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1 *Letter to Ecology Letters*

2 **Microbial responses to warming enhance soil carbon loss following**
3 **translocation across a tropical forest elevation gradient**

4 **Running head: microbial responses enhance soil carbon loss**

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27 tropical forest, montane tropical forest, Q_{10} , soil carbon cycle, translocation

28 **Author contributions:** ATN and PM conceived the study, with help in design and analysis from JW,
29 BLT, NJO, RDB, NPM, NS and NF. ATN performed the study and analysed the data. AJQC assisted
30 with fieldwork. ATN, NF, JW and BLT performed the laboratory analyses. ATN wrote the paper,
31 with primary input from PM and BLT, and further input from all authors.

32 **Data accessibility statement:** The data that support the findings of this study are available in
33 Figshare at doi.org/10.6084/m9.figshare.8956481.v1.

34

35 **ABSTRACT**

36 Tropical soils contain huge carbon stocks, which climate warming is projected to reduce by
37 stimulating organic matter decomposition, creating a positive feedback that will promote further
38 warming. Models predict that the loss of carbon from warming soils will be mediated by microbial
39 physiology, but no empirical data are available on the response of soil carbon and microbial
40 physiology to warming in tropical forests, which dominate the terrestrial carbon cycle. Here we show
41 that warming caused a considerable loss of soil carbon that was enhanced by associated changes in
42 microbial physiology. By translocating soils across a 3000 m elevation gradient in tropical forest,
43 equivalent to a temperature change of $\pm 15^{\circ}\text{C}$, we found that soil carbon declined over 5 years by 4%
44 in response to each 1°C increase in temperature. The total loss of carbon was related to its quantity
45 and lability, and was enhanced by changes in microbial physiology including increased microbial
46 carbon-use-efficiency, shifts in community composition towards microbial taxa associated with
47 warmer temperatures, and increased activity of hydrolytic enzymes. These findings suggest that
48 microbial feedbacks will cause considerable loss of carbon from tropical forest soils in response to
49 predicted climatic warming this century.

50

51 INTRODUCTION

52 The response of soil organic matter decomposition to increasing temperature is predicted to
53 contribute a significant positive feedback to climate change (Davidson & Janssens 2006; Crowther *et*
54 *al.* 2016; Melillo *et al.* 2017). This positive feedback is expected because biochemical reaction rates
55 increase exponentially with temperature, and because the global soil carbon (C) stock is of sufficient
56 magnitude that even small fractional increases in organic matter decomposition will cause large
57 corresponding CO₂ emissions, increasing the concentration of atmospheric CO₂ (Davidson &
58 Janssens 2006). However, the nature of this feedback in different ecosystems remains uncertain
59 because organic matter decomposition is mediated by complex biological and physicochemical
60 interactions, including microbial metabolism, enzymatic catabolism, and effects of substrate quality
61 and nutrient availability. In particular, this positive feedback has been hypothesized to be strongly
62 regulated by microbial responses to warming, which could either enhance or reduce the expected
63 increases in CO₂ emissions following increased biochemical reaction rates (Frey *et al.* 2013; Wieder
64 *et al.* 2013; Hagerty *et al.* 2014).

65 Despite the importance of the response of soil C and microbial physiology to warming, this
66 has not been assessed empirically in tropical forests. This knowledge gap is significant because
67 tropical forests represent 42% of forested global land area (Pan *et al.* 2011) and their soils contain a
68 third of global soil C (Jobbagy & Jackson 2000). As a consequence, understanding the potential for
69 feedbacks between climate and soil carbon in tropical forests is urgently needed to improve the
70 parameterization of Earth system models used to predict future atmospheric CO₂ and climate
71 (Cavaleri *et al.* 2015; Koven *et al.* 2015; Luo *et al.* 2016). The temperature response of soil organic
72 matter decomposition is likely to differ between the tropics and higher-latitudes due to differences in
73 nutrient availability, biodiversity, species composition, and in the temperature optima of the biota
74 (Wood *et al.* 2019). The large stocks of relatively labile soil C in tropical montane ecosystems
75 (Zimmermann *et al.* 2012), where thermal niches are often narrow and climate warming projections

76 are steep (Loomis *et al.* 2017; Russell *et al.* 2017; Fadrique *et al.* 2018), are especially vulnerable to
77 warming and could create a globally large soil-climate feedback (Nottingham *et al.* 2015b). Indeed,
78 the response to warming in the tropics remains one of the major gaps in our understanding of
79 terrestrial ecosystem responses to climate change in Earth system models (Huntingford *et al.* 2009;
80 Cavaleri *et al.* 2015; Koven *et al.* 2015), and the size of the soil C-climate feedback is a dominant
81 component of this uncertainty.

82 Soil warming experiments in the field, which have so far been conducted only in mid- to
83 high-latitude ecosystems, have shown that warming generates a considerable short-term soil C loss
84 (Lu *et al.* 2013; Romero-Olivares *et al.* 2017). This loss declines over time (e.g. >2 years) (Romero-
85 Olivares *et al.* 2017), although there is evidence that it can continue for longer (e.g. >20 years)
86 (Melillo *et al.* 2017). The short-term decline in soil C loss with warming has been explained by a
87 limited availability of C-substrates and nutrients to heterotrophs (Knorr *et al.* 2005; Romero-Olivares
88 *et al.* 2017), and an overall decline in microbial C-use efficiency (CUE) (Manzoni *et al.* 2012;
89 Melillo *et al.* 2017). Microbial CUE, defined as the fraction of C incorporated for growth over
90 respiratory losses, generally decreases when greater metabolic C-demand at higher temperatures
91 reduces microbial biomass and enzyme synthesis (termed ‘thermal compensation’) (Manzoni *et al.*
92 2012; Bradford *et al.* 2019). However, a longer-term response of increased CUE under warming has
93 been reported for specific substrates, resulting in sustained or increased microbial biomass and
94 enzyme synthesis (Frey *et al.* 2013), which could have a longer-term negative impact on soil C
95 stocks (i.e. an ‘enhancing’ CUE response) (Wieder *et al.* 2013). The underlying mechanisms for
96 these CUE responses remain unclear, but might include physiological changes within species, shifts
97 in microbial community composition (Oliverio *et al.* 2017), or changes in the temperature sensitivity
98 of enzyme activity (Wallenstein *et al.* 2011; Allison *et al.* 2018).

99 The wide range of microbial feedbacks hypothesized in models reflects limited understanding
100 of this important climate response, and confounds attempts to model the soil C response to

101 temperature (Wieder *et al.* 2013; Hagerty *et al.* 2018). For example, depending on the attributed
102 temperature response of microbial CUE, global soil C losses by 2100 have been predicted to range
103 from negligible (decreased CUE with warming) to 300 Pg C (=20% of global soil C stocks; i.e. with
104 increased CUE with warming) (Wieder *et al.* 2013). Reducing this uncertainty requires
105 understanding of how the temperature sensitivity of soil C responds to resource availability and
106 microbial feedbacks in tropical ecosystems.

107 Here we report the results of a five-year soil translocation experiment along a 3000 m elevation
108 gradient (15°C range in mean annual temperature; MAT) in tropical forests between western lowland
109 Amazonia and the Peruvian Andes (Nottingham *et al.* 2015b) (Fig. S1, Table 1). To isolate the effect
110 of temperature, our principal experimental manipulation, we controlled rainfall inputs to represent an
111 average at the site of origin. We tested the hypotheses that: i) five years of temperature manipulation
112 would systematically change soil C stocks across sites (increased loss with warming/reduced loss
113 with cooling); ii) changes in soil C would be determined by soil chemistry, whereby C loss would be
114 positively correlated with the relative abundance of labile compounds; and iii) microbial CUE would
115 increase over five years of warming, indicating an enhancing effect of microbial physiology and/or
116 community composition changes on soil C loss.

117

118 **MATERIALS AND METHODS**

119 We translocated soil among four tropical forest sites along the elevation gradient. Soil was
120 translocated as intact cores, 10 cm diameter × 50 cm depth (4000 cm³). Three undisturbed soil cores
121 were re-installed at the same site ('control'), and the other cores were translocated to the three other
122 elevations to achieve both warming and cooling (downslope = 'warmed', upslope = 'cooled')
123 (Zimmermann *et al.* 2012), an approach similar to laboratory-based studies of thermal-responses of
124 microbial activity (Karhu *et al.* 2014). To assess changes in soil C and thermal-responses of
125 microbial communities and their physiology after five years in a new temperature regime, we

126 quantified the concentration and composition of soil C (using solid-state ^{13}C -NMR spectroscopy),
127 nutrient concentrations, microbial community characteristics (using 16S and ITS rRNA gene
128 sequencing and phospholipid fatty acid, PLFA, biomarkers), and metrics of soil microbial
129 physiology (CUE, instantaneous respiration temperature-sensitivity RQ_{10} , and enzyme activities, Q_{10}
130 of V_{\max}). Changes in these metrics of soil microbial physiology with temperature may occur through
131 different mechanisms, including acclimation (physiological responses of individuals), adaptation
132 (genetic changes within species) and ecological responses (shifts in community composition).
133 Therefore, rather than refer to acclimation or adaptation, we use the terms ‘CUE response’ and
134 ‘enzyme Q_{10} response’. We evaluated the relationships between relative log-response ratios (RR) for
135 all properties and elevation shifts (to normalize responses among different soil types), while the
136 determinants of changes in soil C and RQ_{10} were evaluated with mixed-effects models. To determine
137 whether soil properties changed in response to temperature manipulation, the respective factors ‘soil-
138 destination’ (effect of new temperature regime) and ‘soil-origin’ (effect of intrinsic soil properties)
139 were included in the models.

140

141 **Study sites**

142 To investigate the effect of temperature on soil C dynamics and soil microbial communities, soil
143 cores were reciprocally translocated among four sites along an elevation gradient of tropical forest in
144 Peru. The sites ranged from lowland rainforest (210 m asl; above sea level), pre-montane rainforest
145 (1000 m asl), lower montane cloud forest (1500 m asl) and upper montane cloud forest (3030 m asl).
146 Site mean annual temperature (MAT) was determined over a 5-year period (2005-2010) and varied
147 from 26°C to 11°C with increasing elevation (Table 1). Dominant tree families ranged from
148 Clusiaceae and Cunoniceae at 3030 m asl, to Clethraceae at 1500 m asl, to Elaeocarpaceae and
149 Fabaceae at 1000 m asl, and Moraceae and Fabaceae at 200 m asl. The sampling sites were adjacent
150 to 1 ha permanent ecological inventory plots (Nottingham *et al.* 2015b). The upper three sites are

151 situated predominantly on Paleozoic (~450 Ma) meta-sedimentary mudstones (Sandia formation)
152 and the lowland forest site is on Pleistocene sediments, consisting of typical terra firma clay
153 substrates. Soils are Haplic Cambisols (Inceptisols) at 210 m asl; Cambisols (Inceptisols) at 1000 m
154 asl and 1500 m asl; and Umbrisols (Inceptisols) at 3030 m asl (according to FAO, with USDA Soil
155 Taxonomy in parentheses). Further descriptions of soil, climate and floristic composition of these
156 sites are reported elsewhere (Girardin *et al.* 2010; Rapp *et al.* 2012; Whitaker *et al.* 2014;
157 Nottingham *et al.* 2015b).

158

159 **Soil translocation**

160 At each site, we excavated twelve 50 cm deep, 10 cm diameter cores of intact mineral soil. Three of
161 these cores were re-installed at the same site (hereafter referred to as ‘control’), and the other cores
162 translocated to the three other elevations (hereafter referred to as ‘warmed’ if translocated down the
163 gradient, or ‘cooled’ if translocated up the gradient) (Zimmermann *et al.* 2009). The length of 50 cm
164 was chosen because this was the total depth of the mineral horizon at the highest elevation,
165 shallowest soil profile, sampling site. To maintain the same rainfall per m² as at the site of origin,
166 translocated tubes were capped with reduction collars or expansion funnels, which maintained a
167 similar moisture content in translocated soil compared to soil at the site of origin (Zimmermann *et al.*
168 2010). Temperature was, therefore, our principal experimental manipulation although we
169 acknowledge that under future climate scenarios changes in temperature and rainfall regimes
170 together will be important determinants of the overall tropical forest C cycle (Meir *et al.* 2015). New
171 litter input was excluded and root ingrowth prevented by installing a 63 µm nylon mesh at the base
172 of the tubes. A detailed description of the experimental setup is given in Zimmermann *et al.* (2009).
173 Soil cores were translocated in 2008 and, exactly five years later in 2013, mineral soil was sampled
174 from each core using an auger to 20 cm depth. Soil samples were stored for < 14 days at < 4 °C until
175 DNA extraction, respiration assays, and determination of nutrient content and enzyme activities; this

176 method has been shown to have negligible effects on soil microbial and enzymatic properties
177 (Lauber *et al.* 2010; Turner & Romero 2010). Soil samples were freeze-dried and stored for < 3
178 months prior to PLFA extraction.

179

180 **Soil analyses**

181 ***Soil characteristics:*** We determined the following edaphic variables: total carbon (C), total
182 nitrogen (N), total phosphorus (P), organic P, resin-extractable P (resin P), cation exchange capacity
183 (ECEC) and exchangeable cations (Al, Ca, Cl, Fe, K, Mn, Mg, Na), soil pH, bulk density and
184 moisture content. The C composition of soils was analysed by solid-state cross polarization magic
185 angle spinning (CP/MAS) ^{13}C NMR spectroscopy.

186 ***Enzyme activities and Q_{10} of enzyme activities:*** Soil enzyme activity (V_{\max}) and the
187 temperature sensitivity of enzyme activity (Q_{10} of V_{\max}) was determined for seven enzymes involved
188 in carbon and nutrient cycling. We used microplate fluorimetric assays with 100 μM
189 methylumbelliferone (MU)-linked substrates to measure activity of β -glucosidase (degradation of β -
190 bonds in glucose), cellobiohydrolase (degradation of cellulose), *N*-acetyl β -glucosaminidase
191 (degradation of *N*-glycosidic bonds), phosphomonoesterase (degradation of monoester-linked simple
192 organic phosphates), sulfatase (degradation of ester sulfates), and β -xylanase (degradation of
193 hemicellulose). Phenol oxidase (degradation of phenolic compounds) was measured using 5 mM L-
194 dihydroxyphenylalanine (L-DOPA) as substrate. Further information on protocols for enzyme
195 analyses is reported elsewhere (Nottingham *et al.* 2015a). For each soil sample, five replicate micro-
196 plates were prepared and incubated at 2°C, 10°C, 22°C, 30°C and 40°C respectively, for calculation
197 of Q_{10} of V_{\max} (see below).

198 ***DNA sequencing and phospholipid fatty acid (PLFA) biomarkers:*** Soil microbial
199 community composition, including the relative abundances of bacterial and fungal groups, was
200 determined using phospholipid fatty acid (PLFA) biomarkers (Whitaker *et al.* 2014). Further

201 assessment of the relative abundances of specific bacterial and fungal phylotypes was made using
202 high-throughput sequencing to characterise the variation in marker gene sequences (Leff *et al.* 2015).
203 For bacterial community composition, the 16S rRNA gene was amplified in triplicate PCR reactions
204 using the 515f and 806r primers for bacterial and archaeal taxa. For fungal community composition,
205 the first internal transcribed spacer region (ITS1) of the rRNA gene was amplified using the ITS1-F
206 and ITS2 primer pair. For each soil sample, DNA was extracted using the MoBio PowerSoil DNA
207 isolation kit (MoBio Laboratories, Carlsbad, CA) following manufacturer instructions. Primers were
208 modified to incorporate 12 bp error-correcting barcodes, and 16S rRNA amplicons and ITS
209 amplicons were pooled separately prior to sequencing with two separate runs on an Illumina MiSeq
210 instrument at the University of Colorado at Boulder. Raw sequence data were processed using the
211 QIIME v1.7 pipeline, where sequences were de-multiplexed using their unique barcode specific to
212 individual samples and assigned to phylotypes (operational taxonomic units, OTUs, at 97%
213 similarity) using the 'open reference' clustering approach recommended in the pipeline (Caporaso *et al.*
214 *al.* 2012). Taxonomy was determined for each phylotype using the RDP classifier (Wang *et al.* 2007)
215 trained on the Greengenes (McDonald *et al.* 2012) and UNITE (Abarenkov *et al.* 2010) databases for
216 bacterial and fungal sequences. Relatively abundant phylotypes were checked using BLAST and
217 comparison against sequences contained within GenBank.

218 ***Temperature sensitivity of microbial respiration (RQ₁₀):*** Soil samples (8 g) from each soil
219 core (n = 3) were incubated in bottles at 5 temperatures (5, 12, 19, 26, 33°C), selected to span the
220 range of site mean annual temperatures (48 soil core samples at 5 temperatures, yielding 240 soil
221 incubations in total). All soils were adjusted to 80% water holding capacity. Soils were pre-incubated
222 at 20°C for 24 h and then the temperature was adjusted to specified incubation temperatures.
223 Following an initial incubation period of 2 h, bottle headspace was flushed with compressed air and
224 sealed. Soil incubations lasted for 48 h; air samples (5 ml) from bottle headspace was taken at 24 h
225 and 48 h for CO₂ analyses.

226

227 **Calculations**

228 **Determination of Q_{10} values:** We determined Q_{10} of enzyme activities (Q_{10} of V_{\max}) and
229 microbial respiration (RQ_{10}) according to:

230
$$Q_{10} = \exp(10 \times k) \quad (\text{equation 1})$$

231 and
$$k = \frac{\ln(a)}{t} \quad (\text{equation 2})$$

232 Where k is the exponential rate at which activity (a) increases with temperature (t) (Nottingham *et*
233 *al.* 2016). To calculate k (and thus Q_{10}) we used linear regression of $\ln(\text{activity})/\text{temperature}$, for $n =$
234 5 temperatures and $n = 3$ replicates per temperature.

235 **Determination of carbon and nutrient use efficiencies:** Microbial CUE is defined as the
236 fraction of C incorporated for growth over respiratory losses. However, it is acknowledged as an
237 emergent property of growth and allocation processes that can vary with the method used for its
238 estimation (Hagerty *et al.* 2018) (see Appendix S1 in Supporting Information). We determined
239 microbial carbon, nitrogen and phosphorus use efficiencies (CUE, NUE and PUE), using a widely-
240 accepted stoichiometric method, whereby the CUE/NUE/PUE of an organism is a function of the
241 difference between its elemental requirements for growth (C, N or P in biomass and enzymatic
242 investment for acquisition) and the abundance of environmental substrate (C, N, P in soil organic
243 matter) (Sinsabaugh *et al.* 2016). Following this approach, NUE and PUE are inversely related to
244 $CUE_{C:N}$ or $CUE_{C:P}$ (CUE calculated relative to enzymatic investment for N or P acquisition,
245 respectively). Therefore, we present NUE and PUE results but focus our hypotheses and discussion
246 on the responses of CUE. While acknowledging the assumptions and limitations of this approach
247 (see Appendix S1 in Supporting Information), this method is considered particularly useful for
248 parameterization and testing of models because it quantifies CUE in terms of the underlying
249 microbial processes (Hagerty *et al.* 2018). This approach assumes that enzyme activities scale with
250 microbial production and organic matter concentration, and that microbial communities exhibit

251 optimum resource allocation with respect to enzyme expression and environmental resources; these
252 assumptions are empirically supported by Michaelis-Menten kinetics and metabolic control analysis
253 (Sinsabaugh *et al.* 2016). Based on this underlying assumption, CUE is therefore calculated as
254 follows:

255

$$256 \text{ CUE}_{C:X} = \text{CUE}_{\text{MAX}} \left[\frac{S_{C:X}}{S_{C:X} + K_X} \right], \text{ where } S_{C:X} = (1/\text{EEA}_{C:X})(B_{C:X}/L_{C:X}) \quad (\text{equation 3})$$

257

258 Where $S_{C:X}$ is a scalar that represents the extent to which the allocation of enzyme activities offsets
259 the disparity between the elemental composition of available resources and the composition of
260 microbial biomass; K_X and CUE_{MAX} are constants: half-saturation constant (K_X) = 0.5; and the upper
261 limit for microbial growth efficiency based on thermodynamic constraints, $\text{CUE}_{\text{MAX}} = 0.6$. EEA is
262 extracellular enzyme activity ($\text{nmol g}^{-1} \text{ h}^{-1}$); $\text{EEA}_{C:N}$ was calculated as BG/NAG , where $\text{BG} = \beta$ -
263 glucosidase and $\text{NAG} = N$ -acetyl β -glucosaminidase; and $\text{EEA}_{C:P}$ was calculated as BG/P , where BG
264 = β -glucosidase and $\text{P} = \text{phosphomonoesterase}$. Molar ratios of soil organic C : total N : total P were
265 used as estimates of $L_{C:N}$ or $L_{C:P}$. Microbial biomass ($B_{C:X}$) C:N and C:P were also calculated as
266 molar ratios.

267 Nutrient use efficiencies (NUE and PUE), which are inversely related to CUE, were
268 calculated according to:

269

$$270 \text{ XUE}_{X:C} = \text{XUE}_{\text{MAX}} \left[\frac{S_{X:C}}{S_{X:C} + K_C} \right], \text{ where } S_{X:C} = (1/\text{EEA}_{X:C})(B_{X:C}/L_{X:C}) \quad (\text{equation 4})$$

271

272 Where X represents N or P, $K_C = 0.5$, and $\text{XUE}_{\text{MAX}} = 1.0$ (Sinsabaugh *et al.* 2016).

273

274 **Statistical analyses**

275 Our first hypothesis, that 5 years of temperature perturbation resulted in consistent changes in soil
276 organic matter cycling and soil C storage across sites (relative decreases under warming and relative
277 increases under cooling), was tested using ANOVA and by evaluating the relationships between the
278 translocation treatment and the relative response ratios of soil C parameters (total soil C and its
279 chemical fractions by ^{13}C -NMR). Our second hypothesis, that changes in soil C were determined by
280 specific soil physical, chemical or biological properties, was tested by using mixed effects models
281 with the relative response ratio of soil C as the response variable and the relative response ratios of
282 environmental and soil properties as explanatory variables. Our third hypothesis, that microbial
283 responses to temperature affected soil C change was tested by measuring: i) microbial community
284 composition, by determining the relative responses of individual bacterial and fungal phylotypes to
285 the elevation-shift treatment; and ii) microbial function, by determining the relative responses of Q_{10}
286 of V_{\max} for 7 soil enzymes to the elevation-shift treatment; by determining the relative responses of
287 substrate use efficiency parameters ($\text{CUE}_{\text{C:N}}$, $\text{CUE}_{\text{C:P}}$, NUE and PUE) to the elevation-shift
288 treatment; and by using mixed effects models with the relative response ratio of RQ_{10} as the response
289 variable and the relative response ratios of environmental and soil properties, including the Q_{10} of
290 V_{\max} for 7 soil enzymes, as explanatory variables. Relative response ratios were determined by: RR
291 of $X = \ln [(X(i=1-3) \text{ at destination} / X(\text{mean}) \text{ at origin})]$, where $n = 3$. Further details on these
292 approaches are provided in Supporting Information (Appendix S1). All statistical analyses were
293 performed in R (version 3.5.2).

294

295 **RESULTS**

296 The translocation of soil upslope (cooling) and downslope (warming) consistently increased
297 and decreased soil C respectively compared to controls. The change in soil C was equivalent to a
298 3.86% decline for each 1°C increase in temperature (Fig. 1; $p < 0.001$). Beyond temperature, the soil
299 properties that were most strongly related to the magnitude of this change were the concentration and

300 chemical composition of the initial soil organic matter (i.e. significant effects of soil-origin,
301 microbial biomass and alkyl:*O*-alkyl ratios; Table 2A). Across all soil properties, warming decreased
302 organic matter content (total C; *O*-alkyl and *di*-alkyl groups), acidified the soil, and increased the
303 availability of base cations (K, Na), potential toxins (extractable Al), microbial biomass (microbial C
304 and total PLFA), specific microbial groups (gram-positive bacteria) and enzyme activities (β -
305 glucosidase, *N*-acetyl β -glucosaminidase, phosphomonoesterase); and *vice versa* for cooling (Fig. 2).
306 These findings were supported by the overall effect of temperature on soil properties: warming
307 increased alkyl:*O*-alkyl ratios (an index of the degree of organic matter decomposition) and
308 microbial C:N and C:P ratios, and decreased available soil P and the temperature sensitivity of
309 phenol oxidase activity (Q_{10} of V_{\max} ; 'destination' effects; Tables S1-S2).

310 Microbial community composition and physiology responded to temperature manipulation.
311 Microbial community composition varied naturally along the gradient (Nottingham *et al.* 2018), but
312 a consistent subset of taxa within each community responded to temperature change across soil
313 types. The temperature response analysis (RR) of common microbial taxa revealed 30 warm-
314 responsive and 18 cold- responsive taxa (Fig. 3D, Figs. S2-S3), although the majority of taxa were
315 unaffected by the temperature change or were influenced by intrinsic soil properties (effect of soil
316 origin; Table S2).

317 Microbial physiology also responded to temperature. There were positive relationships
318 between temperature and the RR of $CUE_{C:N}$ and $CUE_{C:P}$ and a negative relationship for the RR of
319 NUE (Fig. 3A-3B), while microbial CUE was significantly affected by soil destination (i.e. the new
320 temperature regime) and not soil origin (Table S3). The instantaneous temperature-response of
321 respiration (RQ_{10}) at the microbial community-level (Karhu *et al.* 2014), was primarily determined
322 by soil destination (i.e. the new temperature regime; Table 2B), also consistent with the temperature
323 response being the result of a physiological or compositional change in microbial communities.

324

325 DISCUSSION

326 Across the range of tropical lowland-to-montane forests studied here, the change in soil C
327 with temperature was primarily determined by the size and chemical composition of soil C stocks.
328 Importantly, this change in soil C with temperature manipulation occurred alongside physiological
329 and compositional changes in soil microbial communities, in a manner consistent with the prediction
330 of enhanced soil C loss with warming (Wieder *et al.* (2013); see below). Scaling the observed 3.86%
331 change in total soil C per 1°C (Fig. 1) with the projected warming in these ecosystems over the next
332 century (Russell *et al.* 2017) yields a 16–32% decline in soil C with a 4–8°C warming. This loss in
333 soil C is greater than reported from field-based warming experiments in non-tropical ecosystems (Lu
334 *et al.* 2013; Crowther *et al.* 2016; Romero-Olivares *et al.* 2017), including a 17% decline in soil C
335 following 26 years of 5°C warming in a temperate forest (i.e., for comparison 0.7% loss per 1°C
336 warming per 5 year interval) (Melillo *et al.* 2017), and an average 1% decline calculated in meta-
337 analyses of soil warming experiments, based predominantly on data from temperate soils and
338 experiments that only warm the soil surface (Lu *et al.* 2013; Romero-Olivares *et al.* 2017). Our
339 extrapolation assumes that C loss (3.86% C per 1°C warming) would linearly scale over a 4–8°C
340 range and would not have increased if our study continued beyond 5 years and the specified amount
341 of warming. These assumptions may have yielded an underestimation of actual C loss over a longer
342 time period, given that sustained C loss occurred following 26 years of warming in temperate forest
343 (Melillo *et al.* 2017).

344 The soil C losses primarily originated from labile C pools, because the alkyl:*O*-alkyl ratio
345 explained most variation in soil C change with temperature manipulation (Table 1A). Specifically,
346 alkyl:*O*-alkyl and aryl:*O*-alkyl ratios increased with warming (Fig. 2; Table S3), indicating an
347 increased chemical recalcitrance of the residual soil C. Increases in these ratios with warming were
348 also detected two years after translocation (Zimmermann *et al.* 2012) and were related to a decrease
349 in *O*-alkyl groups (Fig. 2; Table S3), which are relatively labile and comprise a major component of

350 carbohydrates in plant debris. Thus, although more chemically recalcitrant compounds have a higher
351 intrinsic temperature sensitivity (Davidson & Janssens 2006), we demonstrate that labile compounds
352 in the montane forests studied here give a high apparent temperature sensitivity because of their
353 availability and abundance (total stocks of 11.8 kg C m⁻² at 0-10 cm depth) (Zimmermann *et al.*
354 2012). This study describes one of the largest soil C stocks represented in any soil warming study; in
355 recent meta-analyses only four out of 143 warming studies had >11 kg C m⁻² and three of those
356 reported large C loss with warming (Crowther *et al.* 2016; van Gestel *et al.* 2018), although there
357 was no relationship between C loss and a broader range of soil C stocks (van Gestel *et al.* 2018). Our
358 findings provide a key advance on results reported from global analyses of soil warming
359 experiments, which remain limited in their ability to make global predictions due to the lack of
360 information for tropical systems (van Gestel *et al.* 2018).

361 The large changes in soil C observed as a result of temperature manipulation occurred
362 alongside changes in the composition and physiology of microbial communities (Fig. 3C-D). A
363 previous short-term laboratory incubation study using soil from the same tropical elevation gradient
364 showed that microbial responses to warming would result in increased growth, potentially decreasing
365 soil C (Nottingham *et al.* 2019). Results from this five year field-translocation study provide long-
366 term data consistent with this, and show that warming changed microbial physiology by increasing
367 CUE, with a concomitant decrease in soil C. Temperature-responsive change in microbial CUE was
368 demonstrated by the positive correlation of the RR of CUE with temperature (Fig. 3A) and because
369 CUE was determined by soil-destination (i.e. new temperature; Table S3). In contrast to reports of
370 short-term decreases in CUE with warming (Tucker *et al.* 2013; Sinsabaugh *et al.* 2016), a longer-
371 term increase in CUE may occur following physiological or community-wide changes through
372 evolutionary processes (Wieder *et al.* 2013). For example, in a 5°C soil warming manipulation in
373 temperate forest, CUE decreased after five years, but increased after 18 years for more recalcitrant
374 substrates (Frey *et al.* 2013). The increased CUE in our study (Fig. 3A) occurred alongside increased

375 microbial biomass and enzyme activities (Fig. 2), contrary to the hypothesis of reduced biomass and
376 activity through thermal compensation (Manzoni *et al.* 2012). Similarly, in a global study of thermal
377 compensation of respiration following 90 days of laboratory incubation, no evidence was found for
378 thermal-compensation of respiration for samples from the same Peru forest sites (Karhu *et al.* 2014).
379 although Karhu *et al.* (2014) also found some geographical variation in thermal compensation of
380 microbial activity under warming. This global variability has been reflected in extra-tropical
381 warming experiments (Melillo *et al.* 2017; Romero-Olivares *et al.* 2017), although some of the
382 variability among studies may also result from the different methods and scales by which CUE and
383 thermal compensation has been defined (Geyer *et al.* 2016; Hagerty *et al.* 2018). While the
384 underlying mechanisms invite further investigation, our results suggest that the experimental
385 warming imposed here induced changes in microbial physiology and community composition that
386 accelerated soil C loss, with no thermal compensation of microbial activity, consistent with model
387 predictions of increased CUE under warming accelerating soil C loss (Wieder *et al.* 2013).

388 The changes in CUE in response to temperature occurred alongside changes in microbial
389 community composition. Although we cannot rule out dispersal as a factor affecting these microbial
390 community shifts (i.e. migration of microbes via aerial dispersal from the surrounding destination
391 site; see SI), which could only have been controlled for using an *in situ* soil warming experiment, a
392 dominant role for temperature shifts in driving these changes is suggested by the consistency
393 between our results and a recent global study of temperature-responsive bacterial taxa (Oliverio *et al.*
394 2017). The responsive taxa in our study overlapped with those identified in the global study, with
395 members of the Actinobacteria and Rhizobiales being more abundant in warmed soils (together, 75%
396 consistent with Oliverio *et al.*, 2017) and Acidobacteria becoming more abundant in colder soils
397 (71% consistent with Oliverio *et al.*, 2017), with the latter associated with oligotrophic N-limited
398 conditions such as those found in cooler montane ecosystems (Oliverio *et al.* 2017). Thus, microbial

399 taxa responded to temperature manipulation in a manner consistent with their previously-observed
400 thermal responses across global ecosystems.

401 Temperature adaptation of enzyme function across natural temperature gradients has been
402 associated with differences in the temperature sensitivity (Q_{10} response) of activity (V_{\max}), with
403 decreased Q_{10} of V_{\max} at higher temperature ranges (Brzostek & Finzi 2012; Nottingham *et al.* 2016),
404 although there is also evidence for the insensitivity of Q_{10} of V_{\max} for soil enzymes across natural
405 temperature gradients (Allison *et al.* 2018). This pattern of long-term temperature response of
406 enzyme activity was supported for only one out of seven measured enzymes (phenol oxidase)
407 following the five years of temperature manipulation. This finding implies that the temperature
408 sensitivity of phenolic oxidation, and the decomposition rate of recalcitrant C compounds, decreases
409 under warming. Several mechanisms might underlie this response, including changes in the
410 abundances of iso-enzymes with different temperature optima (Wallenstein *et al.* 2011), shifts in the
411 relative abundance of microbial taxa with different functional capabilities (Fig. 3D) and
412 physiological, and/or evolutionary changes in microbial function (e.g. increased selective pressure
413 for lignin-degrading microbial groups or capability). The response could also arise from abiotic
414 factors. For instance, soil acidification with warming (Fig. 2), which can reduce potential enzyme
415 activity (Burns & Staunton 2013), may have played a role. The response could further be related to a
416 change in the abundance of metal oxides (Mn, Fe, and Al), which contribute to humification
417 reactions by providing electron acceptors that catalyze the formation of reactive species from
418 phenols (Keiluweit *et al.* 2015). However, although amorphous manganese (Mn) oxide concentration
419 was positively correlated with phenol oxidase activity, it was not affected by translocation and was
420 not related to differences in the Q_{10} of activity (Fig. S6). Overall, despite the result for phenol
421 oxidase, the Q_{10} of V_{\max} for the remaining six enzymes was not affected by warming (Figs. S4-S5),
422 consistent with a recent global study showing an insensitivity of Q_{10} of V_{\max} to temperature for the
423 majority of enzymes (Allison *et al.* 2018). These results indicate that the dominant effect of

424 enzymatic responses to warming on soil C result from changes in V_{\max} , whether reduced (by thermal
425 compensation) or increased as shown here (Fig. 2).

426 Because our study is a soil translocation rather than an *in situ* warming experiment, it has
427 associated caveats. First, plants and hence plant-inputs to soil were absent from the translocated soil
428 monoliths, which could offset the change in soil C (Koven *et al.* (2015); see S1). Second, the
429 translocation design did not allow a test of the response of lowland tropical forest soils to novel
430 warm temperature regimes predicted this century (Cavaleri *et al.* 2015; Wood *et al.* 2019), and has a
431 principal focus on temperature responses between 11 and 26°C. However, because the translocation
432 approach tests the common soil and microbial responses that are shared among different soil types
433 (Table 1), it does enable generalisation across tropical forest soils. Notwithstanding these caveats,
434 our results clearly demonstrate the potential vulnerability of tropical forest soil C to warming, and
435 reveal the microbial responses that may be associated with this loss, especially where soil C stocks
436 are large and relatively labile.

437 In summary, we provide new evidence that long-term (five-year) warming induced
438 fundamental changes in microbial community physiology in tropical forest soils through increased
439 CUE, leading to reduced soil C stocks. This occurred alongside an underlying change in microbial
440 community composition and with no compensatory effect for the majority of soil enzymes. Our
441 findings provide field-based evidence for tropical forests to link changes in soil C under warming to
442 changes in microbial physiology and communities, resulting in increased CUE. This is a complex
443 process that has been conceptualized in models and shown to result in very large differences in the
444 potential impact on the future terrestrial carbon cycle depending on the nature of the response
445 (Wieder *et al.* 2013), and has not previously been studied in the tropics (Cavaleri *et al.* 2015). By
446 accounting for the response of microbial community physiology to temperature change, we: (i) show
447 that tropical forest soil C stocks are highly sensitive to short-term warming, imposing a positive

448 feedback on climatic warming; and (ii) demonstrate the fundamental need to account for microbial
449 responses in order to understand climate-induced changes in the tropical forest C cycle.

450

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668 **Figure legends:**

669

670 **Figure 1. The relative change in total soil C (%) in mineral soils following five years of**
671 **translocation.** Translocation represented an elevation shift of up to ± 3000 m, which was equivalent
672 to a warming or cooling treatment of up to $\pm 15^\circ\text{C}$. Calculations for log response ratio of soil C (RR
673 of %C) and description of the translocation design are provided in Supplementary Materials. The
674 linear relationship, $\% \text{ C RR} = 0.00703 + (0.0000824 * \text{elevation shift})$, equates to 0.021 %C RR for
675 every 1°C (or 170 m elevation), or 3.86% decrease in total soil C per 1°C increase in temperature (R^2
676 $= 0.23$; $p < 0.001$).

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678 **Figure 2. The effects of elevation shift (warming/cooling) on the log response ratios (RR) of soil**
679 **and microbial properties following 5 years of translocation.** For each soil and microbial property
680 (Extended Data Table 1), RR values were calculated (see SI) and regressions between RR value and
681 elevation shift (m) were determined. A negative relationship represents an increase in RR with
682 warming (or decrease in RR with cooling) and a positive relationship represents a decrease in RR
683 with warming (or increase in RR with cooling). Significant relationships are highlighted by asterisks
684 ($p < 0.05$).

685

686 **Figure 3. Temperature adaptive responses of microbial communities and physiology following**
687 **five years of translocation: carbon-use-efficiency (CUE) (A) nutrient-use-efficiency (B), phenol**
688 **oxidase activity (C) and community composition (D).** For A-B, CUE was calculated according to
689 microbial stoichiometry with respect to N ($\text{CUE}_{\text{C:N}}$) and P ($\text{CUE}_{\text{C:P}}$), according to equation 3.
690 Nitrogen (NUE) and phosphorus (PUE) use efficiencies were calculated according to equation 4 (ref.
691 30). For C, the temperature response of Q_{10} of V_{max} for phenol oxidase, we calculated the Q_{10} of V_{max}
692 by determining V_{max} at 2°C , 10°C , 20°C , 30°C , 40°C and fitting a Q_{10} function (equations 1-2). The

693 temperature responses of all 7 enzymes are shown in Figure S3 and the Q_{10} values of V_{\max} are
694 summarized in Extended Data Figure 4. For **D**, ‘Warm-adapted’ taxa significantly increased in their
695 relative abundance when soil was translocated downslope or decreased when translocated upslope
696 (phylotype responses are in Extended Data Figure 2). The temperature responses for all response
697 variables were estimated using linear regression of RR against the elevation shift ($p < 0.05$; error
698 bars are 1 standard error).

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718 **Table 1: Summary of site characteristics along the elevation gradient.** Mean annual temperature
719 and mean annual precipitation were determined over the period 2005-2010.

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Site name	Elevation (m asl)	Lat	Long	Mean annual temp (°C)	Mean annual precipitation (mm yr ⁻¹)	Parent material	Soil classification
Explorer's Inn plot 3 (TP3)	210	-12.830	-69.271	26	3199	Pleistocene alluvial terrace	Inceptisol
Tono	1000	-12.866	-71.401	21	3100	Paleozoic shales- slates	Inceptisol
San Pedro 2	1500	-13.049	-71.537	17	5302	Plutonic intrusion (granite)	Inceptisol
Wayqecha	3025	-13.190	-71.587	11	1706	Paleozoic shales- slates	Inceptisol

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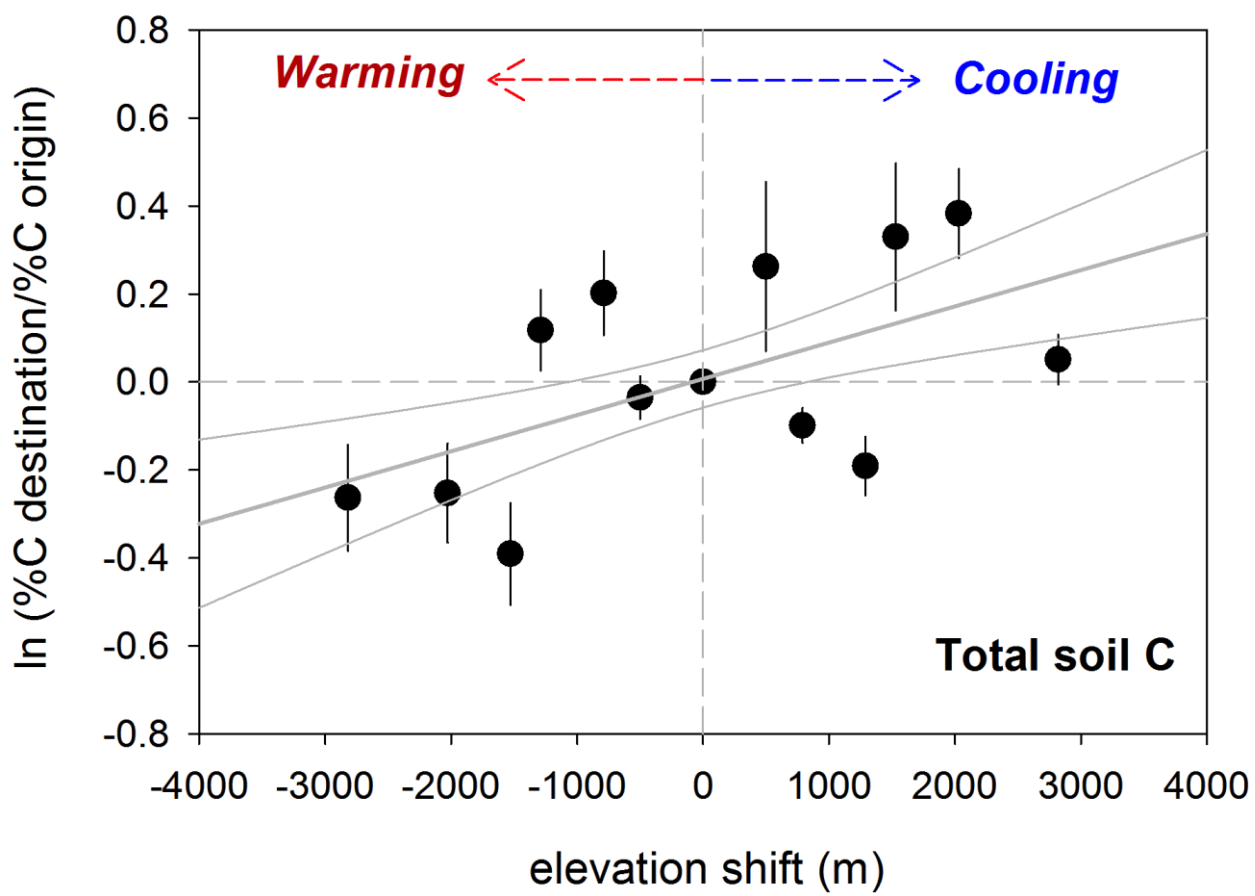
738

739 **Table 2. The effect of soil and environmental properties on the relative response of total soil C**
740 **(A) and on the instantaneous temperature sensitivity of microbial respiration (B).** Mixed-effects
741 models were fitted using maximum likelihood, by beginning with full model (70 variables) and step-
742 wise parameter removal. The final model was determined by lowest AIC value. The significance of
743 fixed effects was determined by AIC likelihood ratio tests comparing the full model against the
744 model without the specified term.
745

A) Relative response of total soil C				
	Parameter	SE	P-value	X ² test
<i>Fixed effects</i>				
Total PLFA	0.00498	0.00264	0.0680	0.0311 *
Alkyl:O-Alkyl	-0.69858	0.30904	0.0311	0.0323 *
<i>Random effects</i>				
Soil Origin	0.40469	0.27731	0.1545	
AIC value				11
R ²				0.631
B) Relative response of RQ₁₀				
	Parameter	SE	P-value	X ² test
<i>Fixed effects</i>				
Al	2.60e-04	7.79e-04	0.7406	0.7392
Microbial C:P	2.38e-03	8.42e-04	0.0071	0.0219 *
Bacteria PLFA	9.82e-03	5.66e-03	0.0901	0.6106
Alkyl:O-Alkyl	1.02e-01	6.29e-02	0.1133	0.1112
Phenol Oxidase	2.67e-02	4.45e-02	0.5517	0.5493
Q ₁₀ V _{max}				
β-Glucosidase Q ₁₀	7.80e-02	3.53e-02	0.0325	0.0315 *
V _{max}				
<i>Random effects</i>				
Soil Destination	7.26e-01	1.12e-01	7.38e-08	
AIC value				-125
R ²				0.277

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Positive temperature response

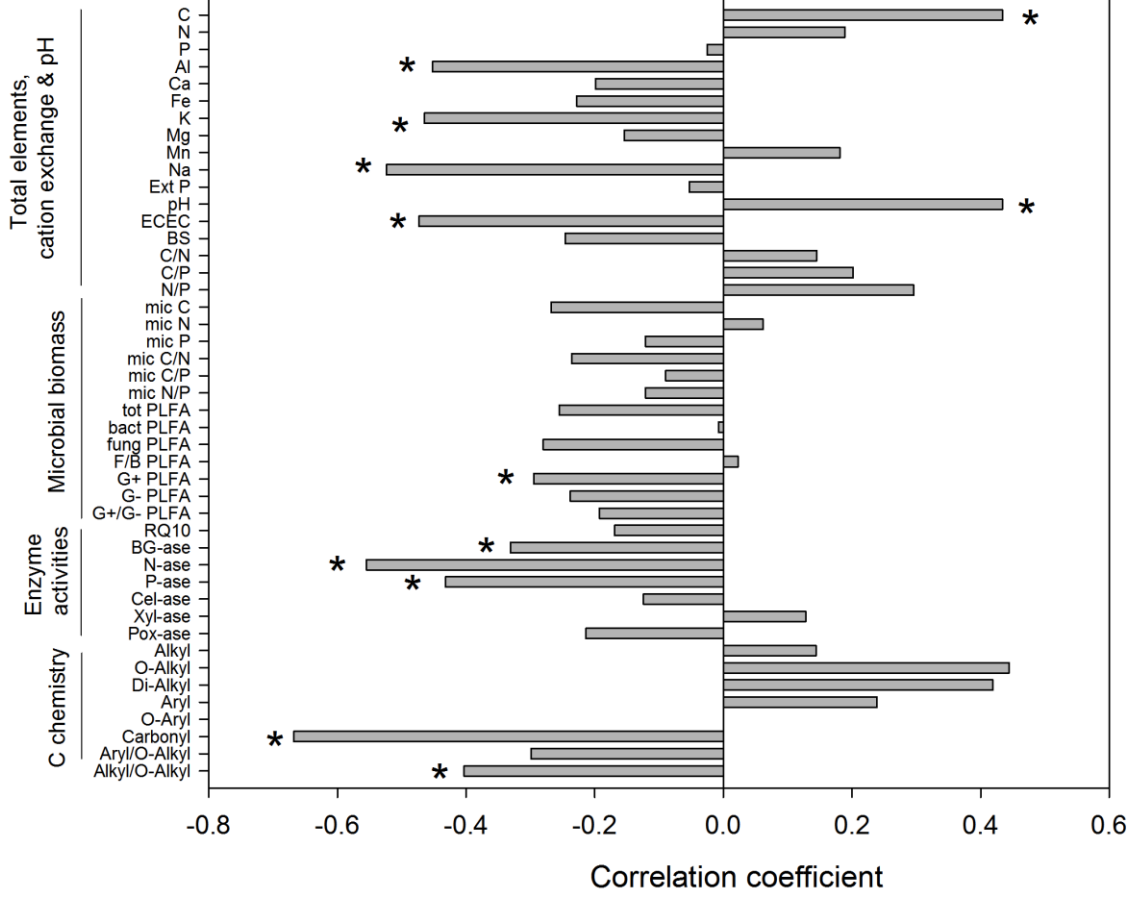
Negative temperature response

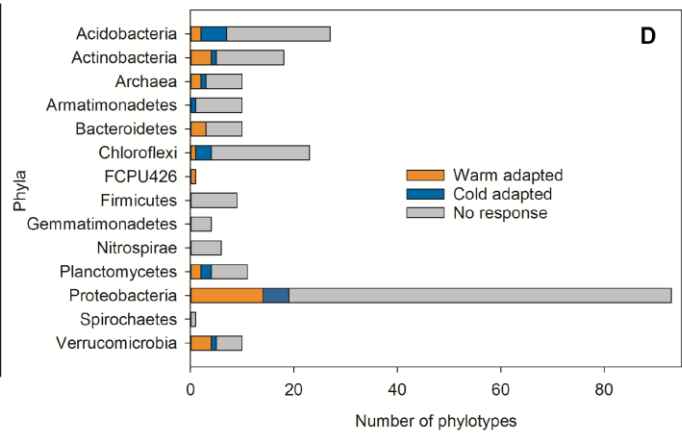
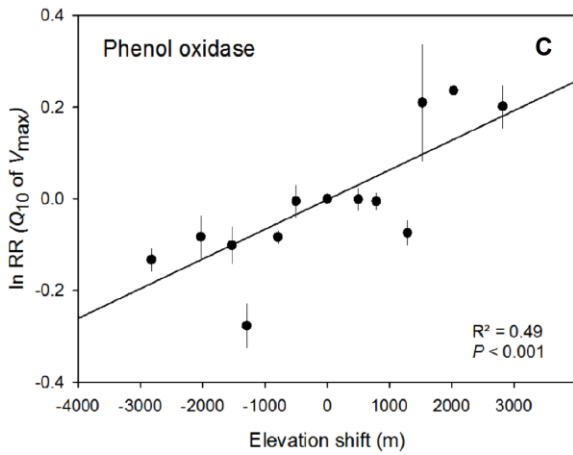
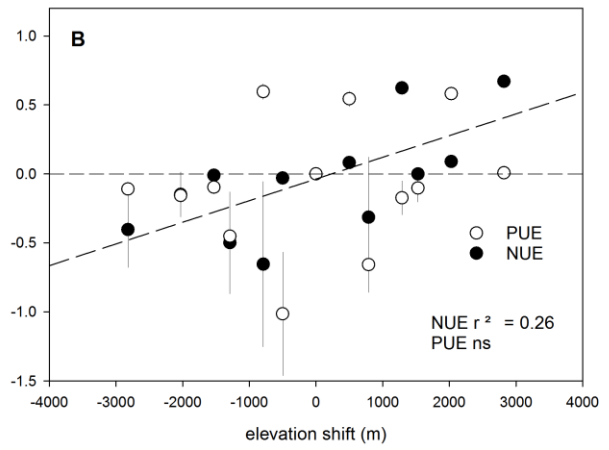
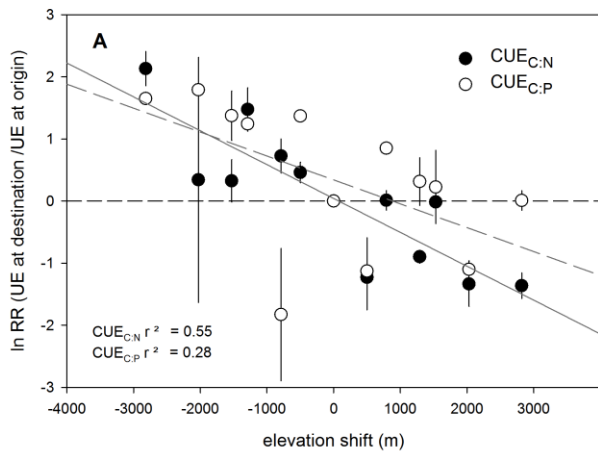
RR decrease with upslope translocation

RR increase with upslope translocation

RR increase with downslope translocation

RR decrease with downslope translocation





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