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Title: Microbial diversity and community response are decoupled from increased respiration in warmed tropical forest soil

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32 Soil microbes form some of the most diverse biological communities on Earth and are
33 fundamental in regulating the terrestrial carbon cycle. Their response to climate warming could
34 therefore have major consequences for future climate, particularly in tropical forests where high
35 biological diversity coincides with a vast store of soil carbon. Here, we show high sensitivity of
36 the tropical forest soil microbial community and growth to two-years of *in situ* soil warming,
37 which was decoupled from large increases in CO₂ emission. Microbial diversity declined
38 markedly, especially of bacteria. As the microbial community composition shifted under
39 warming, many taxa were no longer detected and others, including taxa associated with
40 thermophilic traits, were enriched. This community shift resulted in an adaptation of growth to
41 warmer temperatures, which we used to specify a microbial model to predict changes in soil CO₂
42 emissions. However, the observed *in situ* CO₂ emissions increase exceeded the rates predicted by
43 our model three-fold. The additional emissions were driven by acceleration of enzymatic activity,
44 likely through abiotic processes because activity increased even at temperatures beyond the
45 optimal temperature for microbial growth. Our results suggest that warming of tropical forests
46 will have rapid, detrimental consequences both for soil microbial biodiversity and future climate.

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56 Microbial communities sustain the biosphere by cycling carbon (C) and nutrients between the Earth
57 and the atmosphere. As a result, their response to warming provides a fundamental feedback on the
58 terrestrial C cycle and climate, and will have direct consequences for the function and maintenance of
59 terrestrial biota¹. The nature of this feedback is especially critical for tropical forests, because they
60 exchange more carbon dioxide (CO₂) with the atmosphere than any other ecosystem, contain over a
61 third of global soil C², two-thirds of terrestrial plant biomass³, and represent the apex of global
62 terrestrial biodiversity⁴. Under current emission scenarios, temperatures in the tropics are predicted to
63 warm by 2-5°C by 2100⁵ and to exceed historical precedent more quickly than anywhere else on Earth⁶.
64 Despite this, we have almost no information on the magnitude and direction of soil microbial feedbacks
65 under warming for the huge C stores and biodiversity found in tropical forests⁷.

66

67 Climate warming is predicted to increase soil C mineralisation and CO₂ emission from soil to the
68 atmosphere⁸. Numerous experiments performed outside the tropics have shown that warming increases
69 CO₂ emission from soil⁹, and that changes in the microbial community composition and activity
70 determine the associated soil C loss^{10,11}. In tropical forests where soils contribute a major portion of
71 these ecosystems' globally significant total C exchange with the atmosphere¹², small fractional
72 increases in CO₂ emission from soils will have a large impact on the atmosphere and climate. Warming
73 experiments in tropical forests have only recently been initiated and first results point towards a large
74 response. Two years of *in situ* full-profile soil warming by an average 4°C increased the soil CO₂ efflux
75 by 55% for a tropical forest in Panama¹³, and significantly for a tropical forest in Puerto Rico under
76 infra-red soil warming by 4°C (Wood et al, pers. com.)*. These results provoke key fundamental
77 questions: what are the drivers of the large CO₂ emissions from warmed tropical forest soils – and are

* Prior to publication, we will amend this line to include detail on the % increase and include the full citation of the paper from this Puerto Rico experiment (the authors understand the results are currently in review/press)

78 they related to abiotic or biotic process, including changes in the composition of the microbial
79 community, its diversity and/or its activity, as found in other ecosystems^{10,11,14}.

80

81 The response of soil C to warming is underpinned by changes in soil microbial activity, via the
82 instantaneous sensitivity of microbial growth and respiration, which can be modified over time by
83 adaptive change in the microbial community composition^{10,15}. These microbial responses have been
84 represented in models of soil C temperature sensitivity by the efficiency of growth and respiration¹⁶,
85 while the thermal response of growth and respiration has been described by the square root model^{15,17}.
86 In the square root model, the moderating effect of temperature adaptation is described by a change in
87 the theoretical value of T_{\min} (the minimum temperature for growth), corroborated by observations that
88 T_{\min} is strongly correlated to mean annual temperature differences across climatic gradients globally¹⁸⁻
89 ²⁰. For example, T_{\min} for bacterial growth ranges from approximately -15°C in arctic ecosystems to
90 approximately 0°C for tropical ecosystems, with similar patterns observed for T_{opt} ^{15,19} and for
91 respiration²⁰. Across temperate temperature ranges, T_{\min} has been observed to increase under
92 experimental warming^{21,22} alongside community compositional shifts^{14,23,24}, thus indicating that the
93 observed thermal adaptation occurred via microbial community composition change. Despite the
94 proven importance of this relationship in determining the temperature response of activity and its
95 thermal adaptation^{15,17}, we have no information on whether it holds under warming in the lowland
96 tropics, where the mean annual temperature is already close to the predicted optima for activity¹⁵.

97

98 The effect of warming on tropical forest soil C will depend not only on the response of the soil
99 microbial community activity⁷, but also its community composition and diversity, which may have
100 consequences for other biota²⁵. In a temperate forest, two decades of experimental warming increased
101 bacterial diversity,¹⁴ specifically for lignin-degrading microbes²⁶; this positive temperature-diversity
102 relationship is consistent with observations across natural temperature gradients where soil pH and

103 moisture are held constant^{23,27,28}. It is unknown whether soil microbial diversity will similarly increase
104 under the novel high-temperature regimes predicted for the tropics⁶ and will depend on the thermal
105 tolerance of the microbial taxa present^{24,29}. Nor is it understood how diversity change would affect soil
106 process rates, although the effect might be considerable given phylogenetic evidence for high niche
107 specialization among tropical forest microbial taxa³⁰. The historically-novel high temperature regimes
108 predicted for the tropics this century⁶ (e.g. 2-5°C atmospheric warming⁵ added to 1-3°C warming
109 through land-use change and reduced transpiration³¹) could result in temperature maxima that exceed
110 a metabolic threshold for portions of the tropical forest soil microbial community, with potentially
111 large implications for ecosystem functioning and the climate.

112
113 Here we used an *in situ* warming experiment to test the response of the soil microbial community, and
114 its growth and respiration to warming over a range of 3 to 8°C above ambient – thereby providing the
115 first test of how tropical forest soil communities and function respond across these levels of warming
116 in a field experiment. The experiment, SWELTR (Soil Warming Experiment in Lowland TRopical
117 forest) consists of five pairs of circular control and warmed plots (whole-profile warming, using buried
118 resistance cables) distributed evenly within approximately 1 ha of semi-deciduous moist lowland
119 tropical forest on Barro Colorado Island, Panama¹³. Each warmed plot has a ground surface area of
120 ~20 m² and is heated across the full soil profile, resulting in a total of 120 m³ of warmed soil for the
121 experiment. For this study we established two subplots per treatment plot that differed with distance
122 to the heating source, thus providing two treatments of, on average, 3°C and 8°C warming of surface
123 soils (0–20 cm depth). Two years after the warming treatment was initiated, we conducted field
124 campaigns during the wet season (when moisture was non-limiting) to measure soil CO₂ efflux, to
125 characterise the temperature sensitivity of instantaneous microbial growth, respiration and enzyme
126 activities, and to determine the microbial community composition. We tested the hypotheses that: (1)
127 warming will change the α -diversity and community composition of soil bacteria and fungi; (2) the

128 temperature sensitivity of microbial communities (with respect to growth, T_{\min} , and enzymatic activity)
129 will become ‘adapted’ to the new temperature regime (whether adaptation is via genetic change within
130 species, phenotypic plasticity or community-composition change, *sensu* Pietikäinen et al.; Bradford
131 ^{32,33}); and (3) soil CO₂ emission will increase under 3 to 8°C warming and follow the increase predicted
132 by the temperature sensitivity of microbial growth and respiration.

133

134 Two years of soil warming reduced the diversity of both bacteria and fungi and caused large shifts in
135 the microbial community composition (Fig. 1). The diversity decline was largest for bacteria, occurring
136 via the loss of proportionally-abundant taxa (Shannon and Inverse-Simpson indices declined; Fig. 1,
137 Extended Data Fig. 1). For fungi, our results suggest a diversity decline due to loss of rare taxa (species
138 richness declined but not Shannon and Inverse-Simpson indices), although this result is less definitive
139 that for bacteria, given methodological difficulties when detecting rare taxa (see Supplementary
140 Methods) and our detection of new fungal taxa in warmed soils (see below). Warmed soils also hosted
141 microbial species (defined by Amplicon Sequence Variants, ASVs) that were undetected in soils at
142 ambient temperature, especially among fungi, although the number of newly detected species was too
143 few to offset the number of species no longer detected (Fig. 1). This decline in diversity, especially for
144 the bacteria, may have negative implications for soil functioning, given the prevailing paradigm of a
145 positive relationship between biological diversity and ecosystem functioning³⁴, also supported for
146 soils^{35,36}. Such a decline in soil microbial diversity under warming is also contrary to positive
147 relationships between temperature and diversity observed in a temperate warming experiment¹⁴ and
148 across natural environmental gradients^{27,28,37}. This positive relationship is consistent with metabolic
149 theory of ecology (i.e. positive correlation between energy input, evolutionary rates and diversity)³⁸
150 and is considered to contribute a positive feedback on tropical plant diversity^{39, 40,41}. Our results point
151 towards a breakdown in this energy-diversity relationship for tropical soil bacterial communities after
152 a two-year period where temperatures ranged from 29–34°C. These temperatures may represent a

153 thermal maximum for the persistence of many species, implying that our findings can also provide
154 insight over timescales longer than the duration of our warming treatment.

155

156 Warming also caused large shifts in community composition (Figs. 1–2, Extended Data Figs. 1–5),
157 with many taxa significantly increasing or decreasing in relative abundance with warming by 3°C, and
158 further with warming by 8°C (Fig. 1; Extended Data Figs. 2–3). In warmed soils there was a decrease
159 in the relative abundance of Bacteroidetes, a common non-spore-forming bacterial group which
160 comprise taxa that are primary degraders of polysaccharides⁴². For fungi, there was decrease in the
161 relative abundance of members of the Basidiomycota including the Agaricales, a broad order of
162 saprophytic fungi, and the ecologically diverse yeast order, Sporidiobolales. In contrast, warming
163 increased the relative abundance of Firmicutes, a diverse and stress-tolerant bacterial phylum, able to
164 form endospores resistant to desiccation and high temperatures⁴³. Indeed, taxa within the Firmicutes
165 have been identified as warm-responsive in laboratory studies^{24,29} and in field soil warming
166 experiments outside the tropics^{14,44}. Warming also increased the abundance of the class
167 Thermoleophilia within the Actinobacteria, known to include aerobic thermophiles⁴⁵. For fungi,
168 warming increased the relative abundance of Glomerales—arbuscular mycorrhizae—as also seen in
169 warming experiments outside the tropics⁴⁶. In addition, warming increased the relative abundance of
170 several orders in the phylum Ascomycota, including the Eurotiales, Hypocreales and Pezizales, which
171 include thermotolerant saprophytic and pathogenic species, as well as saprophytic and pathogenic
172 yeast in the Saccharomycetales. Thus, broadly, changes in diversity under warming occurred alongside
173 shifts in communities towards thermotolerant microorganisms.

174

175 Adaptation of the microbial community to warming potentially can have a large influence on long-
176 term change in soil C emissions^{10,16}. To assess this, we used laboratory incubations to determine the
177 instantaneous temperature sensitivity of bacterial growth (T_{\min})^{15,17}. We found T_{\min} to increase under

178 3°C warming and to increase further under 8°C warming (Fig. 2); where the observed magnitude of
179 increase in T_{\min} , of 0.3°C per 1°C warming, is consistent with observations made elsewhere¹⁵.
180 Furthermore, among all the parameters associated with temperature adaptation in the field experiment,
181 T_{\min} was the most significant correlate of the change in bacterial and fungal diversity and community
182 composition (Fig. 2e, Extended Data Tables 1–2). Thus, while acknowledging that we cannot exclude
183 an influence of genetic change within species on this temperature adaptation, our results strongly
184 suggest that adaptation occurred through community compositional change, as found elsewhere²⁴, and
185 the development of a microbial community functionally adapted to the warmer conditions.
186
187 These changes in diversity and community composition occurred alongside altered soil process rates
188 in the field experiment: increased bacterial growth rates, enzyme activity per unit microbial biomass
189 for 7 hydrolytic and oxidative enzymes involved in C, N and P cycling (although microbial biomass
190 remained stable) and, measured *in situ*, increased soil CO₂ emission (Figs. 2–3, Extended Data Figs.
191 5–6). Soil CO₂ emission in the field experiment increased markedly at warmer temperatures: 78%
192 higher than controls under 3°C warming and 337% higher under 8°C warming of surface soils (Fig. 3;
193 Extended Data Table 3). The soil CO₂ efflux response for the wet season was consistent with the
194 previously-reported 55% increase over 2-years of 3°C surface soil warming at this experiment
195 (including dry and wet seasons), which was shown to have arisen predominantly from increased
196 heterotrophic microbial activity¹³. Our observation of increased soil metabolic activity, indicated by
197 increased bacterial growth and enzyme activity with *in situ* soil warming, describes a further
198 acceleration of heterotrophic activity with warming. Enzymatic activity per unit of microbial biomass
199 increased for 7 out of 10 studied enzymes and markedly at +8°C *in situ* warming for enzymes that
200 degrade organic phosphorus, nitrogen, and carbon in phenolic and hemicellulose compounds (Fig. 2,
201 Extended Data Fig. 5–6). Collectively, the observed changes in process rates—of increased respiration,
202 growth and enzymatic activity per unit microbial biomass—corroborate our parallel findings that the

microbial community shifted towards favouring thermotolerant taxa that readily persist and even increase in productivity under warmer conditions.

However, the predicted increase in soil CO₂ efflux based on the measured temperature sensitivity of microbial respiration and growth in control soils (24–68% increase under 3–8°C warming; Fig. 3), was substantially exceeded by the observed *in situ* increase in soil CO₂ efflux (78–337% under 3–8°C warming; Fig. 3). Furthermore, the predicted CO₂ emission was only marginally higher when accounting for adaptation of the microbial community to warmer conditions (measured T_{min} increase; Fig. 2), resulting in a 25–77% increase under 3–8°C warming (Fig. 3). Importantly, we found no evidence to suggest that the observed *in situ* increase in soil CO₂ emission occurred due to decreased microbial metabolic efficiency, a common finding in short-term soil warming experiments where high waste respiration exceeds growth⁴⁷. Reduced metabolic efficiency is inconsistent with our previously reported observation of no decrease in the size of the microbial biomass or in microbial CUE⁴⁸ (measured using a stoichiometric method, see Supplementary Methods for discussion of this method and its assumptions; Extended Data Fig. 5); a result in line with the independent observation of increased microbial biomass under soil warming in tropical forest in Puerto Rico⁴⁹. Similarly, we cannot explain the augmented soil CO₂ emission by reference to accelerated substrate depletion, which would also be expected to cause a decline in microbial biomass⁵⁰. Indeed, microbial biomass remained stable despite evidence for substrate depletion (decreased DOC and available P at 8°C warming; Extended Data Fig. 5). Soil warming can also induce soil drying, potentially influencing CO₂ emission and other community and process rate changes⁸. However, our study here was focused on the tropical rainy season and despite lower moisture content in our +8°C treatment (Extended Data Fig. 5), we expect this had negligible influence on our results because moisture remained non-limiting to microbial activity. Finally, the augmented *in situ* soil CO₂ emission cannot be explained by increased root respiration or substrate supply from root exudates, because by using root-partitioning cores we

found that warming had no effect on the root-derived soil CO₂ efflux¹³. Thus, we show that the temperature response of microbial community metabolism to warming—considered in models to be fundamental in explaining the long-term, and relatively large, response of soil C to climate warming^{16,48}—only accounted for 23–32% of the observed *in situ* soil CO₂ emission.

232

In addition to biotic processes, our data point towards a further influence of abiotic processes in accelerating CO₂ emission at warmer temperatures. By using *ex situ* soil incubations across 2–40°C, we found that microbial growth declined at temperatures exceeding 34°C (Fig. 2); but enzyme activities increased—as did *in situ* soil CO₂ emissions (Figs. 2–3; Extended Data Fig. 6). These results can be explained by the effect of warming on the soil physico-chemical environment, including chemical oxidation/hydrolysis and desorption of mineral-stabilised organic matter and extracellular enzymes (Supplementary discussion)⁵¹. Clay-rich soils, such as those found at our tropical forest site, contain a large pool of stabilised C and inactive extracellular enzymes adsorbed to clay minerals.⁵² At high temperatures desorption reaction rates can overtake adsorption reaction rates⁵³, thereby increasing the pools of active enzymes and labile C, and consequent CO₂ emissions. Consistent with a rapid increase in the pool of active enzymes driven by desorption, under warming we observed increased Q_{10} of V_{\max} for four enzymes including phosphomonoesterase, β -xylanase and β -glucosidase (Fig. 2, Extended Data Fig. 5). A combination of these processes therefore resulted in increased enzyme activity that was uncoupled from growth (Fig. 2), contributing substantially to the observed CO₂ emissions that exceeded the predicted increase based on standard expectations from the observed temperature sensitivity and warm-adaptation response of the microbial community¹⁵ (i.e. it was exceeded by 3.1–4.4 fold; Fig. 3).

250

In summary, our results show a progressive decline in tropical forest soil microbial diversity, especially for bacteria, and clear microbial community compositional shifts with warming (Fig. 1), occurring

alongside community growth-adaptation to temperature (Fig. 2) and resulting in further increased CO₂ emission (Fig. 3). This response of diversity declines under warming is contrary to observations from temperate forest warming studies^{14,26}. Our data thus provide empirical support for the hypothesis that tropical soil communities are highly sensitive to warming and are consistent with independent evidence for deep evolutionary niche specialization in tropical soil microbes³⁰. Further, we note that in view of the widespread evidence for intensive feedbacks among tropical soil microbial communities, plant diversity, and soil processes^{25,41}, declines in diversity may have substantial implications for overall tropical forest functioning, composition, and diversity in a warmer world. Alongside the decline in diversity observed in this experiment, the concurrent increased abundance of thermotolerant species resulted in a stable microbial biomass, accelerated enzymatic activity, and increased soil CO₂ emissions. This finding partially supports prior model-based projections showing increased C loss under climate warming this century due to adaptation of microbial growth¹⁶. However, our results go further by demonstrating that microbial models alone do not accurately predict the change in soil C emissions under warming in tropical ecosystems, especially at high temperatures where abiotic processes may accelerate C loss. Further study is urgently required to understand these combined biotic and abiotic controls on soil C in different tropical soils, the timescales of their effects, and the wider consequences of declines in soil microbial diversity for the functioning and composition of tropical forests in a warmer world.

METHODS

Site and experiment. The experiment is situated in seasonally moist lowland tropical forest on Barro Colorado Island, Panama. Within the experiment area (1 ha) the dominant tree species include *Anacardium excelsum* and *Poulsenia armata*; a full census of tree and understory species composition in this forest is available for a nearby 50 ha forest plot in forest with similar soils, tree species and

278 demographic composition⁵⁴. The soils are Inceptisols (Fine, isohyperthermic, Dystric Eutrudepts) that
279 are rich in clay (~54% profile-weighted clay concentration) and secondary metal oxides. The soils
280 developed on the volcanic facies of the Bohio Formation, a basaltic conglomerate of Oligocene age⁵⁵.
281 Inceptisols account for 14% of total land area in the tropics (Ultisols and Oxisols account for 20% and
282 23%, respectively)⁵⁶.

283

284 The SWELTR experiment consists of 10 circular plots (five paired plots ‘warm’ and ‘control’). Each
285 plot measures 5 m diameter, with approximately 10 m between each plot-pair and a minimum of 20 m
286 between different plot-pairs. The experiment heats approximately 120 m³ soil in total (5 plots x 5 m
287 diameter by 1.2 m depth). Temperature in the internal plot area (~3 m diameter) of each warmed plot
288 was maintained at 4°C above the temperature in each corresponding paired control plot, based on the
289 average temperature from 0–120 cm depth at the mid-radius points in each plot. For this study we
290 established subplots representing a high-temperature treatment, situated in a buffer-zone close to the
291 heating cable. We therefore had two subplots per plot, situated at approximately 10 cm and 1 m
292 distance from the one of the main heating rods, representing two different levels of warming. The
293 average warming for the low-warming subplot was 2.8°C and for the high-warming subplot was 7.9°C
294 (determined at 0–10 cm soil depth), based on the difference in temperature between control plots.
295 Thus, our study consisted of three treatments, soil at 26 ± 1°C (‘Control’), 29 ± 2°C (‘+3°C’) and 34 ±
296 7°C (‘+8°C’), providing a test of moderate (atmospheric warming with moderate fossil fuel emission
297 reduction) to extreme (atmospheric warming plus deforestation) predictions of warming for tropical
298 soils this century^{5,31}. Further information on the plot design, thermostat control and power
299 specifications can be found in Nottingham *et al.* 2020¹³.

300

301 ***Soil gas-exchange and partitioning.*** Soil CO₂ efflux was measured every week at four systematically
302 distributed locations within each plot from June 2018 to September 2018 (representing the 3°C surface

soil-warming treatment); and was measured twice-weekly at two systematically distributed locations within the high-warming subplot from August to September 2018 (representing the 8°C surface soil-warming treatment). Soil CO₂ efflux measurements were made using an infra-red gas analyser (IRGA Li-8100; LI-COR Biosciences, Nebraska, USA) and at the same time we measured soil temperature (using a HI98509 thermometer probe; Hanna Instruments, USA) and soil moisture (using a Thetaprobe; Delta-T, Cambridge, UK) at 0–20 cm soil depth for a random location immediately adjacent to each soil collar.

310

Soil sampling. Soil for this study was sampled during the wet season (June–Sept) in 2018. We sampled during the wet season to ensure that there was no moisture limitation to soil microbial activity and soil processes, and no difference in moisture limitation among treatments. Soil was sampled from 0–10 cm depth from the mineral horizon for each subplot and analysed for properties: total elements, available nutrients, exchangeable cations, microbial C, N and P and enzyme activities using standard procedures (see below and Supplementary Methods). We calculated microbial carbon-use-efficiency (CUE) using microbial CNP and enzyme activity data using a stoichiometric method (see Supplementary Methods). Soil samples were stored at –60°C until DNA extraction (see below). All analyses were performed on replicate soil samples (n = 5).

320

Soil properties. Soil microbial biomass C and N were measured by fumigation-extraction^{57,58} and extractable C and N were determined by fresh soil extraction in 0.5 M K₂SO₄. Extracts were analyzed for extractable organic C and N using a TOC-VCHN analyzer (Shimadzu, Columbia, MD). Microbial C and N were calculated as the difference between fumigated and unfumigated extracts and corrected for unrecovered biomass using a *k* factor of 0.45⁵⁹. Microbial biomass P was determined by hexanol fumigation and extraction with anion-exchange membranes⁶⁰. Extractable P was determined using unfumigated samples and microbial P was calculated as the difference between the fumigated and

328 unfumigated samples, with correction for unrecovered biomass using a k_p factor of 0.4⁶⁰. Exchangeable
329 cations were determined by extraction in 0.1 M BaCl₂ and detection by inductively coupled plasma-
330 optical emission spectrometry (Optima 7300 DV; Perkin-Elmer Ltd, Shelton, CT, USA). Effective
331 cation exchange capacity (ECEC) was calculated as the sum of the charge equivalents of Al, Ca, Fe,
332 K, Mg, Mn and Na. Soil pH was determined in deionized water in a 1:2 soil to solution ratio. All
333 analyses apart from total elements (C, N, P), cations, and pH were determined on fresh soils within 24
334 hours of sampling, and K₂SO₄ extracts within 6 h. All soil chemical properties are expressed on the
335 basis of oven-dry equivalent soil (determined by drying at 105°C for 24 hours).

336

337 ***DNA extraction, sequencing, and processing.*** DNA was extracted using the DNeasy Powersoil kit
338 (Qiagen) and communities (bacterial and fungal) were amplified using a two-stage PCR protocol. For
339 bacteria, we amplified the V4 hypervariable region of the 16S rRNA and for fungi we amplified the
340 first internal transcribed spacer (ITS1) region of the rRNA operon (see SI methods for complete
341 details). Libraries were sequenced on an Illumina MiSeq with 250bp paired end reads. Reads in the
342 16S rRNA and ITS data sets were first trimmed of forward and reverse primers. Based on visual
343 inspection of read quality profiles, we removed the reverse reads from the 16S rRNA analysis due to
344 poor quality. We then used DADA2⁶¹ within the R environment (R Core Team, 2019) (v4.1.0) to filter
345 and trim both datasets (based on quality profiles), error correct, dereplicate, and infer amplicon
346 sequence variants (ASVs). We then merged pair-end reads (ITS only) and constructed sequence tables
347 for both datasets. In the final step, we removed chimeras and assigned taxonomy (see Supplementary
348 Methods for further detail).

349

350 ***Instantaneous temperature response of microbial growth and respiration.*** We used the instantaneous
351 temperature response of microbial growth and respiration to: i) predict the effect of warming on *in situ*
352 soil CO₂ emissions and ii) to determine the temperature adaptation of the bacterial community

353 following two years of *in situ* warming. For the former, we measured the instantaneous temperature
354 response of respiration and bacterial and fungal growth for control soils only. For the latter, we
355 measured the instantaneous temperature response of bacterial community growth for all warming
356 treatments and controls; assuming the temperature adaptation respiration and fungal growth responded
357 similarly as for bacterial growth, as found in tropical soils elsewhere^{19,20}. To determine the temperature
358 response of bacteria growth, we used the leucine incorporation method¹⁹; for the temperature response
359 of fungal community growth, we used the acetate-in-ergosterol method¹⁹; for the temperature response
360 of instantaneous respiration, we used incubation assays of 2 g soil in 20 ml vials for 24–140 hours at
361 10–30°C and measurement of headspace CO₂. For full details, see Supplementary Methods and
362 references therein.

363

364 **Soil enzymes.** Soil enzyme activity (V_{\max}) was determined for ten enzymes involved in carbon and
365 nutrient cycling, We used microplate assays to measure activity of α -glucosidase and β -glucosidase
366 (degradation of α - and β -bonds in glucose), cellobiohydrolase (degradation of cellulose), β -xylanase
367 (degradation of hemicellulose), *N*-acetyl β -glucosaminidase (degradation of *N*-glycosidic bonds),
368 leucine aminopeptidase (degradation of leucine residues; N-rich amino acids), phenol oxidase
369 (degradation of lignin via oxidation of phenols), phosphomonoesterase and phosphodiesterase
370 (degradation of monoester- and diester-linked simple organic phosphates) and sulfatase (degradation
371 of ester sulfates). For hydrolytic enzymes we used fluorometric assays with 100 μ M
372 methylumbelliferone (MU)-linked substrates, except for leucine aminopeptidase for which we used 7-
373 amino-4-methylcoumarin (AMC) substrates. For oxidative enzymes (phenol oxidase) we used
374 absorbance assays with L-3,4-dihydroxyphenylalanine (L-DOPA) substrates. For each soil sample,
375 five replicate micro-plates were prepared and incubated at 2°C, 10°C, 22°C, 30°C and 40°C respectively,
376 for calculation of the temperature sensitivity (Q_{10} of V_{\max}) and determination of V_{\max} at soil

377 temperature. Enzyme activities (V_{\max}) are expressed on the basis activity at soil temperature per unit
378 of microbial biomass C. To determine the Q_{10} of V_{\max} we used:

379

380
$$Q_{10} = \exp (10 \times k); \text{ where } k = \ln \frac{V_{\max}}{t}$$

381

382 Where k is the exponential rate at which V_{\max} increases with temperature (t). To calculate k (and thus
383 Q_{10}) we used linear regression. See Supplementary Methods for detailed protocols.

384

385 ***Treatment effects on soil properties.*** To determine treatment effects on soil CO₂ emissions, soil
386 moisture and temperature we used repeated measures ANOVA fitted by maximum likelihood
387 (repeated measures model with time as random factor). To determine treatment effects (levels: control,
388 +3°C and +8°C) on soil properties we used one-way ANOVA with post-hoc Tukey HSD tests. We
389 used this approach for all soil properties, including enzyme V_{\max} and the Q_{10} of V_{\max} for each enzyme
390 determined at soil temperature. Prior to analyses all data were tested for normality using a Shapiro-
391 Wilk test and log-transformed where non-normally distributed.

392

393 ***Determination of T_{\min} for respiration and growth and the predicted response of CO₂ efflux to in situ***
394 ***warming.*** In the square root model, the effect of temperature on activity is described by a quadratic
395 increase up to an optimal temperature (T_{opt}) and then a sharp decline^{15,17}, where the quadratic phase
396 of the increase is constrained by the minimum temperature for activity (T_{\min} , the y-intercept of the
397 square root of activity plotted against temperature). The T_{\min} of microbial activity was calculated using
398 empirically defined microbial activity across the temperature range 4–28°C (where the increase in the
399 SQRT of activity is linear), according to the Ratkowsky (square root) equation^{15,17}:

400
$$\sqrt{\text{Activity}} = a * (T - T_{\min})$$

401 where T is the measurement temperature, T_{\min} is the minimum temperature for activity (temperature
402 where activity = 0) and a is empirically defined by the slope parameter from the square root of activity
403 plotted against temperature; and where activity is either bacterial or fungal growth rates, or respiration.
404 We determined T_{\min} for each field replicate ($n = 5$ plots).

405 We then used the instantaneous temperature sensitivity of microbial activity (T_{\min}) to model
406 the CO_2 efflux response to warming, both with and without microbial community adaptation. Here we
407 used T_{\min} for bacteria growth only, because there was no significant difference in the T_{\min} for bacterial
408 growth (-1.39 ± 0.8) and respiration (0.3 ± 0.4) in control soils ($P = 0.1$). The T_{\min} values for bacterial
409 growth in control soils were also similar to those determined independently for two lowland tropical
410 forests in Peru with similar mean annual temperature (-1.66 ± 0.7 , -1.77 ± 1.0 ; MAT = 26.4°C)¹⁹.

411 To model the CO_2 efflux response to warming we used the following equation:

412
$$\text{Predicted } \text{CO}_2 = [a * (T - T_{\min})]^2$$

413 where T_{\min} is for control soils. To model the CO_2 efflux response to warming following temperature-
414 adaptation of microbial communities, we refitted the model using the T_{\min} determined for bacterial
415 growth in experimentally warmed soils for two years by 3°C and 8°C ('adapted' communities).

416

417 **Microbial community analysis.** To determine temperature treatment effects on alpha diversity of soil
418 bacterial and fungal communities, we first applied general prevalence filtering using the R package
419 PERFect (PERmutation Filtering test for microbiome data)⁶² (v0.2.4). Here we used the function
420 PERFect_sim with the alpha parameter set to 0.05 for the 16S rRNA data and 0.1 for the ITS data. We
421 also applied two complementary methods of prevalence filtering to determine how filtering influenced
422 alpha diversity estimates (see Supplementary Methods for complete details). We then calculated Hill
423 numbers using the R package hilldiv⁶³ (v1.5.1), specifically Observed richness (q-value = 0), Shannon
424 exponential (q-value = 1), and Simpson multiplicative inverse (q-value = 2). We used Shapiro-Wilk
425 Normality test and Bartlett's test of Homogeneity of Variances to determine whether Hill numbers

426 were normally distributed. In cases where both p-values were greater than 0.05 (parametric data), we
427 used ANOVA followed by Tukey post-hoc analysis to test for significance. For non-parametric data
428 (cases where one or both p-values were less than 0.05), we instead used Kruskal-Wallis followed by
429 Dunn test with Benjamini-Hochberg correction.

430

431 For soil bacterial and fungal beta diversity, we calculated distance matrices for the filtered data sets
432 using unweighted and weighted UniFrac⁶⁴ for the 16S rRNA data and Jensen-Shannon Divergence
433 and Bray-Curtis for the ITS data. To test for temperature treatment effects on beta diversity, we used
434 the vegan package⁶⁵ (v2.5-7) to first calculate beta dispersion for the distance matrices (betadisper
435 function), then perform a Permutation Test for Homogeneity of multivariate dispersions (permutest
436 function), and finally run PERMANOVA (adonis function; assuming equal dispersion) or Analysis of
437 Similarity (ANOSIM; where beta dispersion was significant).

438

439 To identify ASVs from the bacterial and fungal communities that were differentially abundant across
440 temperature treatments, we used Indicator Species Analysis (ISA)⁶⁶ and linear discriminant analysis
441 (LDA) effect size (LEfSe)⁶⁷. Prior to differential abundance analysis, we applied PIME (Prevalence
442 Interval for Microbiome Evaluation)⁶⁸ (v0.1.0) filtering to both complete datasets. PIME is a slightly
443 more aggressive filtering tool specifically designed to work with data sets containing high variation
444 among samples⁶⁸ — a pattern observed in the +8°C warming samples from the 16S rRNA data and all
445 treatments from the ITS data (Extended Data Figs. 1c and 1f). PIME applies prevalence filtering on a
446 per treatment basis and removes a substantial amount of within-group variation by eliminating low
447 abundance ASVs in each treatment and retaining only those ASVs shared at the selected level of
448 prevalence, within a given treatment⁶⁸. Per the developer's recommendation, we first rarefied all
449 samples to even depths (per sample: 16S rRNA = 25,088 reads, ITS = 9172 reads) and then split the
450 data sets by predictor variable (temperature treatment) using the pime.split.by.variable function in R.

451 Next, we calculated all prevalence intervals from 5% to 95% (increments of 5%) with the function
452 `pime.prevalence` and then used the function `pime.best.prevalence` to choose the best prevalence. The
453 best prevalence interval was selected when the out-of-bag (OOB) error rate first reached zero or close
454 to zero. The most prevalent ASVs (at the best prevalence interval) were retained from each split. Splits
455 were then merged to obtain the final, PIME filtered data set. ISA was computed with the R package
456 *labdsv*⁶⁹ (v2.0-1)—ASVs were considered an indicator of a treatment if they had a p-value less than
457 or equal to 0.05. LEfSe analysis was performed within the R package *microbiomeMarker*⁷⁰ (v0.0.1)
458 using the following parameters: pre-sample normalization of the sum of values set to $1e^{+06}$, `lda_cutoff`
459 = 2, `kw_cutoff` = 0.5, and `wilcoxon_cutoff` = 0.5. We used *anvi'o*⁷¹ (v7-dev) to visualize the
460 distribution of PIME-filtered 16S rRNA ASVs represented by more than 100 total reads and PIME-
461 filtered ITS ASVs represented by more than 50 reads. We then overlaid the results of the ISA and
462 LEfSe analyses. Hierarchical clustering of ASVs was performed using Euclidean distance and Ward
463 linkage against the ASV/sample abundance matrix while hierarchical clustering of samples was
464 performed using Bray-Curtis distance and complete linkage.

465

466 To assess potential drivers of change in microbial community composition, we used three subsets of
467 metadata to test correlations with community change; 1) environmental properties, 2) soil functional
468 responses, and 3) temperature adaptive responses. For each of the three metadata subsets, we
469 performed the following steps: i) use Shapiro-Wilk Normality Test to determine which metadata
470 parameters are normally distributed; ii) use the R package *bestNormalize*⁷² to find and execute the best
471 normalization transformation for non-normally distributed parameters; iii) perform autocorrelation
472 tests for all pair-wise comparisons; iv) remove autocorrelated parameters; v) run Mantel Tests to
473 determine if any of the metadata subsets are significantly correlated with microbial community data;
474 and vi) use the `bioenv` function (*vegan* package) to identify metadata parameters that are most strongly
475 correlated with the community data. In last step, vii) we performed distance-based redundancy analysis

(dbRDA) using capscale from the vegan package. First, we ran rankindex (vegan) to select the best community dissimilarity index. Then, we ran capscale for distance-based redundancy analysis. Next, we used envfit (vegan) to fit environmental parameters onto ordinations. And finally, we selected all metadata parameters that were significant for bioenv (see above) and/or envfit analyses for plotting the ordinations and vector overlays. For full details including all references on community analyses methods, see Supplementary Methods.

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667 **End notes**

668
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677

678 **Author contributions** | ATN conceived the study. ATN, JJS, MM, JP, EB, KB and KS performed the study.
679 ATN and JJS analysed the data. ATN wrote the paper with input from JJS, EB, KS, KB and PM.

680
681 **Author Information** | The authors declare no competing financial interests. Mention of trade names or
682 commercial products in this publication is solely for the purpose of providing specific information and does not
683 imply recommendation or endorsement by the USDA. The USDA is an equal opportunity provider and
684 employer. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and
685 requests for materials should be addressed to A.T.N. (A.Nottingham@leeds.ac.uk).

686

687 **Data availability**

688 Trimmed (primers removed) sequence data generated in this study are deposited in the European Nucleotide
689 Archive (ENA) under Project Accession number PRJEB45074 (ERP129199), sample accession numbers
690 ERS6485270–ERS6485284 (16S rRNA) and sample accession numbers ERS6485285– ERS6485299 (ITS).
691 Raw fastq files can be accessed through the Smithsonian figshare, at <https://doi.org/10.25573/data.14686665>
692 (16S rRNA) and <https://doi.org/10.25573/data.14686755> (ITS). Related data and data products for individual
693 analysis workflows are available through the Smithsonian figshare under the collection
694 <https://doi.org/XXXXX>.[†]

695

696 **Code availability**

[†] A figshare DOI for the collection containing related data & data products will be generated upon final manuscript acceptance.

697 All code, reproducible workflows, and further information on data availability can be found on the project
698 website at <https://sweltr.github.io/high-temp/>. The code embedded in the website is available on GitHub
699 [<https://github.com/sweltr/high-temp/>] in R Markdown format. The version of code used in this study is
700 archived under SWELTR Workflows v1.0 (REF) [<https://github.com/sweltr/high-temp/releases/tag/v1.0>],
701 DOI identifier, <https://doi.org/XXX/zenodo.XXXXXX>.[‡]

702

703

704 **Extended Data is available for this manuscript, including the following:**

705 **Extended Data: Tables 1 to 3**

706 **Extended Data: Figs. 1 to 6**

707 **Supplementary Information is available for this manuscript, including the following:**

708 **Supplementary Methods**

709 **Supplementary Discussion**

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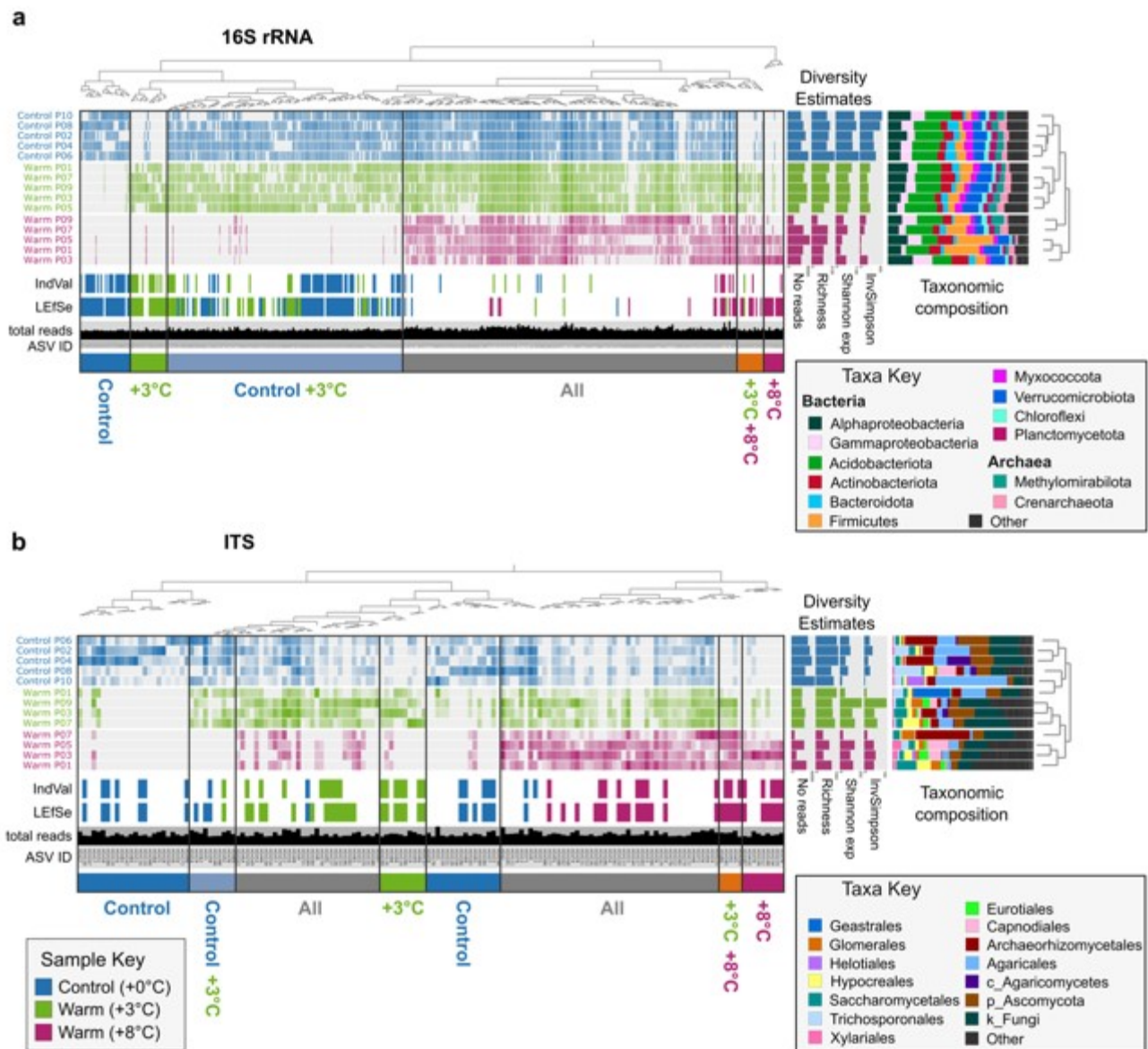
720

[‡] A workflow version number (GitHub) and a DOI for the code (generated through Zenodo) will both be generated upon final manuscript acceptance.

721 **Figures**

722 **Figure 1 | Microbial diversity decline and community change under 3°C and 8°C *in situ* soil warming in**
723 **lowland tropical forest.** Two years of soil warming caused significant decreases in (a) bacterial and (b) fungal
724 diversity, determined by 16S rRNA and ITS sequencing, respectively. Data from the PIME filtered data sets for
725 controls (blue), 3°C warming (green) and 8°C warming (red). Hierarchical clustering of ASVs (top dendrograms)
726 based on Euclidean distance and Ward linkage. Hierarchical clustering of samples (right dendrograms) based
727 on Bray-Curtis distance and complete linkage. Each vertical line in the main plot represents a unique ASV,
728 where colour intensity indicates the log-normalized abundance, and no colour indicates an ASV that was either
729 not detected or removed during prevalence filtering. The coloured bars below indicate ASVs that were enriched
730 in different temperature treatments as determined by either Indicator Species Analysis (IndVal) or Linear
731 discriminant analysis Effect Size (LEfSe). Additional data for each sample are presented in the plots on the
732 right. Diversity estimates charts show the total number of reads, observed richness, Shannon exponential index,
733 and Inverse Simpson index. Taxonomic profiles show the proportion of major classes (16S rRNA data) or orders
734 (ITS data).

735

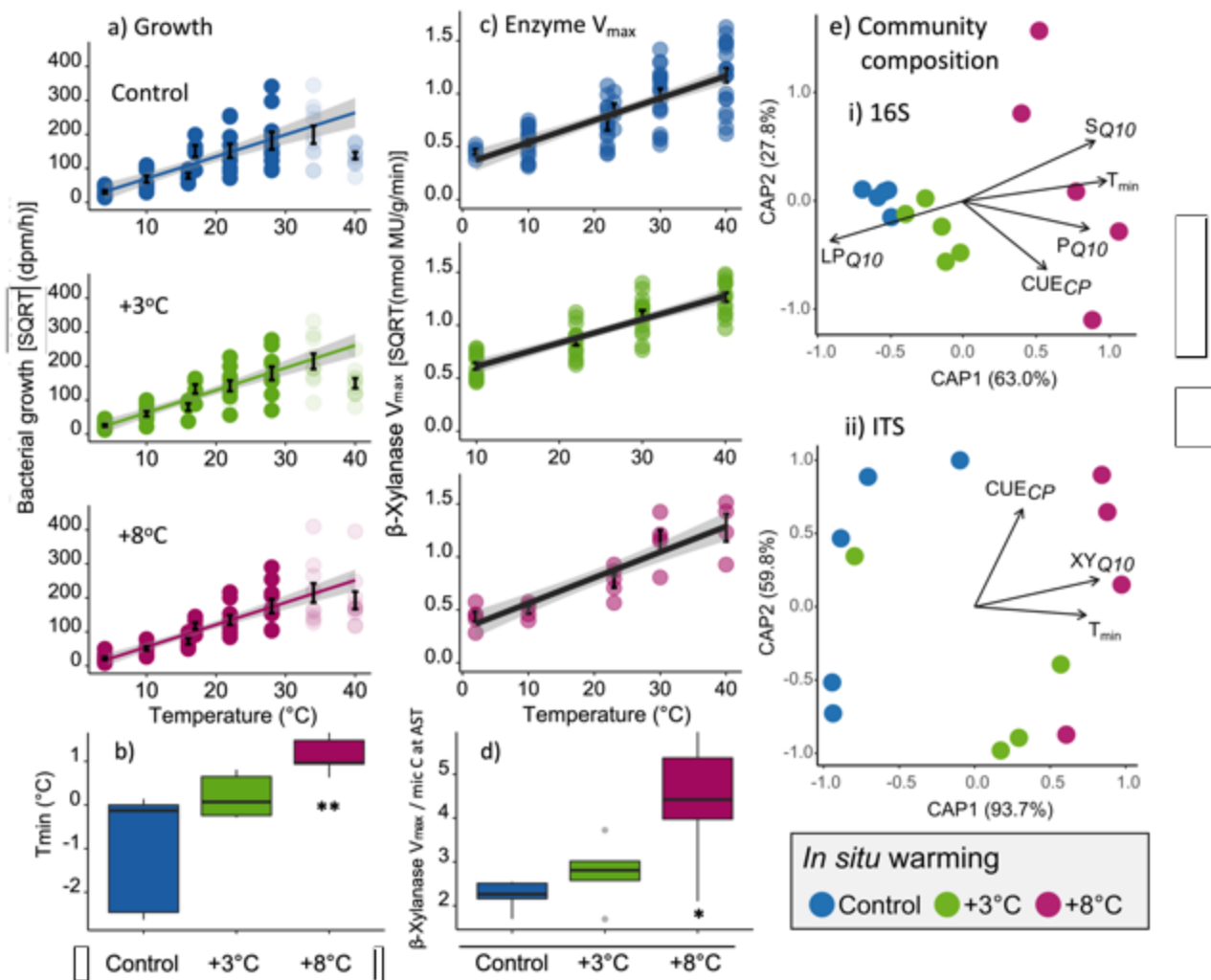


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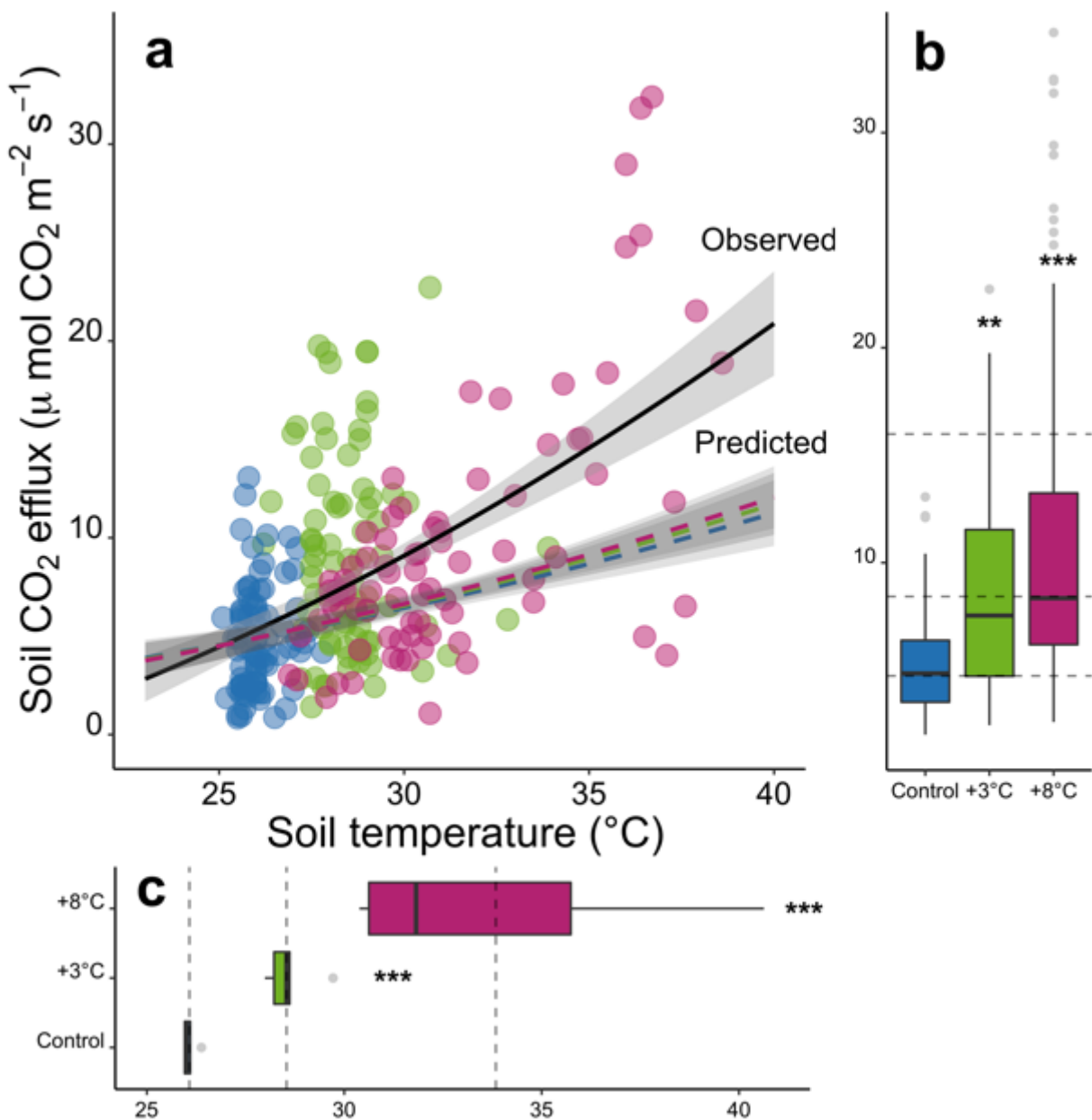
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Figure 2 | The response of (a–b) microbial growth and (c–d) enzyme activity to 3°C and 8°C soil warming, and (e) the relationship between the temperature response of growth and activity with microbial community changes. (a–b) Microbial growth was determined for bacteria for each treatment using Leu-incorporation incubation assays across a temperature range of 4–40°C. The minimum temperature for growth (T_{\min}) increased with warming (see **b**), but growth declined at high temperatures (>30–34°C; see lighter shaded points in **a**); these data were not used for the linear model to determine T_{\min} . **(c)** Activities were determined for 10 enzymes (β -xylanase shown here, six others responded similarly; see SI) across an incubation temperature range of 4–40°C. The maximum potential activity—at soil temperature per unit microbial C—increased with warming for 7 out of 10 enzymes (see **d**) and increased across high temperature ranges (to 40°C) illustrating a decoupling of growth and activity above 30°C. **(e)** The microbial community composition change was related to the temperature response of growth (T_{\min}) and of enzyme activities (Q_{10} of V_{\max}) for **i)** bacteria and **ii)** fungi. Bacterial growth and enzyme activity are plotted using a linear transformation (square root). Microbial community composition change estimated using Distance-based Redundancy Analysis (db-RDA) based on Bray-Curtis dissimilarity; see Extended Data (Table 2, Fig. 4) for relationships between community composition change and other soil properties. All analyses are for $n = 5$ plots.



763 **Figure 3 | The response of soil CO₂ efflux to *in situ* warming by 3 to 8°C is greater than the increase**
764 **predicted by the temperature response of microbial respiration and growth (a).** Data points are
765 measurements of soil CO₂ efflux from control (blue), 3°C warming (green) and 8°C warming (red). The response
766 of CO₂ emission to temperature was described by a square root function ('Observed' line; $\text{CO}_2 = 1.9 \times T^2 - 45$;
767 $R^2 = 0.68$, $P < 0.001$, $F = 556$). The modelled CO₂ efflux responses ('Predicted' lines) are based on measured
768 T_{\min} at ambient temperature (blue dash line = no adaptation; $\text{CO}_2 = 1.21 \times T^2 - 0.17$; $R^2 = 0.87$, $P < 0.001$, $F =$
769 124) and T_{\min} change after two years of warming indicating community adaptation (green dash line = 3°C
770 adaptation, $\text{CO}_2 = 1.24 \times T^2 - 0.18$; $R^2 = 0.87$, $P < 0.001$, $F = 118$; and red dash line = 8°C adaptation, $\text{CO}_2 =$
771 $1.25 \times T^2 - 0.20$; $R^2 = 0.86$, $P < 0.001$, $F = 111$). The box plots show the treatment effects on (b) soil CO₂ efflux
772 and (c) soil temperature (repeated measures ANOVA; ** $P < 0.01$; *** $P < 0.001$). The centre line of each box
773 plot represents the median, the lower and upper hinges represent the first and third quartiles and whiskers
774 represent ± 1.5 the interquartile range; the dashed lines represent means. The soil temperature and soil CO₂
775 efflux by treatment was, for controls: $26 \pm 1^\circ\text{C}$ and $4.74 \pm 0.25 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, 3°C warming: $29 \pm 2^\circ\text{C}$ and
776 $8.42 \pm 0.44 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, 8°C warming: $34 \pm 7^\circ\text{C}$ and $15.98 \pm 1.68 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (mean \pm one standard
777 error, $n = 5$ plots).



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