Temperature drives plant and soil microbial diversity patterns across an elevation gradient from the Andes to the Amazon

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Summary

1. Climate strongly regulates plant community composition and diversity, exemplified by gradients in plant diversity and community structure with elevation. However, we do not know if soil bacteria and fungi, key drivers of terrestrial biogeochemical cycling, follow similar biogeographical patterns determined by the same climatic drivers.

2. We studied an Andean tropical forest transect traversing 3.5 km in elevation. The species richness ($\alpha$-diversity) and compositional dissimilarity of communities ($\beta$-diversity) were determined for plants, bacteria and fungi. We determined the environmental drivers of these patterns, using 31 environmental and edaphic predictor variables, and the relationship between microbial communities and soil organic matter cycling (extracellular enzymes).

3. We found co-ordinated changes with elevation in the species richness and composition of plants, soil bacteria and fungi. Across all groups, $\alpha$-diversity declined significantly as elevation increased, and $\beta$-diversity increased with increased elevation difference. Temperature was the dominant driver of these diversity gradients, with only weak influences of edaphic properties, including soil pH, which did not vary substantially across the study transect. The gradients in microbial diversity were strongly correlated with the activities of enzymes involved in soil organic matter cycling, and were accompanied by a transition in microbial traits, towards slower-growing, more oligotrophic taxa at higher elevations.

4. We provide the first evidence of co-ordinated temperature-driven patterns in the diversity and distribution of plants, soil bacteria and fungi in tropical ecosystems. This finding suggest that, across landscape scales of relatively constant soil pH, shared patterns and environmental drivers of plant and microbial communities can occur, with large implications for tropical forest communities under future climate change.
Introduction

Climate regulates plant community composition and diversity, exemplified by the existence of plant diversity and community structure changes with elevation along mountainsides – first reported in a classical 19th century study of the tropical Andes (von Humboldt and Bonpland 1805). However, we do not know if soil bacteria and fungi, key drivers of terrestrial biogeochemical cycling, follow similar biogeographical patterns determined by the same climatic drivers. Microbes are the most diverse and abundant organisms on Earth (Whitman et al. 1998) and perform vital metabolic processes including the decomposition of organic matter, recycling of nutrients, and formation of root symbioses, all of which generate feedbacks affecting the productivity and diversity of plants (Bardgett and van der Putten 2014). Given their small size, abundance and rapid life cycles relative to plants and animals, microorganisms were long-assumed to be cosmopolitan in their distributions (Baas Becking 1934). Recent work has challenged this paradigm, highlighting the importance of environmental filtering, historical events, stochastic speciation and dispersal processes in shaping microbial biogeography (Fierer and Jackson 2006, Martiny et al. 2006, Tedersoo et al. 2014). Relationships between plant and soil microbes are now starting to be revealed (Barberán et al. 2015, Prober et al. 2015), but important questions concerning their relationships over landscape gradients within the tropics remain open, especially for forests. As tropical rainforests are highly productive (Pan et al. 2011) and more species-rich than any other terrestrial biome (Pianka 1966), strong associations between plant and microbe communities are likely, potentially leading to co-ordinated changes in biota across climatic gradients.
The large temperature gradients on mountains have proven invaluable for understanding how climate influences plant diversity, community composition and productivity (Colwell et al. 2008). They can also help understand the influence of climate on the diversity and functional attributes of soil microbial communities (Bryant et al. 2008) and their role in soil organic matter cycling (Bardgett and van der Putten 2014). However, such studies are scarce (Bryant et al. 2008, Fierer et al. 2011, Singh et al. 2012, Shen et al. 2014), with the diversity and functional attributes of bacteria and fungi along elevation gradients in tropical forests especially poorly resolved. Shifts in the diversity of plant and animal taxa across mountainsides are thought to result principally from differences in energy limitation and/or niche differentiation, leading to a typically monotonic decrease or mid-elevation peak in above-ground species richness with elevation (Rahbek 2005). No coordinated shifts in plant and soil microbial diversity with elevation have yet been identified globally, although this may be a consequence of insufficient sampling intensity (Fierer et al. 2011) or confounding variation in rainfall and soil pH (Bryant et al. 2008, Shen et al. 2014).

In this study we address two fundamental but unresolved questions in tropical forest ecology. First, are biogeographical patterns in plant, bacterial and fungal species diversity ($\alpha$-diversity) and compositional dissimilarity of communities ($\beta$-diversity) related, and do they occur in response to the same drivers across large environmental gradients? Second, do shifts in diversity reflect differences in soil resource quality and organic matter cycling? We would expect the biogeographical patterns of plants and soil microbes to be related, as suggested by studies that associate microbial communities with plant leaf litter traits (Orwin et al. 2010, de Vries et al. 2012, Handa et al. 2014), and a strong relationship between plants and soil microbes has been hypothesised for tropical forests where there is wide inter-specific variation in leaf traits (Hattenschwiler et al. 2008). However, a relationship of this sort was not evident at local scale in a study of a single, albeit large, tropical forest plot (Barberán et
al. 2015), but the issue has not been investigated across tropical forest landscapes. Hence, relevant studies to date are partly contradictory, and although some work points towards the possibility of related biogeographical patterns among plant and microbial communities, strong evidence has been lacking. A global study of grasslands found relationships between plant, bacterial and fungal $\beta$-diversity, but not $\alpha$-diversity (Prober et al. 2015). Plant and fungal $\alpha$-diversity were positively related across a global latitudinal gradient, but this pattern was not observed for bacteria (Bardgett and van der Putten 2014, Tedersoo et al. 2014, Prober et al. 2015), possibly due to the wide variation in soil pH which influences biogeographical patterns in bacteria (Fierer and Jackson 2006).

We used a 3450 m tropical elevation gradient (~ 6.5 to 26.4 °C temperature gradient) in the Peruvian Andes, where variation in the important co-variants of soil pH and moisture was constrained (pH in organic horizons was 3.9 ± 0.1 (± one standard error) and in mineral horizons 4.0 ± 0.1; with no significant seasonal soil moisture limitation; Table S1). We sampled at relatively high density (22 sites in total, with soil data in separate horizons for 14 sites) significantly improving spatial resolution compared with previous studies of elevation gradients (Fierer et al. 2011, Shen et al. 2014).

Materials and Methods

Study sites and sample collection

The elevation transect under study lies on the Eastern flank of the Andes in South Eastern Peru, in the upper Madre de Dios/Madeira watershed. The transect spans 3.4 km in elevation from 194 to 3644 m above sea level (asl) and consists of 22 sites, each with a 1 ha
permanent sampling plot, in old growth tropical forest and one site on high elevation
grassland (soil properties and microbial diversity were determined for 14 sites; plant diversity
was determined for 19 sites; Table S1). Mean annual temperature (MAT) decreases with
increasing elevation (26 to 6 °C) but mean annual precipitation (MAP) does not vary
consistently with elevation, ranging from 1506-5302 mm yr⁻¹, with no evidence of soil
moisture constraints at any site. The sites are situated on predominantly Paleozoic (~450 Ma)
meta-sedimentary mudstone (~80%), with plutonic intrusions (granite) underlying the sites
between 1500 and 2020 m asl. The soils at the sites above 2520 m are Umbrisols
(Inceptisols), while the soils from 1000 to 2020 m are Cambisols (Inceptisols). The soils at
the two lowland sites are Haplic Allisols (Ultisols) (194 m asl) and Haplic Cambisols
(Inceptisols) (210 m asl) (according to FAO, with USDA Soil Taxonomy in parentheses).
Further descriptions of soil, climate and floristic composition of these sites are reported
elsewhere (Rapp et al. 2012, Whitaker et al. 2014). Trees were recorded in 19 of the 1 ha
plots, where every individual tree ≥ 10 cm diameter at breast height, 1.3 m (d.b.h.) was
measured, tagged and identified to species or morphospecies. Soil was collected during
January 2012 from five systematically distributed sampling points within 14 of the 1 ha plots.
These systems are highly aseasonal, with no significant variation in mean annual temperature
and no seasonal soil or plant moisture constraints (Zimmermann et al. 2010, van de Weg et
al. 2014), therefore the comparison of sites at a single time point was not confounded by
strong seasonality. We used composite soil samples representing spatial replication of three
for DNA extraction, or five spatial replicates for all other analyses. Although we sampled and
analysed soil in spatial replicates (within 1 ha), our treatment unit for all analyses is the plot
mean. Organic horizons and the surface 0-10 cm layer of mineral horizons were collected
separately. Soil samples were stored for < 14 days at < 4°C until DNA extraction and
determination of nutrients and enzyme activities, which has been shown to have negligible effect on these soil properties (Lauber et al. 2010, Turner and Romero 2010).

Soil analyses: DNA sequencing, nutrients and extracellular enzyme activities

For each soil sample, DNA was extracted using the MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA) following manufacturer instructions. For bacterial community composition, the 16S rRNA gene was amplified in triplicate PCR reactions using the 515f and 806r primers for bacterial and archaeal taxa. For fungal community composition, the first internal transcribed spacer region (ITS1) of the rRNA gene was amplified using the ITS1-F and ITS2 primer pair. Primers were modified to incorporate 12bp error-correcting barcodes, and 16S rRNA amplicons and ITS amplicons were pooled separately prior to sequencing with two separate runs on an Illumina MiSeq instrument at the University of Colorado at Boulder. Raw sequence data were processed using the QIIME v1.7 pipeline, where sequences were demultiplexed using their unique barcode specific to individual samples and assigned to phylotypes (at 97% similarity) using the 'open reference' clustering approach recommended in the pipeline. Samples were rarefied to 1,850 and 100 sequences per sample for bacteria/archaea and fungi, respectively. The lower rarefaction depth for fungi, which was used to increase sample number and statistical power for the ecological analyses, but was sufficient to characterise diversity and community composition characteristics; and these diversity patterns were highly correlated to those found when using higher rarefaction depth (300; \( \rho = 0.98 \)). Representative sequences for each phylotype were assigned taxonomic classifications using the Ribosomal Database Project classifier trained on the Greengenes and UNITE databases for 16S rRNA and ITS phylotypes, respectively.
Relatively abundant phylotypes were checked using BLAST and comparison against sequences contained within GenBank.

Total C and N were determined for dried, ground soil samples using a TruSpec CN Elemental Determinator (LECO, USA). Total P was determined by ignition (550°C, 1 h) followed by extraction in 1 M H₂SO₄, with phosphate detection in neutralised extracts at 880 nm by automated molybdate colorimetry using a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO, USA). Mineral N and P availability were determined using ion exchange resins (Nottingham et al. 2015). Other organic and inorganic phosphorus fractions were determined using a modification of Hedley sequential extraction (in 1M NaOH, 1M HCl) (Hedley et al. 1982) and exchangeable cations extracted in 0.1 M BaCl (Hendershot and Duquette 1986). Soil pH was determined in H₂O (soil solution, 1:2.5 w:v). Gravimetric moisture content, bulk density (dried for 24 h at 105 °C) and water holding capacity (the amount of water remaining in the soil after being saturated and left to drain for 12 h) were calculated for composite soil samples for each site.

Enzyme activities for seven enzymes involved in carbon and nutrient cycling were determined for 14 study sites, using microplate fluorimetric assays with 100 µM methylumbelliferone (MU)-linked substrates to measure activity of β-glucosidase (degradation of β-bonds in glucose), cellobiohydrolase (degradation of cellulose), N-acetyl β-glucosaminidase (degradation of N-glycosidic bonds), phosphomonoesterase (degradation of monoester-linked simple organic phosphates) and β-xylanase (degradation of hemicellulose). Phenol oxidase (degradation of phenolic compounds) was measured using 5 mM L-dihydroxyphenylalanine (L-DOPA) as substrate. Further information on protocols for enzyme analyses is reported elsewhere (Nottingham et al. 2015).
Statistical analyses

We determined species richness (α-diversity) using Shannon diversity index, according to total species abundance for plants or OTUs for soil bacteria and fungi.

Community composition (β-diversity) was determined using Sorrenson indices for plants and Bray Curtis dissimilarity matrices for soil bacteria and fungi. We tested whether patterns in bacterial and fungal diversity and community composition were explained by biotic interactions with plant communities, by using Spearman’s correlation (α-diversity) and Mantel tests (β-diversity) among biotic groups and comparing the relationships between plants and microbes in organic and mineral soil horizons. Permutational MANOVA and Principal Co-ordinates Analyses to explore differences in β-diversity with elevation or soil horizon. The environmental or edaphic drivers of these patterns in plant, bacterial or fungal diversity and community composition were determined univariate and multivariate correlation. Multivariate correlation was performed using the BEST trend correlation function (Primer; version 6.1.12), to show how multivariate biotic data are shaped by 31 predictor variables including soil nutrients (total, organic, exchangeable), soil micronutrients, soil enzymes (obtained by fluorogenic or absorption assays), and climatic and soil abiotic properties. We quantified the functional consequences of changes in soil microbial β-diversity by using Mantel tests of bacterial and fungal community composition and enzymatic activity for seven enzymes (obtained by fluorogenic or absorption assays). For all analyses, we only included sites for which we have combined plant, microbial, soil edaphic and environmental data. The combined methodology allowed us to 1) determine whether diversity patterns in plants, bacteria and fungi are co-related; 2) identify the β-diversity principle environmental or edaphic drivers of these patterns and 3) test whether the diversity and community composition of soil microorganisms influence soil processes along a tropical
environmental gradient. All statistical analyses were performed in either Primer (version 6.1.12) or R (version 2.15.2).

Results

The \( \alpha \)-diversity of plants, soil bacteria and fungi declined as elevation increased along our study transect (Fig. 1). The \( \alpha \)-diversity of plants declined most steeply, followed by bacteria in organic horizons and fungi in mineral horizons. The decline in diversity was linear for plants and fungi, but non-linear for bacteria. Bacterial \( \alpha \)-diversity declined more steeply at higher elevations, especially in the mineral horizon, whereas the linear decline in fungal \( \alpha \)-diversity with elevation was partly driven by the high fungal diversity in the two lowland forest sites. There was a stronger coupling of plant and bacterial diversity compared to plant and fungal diversity (Fig. 2). Mean annual temperature (MAT) was the strongest predictor of the patterns in \( \alpha \)-diversity for plants and bacteria in both soil horizons and for fungi in the mineral horizon (Table S2).

The composition of plant, bacterial and fungal communities also differed with elevation (Figs. 3 & S1), and between organic and mineral horizons for soil microorganisms (Fig. S2), although fungi differed to a lesser extent between horizons than bacteria. For bacteria, increased elevation was associated with an increased dominance of Acidobacteria and Betaproteobacteria, and decreased dominance of Actinobacteria and Deltaproteobacteria; the patterns occurred in both horizons, although mineral horizons contained a greater proportion of Acidobacteria and Archaea (Fig. 3). For fungi, increased elevation was associated with increased dominance of Ascomycota (Archaeorhizomycetes, Leotiomycetes), Basidiomycota (Microbotryomycetes), and decreased dominance of other Ascomycota (Sodariomycetes, Dothideomycetes, Eurotiomycetes), Glomeromycota and...
Zygomycota (Fig. 3; Table S3). Bacteria exhibited the largest compositional dissimilarities of communities with elevation (β-diversity) followed by plant and then fungal communities, and the β-diversity patterns observed for bacteria and fungi were correlated with those observed for plants (Fig. 4). Thus, plants and several major taxonomic groups of both bacteria and fungi showed clear changes in composition with elevation, suggesting shared environmental drivers in community structure.

As with α-diversity, MAT was the strongest correlate of patterns in β-diversity. MAT was the most significant parameter in multivariate models for β-diversity of plants, bacteria in both organic and mineral horizons, and fungi in mineral horizons (Table 1). There were additional significant correlations between the β-diversity of bacteria and fungi, and organic nutrient concentrations and their ratios; these were stronger in the organic compared to mineral horizons (Fig. S4). Nutrients other than nitrogen and phosphorus also influenced β-diversity, including potassium for plants and sodium for bacteria (Table 1). Soil pH was correlated with bacterial β-diversity but not fungal β-diversity.

To assess whether soil microbial distributions were related to differences in rates of organic matter cycling, we determined the activities of seven enzymes involved in the degradation of different organic compounds. The activity of the different soil enzymes decreased with increased elevation but at different rates, and independently of differences in ambient temperature (Fig. S5). These patterns reflected responses in the microbial community to shifts in substrate availability. For example, relative microbial investment into different enzymes shifted with increased elevation, from enzymes that degrade phosphorus- to nitrogen– containing organic compounds (Nottingham et al. 2015). Strong relationships between the differential activity of these seven enzymes and differences in β-diversity were found for bacteria (ρ = 0.75) and fungi (ρ = 0.74) in organic horizons (Fig. S6). Together
these findings suggest that, in addition to temperature, differences in organic nutrient cycling are related to the β-diversity of bacteria and fungi in organic soil horizons.

Discussion

Overall, our results demonstrate a fundamental role for environment, principally temperature, in co-ordinating the diversity and community composition of plants, soil bacteria and fungi. Although environmental filtering at large geographic scales has been suggested to shape community composition for plants and soil bacteria and fungi independently (Tedersoo et al. 2014), this has not been observed for both diversity (species richness) and community composition across all three biotic groups. Evidence for this environmental filtering comes from both multivariate models and correlations between distance matrices, where temperature and, to a lesser extent, organic nutrient concentrations, were strongly associated with variation in bacterial and fungal α- and β-diversity (Table 1).

The role of temperature in determining microbial β-diversity is also illustrated by an increased relative abundance of Acidobacteria and the fungi Archaerhizomycetes with increased elevation, but a decreased relative abundance of Actinobacteria and Alphaproteobacteria (Fig. 3). These major taxonomic groups have been associated with oligotrophic (Acidobacteria, Archaerhizomycetes) and copiotrophic (Actinobacteria, Alphaproteobacteria) life history strategies, respectively (Fierer et al. 2007, Rosling et al. 2011), which is consistent with evidence for increased energy limitation of microbial activity at higher elevations, favoring slower growth (Nottingham et al. 2015). The strong correlations between the β-diversity of plants, soil bacteria and fungi (Fig. 4) further indicated that similar environmental factors, primarily temperature (Table 1), drive these patterns across the three biotic groups. The high relative abundance of the Ascomycota,
Archaerhizomycetes at higher elevations (Fig. 3) is of particular interest because this class of fungi was identified only very recently, in tundra soils (Schadt et al. 2003) and their global distribution is poorly understood because many previous analyses failed to identify them because of amplification biases (Rosling et al. 2011). They are understood to be typically oligotrophic and root-associated fungi, colonizing typical ectomycorrhizal habitats beneath pine and ericaceous plants (Rosling et al. 2011), which is consistent with our current understanding of tropical montane forest habitats being energy-limited (Bruijnzeel et al. 2011). This important class of fungi, which until very recently was unknown, dominate the fungal biomass in these tropical montane forests.

Temperature was also the principal correlate of plant $\beta$-diversity (Table 1). Temperature has previously been shown to be a major determinant of tree community composition across this transect (Rapp et al. 2012) and up-slope movement of tree species’ ranges has been observed under recent climatic warming (Duque et al. 2015). Patterns in plant species composition and richness on tropical mountains are thought to be driven mainly by the effect of narrow temperature ranges on niche separation (by directly affecting metabolism and indirectly affecting resource availability), while further constrained by land area, lithology and disturbance history (Janzen 1967, Colwell et al. 2008). Although the high landslide activity and soil erosion in the humid Eastern Andean Cordillera (Clark et al. 2013) may be significant factors in constraining diversity at higher elevations in this region, our study identifies a central underlying role for temperature. Analogous observations have been made along latitudinal gradients where plant/fungal species richness ratios decrease with distance from the equator (Tedersoo et al. 2014). Our data indicate a stronger coupling between the $\alpha$-diversity of plants and bacteria compared to fungi (Fig. 2), but a stronger coupling between the $\beta$-diversity of plants and fungi as compared to bacteria (Fig. 4).
There was a secondary role for edaphic properties in shaping these diversity patterns, with direct influences of nutrient ratios on microbial $\beta$-diversity and potassium on plant $\beta$-diversity (Table 1). Our data also suggest that this role of edaphic processes on microbial $\alpha$- and $\beta$-diversity is more significant in organic horizons, where the community-wide differences in plant litter traits is largest (Hattenschwiler et al. 2008). Multiple lines of evidence suggest an influence of plant organic matter inputs on soil microbes: (i) the large difference in microbial diversity between organic and mineral soil horizons (Figs. S1 & S2); (ii) the existence of stronger correlations between microbial $\beta$-diversity and soil organic nutrients in organic horizons compared to mineral horizons (Table 1; Fig. S3); (iii) the overall strong correlation between plant and soil microbial diversity (Figs. 2 & 4); and (iv) the correlation between soil microbial $\beta$-diversity and the dissimilarity among sites in enzymatic activity, indices of organic nutrient degradation (Nottingham et al. 2015) (Fig. S6).

Laboratory incubations of soils from this transect also support the link between differences in microbial community composition and the rate at which different organic substrates undergo degradation (Whitaker et al. 2014). Together these findings point towards a relationship between the high soil microbial diversity in lowland forests and the diverse complexity and stoichiometry of plant organic matter inputs to soil, through the high inter- and intra-species chemical diversity in leaf-litter (Hattenschwiler et al. 2008).

Our results, from a 3450 m elevation range, contrast with findings from studies of other elevation gradients that only examined plant and bacterial diversity, and have not found such strongly-related diversity gradients. The fundamental climate-diversity relationships we observed here were probably obscured in previous studies because of insufficient sampling density and wider variation in soil pH and soil moisture. For example, the importance of sampling intensity is demonstrated by the contrast between findings from this study of 14 sites with an earlier report from 6 locations along the same Andean transect where no
elevation gradient in soil bacterial diversity was found (Fierer et al. 2011): if we reduce our
dataset to include only those sites represented in the earlier study, no strong elevation trends
are apparent (Fig S6). While the importance of soil pH and rainfall variation in determining
patterns in bacterial diversity was demonstrated for a 1850 m elevation gradient in South
Korea (Singh et al. 2014). Similarly, these factors also likely accounted for the lack of clear
patterns for two temperate zone elevation transect studies which sampled six locations over
1670 m in Northeast China (Shen et al. 2014) and five locations over 920 m in the Rocky
mountains, the latter indicating a small single-taxon increase with elevation, but no
community-wide trend (Bryant et al. 2008).

This elevation gradient study in the Peruvian Andes demonstrates how temperature
fundamentally shapes plant, bacterial and fungal diversity in tropical forests. Consistent
trends in both α- and β-diversity were observed across the principal organismal groups of
plants, bacteria and fungi, also suggesting that stronger interactions occur among these
groups than has been recognised previously. Our findings imply that, where other potential
influences such as soil pH and moisture remain relatively constrained, anticipated future
temperature change will have significant co-ordinated impacts on the identity and functioning
(above- and below-ground) of tropical biota.

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References

Baas Becking, L. G. M. 1934. Geobiologie of inleiding tot de milieukunde. W. P. Van Stockum & Zoon, The Hague, the Netherlands.

Barberán, A., K. L. McGuire, J. A. Wolf, F. A. Jones, S. J. Wright, B. L. Turner, A. Essene, S. P. Hubbell, B. C. Faircloth, and N. Fierer. 2015. Relating belowground microbial composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest. Ecology letters 18:1397-1405.

Bardgett, R. D., and W. H. van der Putten. 2014. Belowground biodiversity and ecosystem functioning. Nature 515:505-511.

Bruijnzeel, L. A., F. N. Scatena, and L. S. Hamilton. 2011. Tropical Montane Cloud Forests. Cambridge University Press, Cambridge, UK.

Bryant, J. A., C. Lamanna, H. Morlon, A. J. Kerkhoff, B. J. Enquist, and J. L. Green. 2008. Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity. Proceedings of the National Academy of Sciences of the United States of America 105:11505-11511.

Clark, K. E., R. G. Hilton, A. J. West, Y. Malhi, D. R. Gröcke, C. L. Bryant, P. L. Ascough, A. Robles Caceres, and M. New. 2013. New views on “old” carbon in the Amazon River: Insight from the source of organic carbon eroded from the Peruvian Andes. Geochemistry, Geophysics, Geosystems 14:1644-1659.

Colwell, R. K., G. Brehm, C. L. Cardelus, A. C. Gilman, and J. T. Longino. 2008. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. Science 322:258-261.

de Vries, F. T., P. Manning, J. R. Tallowin, S. R. Mortimer, E. S. Pilgrim, K. A. Harrison, P. J. Hobbs, H. Quirk, B. Shipley, J. H. Cornelissen, J. Kattge, R. D. Bardgett, and N. Johnson. 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecology letters 15:1230-1239.

Duque, A., P. R. Stevenson, and K. J. Feeley. 2015. Thermophilization of adult and juvenile tree communities in the northern tropical Andes. Proceedings of the National Academy of Sciences of the United States of America 112:10744-10749.

Fierer, N., M. A. Bradford, and R. B. Jackson. 2007. Toward an ecological classification of soil bacteria. Ecology 88:1354-1364.

Fierer, N., and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 103:626-631.

Fierer, N., C. M. McCain, P. Meir, M. Zimmermann, J. M. Rapp, M. R. Silman, and R. Knight. 2011. Microbes do not follow the elevational diversity patterns of plants and animals. Ecology 92:797-804.
Handa, I. T., R. Aerts, F. Berendse, M. P. Berg, A. Bruder, O. Butenschoen, E. Chauvet, M. O. Gessner, J. Jabiol, M. Makkonen, B. G. McKie, B. Malmqvist, E. T. H. M. Peeters, S. Scheu, B. Schmid, J. van Ruijven, V. C. A. Vos, and S. Hattenschwiler. 2014. Consequences of biodiversity loss for litter decomposition across biomes. Nature 509:218-221.

Hattenschwiler, S., B. Aeschlimann, M. M. Couteaux, J. Roy, and D. Bonal. 2008. High variation in foliage and leaf litter chemistry among 45 tree species of a neotropical rainforest community. The New phytologist 179:165-175.

Hedley, M. J., J. W. B. Stewart, and B. S. Chauhan. 1982. Changes in Inorganic and Organic Soil-Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations. Soil Science Society of America Journal 46:970-976.

Hendershot, W. H., and M. Duquette. 1986. A Simple Barium-Chloride Method for Determining Cation-Exchange Capacity and Exchangeable Cations. Soil Science Society of America Journal 50:605-608.

Janzen, D. H. 1967. Why Mountain Passes Are Higher in Tropics. American Naturalist 101:233-8.

Lauber, C. L., N. Zhou, J. I. Gordon, R. Knight, and N. Fierer. 2010. Effect of storage conditions on the assessment of bacterial community structure in soil and human-associated samples. Fems Microbiology Letters 307:80-86.

Martiny, J. B. H., B. J. M. Bohannan, J. H. Brown, R. K. Colwell, J. A. Fuhrman, J. L. Green, M. C. Horner-Devine, M. Kane, J. A. Krumins, C. R. Naeem, L. Ovreas, A. L. Riesenberg, V. H. Smith, and J. T. Staley. 2006. Microbial biogeography: putting microorganisms on the map. Nature Reviews Microbiology 4:102-112.

Nottingham, A. T., B. L. Turner, J. Whitaker, N. Ostle, N. P. McNamara, R. D. Bardgett, N. Salinas, and P. Meir. 2015. Soil microbial nutrient constraints along a tropical forest elevation gradient: a belowground test of a biogeochemical paradigm. Biogeosciences 12:6489-6523.

Orwin, K. H., S. M. Buckland, D. Johnson, B. L. Turner, S. Smart, S. Oakley, and R. D. Bardgett. 2010. Linkages of plant traits to soil properties and the functioning of temperate grassland. Journal of Ecology 98:1074-1083.

Pan, Y., R. A. Birdsey, J. Fang, R. Houghton, P. E. Kauppi, W. A. Kurz, O. L. Phillips, A. Shvidenko, S. L. Lewis, J. G. Canadell, P. Ciais, R. B. Jackson, S. W. Pacala, A. D. McGuire, S. Piao, A. Rautiainen, S. Sitch, and D. Hayes. 2011. A large and persistent carbon sink in the world’s forests. Science 333:988-993.

Pianka, E. R. 1966. Latitudinal Gradients in Species Diversity - a Review of Concepts. American Naturalist 100:33-8.

Prober, S. M., J. W. Leff, S. T. Bates, E. T. Borer, J. Firn, W. S. Harpole, E. M. Lind, E. W. Seabloom, P. B. Adler, J. D. Bakker, E. E. Cleland, N. M. DeCrappeo, E. DeLorenze, N. Houtier, K. S. Hofmockel, K. P. Kirby, M. H. Knops, K. J. LaPier, A. S. MacDougall, R. L. McCulley, C. E. Mitchell, A. C. Risch, M. Schuetz, C. J. Stevens, R. J. Williams, and N. Fierer. 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. Ecology letters 18:85-95.

Rahbek, C. 2005. The role of spatial scale and the perception of large-scale species-richness patterns. Ecology letters 8:224-239.

Rapp, J. M., M. R. Silman, J. S. Clark, C. A. J. Girardin, D. Galiano, and R. Tito. 2012. Interspecific variation in interspecific tree growth across a long altitudinal gradient in the Peruvian Andes. Ecology 93:2061-2072.

Rosling, A., F. Cox, K. Cruz-Martinez, K. Ihrmark, G. A. Grelet, B. D. Lindahl, A. Menkis, and T. Y. James. 2011. Archaeorhizomycetes: Unearting an Ancient Class of Ubiquitous Soil Fungi. Science 333:876-879.

Schadt, C. W., A. P. Martin, D. A. Lipson, and S. K. Schmidt. 2003. Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301:1359-1361.
Shen, C. C., W. J. Liang, Y. Shi, X. G. Lin, H. Y. Zhang, X. Wu, G. Xie, P. Chain, P. Grogan, and H. Y. Chu. 2014. Contrasting elevational diversity patterns between eukaryotic soil microbes and plants. Ecology 95:3190-3202.

Singh, D., L. Lee-Cruz, W. S. Kim, D. Kerfahi, J. H. Chun, and J. M. Adams. 2014. Strong elevational trends in soil bacterial community composition on Mt. Halla, South Korea. Soil Biology & Biochemistry 68:140-149.

Singh, D., K. Takahashi, M. Kim, J. Chun, and J. M. Adams. 2012. A Hump-Backed Trend in Bacterial Diversity with Elevation on Mount Fuji, Japan. Microbial Ecology 63:429-437.

Tedersoo, L., M. Bahram, S. Polme, U. Koljalg, N. S. Yorou, R. Wijesundera, L. V. Ruiz, A. M. Vasco-Palacios, P. Q. Thu, A. Suija, M. E. Smith, C. Sharp, E. Saluveer, A. Saitta, M. Rosas, T. Riit, D. Ratkowsky, K. Pritsch, K. Poldmaa, M. Piepenbring, C. Phosri, M. Peterson, K. Parts, K. Partel, E. Ötsing, E. Nouhra, A. L. Njouonkou, R. H. Nilsson, L. N. Mordago, J. Mayor, T. W. May, L. Majuakim, D. J. Lodge, S. S. Lee, K. H. Larsson, P. Kohout, K. Hosaka, I. Hiiesalu, T. W. Henkel, H. Harend, L. D. Guo, A. Greslebin, G. Grelet, J. Geml, G. Gates, W. Dunstan, C. Dunk, R. Drenkhan, J. Dearnaley, A. De Kesel, T. Dang, X. Chen, F. Buegger, F. Q. Brearley, G. Bonito, S. Anslan, S. Abell, and K. Abarenkov. 2014. Global diversity and geography of soil fungi. Science 346:1078.

Turner, B. L., and T. E. Romero. 2010. Stability of hydrolytic enzyme activity and microbial phosphorus during storage of tropical rain forest soils. Soil Biology and Biochemistry 42:459-465.

van de Weg, M. J., P. Meir, M. Williams, C. Girardin, Y. Malhi, J. Silva-Espejo, and J. Grace. 2014. Gross primary productivity of a high elevation tropical montane cloud forest. Ecosystems 17:751-764.

von Humboldt, A., and A. Bonpland. 1805. Essai sur la géographie des plantes. Chez Levrault, Scoell et Campagnie, Librarie, Paris.

Whitaker, J., N. Ostle, A. T. Nottingham, A. Ccahuana, N. Salinas, R. D. Bardgett, P. Meir, and N. P. McNamara. 2014. Microbial community composition explains soil respiration responses to changing carbon inputs along an Andes-to-Amazon elevation gradient. Journal of Ecology 102:1058-1071.

Whitman, W. B., D. C. Coleman, and W. J. Wiebe. 1998. Prokaryotes: The unseen majority. Proceedings of the National Academy of Sciences of the United States of America 95:6578-6583.

Zimmermann, M., P. Meir, M. I. Bird, Y. Malhi, and A. J. Q. Ccahuana. 2010. Temporal variation and climate dependence of soil respiration and its components along a 3000 m altitudinal tropical forest gradient. Global Biogeochemical Cycles 24:GB4012.

Figure legends
Figure 1. The α-diversity (Shannon diversity index) of bacteria and fungi declined with increased elevation in Andean tropical forests. There were significant relationships between elevation and the α-diversity of plants, and each of bacteria and fungi in both organic and mineral soils. Models were selected based on AIC values; linear models were the best fit in all cases except for logistic models for bacteria (where $p < 0.001$).

Figure 2. The relationships between the ratios of plant to bacterial and plant to fungal α-diversity and elevation, in organic and mineral soil horizons. Regression lines are shown with increasing number of dashes for bacterial mineral (solid line), bacterial organic, fungal mineral and fungal organic (shortest dashes). The stronger coupling of plant and bacterial diversity (Spearman’s correlation: $\rho = 0.76, 0.70$; organic and mineral horizons, respectively) compared to plant and fungal diversity ($\rho = 0.14, 0.64$), was further reflected in a greater decline with elevation for the species richness ratio of plants-to-fungi (slope of 1.02) compared to plants-to-bacteria (slope of 0.59).

Figure 3. The (A) positive and (B) negative trends in the relative abundances of specific bacterial and fungal taxa in soils along an elevation gradient in Andean tropical forest. Bacteria taxa are Acidobacteria, Actinobacteria, Beta-Proteobacteria and Delta-Proteobacteria; and fungal taxa are Actinomycetes, Sordariomycetes and Archaeorhizomycetes (by phyla or, where italicised, by class). All data are for organic horizons except for Acidobacteria, which is for mineral horizon. The full data for all taxa in organic and mineral horizons (which follow similar trends) are in Fig. S1 and Table S3 (Fig. S1 also shows dissimilarity of communities among sites using heat-maps and Table S3 shows correlations between relative abundance of taxa and elevation).
Figure 4. Elevation comparisons of indices of plant β-diversity, bacteria and fungal β-
diversity in organic horizons. The overall decline with increased elevation indicates
increased dissimilarity in β-diversity between sites with greater difference in elevation. β-
diversity was determined by Sorensen’s indices for plants, and by Bray Curtis indices for
bacteria and fungi. Patterns in β-diversity were correlated between plants and bacteria
(organic horizon $\rho = 0.81$; mineral horizon $\rho = 0.88$) and plants and fungi (organic horizon $\rho$
= 0.67; mineral horizon $\rho = 0.79$; by Mantel tests; $p < 0.001$ for all comparisons
Table 1

The relationship between plant, bacterial and fungal $\beta$-diversity and environmental and edaphic variables.

|                | Plants Organic horizon | Bacteria Organic horizon | Fungi Organic horizon | Bacteria Mineral horizon | Fungi Mineral horizon |
|----------------|------------------------|--------------------------|-----------------------|-------------------------|----------------------|
| MAT            | *** (0.91)             | *** (0.77)               | * (0.67)              | *** (0.88)              | *** (0.59)           |
| Soil pH        | ns                     | ns                       | ns                    | ns                      | ns                   |
| Total C:N      | ns                     | ** (0.68)                | ns                    | ns                      | ** (0.38)           |
| Total C:P      | ns                     | ns                       | ns                    | ns                      | ns                   |
| Na             | ns                     | ns                       | ns                    | ns                      | ns                   |
| K              | ns                     | ns                       | ns                    | ns                      | ns                   |
| N-acetyl $\beta$-glucosaminidase | ns | ns | *** (0.74) | ns | ns |
| Complete model | 0.93                   | 0.88                     | 0.80                  | 0.91                    | 0.65                 |

Models were determined using step-wise selection based on dissimilarity between variables by Euclidean distance (biotic and environmental matching function in Primer). A total of 31 properties were included in models for plants and bacteria and fungi in organic soils (25 for bacteria and fungi in mineral soils), including MAP, soil pH, total soil nutrients (CNP) and their ratios, available soil nutrients, soil phosphorus fractions, cations, cation exchange capacity; and the activities of seven enzymes in organic horizons. For plants, organic horizon soil properties were included in analyses (when we included mineral soil data the model included MAT (0.91) and Fe (0.16). Variables are ranked in order of significance by number of asterisks; the correlation coefficients (Mantel tests) for each individual variable are shown in parentheses.
