

1

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

4

5 Andrew T. Nottingham¹, Noah Fierer², Benjamin L. Turner³, Jeanette Whitaker⁴, Nick J.

6 Ostle⁵, Niall P. McNamara⁴, Richard D. Bardgett⁶, Jonathan W. Leff², Norma Salinas^{7,8}, Adan

7 J. Q. Ccahuana⁸, Miles Silman⁹ & Patrick Meir^{1,10}

8 ¹*School of Geosciences, University of Edinburgh, Crew Building, Kings Buildings, Edinburgh EH9 3FF, UK*

9 ²*Department of Ecology and Evolutionary Biology, Cooperative Institute for Research in Environmental Sciences, University*
10 *of Colorado, Boulder, CO, USA*

11 ³*Smithsonian Tropical Research Institute, 0843-03092, Balboa, Ancon, Republic of Panama*

12 ⁴*Centre of Ecology and Hydrology, Lancaster Environment Centre, Lancaster LA1 4AP, UK*

13 ⁵*Lancaster Environment Centre, Lancaster University, Library Avenue, Lancaster LA1 4YQ, UK*

14 ⁶*School of Earth and Environmental Sciences, Michael Smith Building, The University of Manchester, Oxford Road,*
15 *Manchester M13 9PT, UK*

16 ⁷*Seccion Química, Pontificia Universidad Católica del Peru, Lima, Peru*

17 ⁸*Universidad Nacional de San Antonio Abad del Cusco, Facultad de Biología, Cusco, Peru*

18 ⁹*Wake Forest University, Winston-Salem, NC, USA*

19 ¹⁰*Research School of Biology, Australian National University, Canberra, ACT 0200, Australia*

20 *To whom correspondence should be addressed:

21 Andrew Nottingham, School of Geosciences, University of Edinburgh, Drummond Street, Edinburgh EH8 9XP, UK. email:

22 anotting@staffmail.ed.ac.uk Tel: +44 (0) 131 651 4314 ; Fax: +44 (0) 131 650 2524

23 **Keywords:**

24 biogeography, microbial ecology, plant ecology, plant-soil (below-ground) interactions, Peru, phylogenetic diversity,

25 tropical forests

26 **Summary**

- 27 1. Climate strongly regulates plant community composition and diversity, exemplified by
28 gradients in plant diversity and community structure with elevation. However, we do not
29 know if soil bacteria and fungi, key drivers of terrestrial biogeochemical cycling, follow
30 similar biogeographical patterns determined by the same climatic drivers.
- 31 2. We studied an Andean tropical forest transect traversing 3.5 km in elevation. The species
32 richness (α -diversity) and compositional dissimilarity of communities (β -diversity) were
33 determined for plants, bacteria and fungi. We determined the environmental drivers of these
34 patterns, using 31 environmental and edaphic predictor variables, and the relationship
35 between microbial communities and soil organic matter cycling (extracellular enzymes).
- 36 3. We found co-ordinated changes with elevation in the species richness and composition of
37 plants, soil bacteria and fungi. Across all groups, α -diversity declined significantly as
38 elevation increased, and β -diversity increased with increased elevation difference.
39 Temperature was the dominant driver of these diversity gradients, with only weak influences
40 of edaphic properties, including soil pH, which did not vary substantially across the study
41 transect. The gradients in microbial diversity were strongly correlated with the activities of
42 enzymes involved in soil organic matter cycling, and were accompanied by a transition in
43 microbial traits, towards slower-growing, more oligotrophic taxa at higher elevations.
- 44 4. We provide the first evidence of co-ordinated temperature-driven patterns in the diversity and
45 distribution of plants, soil bacteria and fungi in tropical ecosystems. This finding suggest that,
46 across landscape scales of relatively constant soil pH, shared patterns and environmental
47 drivers of plant and microbial communities can occur, with large implications for tropical
48 forest communities under future climate change.

49

50

51 **Introduction**

52

53 Climate regulates plant community composition and diversity, exemplified by the
54 existence of plant diversity and community structure changes with elevation along
55 mountainsides – first reported in a classical 19th century study of the tropical Andes (von
56 Humboldt and Bonpland 1805). However, we do not know if soil bacteria and fungi, key
57 drivers of terrestrial biogeochemical cycling, follow similar biogeographical patterns
58 determined by the same climatic drivers. Microbes are the most diverse and abundant
59 organisms on Earth (Whitman et al. 1998) and perform vital metabolic processes including
60 the decomposition of organic matter, recycling of nutrients, and formation of root symbioses,
61 all of which generate feedbacks affecting the productivity and diversity of plants (Bardgett
62 and van der Putten 2014). Given their small size, abundance and rapid life cycles relative to
63 plants and animals, microorganisms were long-assumed to be cosmopolitan in their
64 distributions (Baas Becking 1934). Recent work has challenged this paradigm, highlighting
65 the importance of environmental filtering, historical events, stochastic speciation and
66 dispersal processes in shaping microbial biogeography (Fierer and Jackson 2006, Martiny et
67 al. 2006, Tedersoo et al. 2014). Relationships between plant and soil microbes are now
68 starting to be revealed (Barberán et al. 2015, Prober et al. 2015), but important questions
69 concerning their relationships over landscape gradients within the tropics remain open,
70 especially for forests. As tropical rainforests are highly productive (Pan et al. 2011) and more
71 species-rich than any other terrestrial biome (Pianka 1966), strong associations between plant
72 and microbe communities are likely, potentially leading to co-ordinated changes in biota
73 across climatic gradients.

74 The large temperature gradients on mountains have proven invaluable for
75 understanding how climate influences plant diversity, community composition and
76 productivity (Colwell et al. 2008). They can also help understand the influence of climate on
77 the diversity and functional attributes of soil microbial communities (Bryant et al. 2008) and
78 their role in soil organic matter cycling (Bardgett and van der Putten 2014). However, such
79 studies are scarce (Bryant et al. 2008, Fierer et al. 2011, Singh et al. 2012, Shen et al. 2014),
80 with the diversity and functional attributes of bacteria and fungi along elevation gradients in
81 tropical forests especially poorly resolved. Shifts in the diversity of plant and animal taxa
82 across mountainsides are thought to result principally from differences in energy limitation
83 and/or niche differentiation, leading to a typically monotonic decrease or mid-elevation peak
84 in above-ground species richness with elevation (Rahbek 2005). No coordinated shifts in
85 plant and soil microbial diversity with elevation have yet been identified globally, although
86 this may be a consequence of insufficient sampling intensity (Fierer et al. 2011) or
87 confounding variation in rainfall and soil pH (Bryant et al. 2008, Shen et al. 2014).

88 In this study we address two fundamental but unresolved questions in tropical forest
89 ecology. First, are biogeographical patterns in plant, bacterial and fungal species diversity (α -
90 diversity) and compositional dissimilarity of communities (β -diversity) related, and do they
91 occur in response to the same drivers across large environmental gradients? Second, do shifts
92 in diversity reflect differences in soil resource quality and organic matter cycling? We would
93 expect the biogeographical patterns of plants and soil microbes to be related, as suggested by
94 studies that associate microbial communities with plant leaf litter traits (Orwin et al. 2010, de
95 Vries et al. 2012, Handa et al. 2014), and a strong relationship between plants and soil
96 microbes has been hypothesised for tropical forests where there is wide inter-specific
97 variation in leaf traits (Hattenschwiler et al. 2008). However, a relationship of this sort was
98 not evident at local scale in a study of a single, albeit large, tropical forest plot (Barberán et

99 al. 2015), but the issue has not been investigated across tropical forest landscapes. Hence,
100 relevant studies to date are partly contradictory, and although some work points towards the
101 possibility of related biogeographical patterns among plant and microbial communities,
102 strong evidence has been lacking. A global study of grasslands found relationships between
103 plant, bacterial and fungal β -diversity, but not α -diversity (Prober et al. 2015). Plant and
104 fungal α -diversity were positively related across a global latitudinal gradient, but this pattern
105 was not observed for bacteria (Bardgett and van der Putten 2014, Tedersoo et al. 2014, Prober
106 et al. 2015), possibly due to the wide variation in soil pH which influences biogeographical
107 patterns in bacteria (Fierer and Jackson 2006).

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

115

116 **Materials and Methods**

117

118 **Study sites and sample collection**

119 The elevation transect under study lies on the Eastern flank of the Andes in South
120 Eastern Peru, in the upper Madre de Dios/Madeira watershed. The transect spans 3.4 km in
121 elevation from 194 to 3644 m above sea level (asl) and consists of 22 sites, each with a 1 ha

122 permanent sampling plot, in old growth tropical forest and one site on high elevation
123 grassland (soil properties and microbial diversity were determined for 14 sites; plant diversity
124 was determined for 19 sites; Table S1). Mean annual temperature (MAT) decreases with
125 increasing elevation (26 to 6 °C) but mean annual precipitation (MAP) does not vary
126 consistently with elevation, ranging from 1506-5302 mm yr⁻¹, with no evidence of soil
127 moisture constraints at any site. The sites are situated on predominantly Paleozoic (~450 Ma)
128 meta-sedimentary mudstone (~80%), with plutonic intrusions (granite) underlying the sites
129 between 1500 and 2020 m asl. The soils at the sites above 2520 m are Umbrisols
130 (Inceptisols), while the soils from 1000 to 2020 m are Cambisols (Inceptisols). The soils at
131 the two lowland sites are Haplic Allisols (Ultisols) (194 m asl) and Haplic Cambisols
132 (Inceptisols) (210 m asl) (according to FAO, with USDA Soil Taxonomy in parentheses).
133 Further descriptions of soil, climate and floristic composition of these sites are reported
134 elsewhere (Rapp et al. 2012, Whitaker et al. 2014). Trees were recorded in 19 of the 1 ha
135 plots, where every individual tree ≥ 10 cm diameter at breast height, 1.3 m (d.b.h.) was
136 measured, tagged and identified to species or morphospecies. Soil was collected during
137 January 2012 from five systematically distributed sampling points within 14 of the 1 ha plots.
138 These systems are highly aseasonal, with no significant variation in mean annual temperature
139 and no seasonal soil or plant moisture constraints (Zimmermann et al. 2010, van de Weg et
140 al. 2014), therefore the comparison of sites at a single time point was not confounded by
141 strong seasonality. We used composite soil samples representing spatial replication of three
142 for DNA extraction, or five spatial replicates for all other analyses. Although we sampled and
143 analysed soil in spatial replicates (within 1 ha), our treatment unit for all analyses is the plot
144 mean. Organic horizons and the surface 0-10 cm layer of mineral horizons were collected
145 separately. Soil samples were stored for < 14 days at < 4°C until DNA extraction and

146 determination of nutrients and enzyme activities, which has been shown to have negligible
147 effect on these soil properties (Lauber et al. 2010, Turner and Romero 2010).

148

149 **Soil analyses: DNA sequencing, nutrients and extracellular enzyme activities**

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

169 Relatively abundant phylotypes were checked using BLAST and comparison against
170 sequences contained within GenBank.

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

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

192

193 **Statistical analyses**

194 We determined species richness (α -diversity) using Shannon diversity index,
195 according to total species abundance for plants or OTUs for soil bacteria and fungi.
196 Community composition (β -diversity) was determined using Sorrenson indices for plants and
197 Bray Curtis dissimilarity matrices for soil bacteria and fungi. We tested whether patterns in
198 bacterial and fungal diversity and community composition were explained by biotic
199 interactions with plant communities, by using Spearman's correlation (α -diversity) and
200 Mantel tests (β -diversity) among biotic groups and comparing the relationships between
201 plants and microbes in organic and mineral soil horizons. Permutational MANOVA and
202 Principal Co-ordinates Analyses to explore differences in β -diversity with elevation or soil
203 horizon. The environmental or edaphic drivers of these patterns in plant, bacterial or fungal
204 diversity and community composition were determined univariate and multivariate
205 correlation. Multivariate correlation was performed using the BEST trend correlation
206 function (Primer; version 6.1.12), to show how multivariate biotic data are shaped by 31
207 predictor variables including soil nutrients (total, organic, exchangeable), soil micronutrients,
208 soil enzymes (obtained by fluorogenic or absorption assays), and climatic and soil abiotic
209 properties. We quantified the functional consequences of changes in soil microbial β -
210 diversity by using Mantel tests of bacterial and fungal community composition and enzymatic
211 activity for seven enzymes (obtained by fluorogenic or absorption assays). For all analyses,
212 we only included sites for which we have combined plant, microbial, soil edaphic and
213 environmental data. The combined methodology allowed us to 1) determine whether diversity
214 patterns in plants, bacteria and fungi are co-related; 2) identify the β -diversity principle
215 environmental or edaphic drivers of these patterns and 3) test whether the diversity and
216 community composition of soil microorganisms influence soil processes along a tropical

217 environmental gradient. All statistical analyses were performed in either Primer (version
218 6.1.12) or R (version 2.15.2).

219

220 **Results**

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

231 The composition of plant, bacterial and fungal communities also differed with
232 elevation (Figs. 3 & S1), and between organic and mineral horizons for soil microorganisms
233 (Fig. S2), although fungi differed to a lesser extent between horizons than bacteria. For
234 bacteria, increased elevation was associated with an increased dominance of *Acidobacteria*
235 and *Betaproteobacteria*, and decreased dominance of Actinobacteria and
236 *Deltaproteobacteria*; the patterns occurred in both horizons, although mineral horizons
237 contained a greater proportion of *Acidobacteria* and *Archaea* (Fig. 3). For fungi, increased
238 elevation was associated with increased dominance of Ascomycota (*Archaeorhizomycetes*,
239 *Leotiomycetes*), Basidiomycota (*Microbotryomycetes*), and decreased dominance of other
240 Ascomycota (*Sodariomycetes*, *Dothideomycetes*, *Eurotiomycetes*), Glomeromycota and

241 Zygomycota (Fig. 3; Table S3). Bacteria exhibited the largest compositional dissimilarities of
242 communities with elevation (β -diversity) followed by plant and then fungal communities, and
243 the β -diversity patterns observed for bacteria and fungi were correlated with those observed
244 for plants (Fig. 4). Thus, plants and several major taxonomic groups of both bacteria and
245 fungi showed clear changes in composition with elevation, suggesting shared environmental
246 drivers in community structure.

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

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

265 these findings suggest that, in addition to temperature, differences in organic nutrient cycling
266 are related to the β -diversity of bacteria and fungi in organic soil horizons.

267

268 **Discussion**

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

278 The role of temperature in determining microbial β -diversity is also illustrated by an
279 increased relative abundance of *Acidobacteria* and the fungi *Archaeorhizomycetes* with
280 increased elevation, but a decreased relative abundance of Actinobacteria and
281 *Alphaproteobacteria* (Fig. 3). These major taxonomic groups have been associated with
282 oligotrophic (*Acidobacteria*, *Archaeorhizomycetes*) and copiotrophic (*Actinobacteria*,
283 *Alphaproteobacteria*) life history strategies, respectively (Fierer et al. 2007, Rosling et al.
284 2011), which is consistent with evidence for increased energy limitation of microbial activity
285 at higher elevations, favoring slower growth (Nottingham et al. 2015). The strong
286 correlations between the β -diversity of plants, soil bacteria and fungi (Fig. 4) further
287 indicated that similar environmental factors, primarily temperature (Table 1), drive these
288 patterns across the three biotic groups. The high relative abundance of the Ascomycota,

289 *Archaeorhizomycetes* at higher elevations (Fig. 3) is of particular interest because this class of
290 fungi was identified only very recently, in tundra soils (Schadt et al. 2003) and their global
291 distribution is poorly understood because many previous analyses failed to identify them
292 because of amplification biases (Rosling et al. 2011). They are understood to be typically
293 oligotrophic and root-associated fungi, colonizing typical ectomycorrhizal habitats beneath
294 pine and ericaceous plants (Rosling et al. 2011), which is consistent with our current
295 understanding of tropical montane forest habitats being energy-limited (Bruijnzeel et al.
296 2011). This important class of fungi, which until very recently was unknown, dominate the
297 fungal biomass in these tropical montane forests.

298 Temperature was also the principal correlate of plant β -diversity (Table 1).
299 Temperature has previously been shown to be a major determinant of tree community
300 composition across this transect (Rapp et al. 2012) and up-slope movement of tree species'
301 ranges has been observed under recent climatic warming (Duque et al. 2015). Patterns in
302 plant species composition and richness on tropical mountains are thought to be driven mainly
303 by the effect of narrow temperature ranges on niche separation (by directly affecting
304 metabolism and indirectly affecting resource availability), while further constrained by land
305 area, lithology and disturbance history (Janzen 1967, Colwell et al. 2008). Although the high
306 landslide activity and soil erosion in the humid Eastern Andean Cordillera (Clark et al. 2013)
307 may be significant factors in constraining diversity at higher elevations in this region, our
308 study identifies a central underlying role for temperature. Analogous observations have been
309 made along latitudinal gradients where plant/fungal species richness ratios decrease with
310 distance from the equator (Tedersoo et al. 2014). Our data indicate a stronger coupling
311 between the α -diversity of plants and bacteria compared to fungi (Fig. 2), but a stronger
312 coupling between the β -diversity of plants and fungi as compared to bacteria (Fig. 4).

313 There was a secondary role for edaphic properties in shaping these diversity patterns,
314 with direct influences of nutrient ratios on microbial β -diversity and potassium on plant β -
315 diversity (Table 1). Our data also suggest that this role of edaphic processes on microbial α -
316 and β -diversity is more significant in organic horizons, where the community-wide
317 differences in plant litter traits is largest (Hattenschwiler et al. 2008). Multiple lines of
318 evidence suggest an influence of plant organic matter inputs on soil microbes: (i) the large
319 difference in microbial diversity between organic and mineral soil horizons (Figs. S1 & S2);
320 (ii) the existence of stronger correlations between microbial β -diversity and soil organic
321 nutrients in organic horizons compared to mineral horizons (Table 1; Fig. S3); (iii) the overall
322 strong correlation between plant and soil microbial diversity (Figs. 2 & 4); and (iv) the
323 correlation between soil microbial β -diversity and the dissimilarity among sites in enzymatic
324 activity, indices of organic nutrient degradation (Nottingham et al. 2015) (Fig. S6).
325 Laboratory incubations of soils from this transect also support the link between differences in
326 microbial community composition and the rate at which different organic substrates undergo
327 degradation (Whitaker et al. 2014). Together these findings point towards a relationship
328 between the high soil microbial diversity in lowland forests and the diverse complexity and
329 stoichiometry of plant organic matter inputs to soil, through the high inter- and intra-species
330 chemical diversity in leaf-litter (Hattenschwiler et al. 2008).

331 Our results, from a 3450 m elevation range, contrast with findings from studies of
332 other elevation gradients that only examined plant and bacterial diversity, and have not found
333 such strongly-related diversity gradients. The fundamental climate-diversity relationships we
334 observed here were probably obscured in previous studies because of insufficient sampling
335 density and wider variation in soil pH and soil moisture. For example, the importance of
336 sampling intensity is demonstrated by the contrast between findings from this study of 14
337 sites with an earlier report from 6 locations along the same Andean transect where no

338 elevation gradient in soil bacterial diversity was found (Fierer et al. 2011): if we reduce our
339 dataset to include only those sites represented in the earlier study, no strong elevation trends
340 are apparent (Fig S6). While the importance of soil pH and rainfall variation in determining
341 patterns in bacterial diversity was demonstrated for a 1850 m elevation gradient in South
342 Korea (Singh et al. 2014). Similarly, these factors also likely accounted for the lack of clear
343 patterns for two temperate zone elevation transect studies which sampled six locations over
344 1670 m in Northeast China (Shen et al. 2014) and five locations over 920 m in the Rocky
345 mountains, the latter indicating a small single-taxon increase with elevation, but no
346 community-wide trend (Bryant et al. 2008).

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

355

356 **Acknowledgements**

357 This study is a product of the Andes Biodiversity and Ecosystem Research Group consortium
358 (www.andesconservation.org) and was financed by the UK Natural Environment Research
359 Council (NERC), grant numbers NE/G018278/1 and NE/F002149/1 and also supported by an
360 Australian Research Council grant FT110100457 to PM and a European Union Marie-Curie
361 Fellowship FP7-2012-329360 to ATN. We thank the Asociacion para la Conservacion de la

362 Cuenca Amazonica (ACCA) in Cusco and the Instituto Nacional de Recursos Naturales
363 (INRENA) in Lima for access to the study sites. For their logistical support we thank Dr. Eric
364 Cosio and Eliana Esparza Ballón at Pontificia Universidad Católica del Perú (PUCP). For
365 their support in the laboratory we thank Tania Romero and Dayana Agudo. For their support
366 in the field we thank Walter H. Huasco, William Farfan Rios and Javier E. S. Espejo.

367

368 **References**

- 369 Baas Beeking, L. G. M. 1934. Geobiologie of inleiding tot de milieukunde. W.P. Van Stockum & Zoon,
370 The Hague, the Netherlands.
- 371 Barberán, A., K. L. McGuire, J. A. Wolf, F. A. Jones, S. J. Wright, B. L. Turner, A. Essene, S. P. Hubbell,
372 B. C. Faircloth, and N. Fierer. 2015. Relating belowground microbial composition to the
373 taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest.
374 *Ecology letters* **18**:1397-1405.
- 375 Bardgett, R. D., and W. H. van der Putten. 2014. Belowground biodiversity and ecosystem
376 functioning. *Nature* **515**:505-511.
- 377 Bruijnzeel, L. A., F. N. Scatena, and L. S. Hamilton. 2011. Tropical Montane Cloud Forests. Cambridge
378 University Press, Cambridge, UK.
- 379 Bryant, J. A., C. Lamanna, H. Morlon, A. J. Kerkhoff, B. J. Enquist, and J. L. Green. 2008. Microbes on
380 mountainsides: Contrasting elevational patterns of bacterial and plant diversity. *Proceedings*
381 *of the National Academy of Sciences of the United States of America* **105**:11505-11511.
- 382 Clark, K. E., R. G. Hilton, A. J. West, Y. Malhi, D. R. Gröcke, C. L. Bryant, P. L. Ascough, A. Robles
383 Caceres, and M. New. 2013. New views on “old” carbon in the Amazon River: Insight from
384 the source of organic carbon eroded from the Peruvian Andes. *Geochemistry, Geophysics,*
385 *Geosystems* **14**:1644-1659.
- 386 Colwell, R. K., G. Brehm, C. L. Cardelus, A. C. Gilman, and J. T. Longino. 2008. Global warming,
387 elevational range shifts, and lowland biotic attrition in the wet tropics. *Science* **322**:258-261.
- 388 de Vries, F. T., P. Manning, J. R. Tallwin, S. R. Mortimer, E. S. Pilgrim, K. A. Harrison, P. J. Hobbs, H.
389 Quirk, B. Shipley, J. H. Cornelissen, J. Kattge, R. D. Bardgett, and N. Johnson. 2012. Abiotic
390 drivers and plant traits explain landscape-scale patterns in soil microbial communities.
391 *Ecology letters* **15**:1230-1239.
- 392 Duque, A., P. R. Stevenson, and K. J. Feeley. 2015. Thermophilization of adult and juvenile tree
393 communities in the northern tropical Andes. *Proceedings of the National Academy of*
394 *Sciences of the United States of America* **112**:10744-10749.
- 395 Fierer, N., M. A. Bradford, and R. B. Jackson. 2007. Toward an ecological classification of soil
396 bacteria. *Ecology* **88**:1354-1364.
- 397 Fierer, N., and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities.
398 *Proceedings of the National Academy of Sciences of the United States of America* **103**:626-
399 631.
- 400 Fierer, N., C. M. McCain, P. Meir, M. Zimmermann, J. M. Rapp, M. R. Silman, and R. Knight. 2011.
401 Microbes do not follow the elevational diversity patterns of plants and animals. *Ecology*
402 **92**:797-804.

- 403 Handa, I. T., R. Aerts, F. Berendse, M. P. Berg, A. Bruder, O. Butenschoen, E. Chauvet, M. O. Gessner,
404 J. Jabiol, M. Makkonen, B. G. McKie, B. Malmqvist, E. T. H. M. Peeters, S. Scheu, B. Schmid, J.
405 van Ruijven, V. C. A. Vos, and S. Hattenschwiler. 2014. Consequences of biodiversity loss for
406 litter decomposition across biomes. *Nature* **509**:218-221.
- 407 Hattenschwiler, S., B. Aeschlimann, M. M. Couteaux, J. Roy, and D. Bonal. 2008. High variation in
408 foliage and leaf litter chemistry among 45 tree species of a neotropical rainforest
409 community. *The New phytologist* **179**:165-175.
- 410 Hedley, M. J., J. W. B. Stewart, and B. S. Chauhan. 1982. Changes in Inorganic and Organic Soil-
411 Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations. *Soil*
412 *Science Society of America Journal* **46**:970-976.
- 413 Hendershot, W. H., and M. Duquette. 1986. A Simple Barium-Chloride Method for Determining
414 Cation-Exchange Capacity and Exchangeable Cations. *Soil Science Society of America Journal*
415 **50**:605-608.
- 416 Janzen, D. H. 1967. Why Mountain Passes Are Higher in Tropics. *American Naturalist* **101**:233-&.
- 417 Lauber, C. L., N. Zhou, J. I. Gordon, R. Knight, and N. Fierer. 2010. Effect of storage conditions on the
418 assessment of bacterial community structure in soil and human-associated samples. *Fems*
419 *Microbiology Letters* **307**:80-86.
- 420 Martiny, J. B. H., B. J. M. Bohannan, J. H. Brown, R. K. Colwell, J. A. Fuhrman, J. L. Green, M. C.
421 Horner-Devine, M. Kane, J. A. Krumins, C. R. Kuske, P. J. Morin, S. Naeem, L. Ovreas, A. L.
422 Reysenbach, V. H. Smith, and J. T. Staley. 2006. Microbial biogeography: putting
423 microorganisms on the map. *Nature Reviews Microbiology* **4**:102-112.
- 424 Nottingham, A. T., B. L. Turner, J. Whitaker, N. Ostle, N. P. McNamara, R. D. Bardgett, N. Salinas, and
425 P. Meir. 2015. Soil microbial nutrient constraints along a tropical forest elevation gradient: a
426 belowground test of a biogeochemical paradigm. *Biogeosciences* **12**:6489-6523.
- 427 Orwin, K. H., S. M. Buckland, D. Johnson, B. L. Turner, S. Smart, S. Oakley, and R. D. Bardgett. 2010.
428 Linkages of plant traits to soil properties and the functioning of temperate grassland. *Journal*
429 *of Ecology* **98**:1074-1083.
- 430 Pan, Y., R. A. Birdsey, J. Fang, R. Houghton, P. E. Kauppi, W. A. Kurz, O. L. Phillips, A. Shvidenko, S. L.
431 Lewis, J. G. Canadell, P. Ciais, R. B. Jackson, S. W. Pacala, A. D. McGuire, S. Piao, A.
432 Rautiainen, S. Sitch, and D. Hayes. 2011. A large and persistent carbon sink in the world's
433 forests. *Science* **333**:988-993.
- 434 Pianka, E. R. 1966. Latitudinal Gradients in Species Diversity - a Review of Concepts. *American*
435 *Naturalist* **100**:33-&.
- 436 Prober, S. M., J. W. Leff, S. T. Bates, E. T. Borer, J. Firn, W. S. Harpole, E. M. Lind, E. W. Seabloom, P.
437 B. Adler, J. D. Bakker, E. E. Cleland, N. M. DeCrappeo, E. DeLorenze, N. Hagenah, Y. Hautier,
438 K. S. Hofmockel, K. P. Kirkman, J. M. H. Knops, K. J. La Pierre, A. S. MacDougall, R. L.
439 McCulley, C. E. Mitchell, A. C. Risch, M. Schuetz, C. J. Stevens, R. J. Williams, and N. Fierer.
440 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands
441 worldwide. *Ecology letters* **18**:85-95.
- 442 Rahbek, C. 2005. The role of spatial scale and the perception of large-scale species-richness patterns.
443 *Ecology letters* **8**:224-239.
- 444 Rapp, J. M., M. R. Silman, J. S. Clark, C. A. J. Girardin, D. Galiano, and R. Tito. 2012. Intra- and
445 interspecific tree growth across a long altitudinal gradient in the Peruvian Andes. *Ecology*
446 **93**:2061-2072.
- 447 Rosling, A., F. Cox, K. Cruz-Martinez, K. Ihrmark, G. A. Grelet, B. D. Lindahl, A. Menkis, and T. Y.
448 James. 2011. Archaeorhizomycetes: Unearthing an Ancient Class of Ubiquitous Soil Fungi.
449 *Science* **333**:876-879.
- 450 Schadt, C. W., A. P. Martin, D. A. Lipson, and S. K. Schmidt. 2003. Seasonal dynamics of previously
451 unknown fungal lineages in tundra soils. *Science* **301**:1359-1361.

- 452 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.
453 2014. Contrasting elevational diversity patterns between eukaryotic soil microbes and
454 plants. *Ecology* **95**:3190-3202.
- 455 Singh, D., L. Lee-Cruz, W. S. Kim, D. Kerfahi, J. H. Chun, and J. M. Adams. 2014. Strong elevational
456 trends in soil bacterial community composition on Mt. Ha Ilia, South Korea. *Soil Biology &*
457 *Biochemistry* **68**:140-149.
- 458 Singh, D., K. Takahashi, M. Kim, J. Chun, and J. M. Adams. 2012. A Hump-Backed Trend in Bacterial
459 Diversity with Elevation on Mount Fuji, Japan. *Microbial Ecology* **63**:429-437.
- 460 Tedersoo, L., M. Bahram, S. Polme, U. Koljalg, N. S. Yorou, R. Wijesundera, L. V. Ruiz, A. M. Vasco-
461 Palacios, P. Q. Thu, A. Suija, M. E. Smith, C. Sharp, E. Saluveer, A. Saitta, M. Rosas, T. Riit, D.
462 Ratkowsky, K. Pritsch, K. Poldmaa, M. Piepenbring, C. Phosri, M. Peterson, K. Parts, K. Partel,
463 E. Otsing, E. Nouhra, A. L. Njouonkou, R. H. Nilsson, L. N. Morgado, J. Mayor, T. W. May, L.
464 Majuakim, D. J. Lodge, S. S. Lee, K. H. Larsson, P. Kohout, K. Hosaka, I. Hiiesalu, T. W. Henkel,
465 H. Harend, L. D. Guo, A. Greslebin, G. Grelet, J. Geml, G. Gates, W. Dunstan, C. Dunk, R.
466 Drenkhan, J. Dearnaley, A. De Kesel, T. Dang, X. Chen, F. Buegger, F. Q. Brearley, G. Bonito, S.
467 Anslan, S. Abell, and K. Abarenkov. 2014. Global diversity and geography of soil fungi.
468 *Science* **346**:1078.
- 469 Turner, B. L., and T. E. Romero. 2010. Stability of hydrolytic enzyme activity and microbial
470 phosphorus during storage of tropical rain forest soils. *Soil Biology and Biochemistry* **42**:459-
471 465.
- 472 van de Weg, M. J., P. Meir, M. Williams, C. Girardin, Y. Malhi, J. Silva-Espejo, and J. Grace. 2014.
473 Gross primary productivity of a high elevation tropical montane cloud forest. *Ecosystems*
474 **17**:751-764.
- 475 von Humboldt, A., and A. Bonpland. 1805. *Essai sur la géographie des plantes*. Chez Levrault, Scoell
476 et Campagnie, Librairie, Paris.
- 477 Whitaker, J., N. Ostle, A. T. Nottingham, A. Ccahuana, N. Salinas, R. D. Bardgett, P. Meir, and N. P.
478 McNamara. 2014. Microbial community composition explains soil respiration responses to
479 changing carbon inputs along an Andes-to-Amazon elevation gradient. *Journal of Ecology*
480 **102**:1058-1071.
- 481 Whitman, W. B., D. C. Coleman, and W. J. Wiebe. 1998. Prokaryotes: The unseen majority.
482 *Proceedings of the National Academy of Sciences of the United States of America* **95**:6578-
483 6583.
- 484 Zimmermann, M., P. Meir, M. I. Bird, Y. Malhi, and A. J. Q. Ccahuana. 2010. Temporal variation and
485 climate dependence of soil respiration and its components along a 3000 m altitudinal
486 tropical forest gradient. *Global Biogeochemical Cycles* **24**:GB4012.

487

488

489

490

491

492

493

494 **Figure legends**

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

500

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

509

510 **Figure 3. The (A) positive and (B) negative trends in the relative abundances of specific**
511 **bacterial and fungal taxa in soils along an elevation gradient in Andean tropical forest.**
512 Bacteria taxa are Acidobacteria, Actinobacteria, *Beta-Proteobacteria* and *Delta-*
513 *Proteobacteria*; and fungal taxa are Actinomycetes, *Sordariomycetes* and
514 *Archaeorhizomycetes* (by phyla or, where italicised, by class). All data are for organic
515 horizons except for Acidobacteria, which is for mineral horizon. The full data for all taxa in
516 organic and mineral horizons (which follow similar trends) are in Fig. S1 and Table S3 (Fig.
517 S1 also shows dissimilarity of communities among sites using heat-maps and Table S3 shows
518 correlations between relative abundance of taxa and elevation).

519

520 **Figure 4. Elevation comparisons of indices of plant β -diversity, bacteria and fungal β -**

521 **diversity in organic horizons.** The overall decline with increased elevation indicates

522 increased dissimilarity in β -diversity between sites with greater difference in elevation. β -

523 diversity was determined by Sorensen's indices for plants, and by Bray Curtis indices for

524 bacteria and fungi. Patterns in β -diversity were correlated between plants and bacteria

525 (organic horizon $\rho = 0.81$; mineral horizon $\rho = 0.88$) and plants and fungi (organic horizon ρ

526 $= 0.67$; mineral horizon $\rho = 0.79$; by Mantel tests; $p < 0.001$ for all comparisons

1

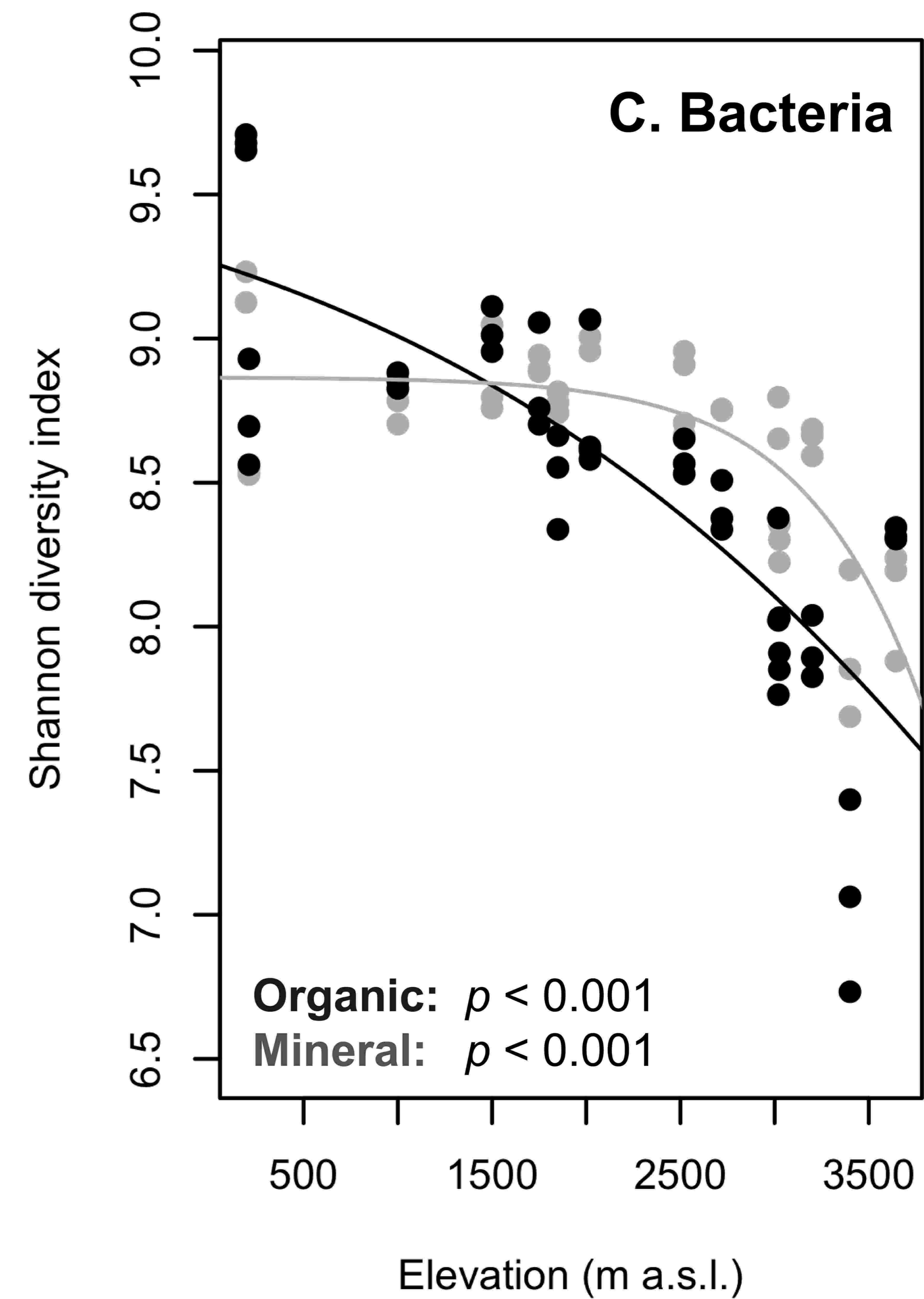
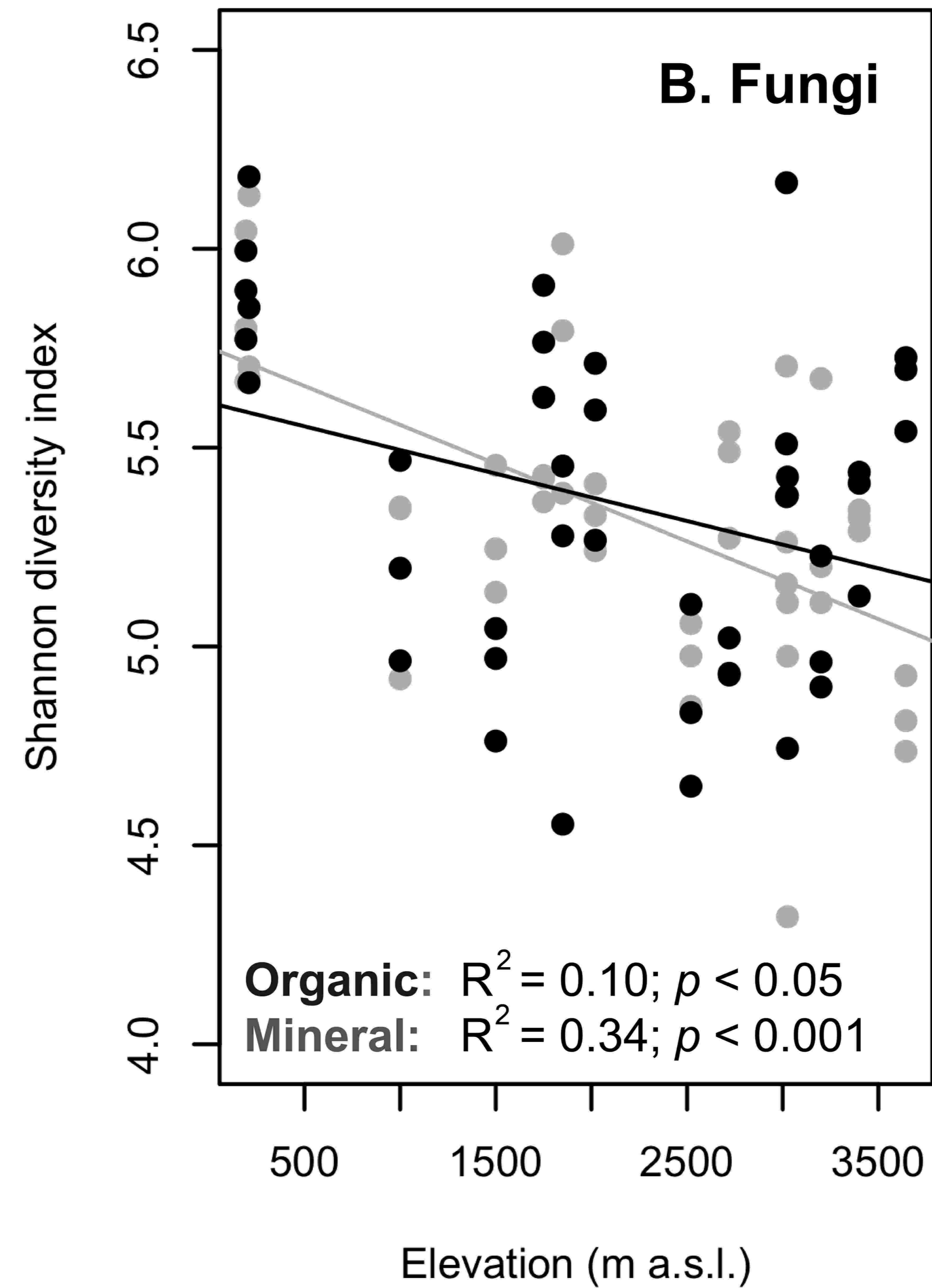
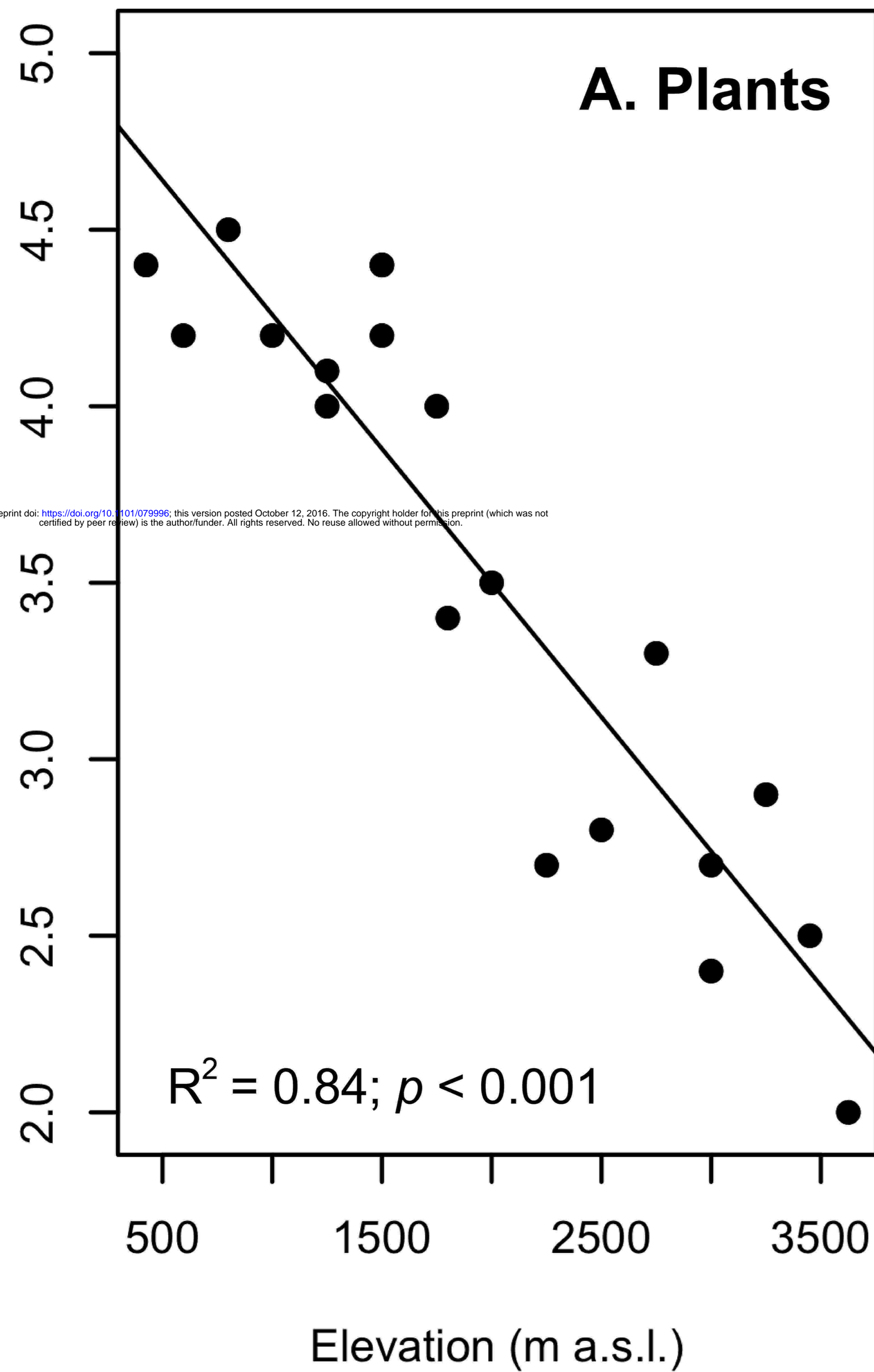
2 **Table 1**

3 **The relationship between plant, bacterial and fungal β -diversity and environmental and**
 4 **edaphic variables.**

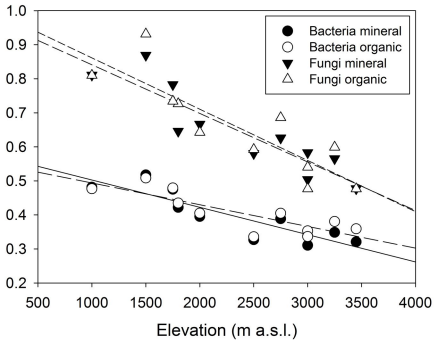
	Plants	Bacteria	Fungi	Bacteria	Fungi
	Organic horizon			Mineral horizon	
MAT	*** (0.91)	*** (0.77)	* (0.67)	*** (0.88)	*** (0.59)
Soil pH	ns	* (0.57)	ns	** (0.47)	ns
Total C:N	ns	** (0.68)	ns	ns	** (0.38)
Total C:P	ns	ns	** (0.70)	ns	ns
Na	ns	ns	ns	* (0.22)	ns
K	** (0.44)	ns	ns	ns	ns
<i>N</i> -acetyl β -glucosaminidase	ns	ns	*** (0.74)	ns	ns
Complete model	0.93	0.88	0.80	0.91	0.65

5 Models were determined using step-wise selection based on dissimilarity between variables by Euclidean
 6 distance (biotic and environmental matching function in Primer). A total of 31 properties were included in
 7 models for plants and bacteria and fungi in organic soils (25 for bacteria and fungi in mineral soils), including
 8 MAP, soil pH, total soil nutrients (CNP) and their ratios, available soil nutrients, soil phosphorus fractions,
 9 cations, cation exchange capacity; and the activities of seven enzymes in organic horizons. For plants, organic
 10 horizon soil properties were included in analyses (when we included mineral soil data the model included MAT
 11 (0.91) and Fe (0.16). Variables are ranked in order of significance by number of asterisks; the correlation
 12 coefficients (Mantel tests) for each individual variable are shown in parentheses.

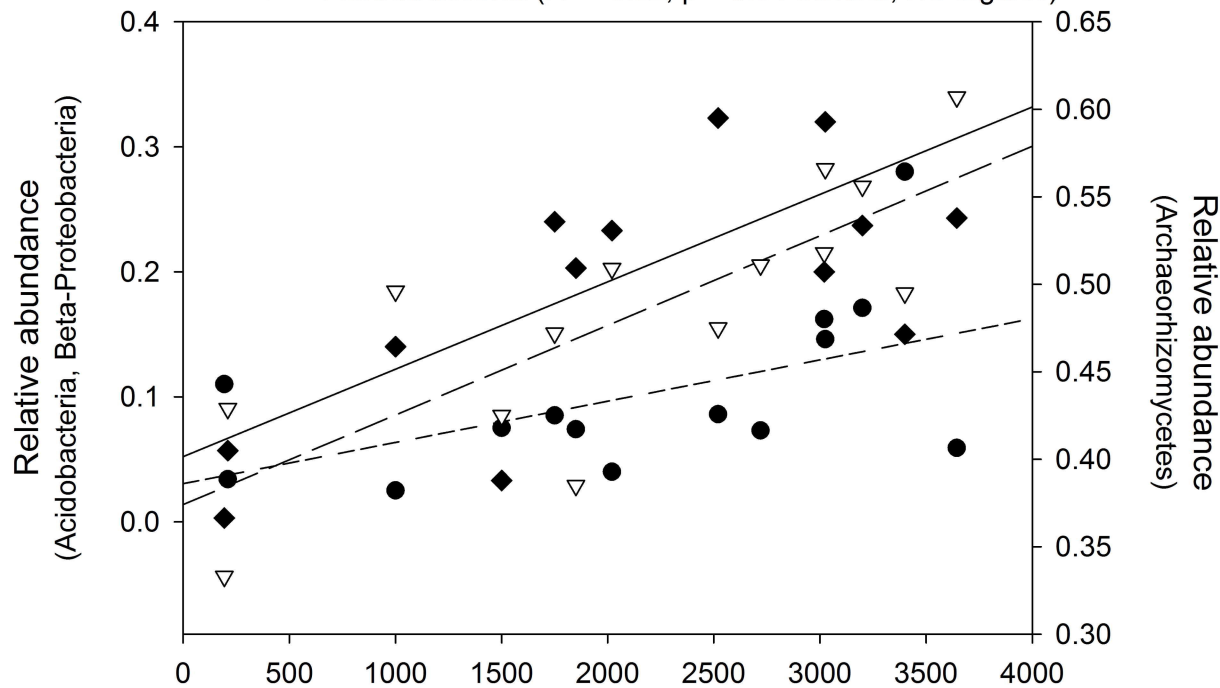
Shannon diversity index



Ratio of plant to microbial Shannon diversity



- ◆ Archaeorhizomycetes ($R^2 = 0.42$, $p = 0.01$)
- Beta-Proteobacteria ($R^2 = 0.40$, $p = 0.05$)
- ▽ Acidobacteria ($R^2 = 0.62$, $p < 0.01$ mineral; NS organic)



- ◆ Sordariomycetes ($R^2 = -0.36$, $p = 0.02$)
- Actinobacteria ($R^2 = -0.40$, $p = 0.02$)
- ▽ Delta-Proteobacteria ($R^2 = -0.27$, $p = 0.05$)

