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Microbial death in the Andes: necromass declines despite growth and carbon-use-efficiency increases with decadal soil warming

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ABSTRACT

The growth and death of soil microbes are important drivers of soil carbon formation. A warming climate is predicted to affect both the production of microbial biomass and the stability of microbial residues (necromass) held in soils. However, we have very little information on how warming in tropical soils will affect these processes, and on the effect of temperature on microbial production and turnover over different time-scales. To address this, we studied temperature effects on microbial-mediated C cycling across two different time-scales, using a 20 °C mean annual temperature gradient in the Peruvian Andes (long-term effects) and decadal experimental-warming via soil translocation (11-years of temperature effects). At long-term timescales, a legacy of warmer temperatures decreased microbial carbon use efficiency (CUE), microbial biomass C, and decreased fungal and bacterial necromass concentration in soils. At decadal timescales, experimental warming increased CUE, microbial production and microbial biomass concentration (likely the result of concomitant changes in substrate availability). However, this did not translate into increased microbial necromass concentration, which generally declined with warming across all temporal scales. Together, we show that warmer temperatures over decadal (11-year) timescales affect soil microbial processes to potentially increase their C input to soil (increased CUE, microbial production, and biomass) but we find no evidence that this C became stabilized as the necromass C pool decreased. Our results indicate that warming can alter microbial community metabolism to potentially increase necromass C inputs to soil, although we find no evidence to show that this offset overall soil C loss with warming.

1. Introduction

As soil microbes grow and die, their residues accumulate as organic carbon (C), forming a major portion of soil organic matter (15–80 %) (Liang et al., 2019; Angst et al., 2021). However, climatic perturbations (such as increasing temperatures) can affect this microbial C pump (Liang et al., 2017), i.e. the transformation of plant organic matter into microbial organic matter, either through influencing microbial growth, or through 'necromass' C production and its degradation, with a potentially large impact on the major C pool held in soils. If only a minor fraction of the soil C pool is destabilised under warmer temperatures predicted this century (IPCC, 2023), this could significantly accelerate climate change (Melillo et al., 2017; Garcia-Palacios et al., 2021). However, if climate perturbations result in increased efficiency of microbial growth the soil necromass C pool may increase (Kallenbach et al., 2016; Tao et al., 2023). The consequences of these interactions may be especially important in tropical soils, which contain a third of global soil organic C stores (Jackson et al., 2017), have large stocks of microbial biomass C (Serna-Chavez et al., 2013), high abundance of organo-mineral associations that may protect C from decomposition (Kirsten et al., 2021), and where soil C turnover is rapid as characterised by high rates of gross primary production, decomposition and

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heterotrophic respiration (Malhi, 2012; Hashimoto et al., 2015; Steidinger et al., 2019). Thus, climate induced change in tropical soil microbial C cycling could have major consequences for the global C cycle.

The processing of C by the soil microbial community can be understood in terms of its carbon use efficiency (CUE), and of microbial necromass formation and persistence. Microbial CUE is the ratio of C incorporated into new biomass over that taken up, and therefore encompasses microbial growth or gains (C production, anabolism) and losses (C respiration, catabolism) (Manzoni et al., 2012; He et al., 2024b). Consequently, studies have shown that soils with high microbial biomass stocks and high microbial CUE are associated with high rates of necromass formation (Kallenbach et al., 2016) and that microbial CUE is positively correlated to soil C stores globally (Tao et al., 2023). However, whether the dominant influence on soil C storage is microbial processing (growth, CUE, turnover) or plant inputs via primary productivity remains contested (He et al., 2024a). Warming can affect microbial CUE and necromass formation in different ways. There is strong evidence for a negative relationship between temperature and CUE, with CUE declining as soils warm and respiration rates increasing disproportionally to the formation of new biomass (Manzoni et al., 2012). This partly explains observations of increased CO_2 emissions over the first 10 years of experimental warming (Romero-Olivares et al., 2017). However, a change or adaptation of the soil microbial community composition to longer-term warming can affect CUE and respiration rates (Frey et al., 2013; Melillo et al., 2017; Guo et al., 2020), even resulting in higher CUE at warmer temperatures (Ye et al., 2019). Any change in microbial CUE under warming may have consequences both for microbial necromass formation and its stabilization. For example, increased CUE may contribute more C to the microbial necromass stock, counterbalanced by potentially increased enzymatic capacity and priming of existing soil C (Tao et al., 2023). Meanwhile, decreased CUE may increase metabolic rates with both decreased input to, and increased degradation of, the necromass C stock (Wang et al., 2021; Liu et al., 2024).

While our understanding of temperature effects on microbial CUE remains unclear, contextualisation of our current knowledge is constrained by the different methods used to define CUE and the different assumptions associated with each method (Hagerty et al., 2018). Because microbial CUE is an emergent property it cannot be directly measured, where the indirect methods available all carry with them certain assumptions and measure a slightly different aspect/or definition of CUE (Hagerty et al., 2018). The ^{18}O-CUE method, used in the present study, determines microbial CUE under native soil conditions without changing substrate availability (Spohn et al., 2016). Other widely used methods determine CUE under substrate-specific and saturating conditions. Determination of CUE by adding ^{13}C substrates provides substrate-specific metrics of CUE to assess how it varies according to substrate identity and complexity (Whitaker et al., 2014; Wu et al., 2022b). Similarly, CUE can be estimated using a stoichiometric approach by assessment of the stoichiometric ratio in synthesised exoenzymes relative to microbial biomass stoichiometry (Sinsabaugh et al., 2016), which is also determined under saturating enzyme substrate conditions and for a specific nutrient relative to C (e.g. CUE_{CP} is based on phosphatase for organic P acquisition, compared to C mining exoenzymes). This stoichiometric approach assumes that enzyme synthesis is correlated to substrate acquisition, which is generally supported for phosphatases while the relationship is less clear for other soil enzymes (Treseder and Vitousek, 2001; Nottingham et al., 2012; Mori et al., 2023). These ^{13}C- or enzyme-based metrics thus give a substrate-specific and substrate-saturated estimation of microbial CUE and require careful interpretation in the context of the substrate under study, whereas by estimating CUE in the context of native soil organic matter turnover, the ^{18}O-method is considered a more direct approach that enables greater generalization across different soils (Spohn et al., 2016).

There is now large body of evidence showing that change in CUE can affect soil C stocks by altering the rate of production of dead microbial residues (necromass) (Buckeridge et al., 2022; Hu et al., 2023). Consequently, there is increasing interest in methods for the accurate estimation of microbial necromass C. Amino sugar biomarkers are components of microbial cell walls that are relatively stable in soil, such that their extraction and quantification can provide an index of the relative of abundance of either bacterial or fungal necromass (by quantification of muramic acid or glucosamine, respectively) (Liang et al., 2019). Thus, the determination of CUE and necromass C together, provide powerful indicators of C-processing through the growth and death of soil microbial communities and on the response of microbial-mediated soil C dynamics to climatic change.

Natural temperature gradients across latitude or elevation offer natural experiments to understand the long-term effects of temperature on ecosystem dynamics including soil microbial C cycling (Sundqvist et al., 2013; Nottingham et al., 2015b). Microbial community growth rates, one important component of CUE, have been demonstrated to be temperature-adapted across latitudinal transects (Rinnan et al., 2009; Bååth, 2018) and elevational gradients (Nottingham et al., 2019a), including the experimental system under study here (Nottingham et al., 2021). Previous studies of soil microbial communities across this Andean elevational transect examined the temperature dependency of microbial growth under long-term temperature differences (natural differences in elevation) and following 5–11 years of temperature change (via experimental soil translocation). Long-term temperature difference with elevation resulted in decreased CUE with increased temperature, based on ^{13}C methods (Whitaker et al., 2014). In contrast, five years of warming increased CUE based on the enzyme stoichiometric approach (Nottingham et al., 2019d), a finding that could be explained both by a temperature adaptive shift of the microbial community and by increasing substrate availability in warmed soils (e.g. shifting energy investment from enzymes for resource acquisition to biomass and growth). Another study using a ^{14}C-assay of microbial growth found increased growth with warming and a temperature adaptation of growth. Relatively faster growth was observed in warmer soils under long-term change (Nottingham et al., 2019a) and following 2–11 years of temperature change (Nottingham et al., 2021), consistent with a microbial community compositional shift with temperature (Donhauser et al., 2020). Together these results demonstrate temperature adaptation of tropical soil microbial communities, with increasing microbial growth at warmer temperatures. However, we lack information on CUE responses asssessed under native soil conditions and in the absence of new substrate additions (i.e. using the ^{18}O method) to conclusively infer how warming may impact microbial CUE and the soil C balance.

Ecosystem properties other than temperature can also exert strong influences on soil microbial CUE and necromass formation and stabilization. For example, it is well understood that resource availability can positively affect microbial CUE (Qiao et al., 2019; Oliver et al., 2021; Duan et al., 2023), likely by increasing microbial growth rates. The increased microbial growth rates and activities often observed under warming (Fanin et al., 2022) could consequentially increase rather than decrease CUE, at least in the short-term. Other studies have shown positive correlations between soil microbial diversity and microbial community CUE, implying that increased diversity can increase the complementary use and incorporation of C into microbial biomass at the community level (Domeignoz-Horta et al., 2020). Such relationships between microbial community diversity and CUE may be important in the context of climate change, as some warming experiments have reported decreased microbial diversity (Nottingham et al., 2022; Wu et al., 2022a), which may have a longer-term negative effect on CUE as the community composition is affected by temperature. It is, therefore, important to explore how other interacting soil properties are affected by temperature, and their resulting feedback on soil microbial C dynamics.

Here, we studied an elevation transect in the Peruvian tropical Andes to understand how temperature affects soil microbial CUE and microbial

necromass content across different timescales, from a decade to centuries. We investigated (1) their long-term legacy-temperature response (i.e. responses to centuries of current climate regime) by studying soil microbial communities across a 20 °C mean annual temperature (MAT) gradient and (2) the response of soil microbial communities to decadal temperature change, following 11 years of experimental temperature manipulation by translocation of soil cores. The use of temperature gradients on tropical mountains is a powerful tool to understand long-term (centuries) of biotic community and process rate temperature responses, provided the gradient is carefully selected to mitigate large confounding influences of soil type or rainfall (Sundqvist et al., 2013), as demonstrated for the transect under study here (Nottingham et al., 2015b). To assess these 'legacy' temperature responses of CUE, rather than its instantaneous temperature response, it was determined at a standard temperature for all sites and treatments. We hypothesised that microbial CUE decreases with increasing legacy-temperature under both long-term conditions (natural temperature gradient) and with 11 years of temperature change (following hypothesised decrease in CUE with warming, Manzoni et al. (2012)). We further hypothesised that necromass C stocks decrease with increasing temperature, due to (i) a positive effect of temperature on necromass C decomposition and (ii) a reduced CUE with warming, following a positive relationship between microbial CUE and necromass C concentration; with warming, more C is allocated to catabolism and respiration thereby less C is channelled into biomass and necromass formation. (3) we further assessed whether microbial CUE and necromass concentrations across the elevation gradient were related to other ecosystem properties, including microbial and plant diversity, and resource availability. Finally, we explored whether our temperature treatments (11-years compared to long-term MAT difference with elevation) would invoke different responses in soil microbial CUE, with the former reflecting the effect of ongoing climate perturbation, and the latter reflecting its long-term adaptation.

2. Materials and methods

2.1. Study sites: elevation transect

The study sites are situated along an elevation transect on the Eastern flank of the Andes in the Madre de Dios/Madeira watershed, Peru (see Extended Data Fig. 1, Table 1). The transect spans 3450 m elevation (194 m–3644 m above sea level, a.s.l.) and 270 km in length. There are 14 sites each with a 1 ha permanent sampling plot, all of which are in old growth tropical forest except for one site (3644 m asl) on high elevation 'Puna' grassland. Mean annual temperature (MAT) decreases with increasing elevation (from 26 °C to 6 °C) with little seasonal temperature variation irrespective of elevation (<4 °C between warmest and coolest months) (Rapp and Silman, 2012). Mean annual precipitation (MAP) is generally high across all sites, ranging from 760 to 5302 mm yr^{-1} among the sites and with no elevational trend, peaking at mid-elevation (5302 mm $year^{-1}$ at 1500 m) (Nottingham et al., 2018). Precipitation is seasonal across the gradient, with a dry season from May–September and a wet season from November–March (Rapp and Silman, 2012), although there is little seasonal variation in soil moisture (Girardin et al., 2013), and evidence to date indicates that plants and soils at all sites are rarely moisture limited over the seasonal cycle (Zimmermann et al., 2010; van de Weg et al., 2014). Further description of climate and floristic composition of these sites are reported elsewhere (Girardin et al., 2010; Rapp and Silman, 2012; Jankowski et al., 2013; Oliveras et al., 2014; Nottingham et al., 2018b)

The plots are predominantly situated on Paleozoic (~450 Ma) metasedimentary mudstone (~80 %), with plutonic intrusions (granite) underlying the sites between 1500 m and 2020 m a.s.l. The soils are Umbrisols (Inceptisols) at the sites above 2520 m, Cambisols (Inceptisols) at the sites from 1000 m to 2020 m, and Haplic Allisols (Ultisols) and Haplic Cambisols (Inceptisols) at the two lowland sites (according to FAO, with USDA Soil Taxonomy in parentheses). Previous work on this gradient has shown that soil pH is relatively uniform (pH = 4 ± 0.1 S.E

Table 1
Summary of site characteristics along the Andean elevation gradient, spanning lowland rainforest (194–210 m a.s.l.), premontane (1000 m a.s.l.), lower montane (1500–2020 m a.s.l.), upper montane cloud forest (2520–3400 m a.s.l.) and Puna grassland (3644 m a.s.l.). (Aragao et al., 2009; Girardin et al., 2010; Quesada et al., 2010; Asner et al., 2013; Clark et al., 2013). Mean annual precipitation (MAP) values here reported in Nottingham et al. (2015a), although variations in these values for some sites have also been reported (Girardin et al. 2010). Where "-" = data not available. The four soil translocation sites are denoted by bold text.

Site name	Site code	Elevation (m a.s.l.)	Lat	Long	Mean annual temp (°C)	Annual precipitation (mm yr^{-1})	Aspect (deg)	Slope (deg)	Parent material	Soil classification
Explorer's Inn plot 4 (TP4)	TAM-06	194	−12.839	−69.296	26.4	1900	169.4	4	Holocene alluvial terrace	Haplic Alisol
Explorer's Inn plot 3 (TP3)	**TAM-05**	**210**	**−12.830**	**−69.271**	**26.4**	1900	**186.2**	**6.9**	**Pleistocene alluvial terrace**	**Haplic Cambisol**
Villa Carmen	**VC**	**1000**	**−12.866**	**−71.401**	**20.7**	3100	**-**	**-**	**-**	**-**
San Pedro 2	**SPD-2**	**1500**	**−13.049**	**−71.537**	**17.4**	5302	**143.5**	**39**	**Plutonic intrusion (granite)**	**Cambisol**
San Pedro 1	SPD-1	1750	−13.047	−71.543	15.8	5302	141.9	40.1	Plutonic intrusion (granite)	Cambisol
Trocha Union 8	TRU-08	1850	−13.071	−71.555	16.0	2472	137.0	41.8	Plutonic intrusion (granite)	Cambisol
Trocha Union 7	TRU-07	2020	−13.074	−71.559	14.9	1827	–	–	Paleozoic shales-slates/Granite intrusion	Cambisol
Trocha Union 5	TRU-05	2520	−13.094	−71.574	12.1	2318	–	–	Paleozoic shales-slates	–
Trocha Union 4	TRU-04	2720	−13.107	−71.589	11.1	2318	189.8	28.6	Paleozoic shales-slates	Umbrisol
Trocha Union 3	TRU-03	3020	−13.109	−71.600	9.5	1776	129.3	37.6	Paleozoic shales-slates	Umbrisol
Wayqecha	**WAY-01**	3030	**−13.190**	**−71.587**	**11.1**	1506	**-**	**-**	**Paleozoic shales-slates**	**Umbrisol**
Trocha Union 2	TRU-02	3200	−13.111	−71.604	8.9	2555	–	–	Paleozoic shales-slates	Umbrisol
Trocha Union 1	TRU-01	3400	−13.114	−71.607	7.7	2555	144.3	34.3	Paleozoic shales-slates	Umbrisol
Tres Cruces	TC	3644	–	–	6.5	760	–	–	–	–

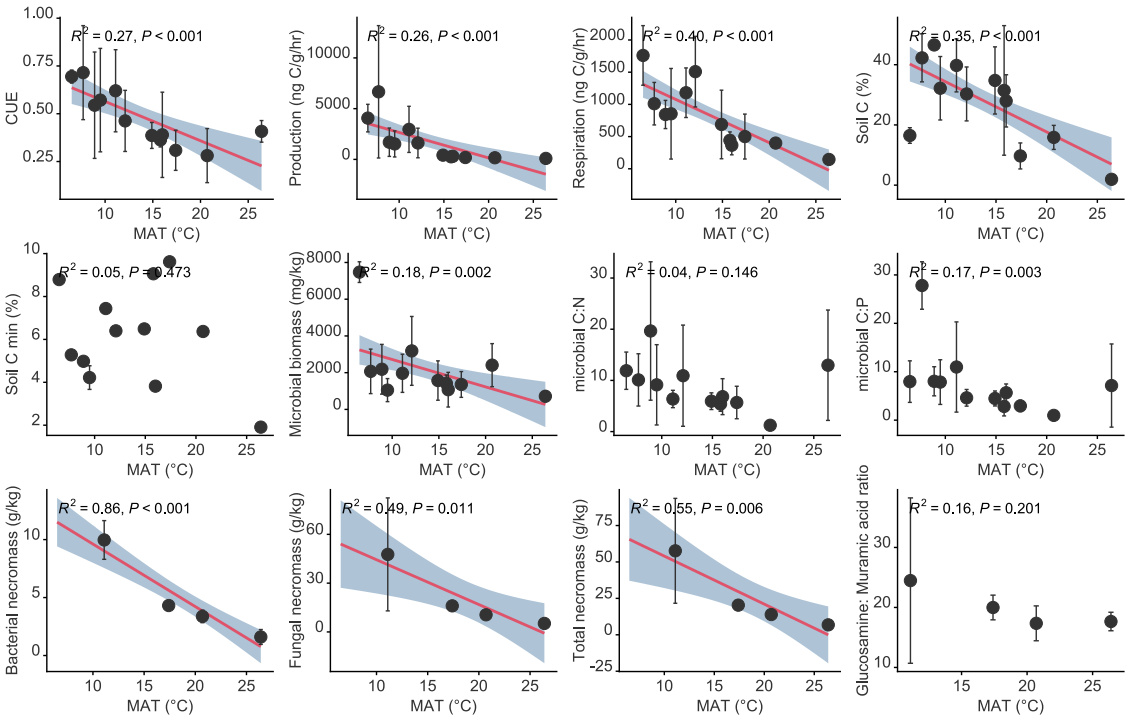

Fig. 1. The relationships between mean annual temperature across an elevational gradient and soil microbial C cycling properties and microbial necromass contents. Variables are carbon use efficiency (CUE) determined by ^{18}O, mass specific production (growth) and respiration; all determined in 24-hr assays performed at standard (16 °C) temperature. Bacterial, fungal and total necromass, determined by the abundance of amino sugars. All soil variables were determined for 0-20 cm mineral soils. Soil C was determined for 0-20 cm mineral soils (soil C min) and for 0-10 surface soils (soil C, including organic horizons); for more information on soils see Nottingham et al. (2015a)). The relationships between CUE and other ecosystem properties are shown in Fig. 4. Points are means with lines representing 1 standard error (n = 4); linear models are plotted if significant where shaded areas are 95 % confidence intervals.

across 14 sites) and there is a shift from N (higher elevation) to P (lower elevation) limitation of soil microbial activity, according to elemental stoichiometry and investment into extracellular enzymes, likely the result of long-term differences in MAT affecting rates of rock weathering (for P) and mineralisation/fixation rates (for N) (Nottingham et al., 2015a). Importantly for the present study, previous work has shown that MAT is the main driver of soil bacterial and fungal community composition and diversity (Nottingham et al., 2018b) and that the temperature sensitivity of bacterial growth is highly correlated to MAT (Nottingham et al., 2019b). Together these findings indicate that for this elevation gradient, temperature is a dominant driver of microbial community and growth rates, and therefore the gradient provides a useful study system to understand long-term MAT effects on other microbial C-cycling metrics such as CUE.

2.2. Translocation experiment

Across the transect, a soil translocation experiment was established in 2008 across a subset of four sites, all in tropical forest (210 m asl, 1000 m asl, 1500 m asl and 3030 m asl) (Zimmermann et al., 2009), with translocation downslope imposing an experimental warming treatment and translocation upslope an experimental cooling treatment (see Extended Data Fig. 2). At each of the four sites, twelve intact monoliths of mineral soil were excavated and placed into PVC tubes (10 cm diameter and 50 cm depth). Soil cores were re-installed at sites across the gradient, by carefully inserting the cores into holes cut into the soil using a 10 cm diameter auger. This included control cores, which comprised soil cores cut and re-installed at the same elevation (i.e. at their site of origin) to control for any disturbance effects. Soil moisture was maintained at contents similar to those experienced at the sites of

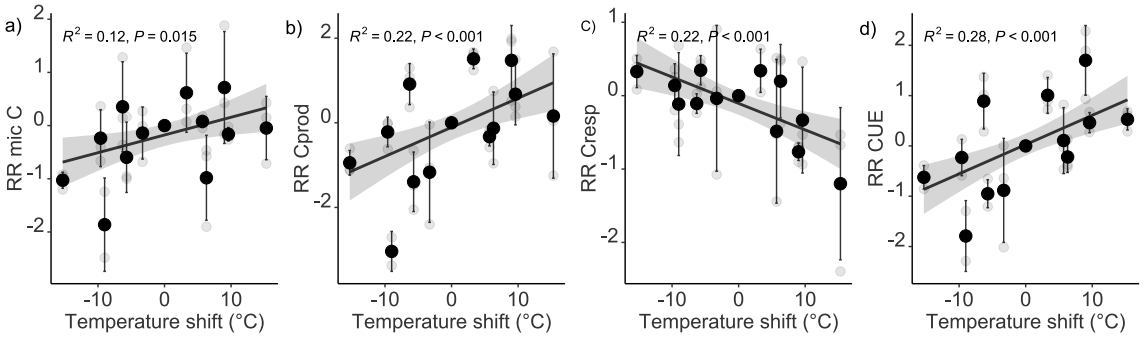

Fig. 2. The response of soil microbial carbon cycling to 11 years of temperature manipulation. Parameters are a) microbial C biomass, b) mass-specific production (growth) and c) respiration, and d) microbial CUE. Temperature treatments are either warming or cooling by 3–15 °C via soil translocation across 500–2820 m elevation difference. Points are the mean of the relative response ratio (RR) per parameter with lines representing 1 standard error (n = 3); linear models are plotted where shaded areas are 95 % confidence intervals. The responses for individual soils are shown in Extended Fig. 3.

origin by using funnels/collars to increase/reduce the total rainfall incident according to its original location (Zimmermann et al., 2009). The cores were located in three separate subplots situated outside the perimeter of 1 ha permanent study plots at each site. These subplots acted as independent spatial replicates, such that twelve cores were re-installed at each elevation site (4 soils x 3 replicates).

2.3. Soil sampling

Soils were collected from the 14 × 1 ha plots, by collecting 4 replicate samples collected from each corner of each 1 ha plot. Soil samples were collected using a soil auger (3 cm diameter) at 0–20 cm mineral soil depth, with five subsamples collected per plot corner, and the subsamples were mixed into one sample per plot corner. We determined soil C for 0-10 cm surface soils (including organic horizons) and for 0-20 cm mineral soils, using a composite sample per plot for the latter. Thus, for all other analyses using soils across the elevation gradient we had a replication of four, representing the four spatial replicates across each 1-ha plot.

At the same time, soils were collected from the translocated soil cores, which had received 11 years of temperature treatment at the time of soil collection. Similarly, we used a 3-cm diameter auger to collect mineral soil at 0–20 cm depth for all translocated samples. By analyzing our data using relative response ratios (which were determined by comparing the treatment and control for each experimental block; see below) we applied a regression approach with 36 experimental observations.

Soil samples were then sieved to 2 mm and stored in a dark/cool (~15 °C) room for up to 2 weeks until transportation to the laboratories for the respective analyses (Smithsonian Tropical Research Institute, Panama; University of Vienna, Austria; University of Helsinki, Finland).

2.4. Amino sugars to determine microbial necromass

Amino sugar extraction was performed following the protocol in Zhang and Amelung (1996). Bacterial C was calculated by multiplying the concentration of muramic acid (MurA) by 45 (Appuhn and Joergensen, 2006). Fungal C was calculated by subtracting bacterial glucosamine (GluN) from total glucosamine, assuming that muramic acid and glucosamine occur at a 1:2 M ratio in bacterial cells (Engelking et al., 2007):

$$\text{Fungal necromass C} = (\text{mmol GluN} - 2 * \text{mmol MurA}) * 179.17 * 9$$

Where 179.17 is the molecular weight of GluN and 9 is the conversion value of fungal GluN to fungal necromass C (Appuhn and Joergensen, 2006; Joergensen, 2018). Total necromass C was calculated as the sum of Bacterial necromass C + Fungal necromass C. The amino sugar ratios GluN:GalN and GluN:MurA were used as indicators of the relative contribution of fungal to bacterial residues (Liang et al., 2016).

2.5. Carbon use efficiency by ^{18}O incorporation into DNA

Microbial growth, respiration and CUE were determined using laboratory assays following the addition of ^{18}O-labelled H_2O and measurement of ^{18}O-incorporation into microbial DNA for growth, and headspace CO_2 accumulation for respiration (Spohn et al., 2016). The incubations, following the addition of ^{18}O-labelled H_2O (97 % ^{18}O, in amounts to achieve 20 at% ^{18}O enrichment in soil water) were for 24 h and were performed at a standard temperature (16 °C, selected to represent an average soil temperature across all sites). We used a standard temperature for CUE determination because our aim was to characterise the long-term legacy effect of temperature on the soil microbial community and its CUE, rather than its intrinsic or instantaneous temperature sensitivity (response to short-term, e.g. hours, of temperature change and driven by reaction rate kinetics) (Davidson and Janssens, 2006). In parallel, control samples were incubated which were amended with the same volume of deionized water (non-labelled, natural abundance ^{18}O) and incubated for 24 h. For each replicate sample we incubated 400 mg of soil in 27 ml flasks. We used a standard temperature to ensure directly comparable results on the legacy of long-term temperature differences on soil microbial CUE across our sites, and a short incubation period to avoid confounding effects arising from changes in microbial community composition that may occur in longer incubations (Geyer et al., 2019).

Microbial respiration was determined by measurement of CO_2 concentration increases in gas sampled from each incubation flask (by gas chromatography; Trace GC Ultra, Thermo Fischer). Respiration rates were estimated by:

$$\text{C in soil } CO_2 \text{ efflux } \left(\text{ng C g}^{-1} \text{ soil d.w. d}^{-1}\right) = \frac{\Delta CO_2}{DW*t} * \frac{p*n}{R*T} * V_{hs} * 1000$$

Where DW is the dry weight of the incubated soil t (hr) is the incubation time, p is the atmosphere pressure (kPa), n is the molecular mass of C (12.01 g/mol), R is the ideal gas constant (8.314 J mol^{-1} K^{-1}), T is the absolute temperature of the gas (295.15 K), V_{hs} is the volume of the vial head space (L), and ΔCO_2 (ppm) is the increase in CO_2 concentration during the 24 h incubation period.

At the end of the incubations, DNA was extracted from ^{18}O-labelled and controls soils (FastDNA™ SPIN Kit for Soil; MP Biomedicals) and DNA concentration was determined fluorimetrically (Sandaa et al., 1998) using a PicoGreen assay (Quant-iT™ PicoGreen® dsDNA Reagent; Life Technologies). DNA oxygen (O) concentration and ^{18}O abundance were measured using a thermo-chemical elemental analyzer (TC/EA Thermo Fisher) coupled with an IRMS (Delta V Advantage; Thermo Fisher).

The amount of new DNA produced by replication and growth was calculated using the following formula:

$$DNA_{produced} = O_{DNA\ extr} * \frac{^{18}O\ at\%_{DNA\ L} - {}^{18}O\ at\%_{DNA\ n.a.}}{^{18}O\ at\%_{soil\ water}} * \frac{100}{31.21}$$

where $O_{DNA\ extr}$ is the total amount of O in the DNA extract, $^{18}O\ at\%_{DNA\ L}$ and $^{18}O\ at\%_{DNA\ n.\ a.}$ are the ^{18}O enrichment in the labelled and unlabeled DNA extracts, respectively, and $^{18}O\ at\%_{soil\ water}$ is the ^{18}O enrichment of the soil water. The fraction at the end of the formula accounts for the average O content of DNA (31.21 %) (Zheng et al., 2019; Canarini et al., 2020). To calculate microbial biomass C produced (C_{Growth}) during the incubation, $DNA_{produced}$ was divided by the total amount of DNA in the sample and multiplied by microbial biomass carbon (MBC) values. Microbial respiration ($C_{Respiration}$) was calculated from the respiration measurements described above.

Microbial CUE was calculated using the following equation (Manzoni et al., 2012):

$$CUE = \frac{C_{Growth}}{C_{Growth} + C_{Respiration}}$$

Extractable organic carbon (EOC) was measured in 1 M KCl extracts (1:7.5 w/v) using a TOC/TN analyzer (TOC-L CPH/CPN, Shimadzu). MBC was determined by chloroform fumigation extraction (Brookes et al., 1985). Samples were fumigated in a desiccator under chloroform atmosphere for 24 h in the dark and subsequently extracted with 1 M KCl and measured on a TOC/TN analyzer. MBC was calculated as the difference in C between fumigated samples and fresh soil samples (EOC). Measured MBC values were divided by 0.45 (Wu et al., 1990) to account for extraction efficiency.

2.6. Plant and soil properties

The following additional soil properties were determined: pH, total organic C, total N, total P, organic P, resin-extractable P, cation exchange capacity and exchangeable cations (Al, Ca, Fe, K, Mn, Mg, Na).

Element contents of soil microbial biomass other than C were determined by chloroform fumigation extraction for microbial biomass N, and by hexanol fumigation for microbial biomass P, following the methods described previously (Nottingham et al., 2015a). Soil microbial diversity was determined by high-throughput sequencing to characterise variation in marker gene sequences for bacteria (by 16S rRNA gene sequencing) and fungi (ITS1-F and ITS2 primer pairs). Methods are described in detail in Nottingham et al. (2018). The plant diversity data have been reported in Nottingham et al. (2018), where all trees ≥10 cm diameter at breast height (1.3 m) were measured in 13 of the 1-ha plots and identified to species or morphospecies. Shannon species diversity indices were used as metrics of plant and soil microbial diversity for each plot, based on the abundance of OTUs for soil bacteria and fungi or of tree species for plants.

2.7. Calculations and statistical analyses

For the translocation experiment, which involved both warming and cooling of different soil types, we used relative response (RR) quotients to assess the average level of change in response variables (growth, respiration, CUE, necromass content). This change was determined across all soils in response to temperature difference (increasing or decreasing temperature). The use of RR allows assessment of relative responses to temperature across all soil types in a single analysis, controling for variation in other potentially confounding soil properties (Nottingham et al., 2021). The RR quotients were determined as follows (here for CUE as example):

$$RR_{CUE} = \log[\text{CUE at destination (i = 1-3)} \ / \ \text{CUE at origin (i = 1-3)}]$$

Errors were determined based on the standard error (SE) of the three replicates, whereby each translocated soil core (i = 1–3 at destination) was paired with a control core (i = 1–3 at origin). To evaluate whether temperature change affected RR (e.g. of RR_{CUE}), we used linear models of RR_{CUE} against the temperature shift (temperature at destination minus temperature at origin).

To determine the effect of MAT difference on soil and soil microbial physiological properties (soil C, biomass C, growth, respiration, CUE) and necromass properties (bacterial, fungal and total necromass) we used linear models with MAT as the explanatory variable and soil microbial properties as response variables. We used MAT as the explanatory variable because our study was designed to understand temperature effects on soil microbial properties. To determine the effect of translocation temperature difference on soil microbial physiological and necromass properties (response variables) we used linear models for each specific soil based on its origin, and we repeated the analyses including all soil types and using RR (of soil or microbial property) as the explanatory variable. Where data were non-normally distributed, we used log transformations.

To test whether other soil and ecosystem properties were directly or indirectly related to CUE changes across the elevation gradient, we used a combination of Spearman's correlations linear regression models and structural equation models (SEM). For the elevation transect soils, we first used Spearman's correlations to provide an overview of correlations among environmental, soil, microbial and plant properties; we then used linear regression to further explore relationships between CUE and identified properties. We included plant species diversity in our correlation analyses (determined for 1-ha plots at each study site), given evidence that plant diversity can affect microbial CUE (Duan et al., 2023). Plant diversity and soil bacterial and fungal diversity data were reported in Nottingham et al. (2018), and soil microbial nutrients and enzyme activities in Nottingham et al. (2015a).

We applied SEM to further understand the climatic and microbial physiological correlates of CUE and necromass C. For both transect and translocated soils, we first specified paths to understand the microbial physiological correlates of CUE (growth, respiration and microbial biomass C); recognising that these variables are colinearly related to CUE but our objective here was to use SEM to determine the relative importance of them (e.g. growth vs respiration) in relating to changes in CUE. Second, having specified the dominant correlate of CUE (growth, in both transect and translocated soils), we specified paths to understand the environmental correlates of microbial growth. For transect soils, our final model included MAT, total C and extractable soil P (after removing total N, MAP, soil pH). For translocation soils, we focussed on temperature effects across timescales, to understand the 'long-term temperature legacy effects' and 'decadal temperature effects' (T_{origin} and T_{dest}, respectively) on each of microbial growth and of necromass C stocks; in addition to assessing the simple correlation between CUE and necromass C. Thus, we tested whether the dominant control on growth and CUE (to drive necromass formation), and on necromass C content, are very long-term temperature effects (by shaping soil and plant properties and the microbial community over centuries) or the relatively short-term effect of 11 years of temperature manipulation. The SEM models were fitted using the piecewiseSEM package (Lefcheck, 2016) and selection of the final model was by step-wise selection removing parameters of low significance and minimizing the Akaike Information Criterion (AIC) score. We used SEM as a tool to assist our hypothesis testing and to provide further detail on path correlation between variables but recognising that the output is highly dependent on our subjective initial model formulation, and that it remains difficult to partition deterministic and indirect relationships in the final model.

The analyses allowed us to: 1) determine whether microbial properties (microbial biomass and necromass) are related to long-term temperature differences across a 3.4 km elevation tropical mountain gradient; 2) determine whether other environmental, plant and soil properties are related to microbial CUE across the elevation gradient; 3) determine whether soil and microbial properties (soil C, microbial CUE and necromass) are altered by 11-years of temperature difference following soil translocation; and 4) ultimately to determine whether the dominant effect of temperature on microbial CUE and necromass C differed between the short-term (11 years of soil translocation) and long-term (MAT change with elevation). All analyses were performed in R (version 4.5.1).

3. Results

3.1. Long-term effects of temperature with elevation

Across the 20 °C MAT gradient, MAT was negatively related, and elevation positively related, to microbial CUE, growth (production), respiration and microbial biomass, the processes being measured at a common temperature in the laboratory (i.e. at 16 °C for all soil samples across all elevations and not at *in situ* temperatures) (Fig. 1). The negative relationship between CUE and MAT indicated that the decline in growth at warmer temperatures was greater than the decline in respiration, resulting in lower CUE at warmer temperatures. Specifically, the respective relationships with MAT were: microbial biomass C (slope: −149 ± 44; R^2: 0.18, F-statistic: 11.3, $p < 0.01$), microbial respiration C (slope: −67 ± 12; R^2: 0.40, F-statistic: 19, $p < 0.001$), microbial growth C (slope: −251 ± 60; R^2: 0.26, F-statistic: 18, $p < 0.001$) and microbial CUE (slope: −0.02 ± 0.005; R^2: 0.27, F-statistic: 19, $p < 0.001$). Soil C increased with increased elevation with the largest effects observed in organic soil horizons (Fig. 1).

Similar patterns were observed for microbial (bacterial, fungal and total) necromass C across the gradient (Fig. 1), with lower necromass at warmer sites, and with fungi contributing a greater proportion to soil microbial necromass C at lower MAT. Specifically, MAT was negatively correlated to total microbial necromass C (slope: −3.3 ± 0.9, R^2: 0.55, F-statistic: 12, $p < 0.01$), fungal necromass C (R^2: 0.49, F-statistic: 10, $p = 0.01$) and bacterial necromass C (R^2: 0.86, F-statistic: 61, $p < 0.001$) (Fig. 1). We further tested for elevational effects on ratios between specific amino acid sugars, but MAT was neither related to GluN: GalN (R^2: 0.05, F-statistic: 0.6, $p = 0.4$) nor to GluN: MurA (R^2: 0.16, F-

statistic: 1.8, $p = 0.2$).

3.2. Decadal effects of temperature with translocation

The patterns we observed in soils from the same sites that had been reciprocally translocated for 11 years did not follow the same pattern as observed in natural soils along the MAT gradient. When soils were warmed (translocated downslope), we generally observed increased microbial biomass C, microbial growth (production) and CUE, while microbial respiration (per soil dry mass) decreased. Thus, the increased CUE with warming resulted from increased growth and decreased respiration. Across all soils, the response ratio (RR) of microbial C increased with warming (R^2: 0.12, $P = 0.015$), C production (growth) increased with warming (R^2: 0.22, $P < 0.001$), and respiration decreased with warming (R^2: 0.22, $P < 0.001$) (Fig. 2). Together these responses translated into an increase in microbial CUE with warming (R^2: 0.28, $P < 0.001$). The responses of individual soils are shown in Extended data Fig. 2.

Soil C and microbial necromass concentrations decreased with warming, with decreases with warming in the RR of total soil C (R^2: 0.15, $P < 0.01$), bacterial necromass (R^2: 0.2, $P = 0.02$), fungal necromass (R^2: 0.14, $P = 0.05$) and total necromass (R^2: 0.18, $P = 0.028$) (Fig. 3). The necromass responses of individual soils are shown in Extended data Fig. 3, where it was evident that the largest declines in necromass were for soils from the highest elevation (3030 m asl) when translocated downslope. We also observed increased dissolved organic C (DOC) with warming (R^2: 0.24, $P < 0.001$) (Fig. 3). Previous data reported from this experiment shows that 5-years of warming increased enzyme activities and exchangeable cation concentrations (Nottingham et al., 2019d).

3.3. Environmental determinants of CUE and necromass over timescales

Finally, we assessed whether ecosystem properties other than temperature were correlated to microbial CUE across the elevation gradient. We observed positive relationships with CUE and microbial biomass stoichiometric ratios of C:N and C:P, pointing towards increased CUE under conditions of high microbial biomass C relative to nutrient contents (Fig. 4). In addition, we observed negative relationships between CUE and plant species richness and soil bacterial species richness (but not fungal richness; Fig. 4), which likely reflected an underlying effect of MAT on CUE. These relationships with CUE were also evident in our correlation analysis of properties across the gradient (Fig. 5). Plant and bacterial diversity were strongly correlated to MAT (Fig. 5), and a previous study of these sites showed that MAT is highly correlated to these diversity patterns (Nottingham et al., 2018b).

Based on the observed relationships (Figs. 4–5) and our hypotheses for determinants of CUE, we selected a subset of variables for SEM analysis which aimed to elucidate dominant direct and indirect drivers of CUE and, in turn, necromass change across timescales (Fig. 6; Extended Data Table 1). Across the elevation transect, we first tested for microbial physiological effects on CUE, which showed a large positive effect of microbial growth on CUE (rather than respiration or biomass) (Fig. 6a). Second, we in turn tested for the environmental drivers of microbial growth, which showed a large negative effect of MAT (rather than soil C or available P); although this may still reflect an effect of total soil C, which was negatively correlated to MAT (Fig. 5). For the translocated soils we determined both the 11-year experimental temperature effect (defined by the new temperature regime, T_{dest}) and the long-term legacy temperature effect (defined by the temperature at site of origin, T_{origin}) on microbial growth and necromass content. We found that T_{origin} had the strongest effect on microbial growth, while T_{dest} had the strongest effect on microbial necromass content. For the translocated soils we observed the same pattern for the elevation transect, that growth rate was the strongest (positive) determinant of CUE. Overall, our SEM analyses show that microbial CUE was determined by temperature effects on microbial growth rates across all scales. It further shows that the long-term legacy effect of MAT (Fig. 6a and b) is negatively related to growth (likely because these soils from low MAT contain higher soil C and substrate; Fig. 5). Finally, it shows that while T_{origin} (long-term legacy temperature effect on soil and soil communities) drove growth rates and CUE, the T_{dest} (experimental warming effect) had the largest influence on necromass C content.

4. Discussion

It is widely predicted that the response of soil microbial CUE to a changing climate will have a large influence on the future terrestrial C balance (Wieder et al., 2013; Tao et al., 2023). However, scarce empirical information on how microbial CUE responds to temperature change across different timescales limits our ability to test these model predictions, especially in the tropics. Here we showed that the climate controls on soil microbial C processing in tropical forests differ across time scales, by comparing the response to 11 years of temperature change (via soil translocation across elevation) with the long-term-scale response to temperature differences (via study of soils across a 3.4 km elevation gradient). In all cases we assessed the legacy effect of temperature on soil microbial community CUE (by assessing CUE at a standard laboratory incubation temperature for 24 h), rather than the intrinsic temperature response (by assessing CUE at different laboratory temperatures). At the long-term-scale with elevation difference, the legacy of warmer temperatures resulted in decreased mass-specific microbial growth, biomass and CUE (Fig. 1), which is consistent with our parallel observation that soil C stocks and soil necromass were lower at warmer temperatures (Fig. 1). Together, these observations are broadly consistent with current understanding on how temperature modifies soil microbial C cycling. For example, several studies have demonstrated that warming increases microbial activity and soil C loss (Melillo et al., 2017; van Gestel et al., 2018), and promotes microbial respiration more than growth, consequentially reducing CUE (Manzoni et al., 2012; Li

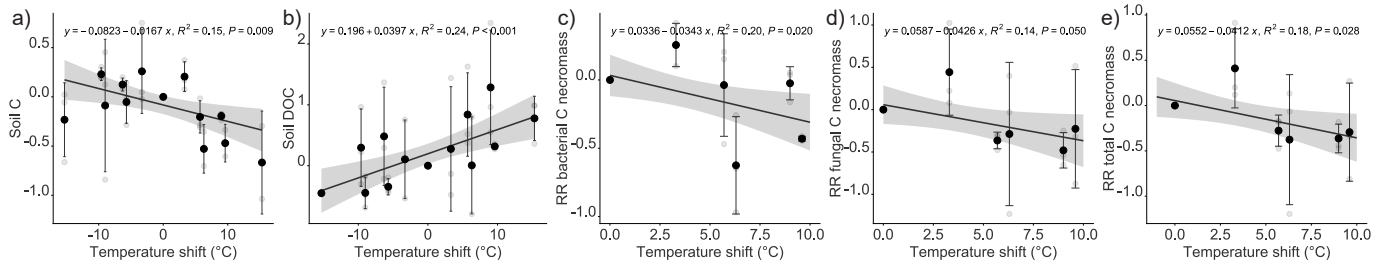

Fig. 3. The response of (a) soil C, (b) dissolved organic C, and (c-e) microbial necromass C contents to 11 years of warming across three different soils. c) Bacterial, d) fungal and e) total necromass were estimated by amino sugar biomarker analysis, with analyses focussed on experimentally warmed (rather than cooled) soils, by 3–15 °C through down-slope translocation by 500–2820 m elevation. Points are the mean of the relative response ratio (RR) per parameter with lines representing 1 standard error (n = 3); linear models are plotted (equations shown) where shaded areas are 95 % confidence intervals. The responses for individual soils are shown in Extended Fig. 4.

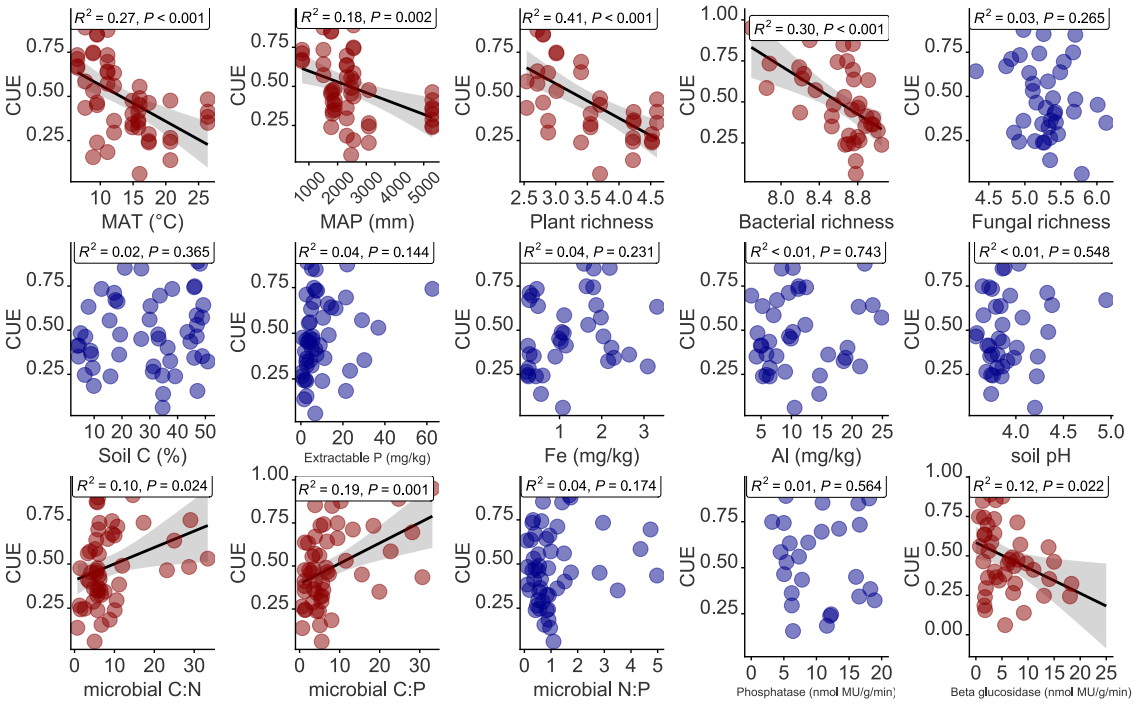

Fig. 4. The relationships between microbial CUE and climate, soil and plant properties across an elevation transect in the Andes. The relationships between CUE and a range of ecosystem and soil properties were evaluated, including: MAT (mean annual temperature), MAP (mean annual precipitation), plant species richness, soil bacterial species richness, soil fungal species richness, soil C content, exchangeable resin P, exchangeable Al , exchangeable Fe , soil pH, elemental stoichiometry of microbial biomass (C:N, C:P, and N:P), and a range of enzyme activities including phosphomonoesterase and β-glucosidase (shown here; there was no relationship between CUE and three other hydrolytic enzymes, i.e. cellobiohydrolase, β-xylanase, and *N*-acetyl glucosaminidase). Significant relationships are illustrated in red with linear fit plotted. Points are means with lines representing 1 standard error (n = 5); linear models are plotted if significant where shaded areas are 95 % confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

et al., 2019); together this is supporting the notion that warming will trigger positive feedbacks releasing C from soil to the atmosphere.

However, the response of microbial CUE to 11 years of temperature change (by experimental translocation) was different to that observed in response to long-term elevational effects and contrasted with results from warming experiments performed at higher latitudes. Warming for 11 years decreased microbial respiration (mass-specific), and increased microbial growth, together resulting in increased CUE (Fig. 2). The finding of increased CUE (determined here using the ^{18}O method) with 11 years of warming is consistent with a previous result showing that microbial biomass C and CUE (determined using an enzyme stoichiometric approach) increased following 5 years of warming for the same soils (Nottingham et al., 2019d). For the present study, the increased CUE with warming arose from increased microbial growth (while respiration declined or did not change) and occurred alongside increased microbial biomass C. Another two independent tropical soil warming studies have also reported increased microbial biomass with warming, following 2 years of *in situ* warming for lowland forests in Panama (Nottingham et al., 2020) and Puerto Rico (Reed et al., 2020). This contrasts with general findings from soil warming studies in higher latitudes where microbial biomass was unaffected (Schindlbacher et al., 2011) or even decreased with warming (Melillo et al., 2017; Romero-Olivares et al., 2017), while microbial CUE decreased (Schindlbacher et al., 2011; Li et al., 2019).

We explain this contrasting result of increased CUE with warming in tropical soils, by the positive effects of warming on substrate availability and thereby on CUE, as observed elsewhere albeit for short-term incubations (Zheng et al., 2019). Evidence that warming of these soils increased the release of C substrates from soil organic matter comes from the observation of increased dissolved organic C (Fig. 3), microbial biomass C (Fig. 2) and increased soil enzyme activities after 5 years, with the largest increase for the N-degrading enzyme *N*-acetyl-β-glucosaminidase (Nottingham et al., 2019d). Tropical forest soil microbial communities are highly metabolically active, with high litterfall substrate turnover, high respiration rates and high rainfall. Given this, we hypothesize that warming - at least in the short term - accelerates decomposition rates to release carbon substrates alongside nutrients for microbial utilization, promoting microbial growth, microbial biomass and CUE. In our study, we observed increased CUE after a relatively long experimental period of 11 years of warming, which we attribute to the high content of soil organic C and particulate organic matter in the warmed montane forest soils in our study (Zimmermann et al., 2012), providing a large resource for provisioning substrates for accelerated microbial growth. This may include organic matter mineralisation for N uptake via priming effects, which may be large for the warmed high-elevation soils in our study that are high in organic C and N; Nottingham et al. (2015a)). This may also include N-rich peptide degradation from microbial necromass as observed in controlled experiments (Meyer et al., 2023), thus explaining why necromass C decreased with warming (Fig. 3). We subsequently hypothesize that over time CUE will eventually decline as substrate availability declines with prolonged warming. Therefore, with further time and with gradual decreases in soil organic matter content, we expect the soil microbial C cycling responses observed after 11 years of warming (Fig. 2) to converge on those observed across the elevational transect (Fig. 1).

These microbial physiological changes, with increased biomass C and CUE following 11 years warming in (montane) tropical soils, raise the question of whether short-term warming could stimulate microbial production, and in turn, the formation of new soil organic C derived from microbial necromass. Indeed, there is a positive correlation between microbial CUE and soil organic C stocks globally (Tao et al., 2023). Moreover, several studies have demonstrated a large

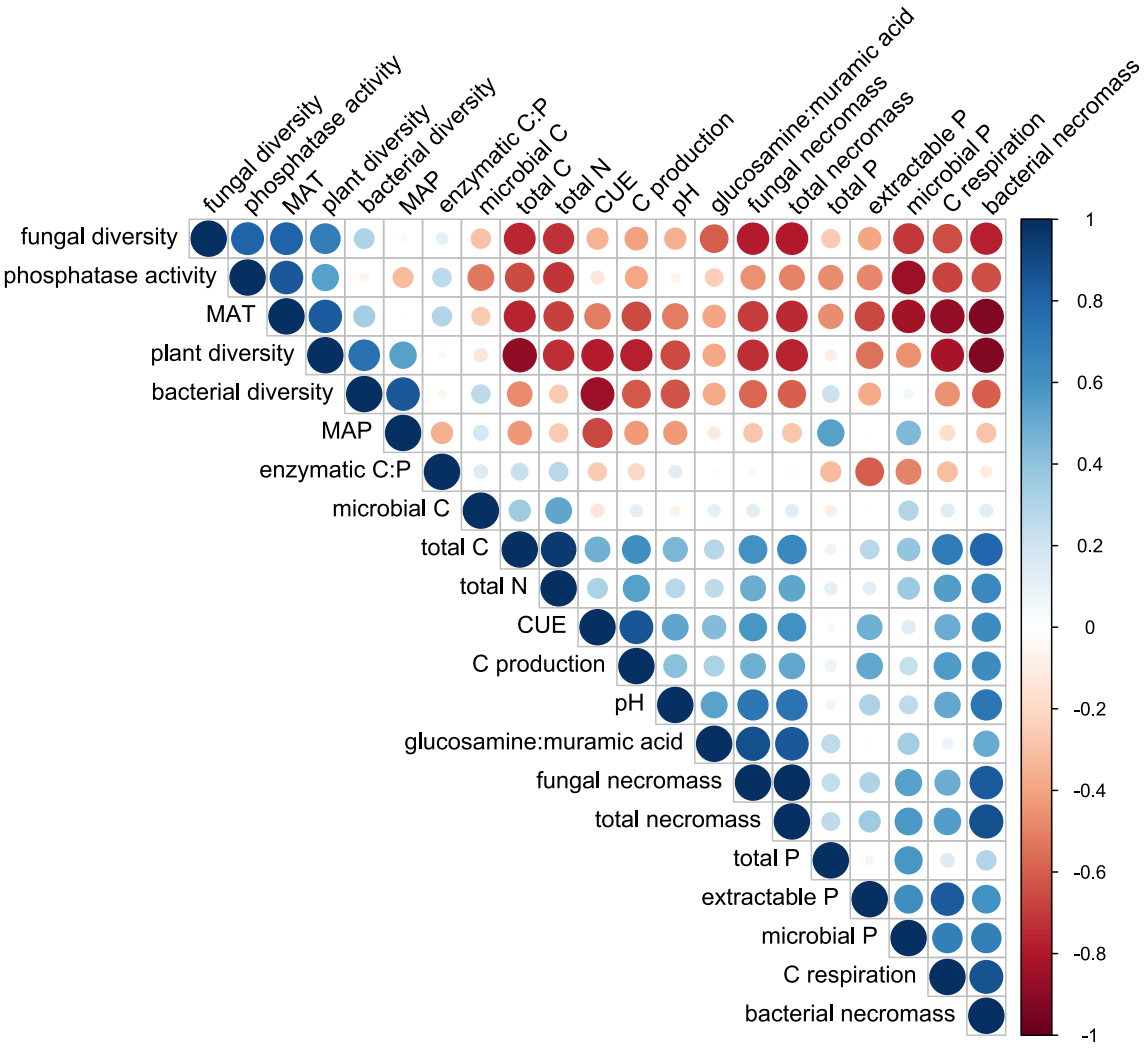

Fig. 5. Correlations between site and soil properties across a 20 °C elevation transect in the Peruvian Andes. Data are for the 14 sites (see Table 1) and properties of the native soils (non-translocated). The plot shows Spearman correlation coefficients, with negative correlations highlighted in red and positive correlations highlighted in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

contribution of microbial turnover and necromass formation as drivers of soil C accumulation (Kallenbach et al., 2016; Buckeridge et al., 2020). However, despite increased CUE with warming in our study, we observed decreased soil C (Fig. 2). The slope of decrease in soil C after 11 years of warming (Fig. 3) was similar to the slope reported after 5 years of warming (Nottingham et al. (2019d)), suggesting that either 1) the majority of soil C loss occurred within the initial five years of warming, or 2) the increased CUE resulted in increased soil microbial necromass inputs to soil C (that was rapidly stabilized by mineral surface interactions and therefore undetected via amino sugar extraction) to offset warming-induced losses, albeit insufficient to prevent a net loss.

In the case of our study, however, the increase in microbial biomass C and CUE following decadal warming did not translate into increased contents of microbial necromass C, which followed the same trend as the soil C decrease with long-term warming (Fig. 3). Elsewhere, it was found that the soil necromass C pool is correlated to the size and turnover rate of the microbial biomass (Chen et al., 2020; Wang et al., 2023). For example, a meta-analysis of warming experiments found that warming resulted in increased bacterial necromass, suggested to be the result of the high sensitivity of bacterial growth (and turnover) to increased temperature (Hu et al., 2023). However, this contrasts with our observation that the necromass C pool declined with warming (Fig. 3), following the same trend as the overall decline in total SOC and suggesting that necromass was decomposed before it became stabilized. This finding is potentially very important: despite short-term increases in the microbial biomass C pool with warming, as found in other tropical soil warming studies (Nottingham et al., 2020; Reed et al., 2020), we found no evidence that this increased the necromass C pool. While it's possible that increased mineral-stabilization of necromass occurred under warming to dampen the decline in soil C, there was clearly no evidence for increased soil C storage. This result is consistent with a mechanism posited by Tao et al. (2023) whereby increased CUE can result in decreased rather than increased soil C storage, due to increased microbial biomass being associated with greater enzymatic capacity to decompose microbial necromass, thereby constraining soil organic matter accumulation. Along these lines we found the lack of necromass accumulation (though at high CUE and microbial biomass C) to be linked to increased activity of N-degrading enzymes in warmed N-rich soils (Nottingham et al., 2019d), further being indicative of priming effects due to increasing N limitation with warming (Bernard et al., 2022). In addition, a large fraction of necromass production is expected to be stabilized in highly weathered lowland tropical soils with high abundance of Fe/Al minerals to form organo-mineral associations (Kirsten et al., 2021). Notably, the soils warmed via translocation here were predominantly tropical montane forest soils, which are rich in soil organic matter and high in particulate organic matter but relatively low in reactive minerals that would be expected to stabilize necromass inputs (Zimmermann et al., 2012). Therefore it is plausible that in more

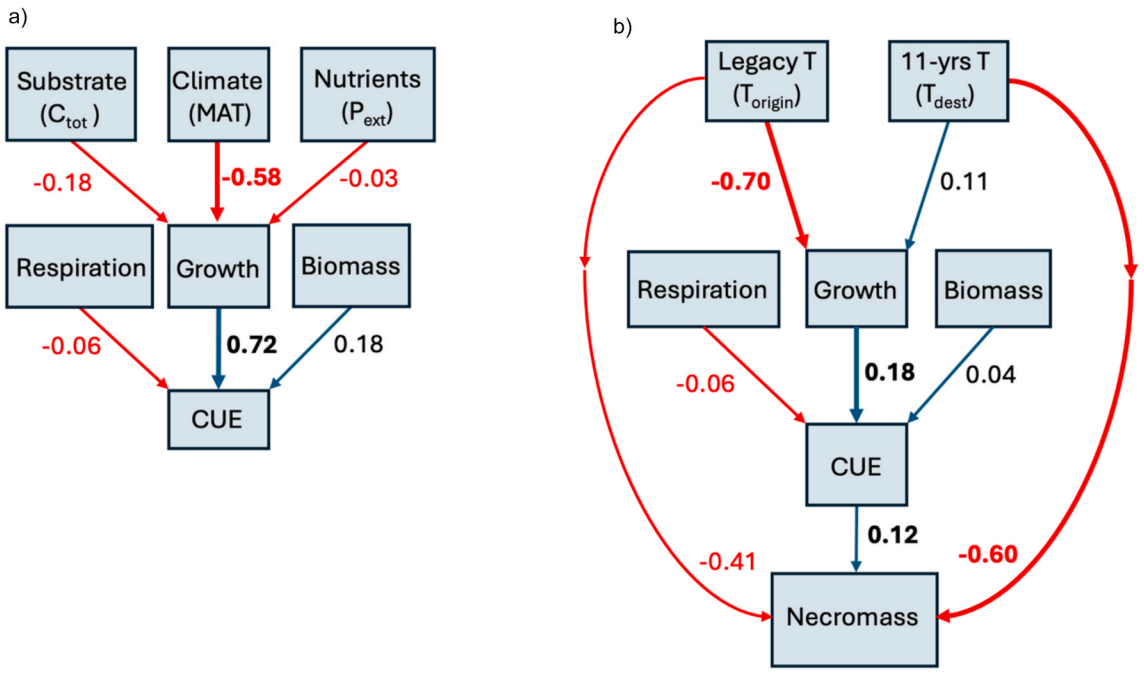

Fig. 6. The determinants of microbial CUE and necromass C with temperature change across temporal scales: a) across the elevation transect (20 °C MAT gradient) and b) in translocated soils (warming by 3–15 °C for 11 years). For a) the elevation transect we determined: i) microbial physiological effects on CUE (respiration, growth or microbial biomass C; necromass was not included in this analysis as it was only determined in a smaller subset of sites; Fig. 1), and ii) the climate or edaphic effects on microbial growth (MAT, total soil C, extractable P). For b) the translocated soils we determined: i) decadal temperature effects (defined by the new temperature regime, T_{dest}) and legacy temperature effects (defined by the temperature at site of origin, T_{origin}) on microbial growth and necromass C content, whereby T_{origin} had the strongest effect on growth, but T_{dest} had the strongest effect on necromass C content; ii) microbial physiological effects on CUE (respiration, growth or microbial biomass C); iii) the correlation between CUE and necromass C content. Correlations are represented by blue lines (positive) and red lines (negative) and increased line thickness where coefficient >0.5. Model coefficients are reported in Extended data Table 1

weathered lowland tropical soils, where increased microbial biomass under warming has also been reported (Nottingham et al., 2020; Reed et al., 2020), increased CUE could indeed result in increased necromass stabilization, offering a mechanism to potentially offset the C losses from stimulated soil organic matter decomposition.

The elevation gradient under study also forms a gradient in species diversity, for plants (trees), and soil bacteria and fungi (Nottingham et al., 2018b). The microbial CUE patterns we observed may therefore also have been influenced by changes in species diversity (which itself is considered a long-term ecosystem response to temperature difference). Several studies have demonstrated a positive relationship between soil microbial CUE and microbial or plant species diversity. For microbes, this relationship can arise through the increased diversity of microbial traits for C processing, or via an interaction with genome size with lower genome size increasing the ability to access a wider C substrate variety to increase CUE (Saifuddin et al., 2019; Domeignoz-Horta et al., 2020). For plants, this relationship may arise through the increased chemical diversity of substrate inputs to soils, as observed along a subtropical forest diversity gradient (Duan et al., 2023) though not reported in a temperate grassland diversity experiment (Prommer et al., 2020). Here we found no evidence for a positive relationship between plant or soil microbial diversity and soil microbial CUE. In fact, CUE was negatively related to these diversity metrics (Fig. 4). Our analyses showed that this pattern resulted from an overriding direct effect of temperature on CUE (Figs. 4–5). Differences in MAT drove changes in both plant and soil microbial diversity (Nottingham et al., 2018b) and simultaneously was the underlying driver of changes in CUE (overriding any smaller effect of microbial or plant diversity; Fig. 5). Together these observations demonstrate that substrate availability and quality and plant and soil microbial community composition have large influences on CUE, although the results from the Peru elevation gradient suggest that impacts of changes in vegetation and soil microbial community on soil microbial CUE may be minor relative to the larger effects of climate change.

Our findings provide important new insights into how temperature affects CUE and necromass in tropical soils, but these results need to be considered in the context of our experimental design. Our elevation gradient study considers the very long-term temperature response of the entire ecosystem (plant communities and soil biogeochemistry together). Our translocation study, in contrast, considers the 11-year warming effect on soil C cycling in predominantly montane forest soils and without the moderating effect of changes in plant-C input to soil to modify substrate availability (in soil cores where root-ingrowth was excluded). However, because CUE and substrate availability are positively related (He et al., 2024b), we suggest that our finding of increased CUE with 11-years of warming is inconsistent with decreased substrate availability (from reduced plant C-inputs), and points to a dominant influence of increased C-availability following warming-induced stimulation of soil organic matter decomposition; further supported by our observation of increased enzyme activity, microbial biomass growth and increased concentration of microbial biomass C and DOC with warming (Figs. 2–3) (Nottingham et al., 2019b, 2021). Another consideration is that we manipulated elevation rather than temperature directly, therefore other elevation-related environmental changes may have affected CUE, particularly in climatic humidity. This may have been the case for MAP, which was negatively correlated to CUE (albeit a weaker relationship than with MAT), suggesting an effect of soil moisture on CUE by affecting decomposition rates and C availability, as observed elsewhere (He et al., 2024b). However, this does not alter the main conclusions from our experimental warming-by-translocation study, which are based on the analysis of relative responses to assess the direct effect of temperature change.

Last, our experiment uses a reductionist approach to test for warming effects on soil heterotrophic communities and organic matter cycling but

does not test for how temperature may further affect plant C inputs to soil. Further work is needed to test how these temperature effects on microbial dynamics will be affected by changes in plant C inputs (driven through changes in productivity, belowground C-allocation and community shifts). Indeed, if warming stimulates productivity in these montane tropical ecosystems as suggested by observed compositional changes (Fadrique et al., 2018), it is possible that increased substrate supply will further increase CUE with warming and accelerate necromass C inputs. Overall, our findings offer robust insights into temperature effects on CUE across different timescales, but there remains a need for further studies to understand the mechanisms by which CUE change affects necromass (de)stabilization with minerals, and how temperature change effects on microbial soil C cycling are influenced by the moderating influences of moisture and plant community change.

In summary, our results demonstrate that the long-term steady state of soil microbial C cycling across temperature (elevation) gradients is not reflected in the dynamic changes in response to decadal warming. Likely, the 11 year temperature responses were dominated by changes in substrate availability (over the first 11 years of warming, the pool of C and nutrients available to microbial decomposers increased, fueling microbial growth), while over time the system may eventually converge on the long-term pattern as observed with elevational difference. Our findings together with recent emerging results from other experiments (Nottingham et al., 2020; Reed et al., 2020, Wood et al., 2025) suggest that, in tropical soils where warming coincides with conditions of high rainfall and high substrate turnover, warming may cause transient increases in microbial growth and biomass (increasing CUE), but it remains to be seen whether this microbial C could become stabilized or can offset any C lost due to increased rates of mineralisation and respiration. Here, for warmed montane forest soils, we find no evidence that this translates into an increased soil necromass C pool. Elucidating the mechanisms by which microbial necromass C may be stabilized in tropical soils will be important to understand the consequences for global climate, in addition to advancing the understanding of how the soil microbiome in different soils may be managed for increased C sequestration.

CRediT authorship contribution statement

Andrew T. Nottingham: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Kristiina Karhu:** Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – review & editing. **Norma Salinas:** Resources, Project administration, Writing – review & editing. **Jörg Schnecker:** Methodology, Resources, Writing – review & editing. **Outi-Maaria Sietiö:** Methodology, Resources, Writing – review & editing. **Angela K. Martin-Vivanco:** Investigation, Methodology, Resources, Writing – review & editing. **Wolfgang Wanek:** Methodology, Resources, Writing – review & editing. **Patrick Meir:** Funding acquisition, Conceptualization, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2025.110002.

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