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

Running head: microbial responses enhance soil carbon loss

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ABSTRACT

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

INTRODUCTION

The response of soil organic matter decomposition to increasing temperature is predicted to contribute a significant positive feedback to climate change (Davidson & Janssens 2006; Crowther *et al.* 2016; Melillo *et al.* 2017). This positive feedback is expected because biochemical reaction rates increase exponentially with temperature, and because the global soil carbon (C) stock is of sufficient magnitude that even small fractional increases in organic matter decomposition will cause large corresponding CO_2 emissions, increasing the concentration of atmospheric CO_2 (Davidson & Janssens 2006). However, the nature of this feedback in different ecosystems remains uncertain because organic matter decomposition is mediated by complex biological and physicochemical interactions, including microbial metabolism, enzymatic catabolism, and effects of substrate quality and nutrient availability. In particular, this positive feedback has been hypothesized to be strongly regulated by microbial responses to warming, which could either enhance or reduce the expected increases in CO_2 emissions following increased biochemical reaction rates (Frey *et al.* 2013; Wieder *et al.* 2013; Hagerty *et al.* 2014).

Despite the importance of the response of soil C and microbial physiology to warming, this has not been assessed empirically in tropical forests. This knowledge gap is significant because tropical forests represent 42% of forested global land area (Pan *et al.* 2011) and their soils contain a

third of global soil C (Jobbagy & Jackson 2000). As a consequence, understanding the potential for feedbacks between climate and soil carbon in tropical forests is urgently needed to improve the parameterization of Earth system models used to predict future atmospheric CO_2 and climate (Cavaleri *et al.* 2015; Koven *et al.* 2015; Luo *et al.* 2016). The temperature response of soil organic matter decomposition is likely to differ between the tropics and higher-latitudes due to differences in nutrient availability, biodiversity, species composition, and in the temperature optima of the biota (Wood *et al.* 2019). The large stocks of relatively labile soil C in tropical montane ecosystems (Zimmermann *et al.* 2012), where thermal niches are often narrow and climate warming projections are steep (Loomis *et al.* 2017; Russell *et al.* 2017; Fadrique *et al.* 2018), are especially vulnerable to warming and could create a globally large soil-climate feedback (Nottingham *et al.* 2015b). Indeed, the response to warming in the tropics remains one of the major gaps in our understanding of terrestrial ecosystem responses to climate change in Earth system models (Huntingford *et al.* 2009; Cavaleri *et al.* 2015; Koven *et al.* 2015), and the size of the soil C-climate feedback is a dominant component of this uncertainty.

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

The wide range of microbial feedbacks hypothesized in models reflects limited understanding of this important climate response, and has confounded attempts to model the change in soil C under warming, leading to hugely divergent modelling outcomes (Wieder *et al.* 2013; Hagerty *et al.* 2018).

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

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

MATERIALS AND METHODS

We translocated soil among four tropical forest sites along the elevation gradient. Soil was translocated as intact cores, 10 cm diameter × 50 cm depth (4000 cm^3). Three undisturbed soil cores were re-installed at the same site ('control'), and the other cores were translocated to the three other elevations to achieve both warming and cooling (downslope = 'warmed', upslope = 'cooled') (Zimmermann *et al.* 2012), an approach similar to laboratory-based studies of thermal-responses of microbial activity (Karhu *et al.* 2014). To assess changes in soil C and thermal-responses of microbial communities and their physiology after five years in a new temperature regime, we quantified the concentration and composition of soil C (using solid-state ^{13}C-NMR spectroscopy), nutrient concentrations, microbial community characteristics (using 16S and ITS rRNA gene sequencing and phospholipid fatty acid, PLFA, biomarkers), and metrics of soil microbial physiology (CUE, instantaneous respiration temperature-sensitivity RQ_{10}, and enzyme activities, Q_{10} of V_{max}). Changes in these metrics of soil microbial physiology with temperature may occur through different mechanisms, including acclimation (physiological responses of individuals), adaptation (genetic changes within species) and ecological responses (shifts in community composition). Therefore, rather than refer to acclimation or adaptation, we use the terms 'CUE response' and 'enzyme Q_{10} response'. We evaluated the relationships between relative log-response ratios (RR) for 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^{o}C to 11^{o}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 m^2 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

together will be important determinants of the overall tropical forest C cycle (Meir *et al.* 2015). New litter input was excluded and root ingrowth prevented by installing a 63 μm nylon mesh at the base of the tubes. A detailed description of the experimental setup is given in Zimmermann et al. (2009). Soil cores were translocated in 2008 and, exactly five years later in 2013, mineral soil was sampled from each core using an auger to 20 cm depth. Soil samples were stored for < 14 days at < 4 °C until DNA extraction, respiration assays, and determination of nutrient content and enzyme activities; this method has been shown to have negligible effects on soil microbial and enzymatic properties (Lauber *et al.* 2010; Turner & Romero 2010). Soil samples were freeze-dried and stored for < 3 months prior to PLFA extraction.

Soil analyses

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

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

DNA sequencing* and *phospholipid fatty acid (PLFA biomarkers): Soil microbial community composition, including the relative abundances of bacterial and fungal groups, was determined using phospholipid fatty acid (PLFA) biomarkers (Whitaker *et al.* 2014). Further assessment of the relative abundances of specific bacterial and fungal phylotypes was made using high-throughput sequencing to characterise the variation in marker gene sequences (Leff *et al.* 2015). For bacterial community composition, the 16S rRNA gene was amplified in triplicate PCR reactions

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

Temperature sensitivity of microbial respiration (RQ_{10}): Soil samples (8 g) from each soil core (n = 3) were incubated in bottles at 5 temperatures (5, 12, 19, 26, 33^{o}C), selected to span the range of site mean annual temperatures (48 soil core samples at 5 temperatures, yielding 240 soil incubations in total). All soils were adjusted to 80% water holding capacity. Soils were pre-incubated at 20^{o}C for 24 h and then the temperature was adjusted to specified incubation temperatures. Following an initial incubation period of 2 h, bottle headspace was flushed with compressed air and sealed. Soil incubations lasted for 48 h; air samples (5 ml) from bottle headspace was taken at 24 h and 48 h for CO_2 analyses.

Calculations

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

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

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

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

Determination of carbon and nutrient use efficiencies: Microbial CUE is defined as the fraction of C incorporated for growth over respiratory losses. However, it is acknowledged as an

emergent property of growth and allocation processes that can vary with the method used for its estimation (Hagerty *et al.* 2018) (see Appendix S1 in Supporting Information). We determined microbial carbon, nitrogen and phosphorus use efficiencies (CUE, NUE and PUE), using a widely-accepted stoichiometric method, whereby the CUE/NUE/PUE of an organism is a function of the difference between its elemental requirements for growth (C, N or P in biomass and enzymatic investment for acquisition) and the abundance of environmental substrate (C, N, P in soil organic matter) (Sinsabaugh *et al.* 2016). Following this approach, NUE and PUE are inversely related to $CUE_{C:N}$ or $CUE_{C:P}$ (CUE calculated relative to enzymatic investment for N or P acquisition, respectively). Therefore, we present NUE and PUE results but focus our hypotheses and discussion on the responses of CUE. While acknowledging the assumptions and limitations of this approach (see Appendix S1 in Supporting Information), this method is considered particularly useful for parameterization and testing of models because it quantifies CUE in terms of the underlying microbial processes (Hagerty *et al.* 2018). This approach assumes that enzyme activities scale with microbial production and organic matter concentration, and that microbial communities exhibit optimum resource allocation with respect to enzyme expression and environmental resources; these assumptions are empirically supported by Michaelis-Menten kinetics and metabolic control analysis (Sinsabaugh *et al.* 2016). Based on this underlying assumption, CUE is therefore calculated as follows:

$$CUE_{C:X} = CUE_{MAX}\,[S_{C:X}/(S_{C:X} + K_X)], \text{ where } S_{C:X} = (1/EEA_{C:X})(B_{C:X}/L_{C:X}) \qquad \text{(equation 3)}$$

Where $S_{C:X}$ is a scalar that represents the extent to which the allocation of enzyme activities offsets the disparity between the elemental composition of available resources and the composition of microbial biomass; K_x and CUE_{MAX} are constants: half-saturation constant (K_x) = 0.5; and the upper limit for microbial growth efficiency based on thermodynamic constraints, CUE_{MAX} = 0.6. EEA is extracellular enzyme activity (nmol g^{-1} h^{-1}); $EEA_{C:N}$ was calculated as BG/NAG, where BG = β-glucosidase and NAG = *N*-acetyl β-glucosaminidase; and $EEA_{C:P}$ was calculated as BG/P, where BG = β-glucosidase and P = phosphomonoesterase. Molar ratios of soil organic C : total N : total P were 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 molar ratios.

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

$$XUE_{X:C} = XUE_{MAX}\,[S_{X:C}/(S_{X:C} + K_C)], \text{ where } S_{X:C} = (1/EEA_{X:C})(B_{X:C}/L_{X:C}) \qquad \text{(equation 4)}$$

Where X represents N or P, K_C = 0.5, and XUE_{MAX} = 1.0 (Sinsabaugh *et al.* 2016).

Statistical analyses

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

RESULTS

The translocation of soil upslope (cooling) and downslope (warming) consistently increased and decreased soil C respectively compared to controls. The change in soil C was equivalent to a 3.86% decline for each 1^{o}C increase in temperature (Fig. 1; $p < 0.001$). Beyond temperature, the soil properties that were most strongly related to the magnitude of this change were the concentration and chemical composition of the initial soil organic matter (i.e. significant effects of soil-origin, microbial biomass and alkyl:*O*-alkyl ratios; Table 2A). Across all soil properties, warming decreased organic matter content (total C; *O*-alkyl and *di*-alkyl groups), acidified the soil, and increased the availability of base cations (K, Na), potential toxins (extractable Al), microbial biomass (microbial C and total PLFA), specific microbial groups (gram-positive bacteria) and enzyme activities (β-

glucosidase, *N*-acetyl β-glucosaminidase, phosphomonoesterase); and *vice versa* for cooling (Fig. 2). These findings were supported by the overall effect of temperature on soil properties: warming increased alkyl:*O*-alkyl ratios (an index of the degree of organic matter decomposition) and microbial C:N and C:P ratios, and decreased available soil P and the temperature sensitivity of phenol oxidase activity (Q_{10} of V_{max}; 'destination' effects; Tables S1-S2).

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

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

DISCUSSION

Across the range of tropical lowland-to-montane forests studied here, the change in soil C with temperature was primarily determined by the size and chemical composition of soil C stocks. Importantly, this change in soil C with temperature manipulation occurred alongside physiological and compositional changes in soil microbial communities, in a manner consistent with the prediction of enhanced soil C loss with warming (Wieder *et al.* (2013); see below). Scaling the observed 3.86% change in total soil C per 1°C (Fig. 1) with the projected warming in these ecosystems over the next century (Russell *et al.* 2017) yields a 16–32% decline in soil C with a 4–8°C warming. This loss in soil C is greater than reported from field-based warming experiments in non-tropical ecosystems (Lu *et al.* 2013; Crowther *et al.* 2016; Romero-Olivares *et al.* 2017), including a 17% decline in soil C following 26 years of 5°C warming in a temperate forest (i.e., for comparison 0.7% loss per 1°C warming per 5 year interval) (Melillo *et al.* 2017), and an average 1% decline calculated in meta-analyses of soil warming experiments, based predominantly on data from temperate soils and experiments that only warm the soil surface (Lu *et al.* 2013; Romero-Olivares *et al.* 2017). Our

extrapolation assumes that C loss (3.86% C per 1°C warming) would linearly scale over a 4–8°C range and would not have increased if our study continued beyond 5 years and the specified amount of warming. These assumptions may have yielded an underestimation of actual C loss over a longer time period, given that sustained C loss occurred following 26 years of warming in temperate forest (Melillo *et al.* 2017).

The soil C losses primarily originated from labile C pools, because the alkyl:*O*-alkyl ratio explained most variation in soil C change with temperature manipulation (Table 1A). Specifically, alkyl:*O*-alkyl and aryl:*O*-alkyl ratios increased with warming (Fig. 2; Table S3), indicating an increased chemical recalcitrance of the residual soil C. Increases in these ratios with warming were also detected two years after translocation (Zimmermann *et al.* 2012) and were related to a decrease in *O*-alkyl groups (Fig. 2; Table S3), which are relatively labile and comprise a major component of carbohydrates in plant debris. Thus, although more chemically recalcitrant compounds have a higher intrinsic temperature sensitivity (Davidson & Janssens 2006), we demonstrate that labile compounds in the montane forests studied here give a high apparent temperature sensitivity because of their availability and abundance (total stocks of 11.8 kg C m^{-2} at 0-10 cm depth) (Zimmermann *et al.* 2012). This study describes one of the largest soil C stocks represented in any soil warming study; in recent meta-analyses only four out of 143 warming studies had >11 kg C m^{-2} and three of those reported large C loss with warming (Crowther *et al.* 2016; van Gestel *et al.* 2018), although there was no relationship between C loss and a broader range of soil C stocks (van Gestel *et al.* 2018). Our findings provide a key advance on results reported from global analyses of soil warming experiments, which remain limited in their ability to make global predictions due to the lack of information for tropical systems (van Gestel *et al.* 2018).

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

temperate forest, CUE decreased after five years, but increased after 18 years for more recalcitrant substrates (Frey *et al.* 2013). The increased CUE in our study (Fig. 3A) occurred alongside increased microbial biomass and enzyme activities (Fig. 2), contrary to the hypothesis of reduced biomass and activity through thermal compensation (Manzoni *et al.* 2012). Similarly, in a global study following 90 days of laboratory incubation, no evidence was found for thermal-compensation of respiration for samples from the same Peru forest sites (Karhu *et al.* 2014). although Karhu *et al.* (2014) did find some geographical variation in this process.. This global variability has been reflected in extra-tropical warming experiments (Melillo *et al.* 2017; Romero-Olivares *et al.* 2017), although some of the variability among studies may also result from the different methods and scales by which CUE and thermal compensation has been defined (Geyer *et al.* 2016; Hagerty *et al.* 2018). While the underlying mechanisms invite further investigation, our results suggest that the experimental warming imposed here induced changes in microbial physiology and community composition that accelerated soil C loss, with no thermal compensation of microbial activity, consistent with model predictions of increased CUE under warming accelerating soil C loss (Wieder *et al.* 2013).

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

Temperature adaptation of enzyme function across natural temperature gradients has been associated with differences in the temperature sensitivity (Q_{10} response) of activity (V_{max}), with decreased Q_{10} of V_{max} at higher temperature ranges (Brzostek & Finzi 2012; Nottingham *et al.* 2016), although there is also evidence for the insensitivity of Q_{10} of V_{max} for soil enzymes across natural temperature gradients (Allison *et al.* 2018). This pattern of long-term temperature response of enzyme activity was supported for only one out of seven measured enzymes (phenol oxidase) following the five years of temperature manipulation. This finding implies that the temperature

sensitivity of phenolic oxidation, and the decomposition rate of recalcitrant C compounds, decreases under warming. Several mechanisms might underlie this response, including changes in the abundances of iso-enzymes with different temperature optima (Wallenstein *et al.* 2011), shifts in the relative abundance of microbial taxa with different functional capabilities (Fig. 3D) and physiological, and/or evolutionary changes in microbial function (e.g. increased selective pressure for lignin-degrading microbial groups or capability). The response could also arise from abiotic factors. For instance, soil acidification with warming (Fig. 2), which can reduce potential enzyme activity (Burns & Staunton 2013), may have played a role. The response could further be related to a change in the abundance of metal oxides (Mn, Fe, and Al), which contribute to humification reactions by providing electron acceptors that catalyze the formation of reactive species from phenols (Keiluweit *et al.* 2015). However, although amorphous manganese (Mn) oxide concentration was positively correlated with phenol oxidase activity, it was not affected by translocation and was not related to differences in the Q_{10} of activity (Fig. S6). Overall, despite the result for phenol oxidase, the Q_{10} of V_{max} for the remaining six enzymes was not affected by warming (Figs. S4-S5), consistent with a recent global study showing an insensitivity of Q_{10} of V_{max} to temperature for the majority of enzymes (Allison *et al.* 2018). These results indicate that the dominant effect of enzymatic responses to warming on soil C result from changes in V_{max}, whether reduced (by thermal compensation) or increased as shown here (Fig. 2).

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

In summary, we provide new evidence that long-term (five-year) warming induced fundamental changes in microbial community physiology in tropical forest soils through increased CUE, leading to reduced soil C stocks. This occurred alongside an underlying change in microbial community composition and with no compensatory effect for the majority of soil enzymes. Our findings provide field-based evidence for tropical forests to link changes in soil C under warming to

changes in microbial physiology and communities, resulting in increased CUE. This is a complex process that has been conceptualized in models and shown to result in very large differences in the potential impact on the future terrestrial carbon cycle depending on the nature of the response (Wieder *et al.* 2013), and has not previously been studied in the tropics (Cavaleri *et al.* 2015). By accounting for the response of microbial community physiology to temperature change, we: (i) show that tropical forest soil C stocks are highly sensitive to short-term warming, imposing a positive feedback on climatic warming; and (ii) demonstrate the fundamental need to account for microbial responses in order to understand climate-induced changes in the tropical forest C cycle.

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Figure legends:

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

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

Figure 3. Temperature adaptive responses of microbial communities and physiology following five years of translocation: carbon-use-efficiency (CUE) (A) nutrient-use-efficiency (B), phenol oxidase activity (C) and community composition (D). For **A-B,** CUE was calculated according to microbial stoichiometry with respect to N ($CUE_{C:N}$) and P ($CUE_{C:P}$), according to equation 3. Nitrogen (NUE) and phosphorus (PUE) use efficiencies were calculated according to equation 4 (ref. 30). For **C,** the temperature response of Q_{10} of V_{max} for phenol oxidase, we calculated the Q_{10} of V_{max} by determining V_{max} at 2^{o}C, 10^{o}C, 20^{o}C, 30^{o}C, 40^{o}C and fitting a Q_{10} function (equations 1-2). The temperature responses of all 7 enzymes are shown in Figure S3 and the Q_{10} values of V_{max} are summarized in Extended Data Figure 4. For **D, '**Warm-adapted' taxa significantly increased in their relative abundance when soil was translocated downslope or decreased when translocated upslope (phylotype responses are in Extended Data Figure 2). The temperature responses for all response variables were estimated using linear regression of RR against the elevation shift ($p < 0.05$; error bars are 1 standard error).

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

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

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

A) Relative response of total soil C				
	Parameter	SE	P-value	X^2 test
Fixed effects				
Total PLFA	0.00498	0.00264	0.0680	0.0311 *
Alkyl:*O*-Alkyl	-0.69858	0.30904	0.0311	0.0323 *
Random effects				
Soil Origin	0.40469	0.27731	0.1545	
AIC value				11
R^2				0.631
B) Relative response of RQ_{10}				
	Parameter	SE	P-value	X^2 test

	r			
Fixed effects				
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 Q_{10} V_{max}	2.67e-02	4.45e-02	0.5517	0.5493
β-Glucosidase Q_{10} V_{max}	7.80e-02	3.53e-02	0.0325	0.0315 *
Random effects				
Soil Destination	7.26e-01	1.12e-01	7.38e-08	
AIC value				-125
R^2				0.277

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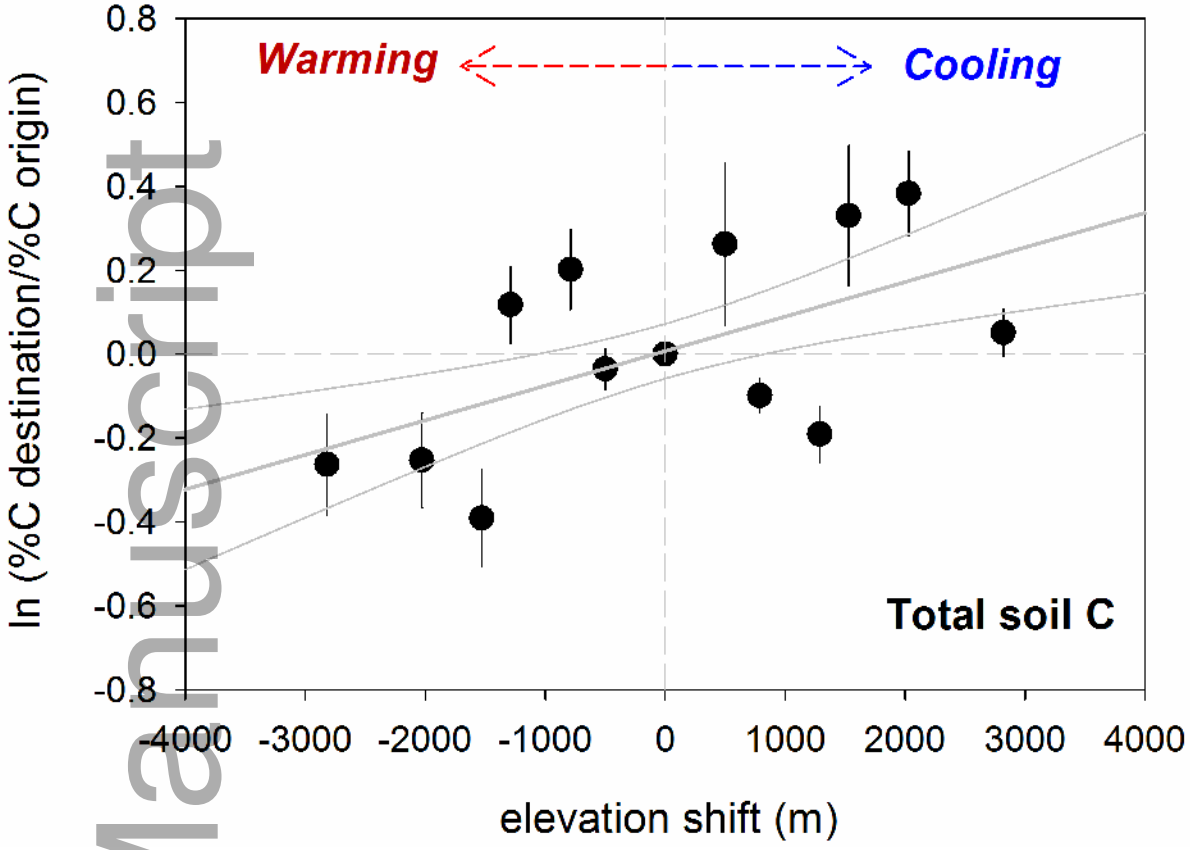

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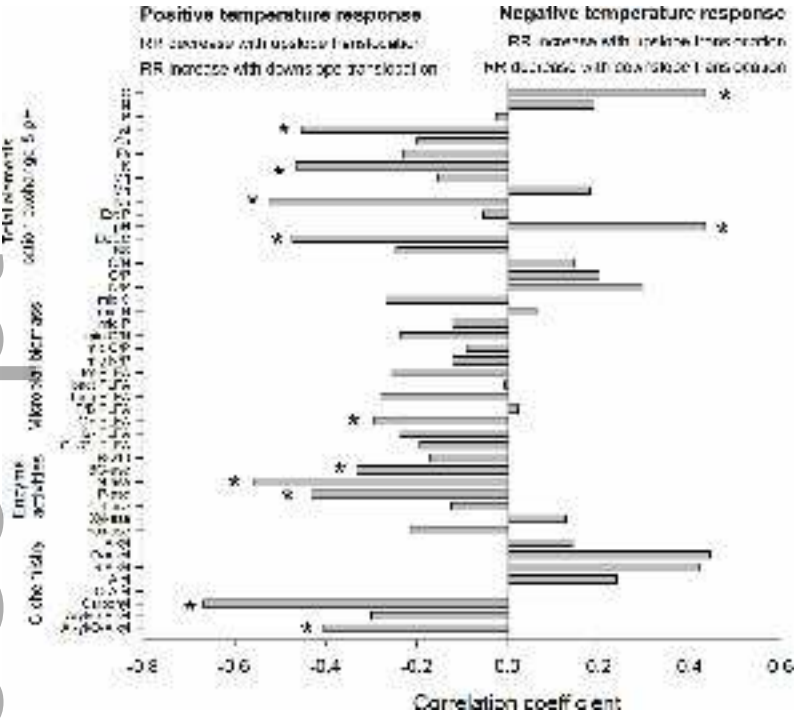

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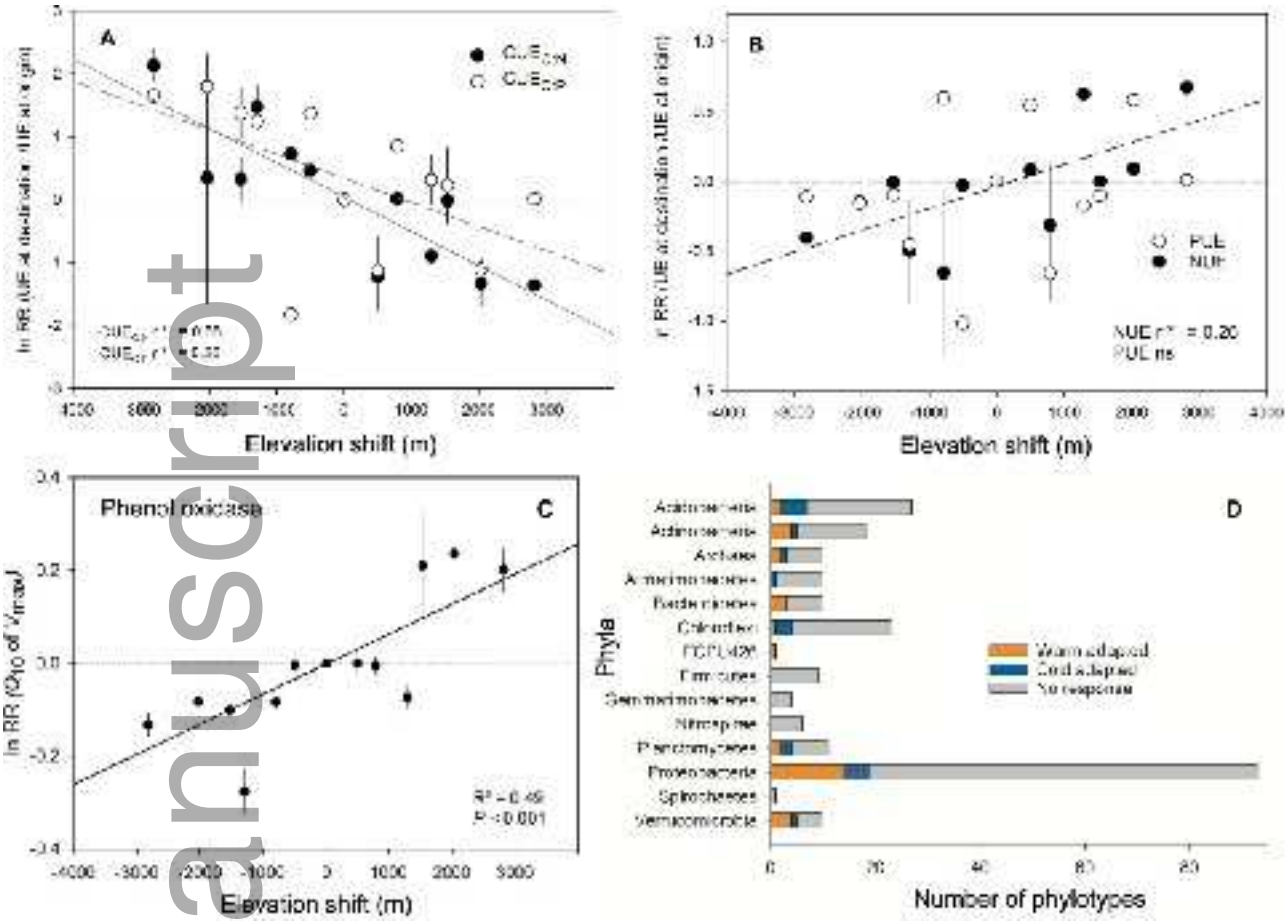

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