < 0.001$) decreased with elevation (Fig. 1a, Fig. 1b). Spearman correlation analysis showed that richness and evenness of the whole bacterial and fungal community were significantly ($p < 0.01$) correlated with Shannon index, with evenness having a higher coefficient with Shannon index (Fig. S1). For bacterial specific phyla, the diversity of nine phyla exhibited significant ($p < 0.05$) elevational patterns, with a U-shaped diversity pattern for *Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, an increasing diversity pattern for *Gemmatimonadetes*, *Verrucomicrobia*, *Betaproteobacteria*, *Deltaproteobacteria*, and a hump-shaped diversity pattern for *Chloroflexi* (Fig. S3). For fungal specific phyla and classes, the diversity of three phyla and eight classes exhibited significant ($p < 0.05$) elevational patterns, with a decreasing diversity pattern for *Ascomycota*, *Archaeorhizomycetes*, *Pezizomycetes*, *Saccharomycetes*, *Tremellomycetes*, a hump-shaped diversity patterns for *Glomeromycota*, *Dothideomycetes*, *Leotiomycetes*, *Sordariomycetes*, *Agaricomycetes*, and a U-shaped diversity pattern for *Chytridiomycota* (Fig. S4).

*Contrasting drivers: pH for bacterial diversity, MAT for fungal diversity*

In terms of Shannon index, bacterial and fungal diversity were linked with environmental factors. Random forest analysis found that pH (12.61%) was the most

important factor affecting bacterial diversity, whereas MAT (9.84%) had the largest impact on fungal diversity (Fig. 1c, Fig. 1d). This result was consistent with results obtained from mixed-effects model (Table 2). According to random forest analysis, MAP (11.35%) and pH (7.98%) were the second important factor for predicting bacterial and fungal diversity, respectively. Classified by climate, energy and local factors, random forest analysis showed a more important role of local factors in explanation of bacterial diversity, whereas climate factors shape fungal diversity (Fig. 1c, Fig. 1d). For bacterial specific phyla, the diversity of 10 phyla showed significant ($p < 0.05$) relationships with pH. For fungal specific phyla and classes, the diversity of three phyla and seven classes exhibited significant ($p < 0.05$) relationships with MAT (Fig. S5).

*Effect of elevation on bacterial and fungal compositional dissimilarities*

The composition of bacterial and fungal communities differed with elevation according to PCoA plots based on Bray-Curtis distance (Fig. 2c, Fig. 2d). Permutational multivariate analysis of variance (PERMANOVA) showed that the compositional dissimilarities among elevations were significant ($p < 0.05$; Table S3). Dissimilarity of bacterial and fungal communities significantly ($p < 0.001$) and exponentially increased with increasing elevation distance (Fig. 2a, Fig. 2b). Specifically, the relative abundance of 10 bacterial phyla *Alphaproteobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Betaproteobacteria*, *Chlorobi*, *Chloroflexi*, *Cyanobacteria*, *Gammaproteobacteria*, *Elusimicrobia and Fibrobacteres* significantly

increased with increasing elevation (Fig. S6, Fig. S7, Table S5). For fungi, increased elevation was associated with increased dominance of *Archaeorhizomycetes*, *Leotiomycetes*, *Tremellomycetes*, and decreased dominance of *Agaricomycetes*, *Blastocladiomycetes*, *Eurotiomycetes*, *Geoglossomycetes*, *Microbotryomycetes*, *Orbiliomycetes*, *Pucciniomycetes*, Saccharomycetes, *Ustilaginomycetes*, *Wallemiomycetes* (Table S5). The beta diversity decomposition analyses showed that bacterial and fungal community compositional dissimilarities among all study sites were dominated by species replacement processes (contributed 80.24% and 74.17% for bacterial and fungal beta diversity, respectively), while richness difference processes only contributed 19.76% and 25.83% on average (Fig. 3a, Fig. 3b). Also, relative contribution of richness difference processes was lower (averagely 15.59% and 21.90% for bacterial and fungal beta diversity, respectively) for beta diversity among sites within elevation (Fig. 3c, Fig. 3d).

*Linkages of bacterial and fungal community composition with environmental factors*

Of all the environmental factors examined, pH showed the highest correlation with bacterial community composition ($\rho = 0.759$, $p = 0.001$), whereas MAT was most significantly correlated with fungal community composition ($\rho = 0.582$, $p = 0.001$) as determined by partial Mantel tests (Table S4). For bacterial community composition, five significant variables – pH, MAT, TC, MAP and C/N explained 88.02% of the total variation ($p < 0.05$), with pH providing the greatest explanatory power (42.98% of the total variation). Most of the variation, 65.75%, in fungal

community composition was explained by MAT, MAP, TN, and TC ($p < 0.05$) and MAT explained the largest percentage, 24.25% of the total variation (Table 3). For specific phyla and classes, the relative abundance of 11 bacterial phyla showed significant ($p < 0.05$) correlations with pH, and the relative abundance of 13 fungal classes were significantly ($p < 0.05$) correlated with MAT (Fig. 5a, Fig. 5b, Table S5). Variation partitioning analysis showed that the joint effects of climate and local factors accounted for the largest explanation for variance of both bacterial and fungal community composition, whereas the pure effects of climate, energy and local were small (Fig. 4c, Fig. 4d).

*Predicted functional groups of bacteria and fungi*

Across all the sampling sites, dominant bacterial functional groups included groups that were involved into chemoheterotrophy, cellulolysis, nitrogen fixation and nitrification (Fig. S8). Dominant fungal functional groups included groups that were involved into wood saprotroph, soil saprotroph, dung saprotroph, plant pathogen, ectomycorrhizal and arbuscular mycorrhizal (Fig. S9). The effects of elevation on the diversity of eight fungal functional groups were tested, with a decreasing diversity pattern for animal pathogens, fungal parasites, and a hump-shaped pattern for arbuscular mycorrhiza, endophytes, saprotrophs being found, while others including ectomycorrhizal, lichenized and plant pathogen showed no apparent diversity pattern with elevation (Fig. S10). The relative abundance of 21 predicted bacterial functional groups and six predicted fungal functional groups showed significant ($p < 0.05$)

relationships with elevation (Fig. S11, Fig. S12). The relative abundance of 15 bacterial functional groups and four fungal functional groups showed significant ($p < 0.05$) correlations with MAT, and the relative abundance of 12 bacterial functional groups and five fungal functional groups were significantly ($p < 0.05$) correlated with pH (Fig. 5c).

**Discussion**

We found that soil bacterial and fungal communities had contrasting diversity patterns along an extensive elevational gradient on Mt. Kilimanjaro. Bacterial diversity patterns were U-shaped, while fungal diversity decreased monotonically with elevation. These results contrast with previous studies, that occurred along shorter gradients or homogenous environmental factors, exploring bacterial and fungal diversity along mountain gradients that found consistent decreasing (Nottingham *et al*., 2018) or no detectable (Shen *et al*., 2014) patterns. Our results were identical with one study on the Kohala Volcano of Hawai'i that found contrasting elevational diversity patterns with a hump-shaped trend for bacteria and an increasing trend for fungi (Peay *et al*., 2017). A recent global-scale study of soil microbes observed contrasting patterns across the latitudinal gradient – fungal diversity decreased with latitude, but bacterial diversity exhibit a hump-shaped pattern with latitude (Bahram *et al*., 2018). Thus, our results, together with the two previous studies, highlight the disparities between bacterial and fungal community diversity patterns along gradients. In fact, when we drill down and explore a finer resolution of

taxonomic diversity (phyla and classes) within bacterial and fungal communities, the patterns of diversity become more complex. For example, much like the entire bacterial community, the diversity of *Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria* and *Gammaproteobacteria* followed a U-shaped elevational pattern, whereas *Gemmatimonadetes*, *Verrucomicrobia*, *Betaproteobacteria* and *Deltaproteobacteria* diversity increased with elevation. Likewise, the diversity of *Archaeorhizomycetes*, *Pezizomycetes*, *Saccharomycetes* and *Tremellomycetes* followed the fungal community trend of decreasing with elevation, however, the diversity of *Dothideomycetes*, *Leotiomycetes*, *Sordariomycetes* and *Agaricomycetes* exhibited a hump-shaped pattern. Undoubtedly, the diversity patterns of dominant (with higher relative abundance) phyla and classes contributed significantly to the overall community pattern – highlighting that generalizing the response of bacterial and fungal community diversity patterns as a whole may hide important responses within less common, but still functionally important taxa (Yeh *et al*., 2019). Nonetheless, these observed microbial diversity patterns indicate niche differentiation (environmental conditions differentiation) among taxa along gradients (Prosser *et al*., 2007; Fierer *et al*., 2007).

Our data indicate that, in general, bacterial and fungal community diversity patterns are influenced by different ecological drivers and the role of local and regional drivers may also differ between these two diverse groups. We found that local factors, such as soil pH, predicted bacterial elevational diversity patterns, not only for the whole community, and many of the phyla as well. Our field sites had a

wide range of soil pH, from 3.82-7.80, resulting in a strong correlation between bacterial diversity and soil pH – a pattern observed in other elevational studies (Shen *et al*., 2013; Wang *et al*., 2015; Shen *et al*., 2019). Additionally, along our gradients of sites, soil pH was highly correlated with the diversity of four dominant bacterial phyla including *Alphaproteobacteria*, *Acidobacteria*, *Actinobacteria* and *Gammaproteobacteria* (Table S5). Identically, pH strongly predicted bacterial diversity across latitudinal gradient (Fierer and Jackson, 2006; Chu *et al*., 2010; Karimi *et al*., 2018). Despite the importance of pH, it should be noted that other factors like MAP, TC, TN, NDVI and C/N, that were significantly correlated with pH, contributed to the variation of bacterial diversity (Fig. S1, Fig. 1c). The results of random forest analysis revealed that MAP (11.35% importance) were the second important factor for predicting bacterial diversity. We infer that precipitation might indirectly affect bacterial diversity by mediating other environmental factors such as pH and NDVI (Angel *et al*., 2010; Tian *et al*., 2018).

While local-scale variation in soil properties, pH, predicted bacterial diversity, larger-scale patterns in climate, temperature (MAT), predicted fungal diversity. This result was supported by both spearman correlation analysis (Table S1) and mixed-effect models, the latter of which found that MAT was a unique key factor compared with other explanatory variables (Table 2). MAT was significantly correlated with the diversity of specific fungal phyla and classes, such as *Ascomycota*, *Archaeorhizomycetes*, *Pezizomycetes*, *Leotiomycetes*, *Saccharomycetes* and *Tremellomycetes*; yet, not all phyla/class diversity were best predicted by MAT. Some

phyla and class diversity, such as *Glomeromycota*, *Chytridiomycota*, *Dothideomycetes*, *Sordariomycetes* and *Agaricomycetes* were correlated with soil pH (Table S2). Our results showed that pH (7.98% importance) was the second contributor for predicting fungal diversity (Fig. 1d). These results are consistent with a recent global study by Bahram *et al*. (2018) that found that MAT was the best predictor for the richness of the entire fungal community as well as the major ascomycete classes; however, the richness of *Glomeromycota* and *Chytridiomycota* was better predicted by soil pH. Fungal species typically have a wider pH optimal growth than bacterial taxa, as revealed by pure culture and gene sequencing studies (Nevarez *et al*., 2009; Rousk *et al*., 2010) – thus, it is not surprising that their diversity patterns are less responsive than bacteria to local-scale shifts in pH. Collectively, our study emphasizes the pivotal role of temperature in driving fungal elevational diversity patterns.

Fungi play important ecological roles as decomposers, mutualists and pathogens of plants, thus we hypothesized that a significant plant productivity-fungal diversity relationship would exist along the elevational gradients we measured. This hypothesis emerged from two complimentary theories developed to understand diversity patterns across landscapes: 1) the species-energy theory that predicts that species richness increases with increasing available energy, such as resource production (Wright, 1983), and 2) the productivity-diversity hypothesis that predicts that the availability of growth-limiting resources in a location limits the diversity of biotic communities at that location (Tilman, 1982; Waldrop *et al*., 2006). A meta-analysis found that plant productivity contributes to species richness patterns across taxa at the regional scale

(Gillman and Wright, 2006). Yet, we unexpectedly found no significant relationship between plant productivity (NDVI) and the diversity of the entire fungal community using both spearman correlation analysis and mixed-effect models (Table 2, Table S1). While surprising, previous studies on fungi conducted at the regional or global scale found that fungal diversity was not related to plant productivity across latitudes (Tedersoo *et al*., 2014; Yang *et al*., 2017). Plant productivity is mainly driven by precipitation at Mt. Kilimanjaro, as revealed by previous studies (Ensslin *et al*., 2015; Peters *et al*., 2016). Our spearman correlation analyses showed that NDVI was highly ($\rho = 0.9$, $P < 0.5$) correlated with MAP, but not significant ($P > 0.5$) with MAT (Fig. S1). In this study, the influence of other factors, such as temperature (this study and Tedersoo et al., 2014) and plant diversity (Yang *et al*., 2017) appear to be larger drivers than plant productivity in predicting fungal diversity patterns at the community scale. However, when we look at specific community member guilds, plant productivity significantly and positively correlated with the diversity of *Glomeromycota*, *Dothideomycetes* and *Sordariomycetes* (Table S1). Not surprisingly, the functional guilds that closely interact with plants – arbuscular mycorrhizal fungi, endophyte fungi and saprotropic fungi – have a positive fungal diversity-plant productivity relationship (Table S3; Hiiesalu *et al*., 2017; Yang *et al*., 2017). Thus, in partial support of our hypothesis, significant productivity-diversity relationships occur for guilds that closely interact with plants, but once again, drivers of patterns at the larger-community scale and the more fine phyla/class/guild scale differ. Together, these findings underline the close interaction of host plant growth with specific fungal

groups, especially arbuscular mycorrhizal fungi, and that the drivers of diversity patterns can shift at different taxonomic scales.

The plant diversity hypothesis states that greater plant diversity increases the range of organic substrates entering soil thus creating niche space for heterotrophic fungi (Lodge, 1997; Hooper *et al*., 2000), and is an alternative hypothesis for what drives soil fungal diversity along gradients. Although plant diversity was not directly assessed in this study, previous studies on the same plots found that plant species richness significantly decreased with increasing elevation on the southern slope of Mt. Kilimanjaro, and the patterns were largely predicted by MAT (Hemp, 2006; Peters *et al*., 2016). Thus, our data together with data shown by Peters *et al*. (2016), indicated a potential coupling of plant and fungal diversity, as we found significant relationships between fungal diversity and MAT. These results also suggest that plant diversity impacts fungal diversity to a greater degree than bacterial diversity, a finding that is consistent with recent results that showed plant richness significantly correlated with soil fungal diversity, but not with bacterial diversity, from a plant richness manipulation experiment (Dassen *et al*., 2017; Chen *et al*., 2019). Actually, the significant plant diversity-fungal diversity relationships have been observed in natural regional-scale grassland ecosystems (Yang *et al*., 2017; Chen *et al*., 2017).

Our data suggest that the combination of temperature and soil pH are the strongest predictor of microbial community composition. Similar to our diversity patterns, – pH and temperature – were the best predictors for bacterial and fungal composition, respectively (partial mantel test in Table S4, DistLM model in Table 3).

Specifically, pH was the best predictor of the relative abundance of 12 bacterial phyla and MAT was the best predictor for the relative abundance of 14 fungal classes (Fig. 5a, Fig. 5b). Variation partitioning analyses found the combined effects of climate and local factors accounted for the largest variance of both bacterial and fungal community composition, whereas the direct effects of climate, energy and local factors contributed less to the variation (Fig. 4c, Fig. 4d). The combined effects were also important when we explored correlations between specific phyla/classes and environmental factors (Fig. 5a, Fig. 5b). For example, MAT was most correlated with the relative abundance of bacterial phyla including *Alphaproteobacteria*, *Firmicutes*, *Cyanobacteria*, *Fibrobacteres*, *Armatimonadetes*, *Chlorobi*, and pH was most correlated with the relative abundance of fungal classes including *Dothideomycetes*, *Eurotiomycetes*, *Sordariomycetes*, *Monoblepharidomycetes*, *Glomeromycetes*. Temperature could alter the composition of microbial communities through a direct effect on individuals' metabolic rates and growth (Brown *et al*., 2004; Zhou *et al*., 2016). Meanwhile, temperature may indirectly affect microbial community composition via plant attributes or soil properties (Delgado-Baquerizo *et al*., 2016; Delgado-Baquerizo *et al*., 2018; Liu *et al*., 2020). The reason that why pH predicted best for the community composition derives from two (but not limited) general explanations (Lauber *et al*., 2008; Rousk *et al*., 2010). First, pH directly imposes a physiological stress for individual's growth (Bárcenas-Moreno *et al*., 2016; Rath *et al*., 2019). Second, pH is not a direct influencing factor, but instead as an integrated functional index, because lots of soil characteristics (e.g., salinity, nutrients

availability and organic matter) are often directly or indirectly related to soil pH (Siciliano *et al*., 2014; Zeng *et al*., 2016; Rath *et al*., 2019). In addition to pH and MAT, other local and climate factors played significant roles in shaping bacterial and fungal community compositions. For example, MAP and TN were significantly correlated with some bacterial phyla (e.g. *Acidobacteria*, *Chloroflexi*) and fungal classes (e.g. *Dothideomycetes, Wallemiomycetes*). Indeed, the roles of precipitation, temperature, pH and nutrients influencing soil bacterial and fungal community composition has been reported in agricultural (Lauber *et al*., 2008, Sun *et al*., 2016), forest (Angel *et al*., 2010; Tian *et al*., 2018), tundra (Shen *et al*., 2015; Shi *et al*., 2015), grassland (Chen *et al*., 2016; Chen *et al*., 2017) and desert ecosystems (Fierer *et al*., 2012; Chu *et al*., 2016). Even at the global scale, the integrated effects of climate and local factors influenced the composition of soil bacterial and fungal communities (Tedersoo *et al*., 2014; Leff *et al*., 2015; Prober *et al*., 2015; Bahram *et al*., 2018). These results suggest that, to a large extent, climate and local factors jointly determined bacterial and fungal community compositions.

## Conclusion

In summary, we found a U-shaped diversity pattern for soil bacteria and a monotonically decreasing diversity pattern for soil fungi across elevational gradients on Mt. Kilimanjaro. The contrasting patterns resulted largely from two different processes, local and climate processes for bacteria and fungi, respectively. These results highlight the disparity of elevational diversity patterns between bacteria and

fungi, which were attributed to different environmental drivers. Further, our results suggest that generalize the response of bacterial and fungal community diversity patterns as a whole may hide important responses within less common, but still functionally important taxa. We found significant productivity-diversity relationships between plant productivity and some fungal specific taxonomic or functional groups. Finally, we found that climate and local factors together influenced bacterial and fungal community composition. These findings enhanced our understanding of the formation and maintenance of microbial diversity along elevation, as well as microbial responses to climate change in montane ecosystems.

## Experimental procedures

*Study site*

Mt. Kilimanjaro (2°45'–3°25'S; 37°00'–37°43'E), located 300 km south of the equator in Tanzania (East Africa), is the highest mountain in Africa and the highest free-standing mountain on the Earth. It rises from savanna plains at 700 m elevation to a snow-clad summit at an elevation of 5895 m a.s.l. The terrain is extremely complex with huge changes of inclinations and slopes. It is an eroded relic of an ancient volcano with three peaks (Shira, Mawenzi and Kibo) and has a diameter of 90 km from northwest to southeast. Mt. Kilimanjaro is characterized by a typical equatorial day-time climate (Hemp, 2006). The precipitation regime follows a bimodal pattern with a long rainy season from March to May and a short rainy season between October and December. The mean annual temperature (MAT), ranges from 5

to 25 °C, decreases almost linearly with elevation. The mean annual precipitation (MAP), ranges from 500 to 3000 mm, shows a unimodal pattern with a peak at ~2200 m a.s.l.

Five elevations, namely at 767, 1920, 2850, 3880 and 4190 m were selected, representing five typical vegetation types with Lowland dry broadleaf forest, lower montane forest, Podocarpus forest, Erica bush forest, and Helichrysum cushion, respectively. All selected sites were natural forest and alpine ecosystems. A detailed description of site characteristics was summarized in Table 1.

*Soil sampling and plant data collection*

We collected soil samples from the southern slope of Mt. Kilimanjaro in October 2014. At each elevation, four independent replicate plots (5 × 5 m; about 100 m apart) were selected. In each plot, five top-soil samples (0-10 cm depth directly below the litter layer) were taken randomly and composited together into a single sample. The fresh soil samples were sieved through a 2 mm sieve after roots and residues were removed. Samples were separated into two portions: one was stored at 4 °C to determine the chemical properties and the other was frozen (-20 °C) until DNA extraction.

Normalized Difference Vegetation Index (NDVI) was used as a proxy for net plant productivity. We collected NDVI data from the Moderate Resolution Imaging Spectroradiometer (MODIS) aboard NASA's Terra satellites (https://ladsweb.nascom.nasa.gov/data/search.html) that were updated once every 8

days with 250 m resolution. Specifically, the NDVI in October 2014 was chosen at the elevation level based on the coordinate of longitude and latitude.

*DNA sequencing and chemical properties*

DNA was extracted using the MoBio PowerSoil DNA isolation kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions. For bacterial community composition, 515f/806r primer sets (515f, GTGYCAGCMGCCGCGGTAA, 806r, GGACTACNVGGGTWTCTAAT) were used to amplify (triplicate reactions for each sample) the 16S rRNA gene (cited by Earth Microbiome Project). For fungal community composition, ITS1f/ITS2 primer pair (ITS1f, CTTGGTCATTTAGAGGAAGTAA, ITS2, GCTGCGTTCTTCATCGATGC) was selected to amplify the ITS1 region of the rRNA gene (cited by Earth Microbiome Project). A unique 10-base pair Golay barcode was included between the 806r/ITS2 primer and the Nextera adapter sequence. All PCR reactions were performed in 25 μL reaction systems including 13 μL of Phusion Master Mix (NewEngland Biolabs, USA), 0.5 μL each of 10 μM forward and reverse primers, 1 μL template DNA (20 ng $\mu L^{-1}$), and 10 μL $H_2O$. Thermal cycling included an initial denaturation step at 95 °C for 1 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension stage of 72 °C for 7 min. 16S rRNA amplicons and ITS amplicons were pooled separately and then sequenced with Illumina MiSeq instrument (Illumina, San Diego, CA, USA). Raw sequence data were processed using the QIIME v1.9 pipeline, where sequences were quality filtered

(script: *split_libraries.py*; parameters: min_seq_length = 200, max_ambig = 0, and min_qual_score = 25), chimera checked, OTU clustered and taxonomy assignment (Caporaso *et al*., 2012). USEARCH algorithm was utilized to conduct chimera detection and OTUs clustering (97% similarity) (Edgar, 2010). Taxonomy was identified for each OTU using the RDP classifier (Wang *et al*., 2007) trained on the Greengenes (McDonald *et al*., 2012) and UNITE (Abarenkov *et al*., 2010) databases for bacterial and fungal sequences. Samples were rarefied to 30,276 and 49,326 sequences per sample for bacteria (30,276-36,913 sequences) and fungi (49,326-58,227 sequences), respectively. Functions were predicted based on bacterial and fungal taxa using the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database (http://www.zoology.ubc.ca/louca/FAPROTAX/) and FUNGuild database (http://www.stbates.org/guilds/app.php). The rarified OTU/taxon tables were first translated into function tables, based on taxon-function annotations in the FAPROTAX and FUNGuild database. Then the relative abundance of each functional group was calculated based on the number of sequences per sample. The sequencing data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the accession number PRJNA525365.

Soil total carbon (TC) and total nitrogen (TN) content was measured using a dry combustion elemental analyzer at 950°C (Vario EL II, Hanau, Germany)( Peters *et al*., 2016). Soil pH was determined in water (soil to water ratio 1:5)( AB15 pH meter, Accumet, Fisher Scientific).

*Statistical analyses*

We calculated OTU richness, Pielou's evenness and Shannon index for the measured bacterial and fungal communities. In addition, we estimated these indices for 11 bacterial phyla and five fungal phyla and 10 fungal classes based on the same sequencing depth (Table 2). To test for the effects of elevation on diversity, the linear or quadratic model was selected based on the lower value of Akaike's information criterion (AIC). Spearman's rank correlations were used to examine the relationships between diversity and environmental variables (elevation, MAT, MAP, NDVI, pH, TC, TN and C/N). The effects of elevation and environmental factors on diversity were tested by linear mixed-effects models using the R package *lmerTest*, with elevation fitted as a random effect in every model, and using maximum likelihood to assess the significance of the fixed effects. We started with full models with all seven variables as fixed effects, and reduced these to final models containing only significant variables. To test the relative importance of environmental variables in driving bacterial and fungal diversity, we used random forest analysis using the R package *randomForest.* We performed the regression using the 'randomForest' function. The importance of variables was determined by the value of %IncMSE (increased in mean squared error) calculated by the 'importance' function. Basically, we classified the measured environmental variables to three categories: climate factors (MAT and MAP), energy factors (NDVI and TC), local factors (pH, TN and C/N).

Community compositional dissimilarities were estimated based on the Bray-Curtis distance of OTU abundance table. To examine the elevational differences

in compositional dissimilarities, principal co-ordinates analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA) were performed in R package *vegan*. An exponential model was selected to test the relationship between dissimilarity of bacterial and fungal communities with elevation difference. Compositional dissimilarities among sites (beta diversity) was partitioned into replacement and richness difference components (Podani family, Sørensen dissimilarities) using the R package *adespatial*. Partial Mantel tests were used to test the correlations between environmental variables and community composition. The percentages of explained variations in community composition by variables were tested through distance-based multivariate analysis for a linear model (DistLM) which were conducted in DISTLM_forward3 software (Anderson, 2003). Variation partitioning analyses (VPA) in R package *vegan* were performed to show the independent or joint effects of three grouping environmental factors (climate, local and energy) on explaining the variations in community composition. Additionally, we also performed VPA based on regional (MAT, MAP and NDVI) and local (pH, TC, TN and C/N) factors partition criterion. Random forest analysis was used to estimate the predictability of environmental variables on the relative abundance of specific phyla or classes.

For the predicted functional groups, a linear or quadratic model was selected to test the relationship between the diversity and the relative abundance of functional groups and elevation. Spearman's rank correlations were used to examine the relationships between the relative abundance of functional groups and environmental

variables. Random forest analysis was used to estimate the predictability of environmental variables on the relative abundance of functional groups.

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**Conflict of Interest**

The authors declare no conflict of interest.

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**Table 1:** Summary of the main characteristics of sampling sites. MAT, mean annual temperature; MAP, mean annual precipitation; NDVI, normalized difference vegetation index; TC, total carbon; TN, total nitrogen.

| Forest type | Longitude | Latitude | Elevation (m) | MAT (ºC) | MAP (mm) | NDVI | TC (g/kg) | TN (g/kg) | C/N | pH ranges |
|---|---|---|---|---|---|---|---|---|---|---|
| Lowland dry broadleaf forest | 37°10' E | 3°22' S | 767 | 23.7 | 844.75 | 0.26 | 95.24±11.38 | 7.42±0.71 | 12.77±0.45 | 7.24–7.80 |
| Lower montane forest | 37°14' E | 3°10' S | 1920 | 15.3 | 2377.52 | 0.87 | 212.24±11.31 | 14.07±0.73 | 15.08±0.09 | 3.99–4.25 |
| Podocarpus forest | 37°15' E | 3°6' S | 2850 | 9.4 | 1773.00 | 0.64 | 325.85±25.16 | 17.71±0.75 | 18.37±0.97 | 3.82–3.95 |
| Erica bush forest | 37°17' E | 3°4' S | 3880 | 4.5 | 1188.00 | 0.45 | 187.22±25.20 | 9.88±1.05 | 18.77±0.77 | 4.73–5.17 |
| Helichrysum cushion | 37°19' E | 3°5' S | 4190 | 4.5 | 961.52 | 0.21 | 47.75±6.72 | 3.13±0.40 | 15.18±0.22 | 4.45–5.65 |

**Table 2:** The effects of environmental factors on diversity were tested using linear mixed-effects models for bacteria and fungi (with elevation as a random effect). A quadratic term of elevation was included to test for non-linear effects of elevation. Marginal $R^2$ gives the proportion of variance accounted for by the fixed effects; Conditional $R^2$ that accounted for by both fixed and random effects. SE, standard error; MAT, mean annual temperature.

| (1) Bacteria | | | | |
|---|---|---|---|---|
| | Estimate | SE | t-value | *P*-value |
| Intercept | 0.6979 | 0.3044 | 2.293 | 0.034 |
| pH | 0.9564 | 0.3037 | 3.149 | 0.005 |
| $pH^2$ | -0.7347 | 0.2536 | -2.897 | 0.01 |
| Random effects variance | | | | |
| Elevation | 0.0264 | | | |
| Residual | 0.6928 | | | |
| Marginal $R^2$ | 0.327 | Conditional $R^2$ | 0.354 | |
| (2) Fungi | | | | |
| | Estimate | SE | t-value | *P*-value |
| Intercept | 0 | 0.1917 | 0 | 1 |
| MAT | 5.511 | 0.1967 | 2.802 | 0.011 |
| Random effects variance | | | | |
| Elevation | 0.0485 | | | |
| Residual | 0.7151 | | | |
| Marginal $R^2$ | 0.243 | Conditional $R^2$ | 0.292 | |

**Table 3:** Results of a distance-based linear model (DistLM) analysis determining the suite of environmental variables that describe significant and independent proportions of the variation in bacterial and fungal community composition. Variables are listed in order of importance, and they are added to the model. Values in bold indicate significant ($p < 0.05$). SS, sum of squares; MAT, mean annual temperature; MAP, mean annual precipitation; NDVI, normalized difference vegetation index; TC, total carbon; TN, total nitrogen.

| Variable | SS(Trace) | Pseudo-F | *P*-value | Proportion | Cumulative |
|---|---|---|---|---|---|
| (1) Bacteria | | | | | |
| pH | 16300.3135 | 13.5691 | **0.001** | 0.4298 | 0.4298 |
| MAT | 12380.1458 | 22.7701 | **0.001** | 0.3265 | 0.7563 |
| TC | 2478.1265 | 5.8612 | **0.001** | 0.0653 | 0.8216 |
| MAP | 1420.9976 | 3.9887 | **0.001** | 0.0375 | 0.8591 |
| C/N | 801.4968 | 2.4703 | **0.023** | 0.0211 | 0.8802 |
| TN | 494.3036 | 1.5874 | 0.097 | 0.013 | 0.8933 |
| NDVI | 195.9825 | 0.6105 | 0.838 | 0.0052 | 0.8984 |
| (2) Fungi | | | | | |
| MAT | 14562.3792 | 5.7628 | **0.001** | 0.2425 | 0.2425 |
| MAP | 12497.197 | 6.4403 | **0.001** | 0.2081 | 0.4506 |
| TN | 9350.5814 | 6.3294 | **0.001** | 0.1557 | 0.6063 |
| TC | 3077.1171 | 2.4031 | **0.012** | 0.0512 | 0.6575 |
| pH | 2439.2934 | 1.7261 | 0.093 | 0.0406 | 0.6981 |
| C/N | 1474.8765 | 1.0469 | 0.373 | 0.0246 | 0.7227 |
| NDVI | 856.5974 | 0.651 | 0.831 | 0.0143 | 0.7371 |

**Figures and legends**

**Figure 1 a**. Elevational patterns for the diversity of bacterial whole community (with green points and blue fitting curve) and specific phyla. Scaled Shannon, z-transformed Shannon index. The quadratic model was selected based on the lower value of AIC (linear, AIC = 2.79; quadratic, AIC = -20.52). **b.** Elevational patterns for the diversity of fungal whole community (with purple points and blue fitting curve) and specific phyla/classes. Scaled Shannon, z-transformed Shannon index. The linear model was selected based on the lower value of AIC (linear, AIC = -7.61; quadratic, AIC = -5.62). **c.** The relative importance of environmental variables for bacterial diversity (Shannon). The percentages were separated by dash line. **d.** The relative importance of environmental variables for fungal diversity (Shannon). The percentages were separated by dash line. MAT, mean annual temperature; MAP, mean annual precipitation; NDVI, normalized difference vegetation index; TC, total carbon; TN, total nitrogen.

**Figure 2 a. b.** The relationship between dissimilarity of bacterial (**a**) and fungal (**b**) communities with elevation distance. The strength of the relationship is based on exponential models {y = a[1-exp(-bx)]+c; with parameter estimates for bacteria (a = 0.6381, b = 0.001, c = 0.1872) and fungi (a = 0.4526, b = 0.0026, c = 0.4207)}. **c. d.** PCoA plots of trends in the composition of bacteria (**c**) and fungi (**d**) based on Bray-Curtis dissimilarity. For bacteria, samples were coded with different shapes and colors according to the elevations and pH. For fungi, samples were coded with

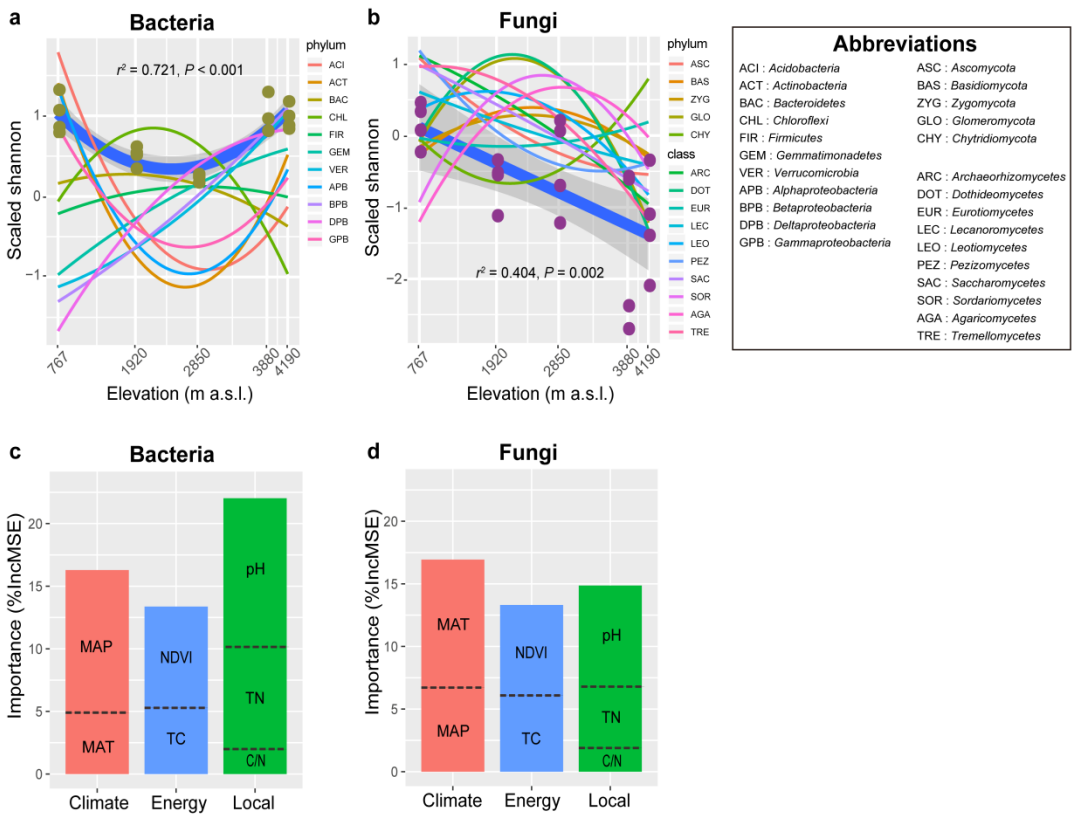
different shapes and colors according to the elevations and MAT. MAT, mean annual temperature.

**Figure 3** Triangular plots of beta diversity comparisons (using Sørensen dissimilarity index) for bacterial and fungal communities among all sites (**a, b**) and among sites within elevation (**c, d**). Each point represents a pair of sites. Its position is determined by a triplet of values from the S (similarity), Repl (replacement) and RichDiff (richness difference) matrices; each triplet sums to 1. Mean values of S, Repl and RichDiff are shown.

**Figure 4** Results of variation partitioning analysis showing the percentages of explained variation for the composition of bacteria (**a**, **c**) and fungi (**b**, **d**). **a**. **b.** Variation was partitioned by regional factors (MAT, MAP and NDVI) and local factors (pH, TC, TN and C/N). **c**. **d.** Variation was partitioned by climate factors (MAT and MAP), energy factors (NDVI and TC) and local factors (pH, TN and C/N). MAT, mean annual temperature; MAP, mean annual precipitation; NDVI, normalized difference vegetation index; TC, total carbon; TN, total nitrogen.

**Figure 5** Correlation and best random forest model for relative abundance of major bacterial phyla (**a,** class for *Proteobacteria*), relative abundance of major fungal classes (**b**), relative abundance of predicted functional groups (**c**, green dash line means predicted functional groups based on bacterial taxa, purple dash line means

predicted functional groups based on fungal taxa). For variable selection and estimating predictability, the random forest machine-learning algorithm was used. Circle size represents the variable importance (that is, decrease in the prediction accuracy (estimated with out-of-bag cross-validation)) as a result of the permutation of a given variable. Colors represent Spearman correlations. MAT, mean annual temperature; MAP, mean annual precipitation; NDVI, normalized difference vegetation index; TC, total carbon; TN, total nitrogen.

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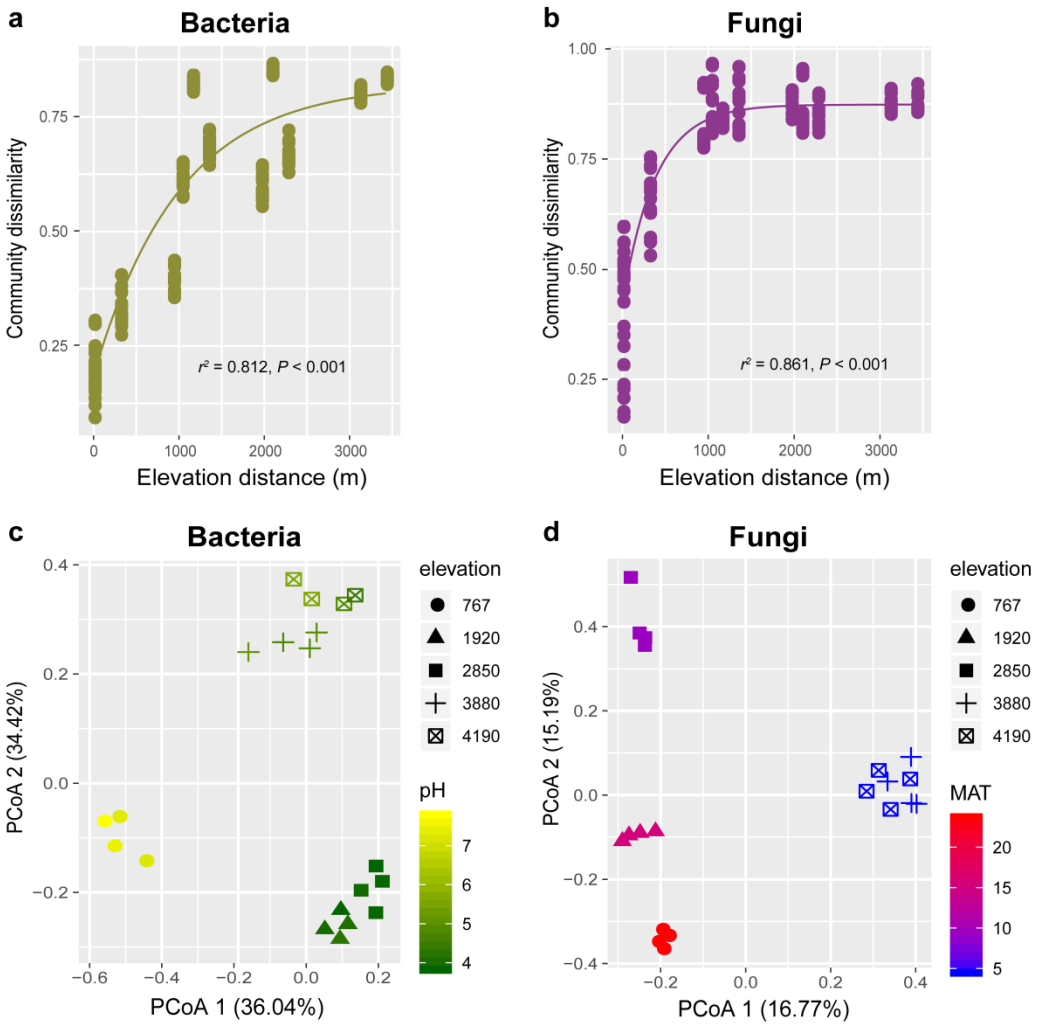


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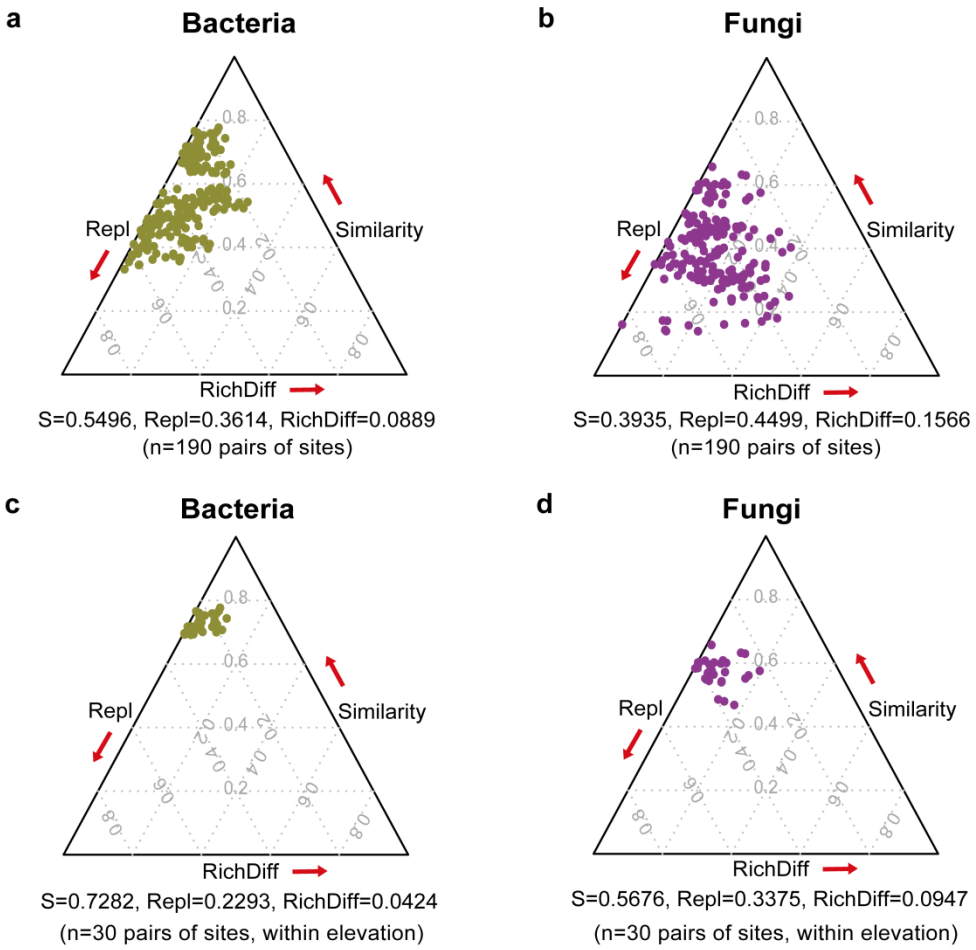


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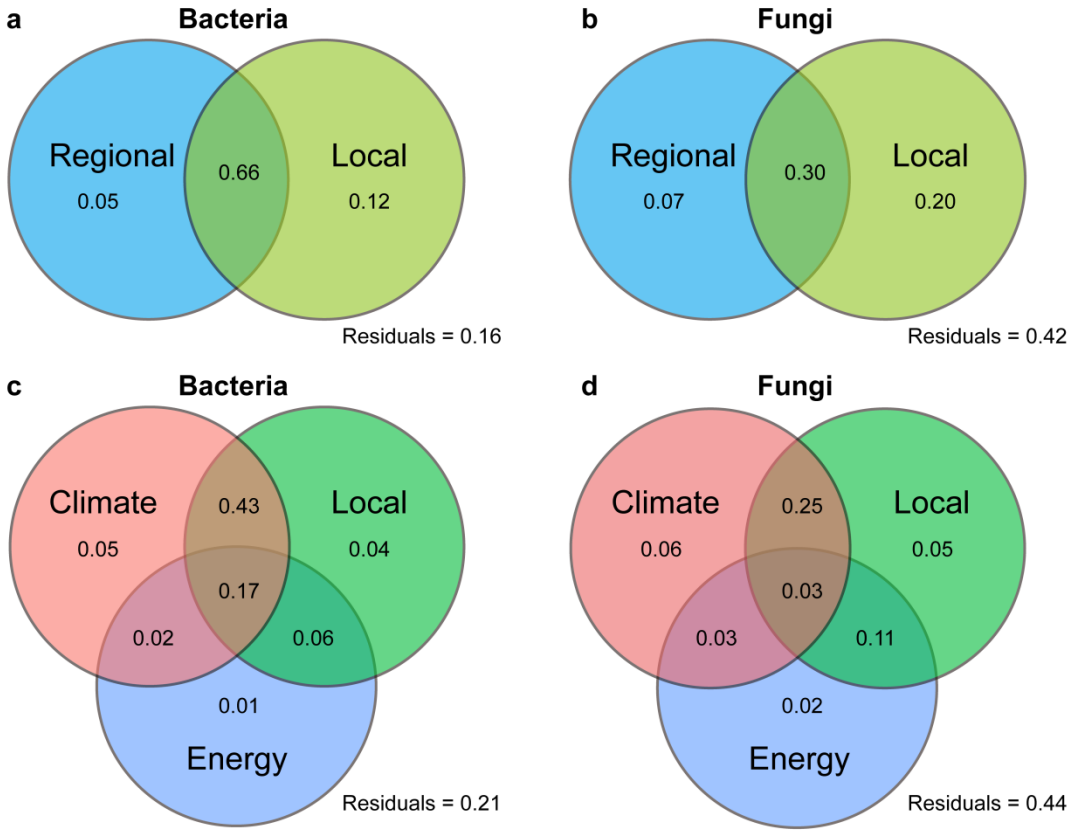


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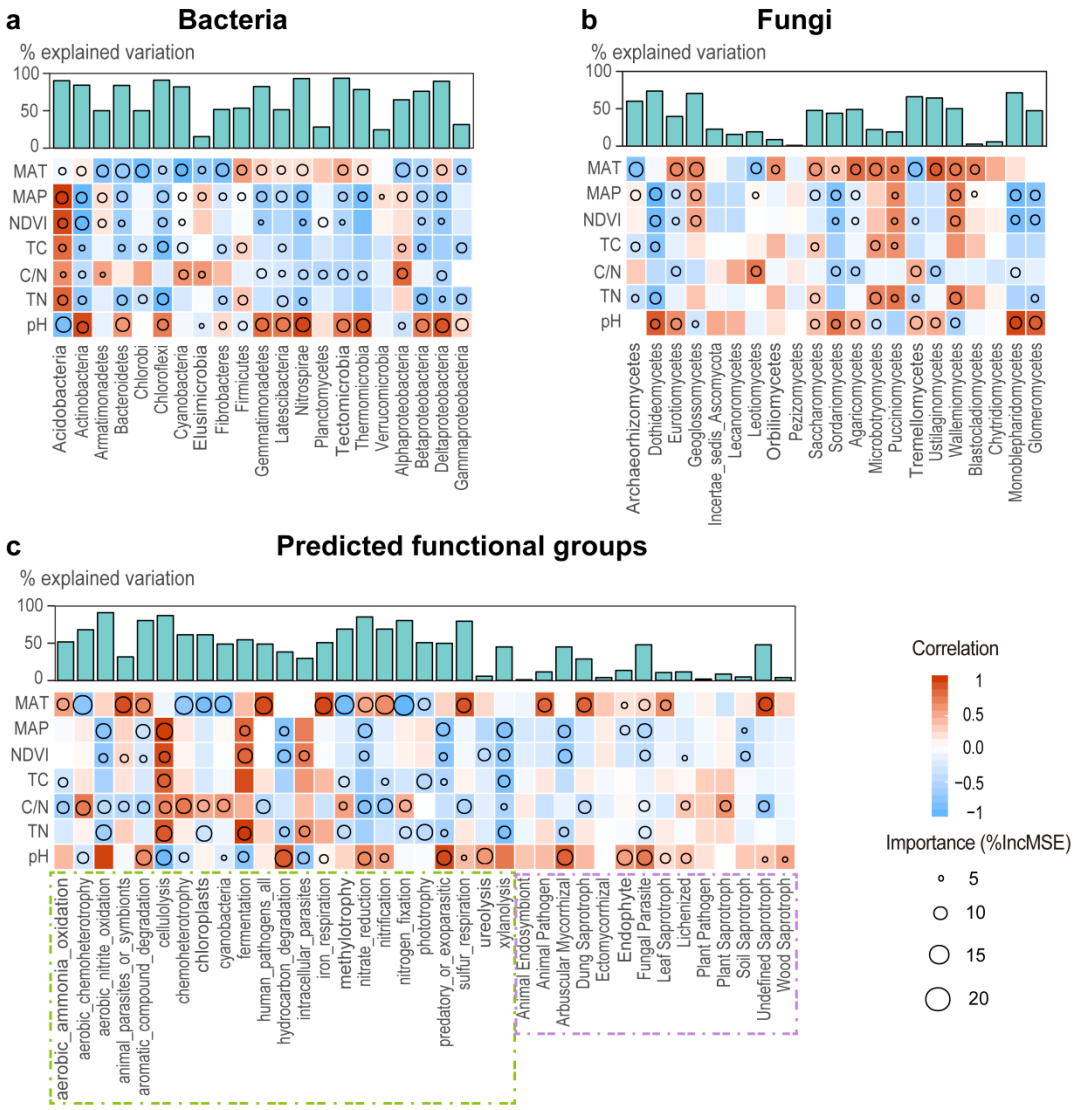


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