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BRIEF REPORT

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Soil microbial communities along elevational gradients in the Madrean Sky Islands

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Abstract

The Madrean Sky Islands are mountain ranges isolated by a 'desert sea'. This area is a biodiversity hotspot currently threatened by climate change. Here, we studied soil microbial communities along elevational gradients in eight Madrean Sky Islands in southeastern Arizona (USA). Our results showed that while elevational microbial richness gradients were weak and not consistent across different mountains, soil properties strongly influenced microbial community composition (overall composition and the abundance of key functional groups) along elevational gradients. In particular, warming is associated with a higher abundance of soil-borne fungal plant pathogens that concomitantly might facilitate upward elevational shifts of plant species released from negative plant–soil feedbacks. Furthermore, projected warming and drought in the area aggravated by anthropogenic nitrogen deposition on mountain tops (and thus, decreasing nitrogen limitation) can enhance a shift from ectomycorrhizal to arbuscular mycorrhizal fungi. Overall, these results indicate that climate change effects on plant–soil interactions might have profound ecosystem consequences.

INTRODUCTION

Mountains are biodiversity hotspots (Rahbek et al., 2019) and provide a natural space-for-time setting to study responses to environmental change (Tito et al., 2020). Current climate change is expected to impact montane ecosystems by causing species ranges to no longer contain conditions suitable for survival, generally favouring upward elevational shifts with uppermost populations at risk of shrinking or even disappearing (Morueta-Holme et al., 2015; Steinbauer et al., 2018). For example, lowland birds have started breeding in montane cloud-forest habitats (Pounds et al., 1999), alpine flora has expanded toward the summits (Grabherr et al., 1994), and butterfly and small mammal species have moved up in elevation (Moritz et al., 2008; Wilson et al., 2005). With warming, decomposition rates will likely increase at higher elevations, causing feedback on soil carbon dynamics, particularly changing mountains from carbon sinks to sources of atmospheric CO_2 (Nottingham et al., 2019; Salazar et al., 2020).

Microorganisms play a vital role in all biogeochemical processes (Falkowski et al., 2008), and thus, they mediate key ecosystem responses to climate change, in particular by controlling the nutrient pools that link plants and ecosystem functions (Pugnaire et al., 2019; Singh et al., 2010). Fungal symbionts have demonstrated their ability to mitigate plant abiotic stressors associated with climate change, and exhibit sensitivity to variations in elevation-related climatic and edaphic factors (Kivlin et al., 2017). In addition, key microbial functional groups involved in soil nutrient cycling are influenced by environmental and edaphic factors associated with altitude (Kou et al., 2021; Xu et al., 2014; Zhang et al., 2009). Interestingly, microbial communities do not present the same diversity patterns along elevation as those observed for plants and animals (Looby & Martin, 2020; Wang et al., 2022). For example, in the first study of microbial communities along a montane gradient, while soil bacterial diversity decreased monotonically with elevation, plant diversity followed an unimodal (i.e., hump-shaped) pattern (Bryant et al., 2008). Understanding the potential mechanisms behind microbial biogeographical patterns along elevation and differences among key microbial functional groups might help provide insights into possible responses to climate change that might cascade to other biotic levels (Bradford et al., 2016; Pugnaire et al., 2019).

Among montane ecosystems, sky islands (i.e., isolated mountains surrounded by drastically different lowland environments) provide an ideal opportunity to study the biogeography of soil microbial communities. The Madrean Sky Islands are bounded by the Sonoran Desert on the west, by the Chihuahuan Desert on the east, and span the gap between the Rocky Mountains in the north and the Sierra Madre Occidental in the south (Shreve, 1922; Yanahan & Moore, 2019). At low elevations, the desert forms a barrier between montane ecosystems (Pelletier et al., 2013; Whittaker & Niering, 1968). Four dominant vegetation biomes in ascending elevation are generally recognized: desert scrub-grassland, encinal (oak woodland or oak-grassland), low forest (pine-oak woodland), and a few mountains tall enough to have the uppermost pine forest or mixed conifer forest (Brusca et al., 2013; Whittaker & Niering, 1965). The Madrean Sky Islands cross from temperate to subtropical latitudes and connect different floristic and faunistic realms, and thus, are home to high levels of biodiversity, endemism, and unique ecological interactions (Yanahan & Moore, 2019).

In this study, we sampled soils along elevational gradients in eight Madrean Sky Islands in southeastern Arizona (USA). Our objective was to investigate the drivers of changes in microbial communities along elevation gradients, both directly and indirectly through alterations in soil parameters. Our research questions were: (1) how do different vegetation biomes along the elevation gradient influence soil microbial community diversity and composition? (2) Is the elevational gradient of soil microbial richness consistent across mountains? and (3) What are the direct and indirect (i.e., mediated by soil characteristics) effects of elevation on soil microbial richness and on the abundance of functional groups? We hypothesized that (i) soil microbial composition in vegetation biomes situated at opposite ends of the elevation gradient (i.e., desert and conifer forest) would exhibit clear distinctions, primarily driven by variations in soil physico-chemistry between these biomes (Looby & Martin, 2020; Wang et al., 2022); (ii) richness would peak at intermediate elevations (i.e., hump-shaped trend) following plant productivity and higher accumulation and diversity of organic matter from plant litter and root deposition (Lange et al., 2015), and (iii) functional groups closely associated with plants such as arbuscular mycorrhizal fungi, ectomycorrhizal fungi, and plant pathogens would be directly influenced by elevation (Kivlin et al., 2017) while the influence of elevation on functional groups involved in nutrient cyclings such as nitrifiers and N fixers would be mostly mediated by soil properties and soil nutrient content (Wang et al., 2022).

EXPERIMENTAL PROCEDURES

Study site

Eight Madrean Sky Islands peaks scattered across southeastern Arizona were selected: Santa Catalina in the Catalinas mountain district, Mica in the Rincon, Mount Hopkins in the Santa Rita, Miller Peak in the Huachuca, Mount Glenn in the Dragoon, Chiricahua Peak in the Chiricahuas, Mount Graham in the Pinaleños, and Bassett Peak in the Galiuros (Figure 1A, and see Table S1 for details on geographic and climatic variables). Dominant vegetation was classified into four biomes found across most mountains: desert scrub-grassland, encinal, low forest, and high forest (Figure 1B). In the desert scrub-grassland biome, desert vegetation such as creosote (*Larrea tridentata*), yellow paloverde (*Parkinsonia microphylla*), mesquites (*Prosopis* sp.), and a mixture of bunch grasses dominates. The encinal biome is mainly composed of broad-leaved evergreen oaks Emory oak (*Quercus emoryi*), Arizona white oak (*Q. arizonica*), silverleaf oak (*Q. hypoleucoides*), and netleaf oak (*Q. rugosa*) with an understory of a mixture of shrubs and grasses. In lower forest zones, there is a mixture of pinyon pine-juniper woodlands, such as Alligator juniper (*Juniperus deppeana*), redberry juniper (*J. coahuilensis*), and Mexican pinyon (*Pinus cembroides*) and oak-pine forest, while high forests are characterized by a mixture of conifers such as Douglas-fir (*Pseudotsuga menziesii*), Arizona pine (*Pinus arizonica*), bristlecone pine (*Pinus aristata*), white fir (*Abies concolor*), and southwestern white pine (*Pinus strobiformis*) (Brusca et al., 2013; Whittaker & Niering, 1965). Sampling locations were first selected based on the US Geological Survey (USGS) GAP-LANDFIRE national terrestrial ecosystem map (Table S2). Final sampling locations were selected after visually identifying the dominant plant species, and avoiding areas that showed clear signs of recent wildfires. The sampling location ranged from an elevation of 1260–2517 msl.

Soil sampling

At each vegetation biome, we collected three equidistant surface soil samples along a 10-m transect during April–June of 2020. To maintain a consistent procedure, we established a transect going from south to north. We brushed off leaf litter and other large material on the surface and shovelled the first 10 cm of soil in a sterile Whirl-Pak bag. Soil bags were then kept on ice until they were transported to the University of Arizona

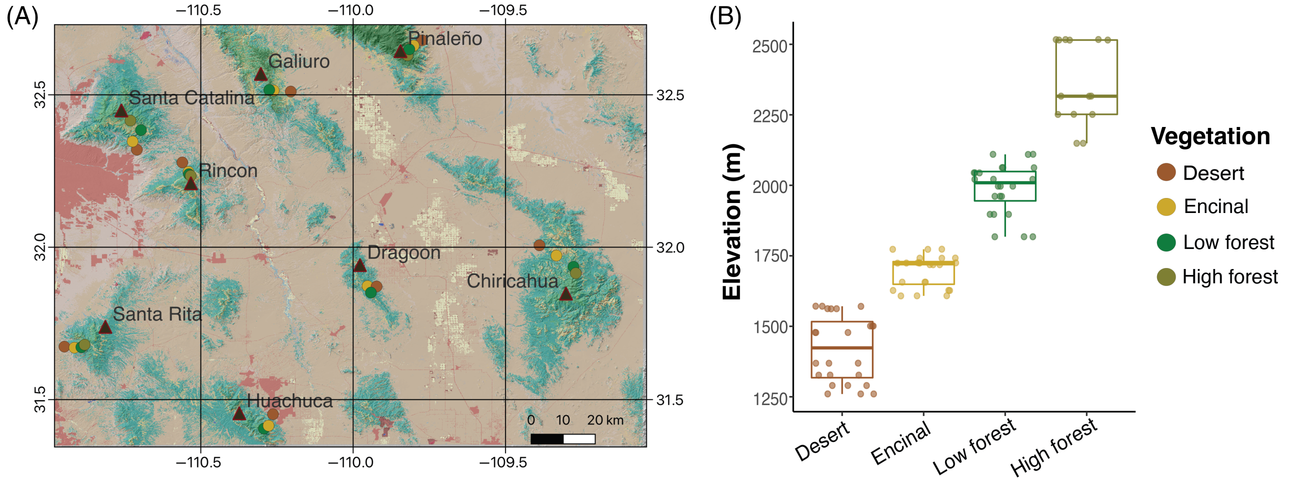

FIGURE 1 (A) Map of the Madrean Sky Islands in Southeastern Arizona. (B) Relationship between elevation and vegetation biomes.

where they were stored in a −80°C freezer. The shovel was sterilized with ethanol between sampling areas to avoid cross-contamination.

Climate variables and soil properties

Mean annual temperature (MAT) and mean annual precipitation (MAP) were obtained from PRISM Climate Group at Oregon State University, while normalized difference vegetation index (NDVI) was gathered from The Terra Moderate Resolution Spectroradiometer (MODIS) Vegetation Indices (MOD13Q1) Version 6 (Figure S1).

Soil subsamples were first sieved to 2 mm and air-dried overnight. Soil moisture was calculated by subtracting the weight of the soil after oven-drying from the initial weight. Soil pH was measured with 1:1 (weight/volume) soil to deionized water using a FiveEasy Plus pH meter (Mettler Toledo, Columbus, OH). Soil electroconductivity (EC) was measured with a 1:5 (weight/volume) soil-to-deionized water ratio using a FiveEasy Plus pH meter combined with a Cond probe LE703 (Mettler Toledo, Columbus, OH). For total carbon (C) and nitrogen (N), samples were oven-dried at 105°C overnight, then manually ground using a mortar, and weighed at 1 g for thermal combustion analysis (Vario MAX Cube, Elementar, Langenselbold, DE) (Table S3). Micronutrient concentrations of iron (Fe), copper (Cu), potassium (K), magnesium (Mg), calcium (Ca), phosphorous (P), Manganese (Mn), and sulphur (S) were measured as follows. First, 3 g of soil was mixed with 30 mL of Menlich III solution in a 50 mL polyethylene bottle. This mixture was shaken for 10 min on a reciprocating shaker at 180 cycles/min, then filtered through quantitative filter paper, and filtrate was analysed for elements of interest using iCAP 7200 inductively coupled plasma-optical emission spectroscopy (ICP-OES) Duo (Thermo Scientific, Waltham, MA) (Table S4).

Molecular analyses

Using the DNeasy PowerLyzer PowerSoil kit (Qiagen, Hilden, Germany), total genomic DNA was extracted following the manufacturer's protocols. To characterize bacterial/archaeal communities, the V4 hypervariable region of 16S RNA was PCR amplified using the primers 515-F (GTGCCAGCMGCCGCGGTAA) and the 806-R (GGACTACHVGGGTWTCTAAT) (Walters et al., 2015). For fungal communities, the first internal transcriber region (ITS1) was PCR amplified using the primers ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) (Walters et al., 2015). Primers included Illumina adapters, and reverse primer pairs contained an error correcting-bp barcode specific to each sample for demultiplexing. PCR was conducted in 40 μL triplicate reactions per sample, using 3 μL of extracted DNA, 3 μL of each primer, 20 μL of MyFi PCR Mix (Bioline, Taunton, MA), and 11 μL of water. PCR consisted of an initial denaturing step at 95°C for 1 min, 35 cycles of amplification (95°C for 15 s, 60°C for 15 s, and 72°C for 15 s), and a final elongation step of 72°C for 3 min. Negative controls were included to detect potential contamination. PCR products were cleaned with an Ultra-Clean PCR Clean-Up kit (MoBio Laboratories, Carlsbad, CA), and quantified with the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Waltham, MA). Purified PCR products were pooled in equimolar concentrations and sequenced on a 2 × 150 bp Illumina MiSeq platform. Sequencing was conducted at the PANDA Core for Genomics and Microbiome Research, University of Arizona.

Sequence processing

Raw sequenced reads were demultiplexed using idemp and processed using the DADA2 pipeline (Callahan et al., 2016). The 16S rRNA reads were trimmed to 140 base pairs (bp). Due to length variation in the ITS region, the last 20 bp were removed from reverse reads and primers were removed with cutadapt (Martin, 2011). Reads that exceeded a maximum error of two or more bp were removed. Filtered reads were used to train the error model and to infer amplicon sequence variants (ASVs). Paired-end reads were merged, and chimera sequences were removed. Using the Ribosomal Database Project (RDP) classifier (Wang et al., 2007), taxonomic identities were associated with each ASV. The SILVA database release 132 (Quast et al., 2013) was used to annotate 16S rRNA and the UNITE database version 8.2 (Nilsson et al., 2019) was used for ITS sequences. Any 16S ASVs without a bacterial or archaeal domain classification, or classified as mitochondria or chloroplasts were removed (31,361 reads), while all ITS amplicons were assigned to Fungi. Samples with less than 10,000 sequences were also removed (1 16S sample). The number of bacterial/archaeal sequences per sample ranged from 37,272 to 192,660. Fungal sequences per sample ranged from 37,156 to 378,269. Functional groups of Bacteria/Archaea were inferred using FAPROTAX (Louca et al., 2016), and fungal guilds with a 'highly probable' or 'probable' confidence ranking were inferred using FUNGuild (Nguyen et al., 2016). A total of 6121 fungal ASVs were assigned to a probable or highly probable functional guild and retained for further functional analysis. Although each step can potentially lead to artefacts (Zinger et al., 2019), we sequenced gene regions demonstrated to provide accurate taxonomic information for a majority of microbial groups, and we employed primers with few biases against specific taxa (Walters et al., 2016). Despite this, ribosomal copy number varies across microbial taxa, and as a result, the variation in the relative abundance of gene reads can be attributed both to variation in the abundance of different taxa and ribosomal copy number variation among taxa (Kembel et al., 2012; Taylor et al., 2016).

Statistical analyses

All data analyses and visualizations were performed in R version 4.3.0. We explored the associations among elevation, climate (MAP, MAT) and soil properties and micronutrients (pH, EC, water content, C, N, P, K, Fe, Cu, Mg, Ca, Mn, S) using Spearman's correlations and principal component analysis (PCA). We assessed the effect of vegetation biomes on soil variables along the elevation gradient using linear mixed-effects models in the lme4 package version 1.1-32 (Bates et al., 2015). All soil variables were log-transformed except for pH. We used biomes as fixed effects and included mountains as random effects. While the vegetation biome is a proxy for elevation and the climatic, topographical, and biotic factors that co-vary along the montane gradient (Körner, 2007), mountain (i.e., Catalina, Chiricahua, Dragoon, Galiuro, Huachuca, Pinaleño, Rincon, Santa Rita) accounts for the factors that co-vary across space. We assessed the explanatory power of the models using marginal R^2 (only fixed effects) and conditional R^2 (fixed and random effects) (Nakagawa & Schielzeth, 2013).

To study soil microbial diversity, we first rarefied the sequence counts to 37,000 for both Bacteria/Archaea and Fungi to control for differences in sequencing depth (see Figure S2 for a comparison with Chao-estimated richness). We used the same linear mixed-effects model approach as described for soil variables to assess the effect of vegetation biomes on soil microbial richness (number of different ASVs) and diversity (measured using the Shannon index). We estimated the relationship between richness and elevation using linear and quadratic models, and we selected the most parsimonious model for each mountain using the Akaike Information Criterion (AIC).

To evaluate soil microbial compositional changes, we calculated Bray–Curtis dissimilarities (see Figure S3 for a comparison with Jaccard dissimilarities). Differences in microbial composition were visualized using non-metric multidimensional scaling (NMDS). To assess the effect of mountains and different vegetation biomes within mountains, we used permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001). Additionally, we studied microbial compositional dissimilarity changes with elevational change and geographical distance using distance correlation *t*-tests (Székely & Rizzo, 2013). Multivariate statistics were performed using the vegan package version 2.6-4.

We evaluated the direct and the indirect (i.e., mediated by soil physico-chemistry) effects of elevation on microbial richness and on the log-transformed abundance of functional groups using structural equation models as implemented in the package piecewiseSEM version 2.3.0 (Lefcheck, 2016). We included mountains as random effects. We represented 'Soil' as a statistical composite (i.e., indicator variables are weighted depending on regression coefficients) using all measured soil variables (pH, EC, water content, C, N, P, K, Fe, Cu, Mg, Ca, Mn, S). We calculated standardized effects with 95% confidence intervals using 1000 bootstrapped estimates.

RESULTS

Temperature and precipitation gradients were closely associated with elevation, as expected (Figure S4). On average, the temperature decreased by 0.006°C * (±0.0001) and precipitation increased by 0.32 mm (±0.19) per meter of gained elevation. In addition, soil

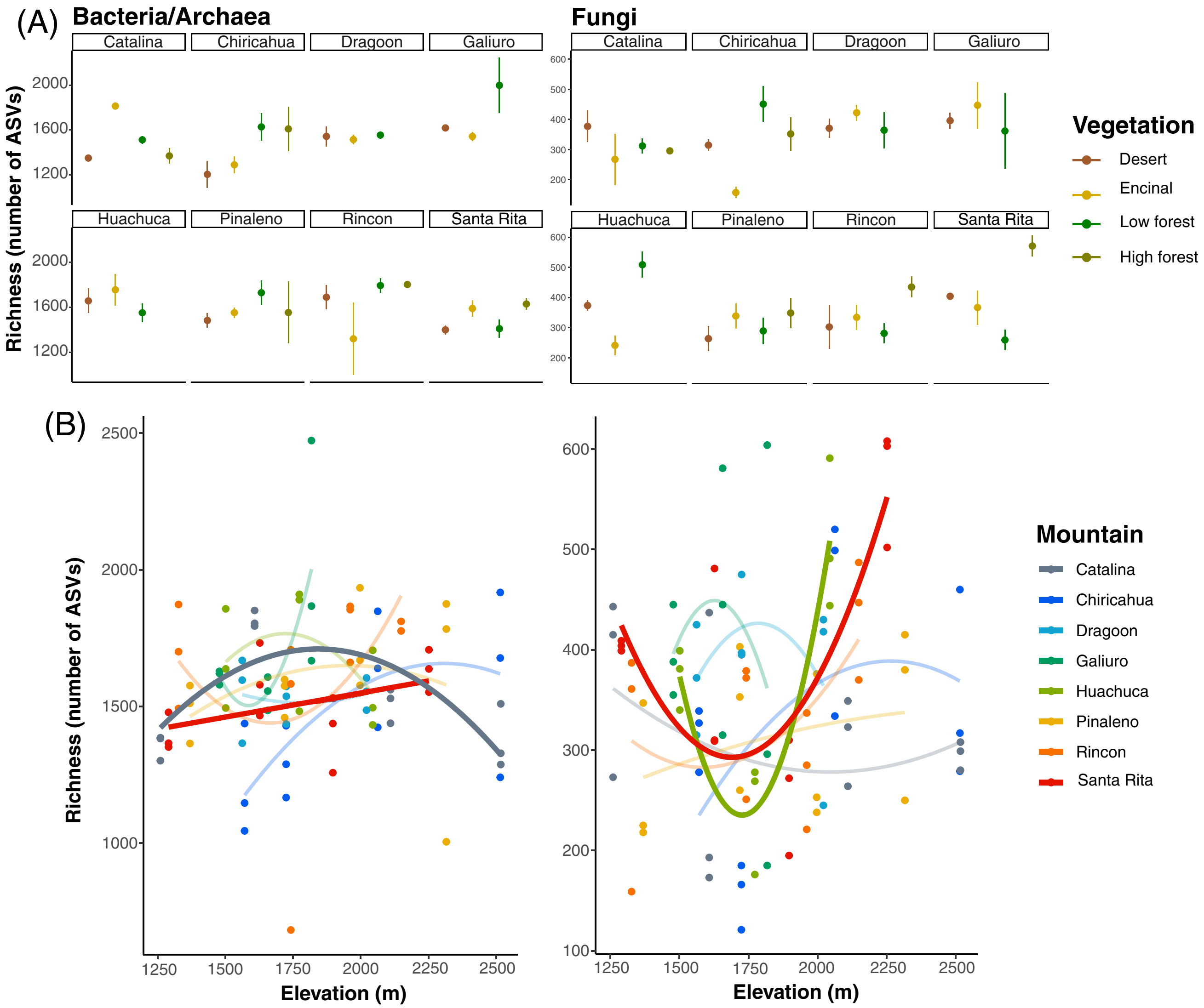

FIGURE 2 (A) Soil microbial richness across vegetation biomes and different mountains. Point ranges show means and standard errors. (B) Soil microbial richness along the elevation of each mountain. The best model (linear or quadratic) based on AIC is represented. Significant relationships ($P < 0.05$) are indicated by bolded lines.

water and iron content consistently increased along elevation (Figure S4). Most soil properties and micronutrients (except for pH, P, and S) were significantly different across vegetation biomes distributed along the elevation gradient, although mountain as a proxy of geographical location generally explained a larger proportion of variation than vegetation biome (Table S5).

The total number of different bacterial/archaeal ASVs after rarefaction was 43,053. Bacterial/archaeal-richness per sample ranged from 681 to 2478. The total number of different fungal ASVs was 13,966. Fungal richness per sample ranged from 121 to 604. Bacterial/archaeal and fungal richness was not significantly different across vegetation biomes (F = 1.83, P = 0.15 for Bacteria/Archaea; F = 2.33, P = 0.08 for Fungi; Figure 2A). Similar patterns were observed for Shannon diversity (Figure S5). Overall, the joint explanatory power of vegetation biomes along elevation and different mountains for microbial richness was low ($R^2_c = 0.12$ for Bacteria/Archaea; $R^2_c = 0.16$ for Fungi). The weak influence of elevational gradients on soil microbial diversity was further confirmed by fitting linear and quadratic models for each mountain (Figure 2B). Only bacterial richness in the Catalina and Santa Rita mountains showed significant hump-shaped and increasing linear trends, respectively. For soil fungal communities, richness showed significant U-shaped trends for the Huachuca and Santa Rita mountains (Figure 2B).

Soil bacterial/archaeal communities were dominated by Actinobacteria (36.53%), Proteobacteria (27.90%), Acidobacteria (12.02%), Chloroflexi (3.97%),

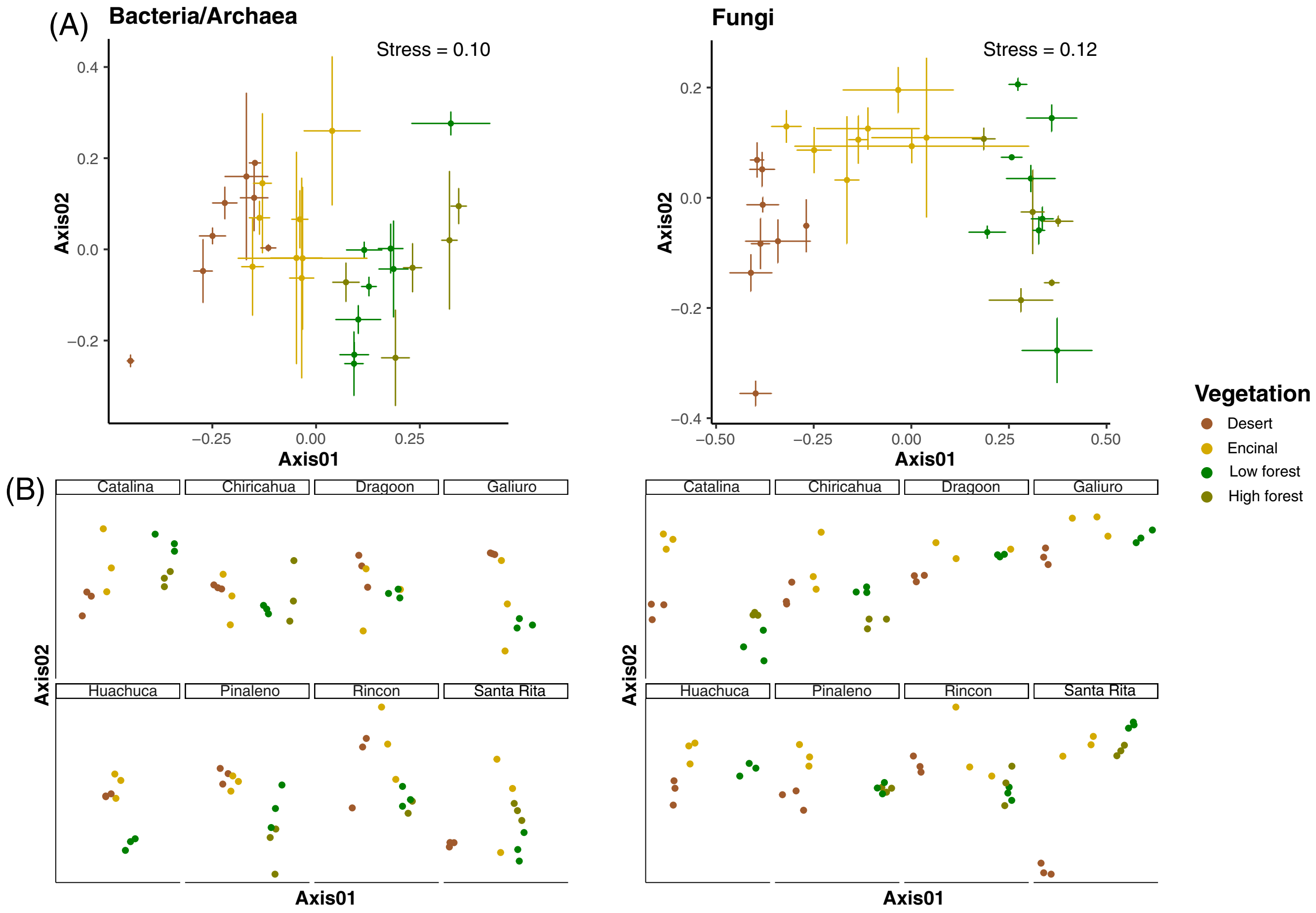

FIGURE 3 Non-metric multidimensional scaling (NMDS) ordination plots of soil microbial community dissimilarity. (A) Soil samples from the same transect plotted together to visualize clustering based on vegetation. (B) Individual soil samples separated by a mountain.

Firmicutes (3.86%), and Gemmatimonadetes (3.68%). Soil fungal communities were dominated by Ascomycota (55.06%), Basidiomycota (39.14%), Mortierellomycota (3.42%), and Mucoromycota (1.05%), also classified as Mucoromycotina (Spatafora et al., 2017) (Figure S6). Compositional differences between low and high vegetation biomes were already visible at broad phylogenetic resolution. Actinobacteria and Ascomycota tended to be more abundant in desert and encinal, while Proteobacteria and Basidiomycota were more abundant in the forest biomes (Figure S6).

Overall soil bacterial/archaeal and fungal compositions were significantly different across vegetation biomes within mountains (PERMANOVA for Bacteria/Archaea: $R^2 = 0.50$, $P < 0.001$; PERMANOVA for Fungi: $R^2 = 0.39$, $P < 0.001$; Figure 3A). This trend was further confirmed by significant positive associations between microbial compositional dissimilarity and elevational distance in each mountain ($r_{\text{cor}} = 0.57$–0.93 for Bacteria/Archaea; $r_{\text{cor}} = 0.57$–0.68 for Fungi; Figure S7A). Soil microbial composition also differed across different mountains, even though the explanatory power was lower than for vegetation (PERMANOVA for Bacteria/Archaea: $R^2 = 0.15$, $P < 0.001$; PERMANOVA for Fungi: $R^2 = 0.12$, $P < 0.001$; Figure 3B). Accordingly, we observed a weak decay pattern of microbial compositional dissimilarity with increasing geographical distance ($r_{\text{cor}} = 0.12$ for Bacteria/Archaea; $r_{\text{cor}} = 0.10$ for Fungi; Figure S7B).

The abundance of soil microbial functional groups along elevation showed distinct patterns (Figures 4A and S8). For example, while ectomycorrhizal fungal abundance increased at upper forested elevations, arbuscular mycorrhizal fungi (AMF) abundance declined with elevation (Figures 4B and S8). Using structural equation models including mountain as random effects (Figure 5A), we were able to tease apart the direct and the indirect (i.e., mediated by soil) effects of elevation (including the climatic, topographical, and biotic factors that co-vary along the gradient) while controlling for different mountain and the factors that co-vary across space. In general, the indirect effect of elevation was mainly positive (except for fermenters, methylotrophs, nitrifiers, oxygenic photoautotrophs, arbuscular mycorrhizal fungi, and fungal plant pathogens) and larger than the direct elevation effect which

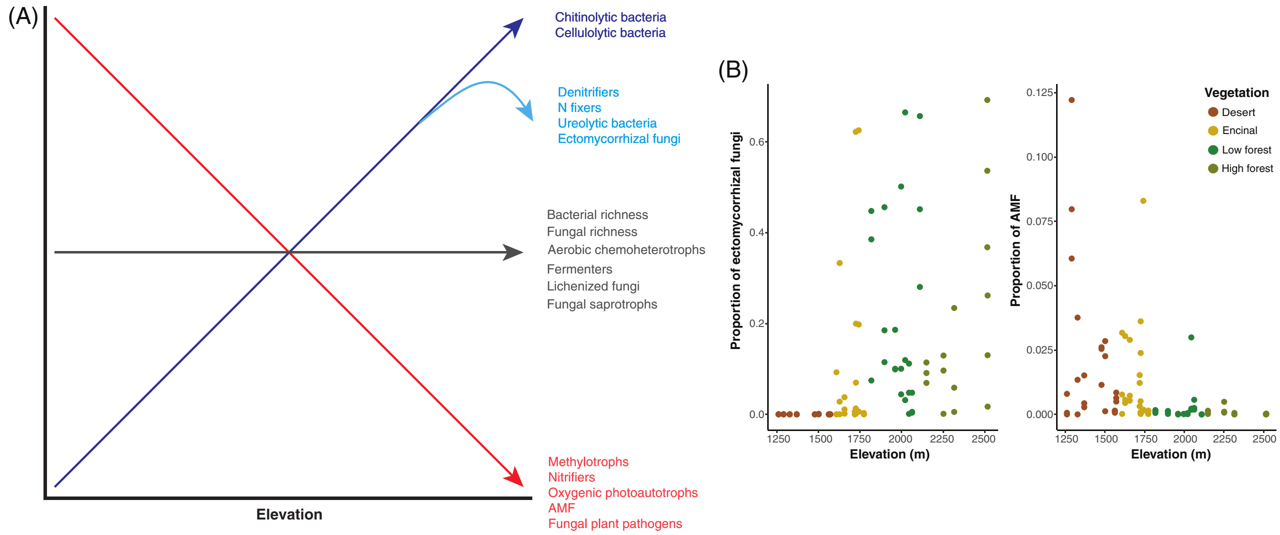

FIGURE 4 (A) Overview of elevational patterns for functional group abundance and richness. (B) Examples of functional groups (i.e., ectomycorrhizal fungi and arbuscular mycorrhizal fungi) abundance patterns.

tended to be close to zero (except for cellulolytic bacteria, denitrifiers, N fixers, and ectomycorrhizal fungi) (Figure 5B). Overall, soil had a stronger significant effect on microbial richness and the abundance of functional groups than elevation (Figure 5B).

DISCUSSION

Climatic models predict higher temperatures and increased drought periods in Southwestern North America (Seager et al., 2007). In addition, the arid Southwestern USA is experiencing one of the fastest population growths in the country (Stehfest et al., 2019). Given that climate change and human impacts are associated with diversity and composition changes in soil microbial communities (Chen et al., 2020; Singh et al., 2010), investigating soil microbial communities along montane gradients and the environmental factors behind these patterns provides insight into microbial biogeography, and the effects of environmental changes on plant–soil interactions and ecosystem functioning (Pugnaire et al., 2019). In the Madrean Sky Islands of southeastern Arizona, we found that while elevational diversity gradients were weak and not consistent across different mountains, elevation and soil characteristics strongly influenced the overall composition and the abundance of key functional groups.

Early naturalists noticed that the number of plant and animal species tended to decrease from low to high mountain elevations (Lomolino, 2001). However, unimodal (i.e., hump-shaped) patterns have been more commonly documented (Rahbek, 1995). In general, patterns of microbial richness along elevation tend to be more varied (i.e., flat, increasing, decreasing, hump-shaped, and U-shaped) and weaker than for plants and animals (Looby & Martin, 2020; Wang et al., 2022). Our results in the Madrean Sky Islands support this general trend. We only found two out of eight mountains with significant elevation trends in bacterial richness, one being an increasing trend and the other hump-shaped. While for fungi, the two significant trends were U-shaped patterns.

Many mechanisms (i.e., climate, productivity, space, topography, biotic interactions, evolutionary history) have been proposed to explain elevational gradients (Nogués-Bravo et al., 2008; Rahbek et al., 2019). Overall, the most important factors for plants and animals appear to be climatic and taxonomy-specific (McCain, 2007, 2009, 2010). Although these drivers certainly also influence microorganisms, soil characteristics (mainly pH) are recognized as the dominant influence of soil microbial communities (Fierer & Jackson, 2006). For instance, a recent meta-analysis showed that soil pH exerts a larger influence than climate on microbial mountain diversity (Wang et al., 2022, but see Singh et al., 2013). In our case, pH did not significantly differ across biomes, although lower pH was expected at higher elevations, probably because of small-scale soil and vegetation variations, which could also cause inconsistent alpha diversity patterns between different mountains. Nevertheless, our SEM results confirm that soil (as a composite variable generated from soil properties, nutrients, and micronutrients) has a stronger effect shaping microbial richness than the total effects of elevation (implicitly including co-varying climatic and other factors) while controlling for geographical location (and factors that co-vary across space). Overall, microbial richness might not be a good indicator of ecological processes (Shade, 2017) and at the same time, experiencing spatial heterogeneity at much smaller scales than

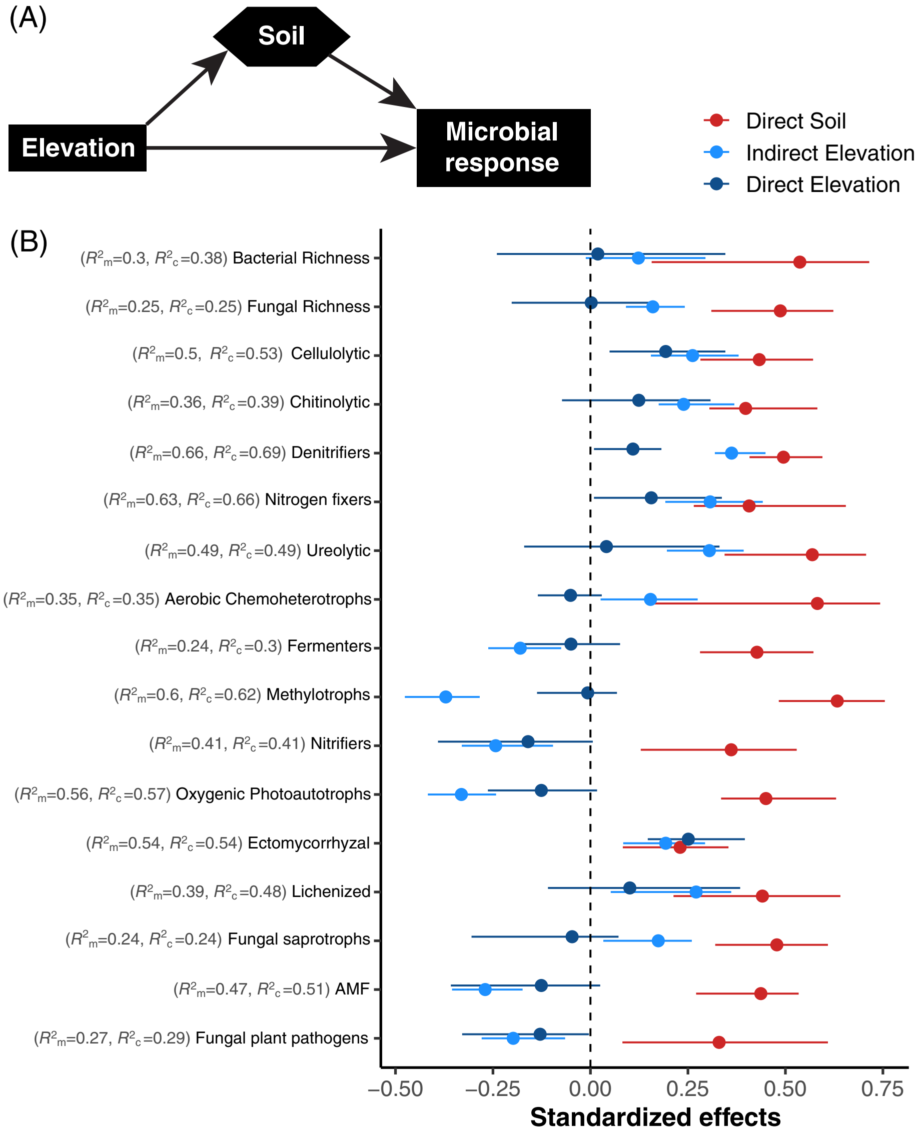

FIGURE 5 (A) Structural causal model showing direct and indirect effects of soil and elevation on microbial response variables (i.e., microbial richness and functional group abundances). (B) Standardized effects of elevation and soil on microbial response variables. Error bars represent 95% confidence intervals. R^2_m refers to marginal R^2 (only fixed effects) and R^2_c refers to conditional R^2 (fixed and random effects).

macroorganisms might blur clear elevational richness patterns for microorganisms (Barberán et al., 2014; Meyer et al., 2018).

By contrast and consistent with the vertical zonation of vegetation, we observed clear and for the most part uniform elevational patterns in overall soil microbial community composition in the Madrean Sky Islands. Elevational changes were far more determinant of microbial composition than geographical distance. That is, based on our results, we expect more distinct communities along a 1000 m elevational gradient within the same mountain than across mountains separated by 150 km. In this arid landscape where soil is exposed to wind erosion due to sparse vegetation, microbial airborne dispersal via dust is an important mechanism for long-distance connectivity (Kellogg & Griffin, 2006; Schiro et al., 2022).

Major phyla followed expected abundance patterns along the elevation gradient. For example, actinobacterial abundance generally declined at higher altitudes, while Basidiomycota increased. Actinobacteria are widespread in desert soils and tolerant to desiccation (Chen et al., 2021; Mohammadipanah & Wink, 2016), while Basidiomycota dominate at cooler temperatures and in high-precipitation areas (Peay et al., 2016). This segregation was further confirmed by assessing changes in the abundance of microbial functional groups along the elevation gradient. Overall, these abundance patterns were mainly driven by soil factors and indirect effects of elevation mediated through soil. This is expected as strong relationships among topography, soil, and vegetation exist in the Madrean Sky Islands with lower elevations characterized by steeper slopes, thinner soils, lower organic matter, and lower plant biomass than higher elevations (Pelletier et al., 2013; Poulos et al., 2007; Whittaker & Niering, 1968).

Although in general, warming will likely increase decomposition rates at higher elevations (Nottingham et al., 2019; Salazar et al., 2020), in Southwestern North America, drying will be more limiting than warming (Seager et al., 2007). Drought can modify plant–soil interactions via decreased litter quantity and quality, causing large changes in nutrient cycling by slowing down mineralization rates (Carrera & Bertiller, 2010; Kaisermann et al., 2017; Karlowsky et al., 2018). For instance, a litter-bag experiment in Arizona mountains reported increased decomposition rates in colder but wetter higher elevations (Murphy et al., 1998). In the Madrean Sky Islands, we observed a general increase of cellulolytic and chitinolytic bacteria along the elevational gradient. Previous studies found monotonic increases in the potential to metabolize recalcitrant carbon compounds along the Tibetan Plateau (Xu et al., 2014), or U-shaped patterns along Mount Kilimanjaro (Dai et al., 2021). Denitrifiers also tended to be more abundant at higher elevations although they peaked at the low forest biome. A similar hump-shaped pattern of denitrifier communities was previously reported along an elevational gradient in China (Kou et al., 2021). This abundance decline at the uppermost elevations could be explained by slower rates of mineralization and decomposition processes due to lower temperatures (Groffman et al., 2009). Plants under drought also change their root exudate composition producing more phytohormones, osmolytes, and antioxidants, changes that are expected to promote fungi to the detriment of bacteria (Preece & Peñuelas, 2016). In addition to warming and drought, in the Madrean Sky Islands region, invasive grasses create a fire hazard by increasing fuel loads and continuity (Fusco et al., 2019), and these changes in the fire regime can directly affect the enzymatic activity associated with soil decomposition (Fairbanks et al., 2020).

Nitrifier abundance decreased monotonically along the Madrean Sky Islands. Several studies have confirmed this general trend and proposed that a combination of altitude-related factors (mainly temperature and soil pH) shape the abundance of nitrifiers (Shen et al., 2020; Zhang et al., 2009). Methylotrophs (i.e., a diverse group of microorganisms that reduce one-carbon compounds) and oxygenic photoautotrophs (i.e., cyanobacteria) also decreased monotonically with elevation. Methylotrophs are present in alkaline soils (such as those found at lower elevations in the Madrean Sky Islands) and have the ability to promote plant growth (Kumar et al., 2019). A previous study similarly reported declining abundances of methylotrophs with altitude (Hofmann et al., 2016). Cyanobacteria are known to survive in arid environments with high temperatures and low water and nutrient availability (such as those in the Madrean Sky Islands' lower elevations) while contributing to primary production and soil stabilization (Chamizo et al., 2018).

In the Madrean Sky Islands, while nitrogen (N) fixers tended to be more abundant at higher elevations (with lower forest peaks), the abundance of saprotroph and lichenized fungi did not change, and fungal plant pathogens abundances tended to decrease with elevation. Increases of N fixers abundance were likewise reported along Mount Kilimanjaro (Shen et al., 2020) and in the Swiss Alps (Jacot et al., 2000). A recent study showed that the proportion of soil-borne pathogens tends to increase with warming (Delgado-Baquerizo et al., 2020). This can explain our observed fungal plant pathogen declining trend with altitude and highlights how more soil-borne pathogens favoured by climate change can benefit range-shifting plant species released from negative plant–soil feedbacks (Engelkes et al., 2008).

In a meta-analysis of plant-associated fungal symbionts along montane gradients, ectomycorrhizal fungal abundance increased at cooler higher elevations, while arbuscular mycorrhizal fungi (AMF) abundance declined (Kivlin et al., 2017). Similarly in our study in the Madrean Sky Islands, the abundance of ectomycorrhizal fungi increased with elevation peaking at lower forest, while AMF abundance decreased with elevation. As AMF can buffer the negative effects of drought (Madouh & Quoreshi, 2023), we might expect their abundances (and those of plant species that form AM) to increase in Southwestern North America. Thus, according to our results and previous studies (Miyamoto et al., 2018; Steidinger et al., 2020), we can expect a shift from ectomycorrhizal fungi (negatively affected by warming) to AMF (positively affected by drought) with crucial implications in soil nutrient cycling and carbon storage (Averill et al., 2014). This shift might be further aggravated because while ectomycorrhizal fungi are limited by dispersal (Bowman & Arnold, 2021), AMF tend to disperse more efficiently including by human mediation (Davison et al., 2018). Moreover, anthropogenic N deposition on mountaintops decreases N limitation and thus the value of ectomycorrhizal fungi, which exchange soil N for plant photosynthate (Lilleskov et al., 2002). This will promote faster N turnover because AMF cannot decompose soil organic matter via extracellular enzymes and is restricted to scavenging mineral forms of N (Read & Perez-Moreno, 2003).

In this study, we analysed the diversity and composition of soil microbial communities along elevational gradients in the Madrean Sky Islands within the context of climate change effects on ecosystem functioning, in particular plant–soil interactions. Overall, we observed substantial variation in the microbial composition along elevation. Our results indicate that global warming might increase the abundance of soil-borne fungal plant pathogens and as a result, might facilitate elevational shifts of invasive plants released from negative plant–soil interactions. Of particular concern are the concerted effects of warning and drought predicted in Southwestern North America on fungal root symbionts (i.e., a shift from ectomycorrhizal fungi to AMF). This shift might have profound ecosystem consequences by generating positive feedbacks further aggravated by anthropogenic N deposition.

AUTHOR CONTRIBUTIONS

Adalee Martinez: Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal); writing – original draft (equal). **Gabriele Schiro:** Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Albert Barberán:** Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Sequencing data are publicly available in NCBI Sequencing Read Archive under BioProject accession PRJNA973661: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA973661

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REFERENCES

Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26, 32–46.

Averill, C., Turner, B.L. & Finzi, A.C. (2014) Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*, 505, 543–545.

Barberán, A., Casamayor, E.O. & Fierer, N. (2014) The microbial contribution to macroecology. *Frontiers in Microbiology*, 5, 203.

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.

Bowman, E.A. & Arnold, A.E. (2021) Drivers and implications of distance decay differ for ectomycorrhizal and foliar endophytic fungi across an anciently fragmented landscape. *The ISME Journal*, 15, 3437–3454.

Bradford, M.A., Wieder, W.R., Bonan, G.B., Fierer, N., Raymond, P.A. & Crowther, T.W. (2016) Managing uncertainty in soil carbon feedbacks to climate change. *Nature Climate Change*, 6, 751–758.

Brusca, R.C., Wiens, J.F., Meyer, W.M., Eble, J., Franklin, K., Overpeck, J.T. et al. (2013) Dramatic response to climate change in the southwest: Robert Whittaker's 1963 Arizona Mountain plant transect revisited. *Ecology and Evolution*, 3, 3307–3319.

Bryant, J.A., Lamanna, C., Morlon, H., Kerkhoff, A.J., Enquist, B.J. & Green, J.L. (2008) Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. *Proceedings of the National Academy of Sciences*, 105, 11505–11511.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. (2016) DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13, 581–583.

Carrera, A.L. & Bertiller, M.B. (2010) Relationships among plant litter, fine roots, and soil organic C and N across an aridity gradient in northern Patagonia, Argentina. *Écoscience*, 17, 276–286.

Chamizo, S., Mugnai, G., Rossi, F., Certini, G. & De Philippis, R. (2018) Cyanobacteria inoculation improves soil stability and fertility on different textured soils: gaining insights for applicability in soil restoration. *Frontiers in Environmental Science*, 6, 49.

Chen, Y., Kuang, J., Wang, P., Shu, W. & Barberán, A. (2020) Associations between human impacts and forest soil microbial communities. *Elementa: Science of the Anthropocene*, 8, 005.

Chen, Y., Neilson, J.W., Kushwaha, P., Maier, R.M. & Barberán, A. (2021) Life-history strategies of soil microbial communities in an arid ecosystem. *The ISME Journal*, 15, 649–657.

Dai, Z., Zang, H., Chen, J., Fu, Y., Wang, X., Liu, H. et al. (2021) Metagenomic insights into soil microbial communities involved in carbon cycling along an elevation climosequences. *Environmental Microbiology*, 23, 4631–4645.

Davison, J., Moora, M., Öpik, M., Ainsaar, L., Ducousso, M., Hiiesalu, I. et al. (2018) Microbial Island biogeography: isolation shapes the life history characteristics but not diversity of root-symbiotic fungal communities. *The ISME Journal*, 12, 2211–2224.

Delgado-Baquerizo, M., Guerra, C.A., Cano-Díaz, C., Egidi, E., Wang, J.-T., Eisenhauer, N. et al. (2020) The proportion of soil-borne pathogens increases with warming at the global scale. *Nature Climate Change*, 10, 550–554.

Engelkes, T., Morriën, E., Verhoeven, K.J.F., Bezemer, T.M., Biere, A., Harvey, J.A. et al. (2008) Successful range-expanding plants experience less above-ground and below-ground enemy impact. *Nature*, 456, 946–948.

Fairbanks, D., Shepard, C., Murphy, M., Rasmussen, C., Chorover, J., Rich, V. et al. (2020) Depth and topographic controls on microbial activity in a recently burned sub-alpine catchment. *Soil Biology and Biochemistry*, 148, 107844.

Falkowski, P.G., Fenchel, T. & Delong, E.F. (2008) The microbial engines that drive Earth's biogeochemical cycles. *Science*, 320, 1034–1039.

Fierer, N. & Jackson, R.B. (2006) The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences*, 103, 626–631.

Fusco, E.J., Finn, J.T., Balch, J.K., Nagy, R.C. & Bradley, B.A. (2019) Invasive grasses increase fire occurrence and frequency across US ecoregions. *Proceedings of the National Academy of Sciences*, 116, 23594–23599.

Grabherr, G., Gottfried, M. & Pauli, H. (1994) Climate effects on mountain plants. *Nature*, 369, 448.

Groffman, P.M., Hardy, J.P., Fisk, M.C., Fahey, T.J. & Driscoll, C.T. (2009) Climate variation and soil carbon and nitrogen cycling processes in a northern hardwood forest. *Ecosystems*, 12, 927–943.

Hofmann, K., Pauli, H., Praeg, N., Wagner, A.O. & Illmer, P. (2016) Methane-cycling microorganisms in soils of a high-alpine altitudinal gradient. *FEMS Microbiology Ecology*, 92, fiw009.

Jacot, K.A., Lüscher, A., Nösberger, J. & Hartwig, U.A. (2000) The relative contribution of symbiotic N2 fixation and other nitrogen sources to grassland ecosystems along an altitudinal gradient in the Alps. *Plant and Soil*, 225, 201–211.

Kaisermann, A., de Vries, F.T., Griffiths, R.I. & Bardgett, R.D. (2017) Legacy effects of drought on plant–soil feedbacks and plant–plant interactions. *New Phytologist*, 215, 1413–1424.

Karlowsky, S., Augusti, A., Ingrisch, J., Hasibeder, R., Lange, M., Lavorel, S. et al. (2018) Land use in mountain grasslands alters drought response and recovery of carbon allocation and plant-microbial interactions. *Journal of Ecology*, 106, 1230–1243.

Kellogg, C.A. & Griffin, D.W. (2006) Aerobiology and the global transport of desert dust. *Trends in Ecology & Evolution*, 21, 638–644.

Kembel, S.W., Wu, M., Eisen, J.A. & Green, J.L. (2012) Incorporating 16S gene copy number information improves estimates of microbial diversity and abundance. *PLoS Computational Biology*, 8, e1002743.

Kivlin, S.N., Lynn, J.S., Kazenel, M.R., Beals, K.K. & Rudgers, J.A. (2017) Biogeography of plant-associated fungal symbionts in mountain ecosystems: a meta-analysis. *Diversity and Distributions*, 23, 1067–1077.

Körner, C. (2007) The use of 'altitude' in ecological research. *Trends in Ecology & Evolution*, 22, 569–574.

Kou, Y., Liu, Y., Li, J., Li, C., Tu, B., Yao, M. et al. (2021) Patterns and drivers of nirK-type and nirS-type denitrifier community assembly along an elevation gradient. *mSystems*, 6, e00667-21.

Kumar, M., Kour, D., Yadav, A.N., Saxena, R., Rai, P.K., Jyoti, A. et al. (2019) Biodiversity of methylotrophic microbial communities and their potential role in mitigation of abiotic stresses in plants. *Biologia*, 74, 287–308.

Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I. et al. (2015) Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications*, 6, 6707.

Lefcheck, J.S. (2016) piecewiseSEM: piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, 7, 573–579.

Lilleskov, E.A., Hobbie, E.A. & Fahey, T.J. (2002) Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. *New Phytologist*, 154, 219–231.

Lomolino, M.V. (2001) Elevation gradients of species-density: historical and prospective views. *Global Ecology and Biogeography*, 10, 3–13.

Looby, C.I. & Martin, P.H. (2020) Diversity and function of soil microbes on montane gradients: the state of knowledge in a changing world. *FEMS Microbiology Ecology*, 96, fiaa122.

Louca, S., Jacques, S.M.S., Pires, A.P.F., Leal, J.S., Srivastava, D.S., Parfrey, L.W. et al. (2016) High taxonomic variability despite stable functional structure across microbial communities. *Nature Ecology & Evolution*, 1, 1–12.

Madouh, T.A. & Quoreshi, A.M. (2023) The function of arbuscular mycorrhizal fungi associated with drought stress resistance in native plants of arid desert ecosystems: a review. *Diversity*, 15, 391.

Martin, M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal*, 17, 10.

McCain, C.M. (2007) Could temperature and water availability drive elevational species richness patterns? A global case study for bats. *Global Ecology and Biogeography*, 16, 1–13.

McCain, C.M. (2009) Global analysis of bird elevational diversity. *Global Ecology and Biogeography*, 18, 346–360.

McCain, C.M. (2010) Global analysis of reptile elevational diversity. *Global Ecology and Biogeography*, 19, 541–553.

Meyer, K.M., Memiaghe, H., Korte, L., Kenfack, D., Alonso, A. & Bohannan, B.J.M. (2018) Why do microbes exhibit weak biogeographic patterns? *The ISME Journal*, 12, 1404–1413.

Miyamoto, Y., Terashima, Y. & Nara, K. (2018) Temperature niche position and breadth of ectomycorrhizal fungi: reduced diversity under warming predicted by a nested community structure. *Global Change Biology*, 24, 5724–5737.

Mohammadipanah, F. & Wink, J. (2016) Actinobacteria from arid and desert habitats: diversity and biological activity. *Frontiers in Microbiology*, 6, 1541.

Moritz, C., Patton, J.L., Conroy, C.J., Parra, J.L., White, G.C. & Beissinger, S.R. (2008) Impact of a century of climate change on small-mammal communities in Yosemite National Park, USA. *Science*, 322, 261–264.

Morueta-Holme, N., Engemann, K., Sandoval-Acuña, P., Jonas, J.D., Segnitz, R.M. & Svenning, J.-C. (2015) Strong upslope shifts in Chimborazo's vegetation over two centuries since Humboldt. *Proceedings of the National Academy of Sciences*, 112, 12741–12745.

Murphy, K.L., Klopatek, J.M. & Klopatek, C.C. (1998) The effects of litter quality and climate on decomposition along an elevational gradient. *Ecological Applications*, 8, 1061–1071.

Nakagawa, S. & Schielzeth, H. (2013) A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4, 133–142.

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J. et al. (2016) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, 20, 241–248.

Nilsson, R.H., Larsson, K.-H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D. et al. (2019) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, 47, D259–D264.

Nogués-Bravo, D., Araújo, M.B., Romdal, T. & Rahbek, C. (2008) Scale effects and human impact on the elevational species richness gradients. *Nature*, 453, 216–219.

Nottingham, A.T., Whitaker, J., Ostle, N.J., Bardgett, R.D., McNamara, N.P., Fierer, N. et al. (2019) Microbial responses to warming enhance soil carbon loss following translocation across a tropical forest elevation gradient. *Ecology Letters*, 22, 1889–1899.

Peay, K.G., Kennedy, P.G. & Talbot, J.M. (2016) Dimensions of biodiversity in the earth mycobiome. *Nature Reviews Microbiology*, 14, 434–447.

Pelletier, J.D., Barron-Gafford, G.A., Breshears, D.D., Brooks, P.D., Chorover, J., Durcik, M. et al. (2013) Coevolution of nonlinear trends in vegetation, soils, and topography with elevation and slope aspect: a case study in the sky islands of southern Arizona. *Journal of Geophysical Research: Earth Surface*, 118, 741–758.

Poulos, H.M., Taylor, A.H. & Beaty, R.M. (2007) Environmental controls on dominance and diversity of woody plant species in a Madrean, Sky Island ecosystem, Arizona, USA. *Plant Ecology*, 193, 15–30.

Pounds, J.A., Fogden, M.P.L. & Campbell, J.H. (1999) Biological response to climate change on a tropical mountain. *Nature*, 398, 611–615.

Preece, C. & Peñuelas, J. (2016) Rhizodeposition under drought and consequences for soil communities and ecosystem resilience. *Plant and Soil*, 409, 1–17.

Pugnaire, F.I., Morillo, J.A., Peñuelas, J., Reich, P.B., Bardgett, R.D., Gaxiola, A. et al. (2019) Climate change effects on plant-soil feedbacks and consequences for biodiversity and functioning of terrestrial ecosystems. *Science Advances*, 5, eaaz1834.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41, D590–D596.

Rahbek, C. (1995) The elevational gradient of species richness: a uniform pattern? *Ecography*, 18, 200–205.

Rahbek, C., Borregaard, M.K., Colwell, R.K., Dalsgaard, B., Holt, B.G., Morueta-Holme, N. et al. (2019) Humboldt's enigma: what causes global patterns of mountain biodiversity? *Science*, 365, 1108–1113.

Read, D.J. & Perez-Moreno, J. (2003) Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist*, 157, 475–492.

Salazar, A., Rousk, K., Jónsdóttir, I.S., Bellenger, J.-P. & Andrésson, Ó.S. (2020) Faster nitrogen cycling and more fungal and root biomass in cold ecosystems under experimental warming: a meta-analysis. *Ecology*, 101, e02938.

Schiro, G., Chen, Y., Blankinship, J.C. & Barberán, A. (2022) Ride the dust: linking dust dispersal and spatial distribution of microorganisms across an arid landscape. *Environmental Microbiology*, 24, 4094–4107.

Seager, R., Ting, M., Held, I., Kushnir, Y., Lu, J., Vecchi, G. et al. (2007) Model projections of an imminent transition to a more arid climate in southwestern North America. *Science*, 316, 1181–1184.

Shade, A. (2017) Diversity is the question, not the answer. *The ISME Journal*, 11, 1–6.

Shen, C., Gunina, A., Luo, Y., Wang, J., He, J.-Z., Kuzyakov, Y. et al. (2020) Contrasting patterns and drivers of soil bacterial and fungal diversity across a mountain gradient. *Environmental Microbiology*, 22, 3287–3301.

Shreve, F. (1922) Conditions indirectly affecting vertical distribution on desert mountains. *Ecology*, 3, 269–274.

Singh, B.K., Bardgett, R.D., Smith, P. & Reay, D.S. (2010) Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nature Reviews Microbiology*, 8, 779–790.

Singh, D., Shi, L. & Adams, J.M. (2013) Bacterial diversity in the mountains of south-West China: climate dominates over soil parameters. *Journal of Microbiology*, 51, 439–447.

Spatafora, J.W., Aime, M.C., Grigoriev, I.V., Martin, F., Stajich, J.E. & Blackwell, M. (2017) The fungal tree of life: from molecular systematics to genome-scale phylogenies. *Microbiology Spectrum*, 5,FUNK-0053-2016. Available from: https://doi.org/10.1128/microbiolspec.funk-0053–2016

Stehfest, E., van Zeist, W.-J., Valin, H., Havlik, P., Popp, A., Kyle, P. et al. (2019) Key determinants of global land-use projections. *Nature Communications*, 10, 2166.

Steidinger, B.S., Bhatnagar, J.M., Vilgalys, R., Taylor, J.W., Qin, C., Zhu, K. et al. (2020) Ectomycorrhizal fungal diversity predicted to substantially decline due to climate changes in North American Pinaceae forests. *Journal of Biogeography*, 47, 772–782.

Steinbauer, M.J., Grytnes, J.-A., Jurasinski, G., Kulonen, A., Lenoir, J., Pauli, H. et al. (2018) Accelerated increase in plant species richness on mountain summits is linked to warming. *Nature*, 556, 231–234.

Székely, G.J. & Rizzo, M.L. (2013) The distance correlation t-test of independence in high dimension. *Journal of Multivariate Analysis*, 117, 193–213.

Taylor, D.L., Walters, W.A., Lennon, N.J., Bochicchio, J., Krohn, A., Caporaso, J.G. et al. (2016) Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for Illumina amplicon sequencing. *Applied and Environmental Microbiology*, 82, 7217–7226.

Tito, R., Vasconcelos, H.L. & Feeley, K.J. (2020) Mountain ecosystems as natural laboratories for climate change experiments. *Frontiers in Forests and Global Change*, 3, 38.

Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A. et al. (2015) Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. *mSystems*, 1, e00009–15.

Wang, J., Hu, A., Meng, F., Zhao, W., Yang, Y., Soininen, J. et al. (2022) Embracing mountain microbiome and ecosystem functions under global change. *New Phytologist*, 234, 1987–2002.

Wang, Q., Garrity, G.M., Tiedje, J.M. & Cole, J.R. (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, 73, 5261–5267.

Whittaker, R.H. & Niering, W.A. (1965) Vegetation of the Santa Catalina mountains, Arizona: a gradient analysis of the South slope. *Ecology*, 46, 429–452.

Whittaker, R.H. & Niering, W.A. (1968) Vegetation of the Santa Catalina mountains, Arizona: IV. Limestone and acid soils. *Journal of Ecology*, 56, 523–544.

Wilson, R.J., Gutiérrez, D., Gutiérrez, J., Martínez, D., Agudo, R. & Monserrat, V.J. (2005) Changes to the elevational limits and extent of species ranges associated with climate change. *Ecology Letters*, 8, 1138–1146.

Xu, M., Li, X., Cai, X., Gai, J., Li, X., Christie, P. et al. (2014) Soil microbial community structure and activity along a montane elevational gradient on the Tibetan plateau. *European Journal of Soil Biology*, 64, 6–14.

Yanahan, A.D. & Moore, W. (2019) Impacts of 21st-century climate change on montane habitat in the Madrean Sky Island Archipelago. *Diversity and Distributions*, 25, 1625–1638.

Zhang, L.-M., Wang, M., Prosser, J.I., Zheng, Y.-M. & He, J.-Z. (2009) Altitude ammonia-oxidizing bacteria and archaea in soils of Mount Everest. *FEMS Microbiology Ecology*, 70, 208–217.

Zinger, L., Bonin, A., Alsos, I.G., Bálint, M., Bik, H., Boyer, F. et al. (2019) DNA metabarcoding—need for robust experimental designs to draw sound ecological conclusions. *Molecular Ecology*, 28, 1857–1862.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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