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

DOI:
10.1111/ele.13379

Document Version
Accepted author manuscript

Letter to Ecology Letters

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

Running head: microbial responses enhance soil carbon loss

Andrew T. Nottingham[1, 2], Jeanette Whitaker[3], Nick J. Ostle[4], Richard D. Bardgett[5], Niall P. McNamara[3], Noah Fierer[6], Norma Salinas[7], Adan J. Q. Ccahuana[8], Benjamin L. Turner[2] & Patrick Meir[1,9]

[1]School of Geosciences, University of Edinburgh, Crew Building, Kings Buildings, Edinburgh EH9 3FF, UK

[2]Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Republic of Panama

[3]Centre for Ecology & Hydrology, Lancaster Environment Centre, Lancaster LA1 4AP, UK

[4]Lancaster Environment Centre, Lancaster University, Library Avenue, Lancaster LA1 4YQ, UK

[5]School of Earth and Environmental Sciences, Michael Smith Building, The University of Manchester, Oxford Road, Manchester M13 9PT, UK

[6]Department of Ecology and Evolutionary Biology, Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO, USA

[7]Seccion Química, Pontificia Universidad Católica del Peru, Lima, Peru

[8]Facultad de Biología, Universidad Nacional de San Antonio Abad del Cusco, Cusco, Peru

[9]Research School of Biology, Australian National University, Canberra, ACT 2601, Australia

*To whom correspondence should be addressed: Andrew Nottingham, School of Geosciences, University of Edinburgh, Drummond Street, Edinburgh EH8 9XP, UK. email: andrew.nottingham@ed.ac.uk. Tel: +44 (0) 131 651 4314 ; Fax: +44 (0) 131 650 2524

Key words: carbon-use-efficiency, climate feedback, climate warming, elevation gradient, lowland tropical forest, montane tropical forest, Q_{10}, soil carbon cycle, translocation

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

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

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 1°C 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

76 are steep (Loomis *et al.* 2017; Russell *et al.* 2017; Fadrique *et al.* 2018), are especially vulnerable to
77 warming and could create a globally large soil-climate feedback (Nottingham *et al.* 2015b). Indeed,
78 the response to warming in the tropics remains one of the major gaps in our understanding of
79 terrestrial ecosystem responses to climate change in Earth system models (Huntingford *et al.* 2009;
80 Cavaleri *et al.* 2015; Koven *et al.* 2015), and the size of the soil C-climate feedback is a dominant
81 component of this uncertainty.
82 Soil warming experiments in the field, which have so far been conducted only in mid- to
83 high-latitude ecosystems, have shown that warming generates a considerable short-term soil C loss
84 (Lu *et al.* 2013; Romero-Olivares *et al.* 2017). This loss declines over time (e.g. >2 years) (Romero-
85 Olivares *et al.* 2017), although there is evidence that it can continue for longer (e.g. >20 years)
86 (Melillo *et al.* 2017). The short-term decline in soil C loss with warming has been explained by a
87 limited availability of C-substrates and nutrients to heterotrophs (Knorr *et al.* 2005; Romero-Olivares
88 *et al.* 2017), and an overall decline in microbial C-use efficiency (CUE) (Manzoni *et al.* 2012;
89 Melillo *et al.* 2017). Microbial CUE, defined as the fraction of C incorporated for growth over
90 respiratory losses, generally decreases when greater metabolic C-demand at higher temperatures
91 reduces microbial biomass and enzyme synthesis (termed 'thermal compensation') (Manzoni *et al.*
92 2012; Bradford *et al.* 2019). However, a longer-term response of increased CUE under warming has
93 been reported for specific substrates, resulting in sustained or increased microbial biomass and
94 enzyme synthesis (Frey *et al.* 2013), which could have a longer-term negative impact on soil C
95 stocks (i.e. an 'enhancing' CUE response) (Wieder *et al.* 2013). The underlying mechanisms for
96 these CUE responses remain unclear, but might include physiological changes within species, shifts
97 in microbial community composition (Oliverio *et al.* 2017), or changes in the temperature sensitivity
98 of enzyme activity (Wallenstein *et al.* 2011; Allison *et al.* 2018).
99 The wide range of microbial feedbacks hypothesized in models reflects limited understanding
100 of this important climate response, and has confounded attempts to model the change in soil C under

101 warming, leading to hugely divergent modelling outcomes (Wieder *et al.* 2013; Hagerty *et al.* 2018).
102 For example, depending on the attributed temperature response of microbial CUE, global soil C
103 losses by 2100 have been predicted to range from negligible (decreased CUE with warming) to 300
104 Pg C (=20% of global soil C stocks; i.e. with increased CUE with warming) (Wieder *et al.* 2013).
105 Reducing this uncertainty requires understanding of how the temperature sensitivity of soil C
106 responds to resource availability and microbial feedbacks in tropical ecosystems.
107 Here we report the results of a five-year soil translocation experiment along a 3000 m elevation
108 gradient (15^{o}C range in mean annual temperature; MAT) in tropical forests between western lowland
109 Amazonia and the Peruvian Andes (Nottingham *et al.* 2015b) (Fig. S1, Table 1). To isolate the effect
110 of temperature, our principal experimental manipulation, we controlled rainfall inputs to represent an
111 average at the site of origin. We tested the hypotheses that: i) five years of temperature manipulation
112 would systematically change soil C stocks across sites (increased loss with warming/reduced loss
113 with cooling); ii) changes in soil C would be determined by soil chemistry, whereby C loss would be
114 positively correlated with the relative abundance of labile compounds; and iii) microbial CUE would
115 increase over five years of warming, indicating an enhancing effect of microbial physiology and/or
116 community composition changes on soil C loss.
117
118 **MATERIALS AND METHODS**
119 We translocated soil among four tropical forest sites along the elevation gradient. Soil was
120 translocated as intact cores, 10 cm diameter × 50 cm depth (4000 cm^3). Three undisturbed soil cores
121 were re-installed at the same site ('control'), and the other cores were translocated to the three other
122 elevations to achieve both warming and cooling (downslope = 'warmed', upslope = 'cooled')
123 (Zimmermann *et al.* 2012), an approach similar to laboratory-based studies of thermal-responses of
124 microbial activity (Karhu *et al.* 2014). To assess changes in soil C and thermal-responses of
125 microbial communities and their physiology after five years in a new temperature regime, we

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 determinants of changes in soil C and RQ_{10} were evaluated with mixed-effects models. To determine whether soil properties changed in response to temperature manipulation, the respective factors 'soil-destination' (effect of new temperature regime) and 'soil-origin' (effect of intrinsic soil properties) were included in the models.

Study sites

To investigate the effect of temperature on soil C dynamics and soil microbial communities, soil cores were reciprocally translocated among four sites along an elevation gradient of tropical forest in Peru. The sites ranged from lowland rainforest (210 m asl; above sea level), pre-montane rainforest (1000 m asl), lower montane cloud forest (1500 m asl) and upper montane cloud forest (3030 m asl). Site mean annual temperature (MAT) was determined over a 5-year period (2005-2010) and varied from 26^{o}C to 11^{o}C with increasing elevation (Table 1). Dominant tree families ranged from Clusiaceae and Cunoniceae at 3030 m asl, to Clethraceae at 1500 m asl, to Elaeocarpaceae and Fabaceae at 1000 m asl, and Moraceae and Fabaceae at 200 m asl. The sampling sites were adjacent 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
170 together will be important determinants of the overall tropical forest C cycle (Meir *et al.* 2015). New
171 litter input was excluded and root ingrowth prevented by installing a 63 μm nylon mesh at the base
172 of the tubes. A detailed description of the experimental setup is given in Zimmermann et al. (2009).
173 Soil cores were translocated in 2008 and, exactly five years later in 2013, mineral soil was sampled
174 from each core using an auger to 20 cm depth. Soil samples were stored for < 14 days at < 4 ^{o}C until
175 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.

226

227 **Calculations**

228 ***Determination of Q_{10} values:*** We determined Q_{10} of enzyme activities (Q_{10} of V_{max}) and

229 microbial respiration (RQ_{10}) according to:

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

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

232 Where k is the exponential rate at which activity (a) increases with temperature (t) (Nottingham *et*

233 *al.* 2016). To calculate k (and thus Q_{10}) we used linear regression of ln(activity)/temperature, for n =

234 5 temperatures and n = 3 replicates per temperature.

235 ***Determination of carbon and nutrient use efficiencies:*** Microbial CUE is defined as the

236 fraction of C incorporated for growth over respiratory losses. However, it is acknowledged as an

237 emergent property of growth and allocation processes that can vary with the method used for its

238 estimation (Hagerty *et al.* 2018) (see Appendix S1 in Supporting Information). We determined

239 microbial carbon, nitrogen and phosphorus use efficiencies (CUE, NUE and PUE), using a widely-

240 accepted stoichiometric method, whereby the CUE/NUE/PUE of an organism is a function of the

241 difference between its elemental requirements for growth (C, N or P in biomass and enzymatic

242 investment for acquisition) and the abundance of environmental substrate (C, N, P in soil organic

243 matter) (Sinsabaugh *et al.* 2016). Following this approach, NUE and PUE are inversely related to

244 $CUE_{C:N}$ or $CUE_{C:P}$ (CUE calculated relative to enzymatic investment for N or P acquisition,

245 respectively). Therefore, we present NUE and PUE results but focus our hypotheses and discussion

246 on the responses of CUE. While acknowledging the assumptions and limitations of this approach

247 (see Appendix S1 in Supporting Information), this method is considered particularly useful for

248 parameterization and testing of models because it quantifies CUE in terms of the underlying

249 microbial processes (Hagerty *et al.* 2018). This approach assumes that enzyme activities scale with

250 microbial production and organic matter concentration, and that microbial communities exhibit

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:

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

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:

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

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 [($X(i=1\text{-}3)$ 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 4–8ºC 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 4–8ºC 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

375 microbial biomass and enzyme activities (Fig. 2), contrary to the hypothesis of reduced biomass and
376 activity through thermal compensation (Manzoni *et al.* 2012). Similarly, in a global study following
377 90 days of laboratory incubation, no evidence was found for thermal-compensation of respiration for
378 samples from the same Peru forest sites (Karhu *et al.* 2014). although Karhu *et al.* (2014) did find
379 some geographical variation in this process.. This global variability has been reflected in extra-
380 tropical warming experiments (Melillo *et al.* 2017; Romero-Olivares *et al.* 2017), although some of
381 the variability among studies may also result from the different methods and scales by which CUE
382 and thermal compensation has been defined (Geyer *et al.* 2016; Hagerty *et al.* 2018). While the
383 underlying mechanisms invite further investigation, our results suggest that the experimental
384 warming imposed here induced changes in microbial physiology and community composition that
385 accelerated soil C loss, with no thermal compensation of microbial activity, consistent with model
386 predictions of increased CUE under warming accelerating soil C loss (Wieder *et al.* 2013).
387 The changes in CUE in response to temperature occurred alongside changes in microbial
388 community composition. Although we cannot rule out dispersal as a factor affecting these microbial
389 community shifts (i.e. migration of microbes via aerial dispersal from the surrounding destination
390 site; see SI), which could only have been controlled for using an *in situ* soil warming experiment, a
391 dominant role for temperature shifts in driving these changes is suggested by the consistency
392 between our results and a recent global study of temperature-responsive bacterial taxa (Oliverio *et al.*
393 2017). The responsive taxa in our study overlapped with those identified in the global study, with
394 members of the Actinobacteria and Rhizobiales being more abundant in warmed soils (together, 75%
395 consistent with Oliverio et al., 2017) and Acidobacteria becoming more abundant in colder soils
396 (71% consistent with Oliverio et al., 2017), with the latter associated with oligotrophic N-limited
397 conditions such as those found in cooler montane ecosystems (Oliverio *et al.* 2017). Thus, microbial
398 taxa responded to temperature manipulation in a manner consistent with their previously-observed
399 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^{o}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.

Acknowledgements:

This study is a product of the Andes Biodiversity and Ecosystem Research Group consortium (www.andesconservation.org) and was led using support from the UK Natural Environment Research Council (NERC), grant numbers NE/G018278/1 and NE/F002149/1 to PM and also supported by an Australian Research Council (ARC) grant DP170104091 to PM, and a European Union Marie-Curie Fellowship FP7-2012-329360 to ATN. We thank the Asociación para la Conservación de la Cuenca Amazónica (ACCA) in Cusco and the Instituto Nacional de Recursos Naturales (INRENA) in Lima for access to the study sites. For support for ^{13}C-NMR analyses we thank Dr David Apperley, Durham University. For their logistical support we thank Dr Eric Cosio and Eliana Esparza Ballón at Pontificia Universidad Católica del Perú (PUCP). For laboratory support we thank Dayana Agudo. For his role in instigating the experiment we thank Michael Zimmermann. For their ongoing support in the field we thank Walter H. Huasco, William Farfan Rios and Javier E. S. Espejo.

References:

1.
Abarenkov, K., Nilsson, R.H., Larsson, K.H., Alexander, I.J., Eberhardt, U., Erland, S. *et al.* (2010). The UNITE database for molecular identification of fungi - recent updates and future perspectives. *New Phytol*, 186, 281-285.

2.
Allison, S.D., Romero-Olivares, A.L., Lu, Y., Taylor, J.W. & Treseder, K.K. (2018). Temperature sensitivities of extracellular enzyme V-max and K-m across thermal environments. *Global Change Biol*, 24, 2884-2897.

3.
Bradford, M.A., McCulley, R.L., Crowther, T.W., Oldfield, E.E., Wood, S.A. & Fierer, N. (2019). Cross-biome patterns in soil microbial respiration predictable from evolutionary theory on thermal adaptation. *Nat Ecol Evol*, 3, 223-+.

4.
Brzostek, E.R. & Finzi, A.C. (2012). Seasonal variation in the temperature sensitivity of proteolytic enzyme activity in temperate forest soils. *Journal of Geophysical Research*, 117, doi: 10.1029/2011JG001688.

5.
Burns, R. & Staunton, S. (2013). Special Issue: Interactions of Soil Minerals with Organic Components and Microorganisms VII and Enzymes in the Environment IV. *Soil Biol Biochem*, 56, 1-2.

6.
Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N. *et al.* (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *Isme J*, 6, 1621-1624.
7.
Cavaleri, M.A., Reed, S.C., Smith, W.K. & Wood, T.E. (2015). Urgent need for warming experiments in tropical forests. *Global Change Biol*, 21, 2111-2121.
8.
Crowther, T.W., Todd-Brown, K.E.O., Rowe, C.W., Wieder, W.R., Carey, J.C., Machmuller, M.B. *et al.* (2016). Quantifying global soil carbon losses in response to warming. *Nature*, 540, 104-108.
9.
Davidson, E.A. & Janssens, I.A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, 440, 165-173.
10.
Fadrique, B., Baez, S., Duque, A., Malizia, A., Blundo, C., Carilla, J. *et al.* (2018). Widespread but heterogeneous responses of Andean forests to climate change. *Nature*, 564, 207-+.
11.
Frey, S.D., Lee, J., Melillo, J.M. & Six, J. (2013). The temperature response of soil microbial efficiency and its feedback to climate. *Nat Clim Change*, 3, 395-398.
12.
Geyer, K.M., Kyker-Snowman, E., Grandy, A.S. & Frey, S.D. (2016). Microbial carbon use efficiency: accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. *Biogeochemistry*, 127, 173-188.
13.
Girardin, C.A.J., Malhi, Y., Aragao, L.E.O.C., Mamani, M., Huaraca Huasco, W., Durand, L. *et al.* (2010). Net primary productivity allocation and cycling of carbon along a tropical forest elevational transect in the Peruvian Andes. *Global Change Biol*, 16, 3176-3192.
14.
Hagerty, S.B., Allison, S.D. & Schimel, J.P. (2018). Evaluating soil microbial carbon use efficiency explicitly as a function of cellular processes: implications for measurements and models. *Biogeochemistry*, 140, 269-283.
15.
Hagerty, S.B., van Groenigen, K.J., Allison, S.D., Hungate, B.A., Schwartz, E., Koch, G.W. *et al.* (2014). Accelerated microbial turnover but constant growth efficiency with warming in soil. *Nat Clim Change*, 4, 903-906.
16.
Huntingford, C., Lowe, J.A., Booth, B.B.B., Jones, C.D., Harris, G.R., Gohar, L.K. *et al.* (2009). Contributions of carbon cycle uncertainty to future climate projection spread. *Tellus Series B-Chemical and Physical Meteorology*, 61, 355-360.
17.
Jobbagy, E.G. & Jackson, R.B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol Appl*, 10, 423-436.
18.
Karhu, K., Auffret, M.D., Dungait, J.A.J., Hopkins, D.W., Prosser, J.I., Singh, B.K. *et al.* (2014). Temperature sensitivity of soil respiration rates enhanced by microbial community response. *Nature*, 513, 81-84.
19.

533 Keiluweit, M., Nico, P., Harmon, M.E., Mao, J.D., Pett-Ridge, J. & Kleber, M. (2015). Long-term
534 litter decomposition controlled by manganese redox cycling. *P Natl Acad Sci USA*, 112,
535 E5253-E5260.
536 20.
537 Knorr, W., Prentice, I.C., House, J.I. & Holland, E.A. (2005). Long-term sensitivity of soil carbon
538 turnover to warming. *Nature*, 433, 298-301.
539 21.
540 Koven, C.D., Chambers, J.Q., Georgiou, K., Knox, R., Negron-Juarez, R., Riley, W.J. *et al.* (2015).
541 Controls on terrestrial carbon feedbacks by productivity versus turnover in the CMIP5
542 Earth System Models. *Biogeosciences*, 12, 5211-5228.
543 22.
544 Lauber, C.L., Zhou, N., Gordon, J.I., Knight, R. & Fierer, N. (2010). Effect of storage conditions on
545 the assessment of bacterial community structure in soil and human-associated samples.
546 *Fems Microbiol Lett*, 307, 80-86.
547 23.
548 Leff, J.W., Jones, S.E., Prober, S.M., Barberan, A., Borer, E.T., Firn, J.L. *et al.* (2015). Consistent
549 responses of soil microbial communities to elevated nutrient inputs in grasslands across
550 the globe. *P Natl Acad Sci USA*, 112, 10967-10972.
551 24.
552 Loomis, S.E., Russell, J.M., Verschuren, D., Morrill, C., De Cort, G., Damste, J.S.S. *et al.* (2017). The
553 tropical lapse rate steepened during the Last Glacial Maximum. *Sci Adv*, 3.
554 25.
555 Lu, M., Zhou, X.H., Yang, Q., Li, H., Luo, Y.Q., Fang, C.M. *et al.* (2013). Responses of ecosystem
556 carbon cycle to experimental warming: a meta-analysis. *Ecology*, 94, 726-738.
557 26.
558 Luo, Y.Q., Ahlstrom, A., Allison, S.D., Batjes, N.H., Brovkin, V., Carvalhais, N. *et al.* (2016). Toward
559 more realistic projections of soil carbon dynamics by Earth system models. *Global*
560 *Biogeochem Cy*, 30, 40-56.
561 27.
562 Manzoni, S., Taylor, P., Richter, A., Porporato, A. & Agren, G.I. (2012). Environmental and
563 stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytol*, 196, 79-
564 91.
565 28.
566 McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A. *et al.* (2012). An
567 improved Greengenes taxonomy with explicit ranks for ecological and evolutionary
568 analyses of bacteria and archaea. *Isme J*, 6, 610-618.
569 29.
570 Meir, P., Wood, T.E., Galbraith, D.R., Brando, P.M., Da Costa, A.C.L., Rowland, L. *et al.* (2015).
571 Threshold Responses to Soil Moisture Deficit by Trees and Soil in Tropical Rain Forests:
572 Insights from Field Experiments. *Bioscience*, 65, 882-892.
573 30.
574 Melillo, J.M., Frey, S.D., DeAngelis, K.M., Werner, W.J., Bernard, M.J., Bowles, F.P. *et al.* (2017).
575 Long-term pattern and magnitude of soil carbon feedback to the climate system in a
576 warming world. *Science*, 358, 101-104.
577 31.
578 Nottingham, A.T., Bååth, E., Reischke, S., Salinas, N. & Meir, P. (2019). Adaptation of soil
579 microbial growth to temperature: using a tropical elevation gradient to predict future
580 changes. *Global Change Biol*.
581 32.

582 Nottingham, A.T., Fierer, N., Turner, B.L., Whitaker, J., Ostle, N.J., McNamara, N.P. *et al.* (2018).
583 Microbes follow Humboldt: temperature drives plant and soil microbial diversity
584 patterns from the Amazon to the Andes. *Ecology*, 99, 2455-2466.
585 33.
586 Nottingham, A.T., Turner, B.L., Whitaker, J., Ostle, N., Bardgett, R.D., McNamara, N.P. *et al.*
587 (2016). Temperature sensitivity of soil enzymes along an elevation gradient in the
588 Peruvian Andes. *Biogeochemistry*, 127, 217-230.
589 34.
590 Nottingham, A.T., Turner, B.L., Whitaker, J., Ostle, N., McNamara, N.P., Bardgett, R.D. *et al.*
591 (2015a). Soil microbial nutrient constraints along a tropical forest elevation gradient: a
592 belowground test of a biogeochemical paradigm. *Biogeosciences*, 12, 6489-6523.
593 35.
594 Nottingham, A.T., Whitaker, J., Turner, B.L., Salinas, N., Zimmermann, M., Malhi, Y. *et al.*
595 (2015b). Climate warming and soil carbon in tropical forests: insights from an elevation
596 gradient in the Peruvian Andes. *Bioscience*, 65, 906-921.
597 36.
598 Oliverio, A.M., Bradford, M.A. & Fierer, N. (2017). Identifying the microbial taxa that
599 consistently respond to soil warming across time and space. *Global Change Biol*, 23,
600 2117-2129.
601 37.
602 Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A. *et al.* (2011). A large and
603 persistent carbon sink in the world's forests. *Science*, 333, 988-993.
604 38.
605 Rapp, J.M., Silman, M.R., Clark, J.S., Girardin, C.A.J., Galiano, D. & Tito, R. (2012). Intra- and
606 interspecific tree growth across a long altitudinal gradient in the Peruvian Andes.
607 *Ecology*, 93, 2061-2072.
608 39.
609 Romero-Olivares, A.L., Allison, S.D. & Treseder, K.K. (2017). Soil microbes and their response to
610 experimental warming over time: A meta-analysis of field studies. *Soil Biol Biochem*,
611 107, 32-40.
612 40.
613 Russell, A.M., Gnanadesikan, A. & Zaitchik, B. (2017). Are the Central Andes Mountains a
614 Warming Hot Spot? *J Climate*, 30, 3589-3608.
615 41.
616 Sinsabaugh, R.L., Turner, B.L., Talbot, J.M., Waring, B.G., Powers, J.S., Kuske, C.R. *et al.* (2016).
617 Stoichiometry of microbial carbon use efficiency in soils. *Ecological Monographs*, 86,
618 172-189.
619 42.
620 Tucker, C.L., Bell, J., Pendall, E. & Ogle, K. (2013). Does declining carbon-use efficiency explain
621 thermal acclimation of soil respiration with warming? *Global Change Biol*, 19, 252-263.
622 43.
623 Turner, B.L. & Romero, T.E. (2010). Stability of hydrolytic enzyme activity and microbial
624 phosphorus during storage of tropical rain forest soils. *Soil Biology and Biochemistry*, 42,
625 459-465.
626 44.
627 van Gestel, N., Shi, Z., van Groenigen, K.J., Osenberg, C.W., Andresen, L.C., Dukes, J.S. *et al.*
628 (2018). Predicting soil carbon loss with warming. *Nature*, 554, E4-E5.
629 45.

Wallenstein, M., Allison, S., Ernakovich, J., Steinweg, J.M. & Sinsabaugh, R. (2011). Controls on the temperature sensitivity of soil enzymes: a key driver of in situ enzyme activity rates. In: *Soil Enzymology* (eds. Shukla, G & Varma, A). Springer Berlin Heidelberg, pp. 245-258.

46.

Wang, Q., Garrity, G.M., Tiedje, J.M. & Cole, J.R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microb*, 73, 5261-5267.

47.

Whitaker, J., Ostle, N., Nottingham, A.T., Ccahuana, A., Salinas, N., Bardgett, R.D. *et al.* (2014). Microbial community composition explains soil respiration responses to changing carbon inputs along an Andes-to-Amazon elevation gradient. *J Ecol*, 102, 1058-1071.

48.

Wieder, W.R., Bonan, G.B. & Allison, S.D. (2013). Global soil carbon projections are improved by modelling microbial processes. *Nat Clim Change*, 3, 909-912.

49.

Wood, T.E., Cavaleri, M.A., Giardina, C., Khan, S., Mohan, J.E., Nottingham, A.T. *et al.* (2019). Soil warming effects on low-latitude forests with highly-weathered soils. In: *Ecosystem Consequences of Soil Warming: Microbes, Vegetation, Fauna and Soil Biogeochemistry* (ed. Mohan, J). Academic Press, pp. 385-439.

50.

Zimmermann, M., Leifeld, J., Conen, F., Bird, M.I. & Meir, P. (2012). Can composition and physical protection of soil organic matter explain soil respiration temperature sensitivity? *Biogeochemistry*, 107, 423-436.

51.

Zimmermann, M., Meir, P., Bird, M.I., Malhi, Y. & Ccahuana, A.J.Q. (2009). Climate dependence of heterotrophic soil respiration from a soil-translocation experiment along a 3000 m tropical forest altitudinal gradient. *Eur J Soil Sci*, 60, 895-906.

52.

Zimmermann, M., Meir, P., Bird, M.I., Malhi, Y. & Ccahuana, A.J.Q. (2010). Temporal variation and climate dependence of soil respiration and its components along a 3000 m altitudinal tropical forest gradient. *Global Biogeochem Cy*, 24, GB4012.

671 ***Figure legends:***

672

673 **Figure 1. The relative change in total soil C (%) in mineral soils following five years of**
674 **translocation.** Translocation represented an elevation shift of up to ±3000 m, which was equivalent
675 to a warming or cooling treatment of up to ±15°C. Calculations for log response ratio of soil C (RR
676 of %C) and description of the translocation design are provided in Supplementary Materials. The
677 linear relationship, % C RR = 0.00703 + (0.0000824 * elevation shift), equates to 0.021 %C RR for
678 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
679 = 0.23; $p < 0.001$).

680

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

688

689 **Figure 3. Temperature adaptive responses of microbial communities and physiology following**
690 **five years of translocation: carbon-use-efficiency (CUE) (A) nutrient-use-efficiency (B), phenol**
691 **oxidase activity (C) and community composition (D).** For **A-B,** CUE was calculated according to
692 microbial stoichiometry with respect to N ($CUE_{C:N}$) and P ($CUE_{C:P}$), according to equation 3.
693 Nitrogen (NUE) and phosphorus (PUE) use efficiencies were calculated according to equation 4 (ref.
694 30). For **C,** the temperature response of Q_{10} of V_{max} for phenol oxidase, we calculated the Q_{10} of V_{max}
695 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).

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

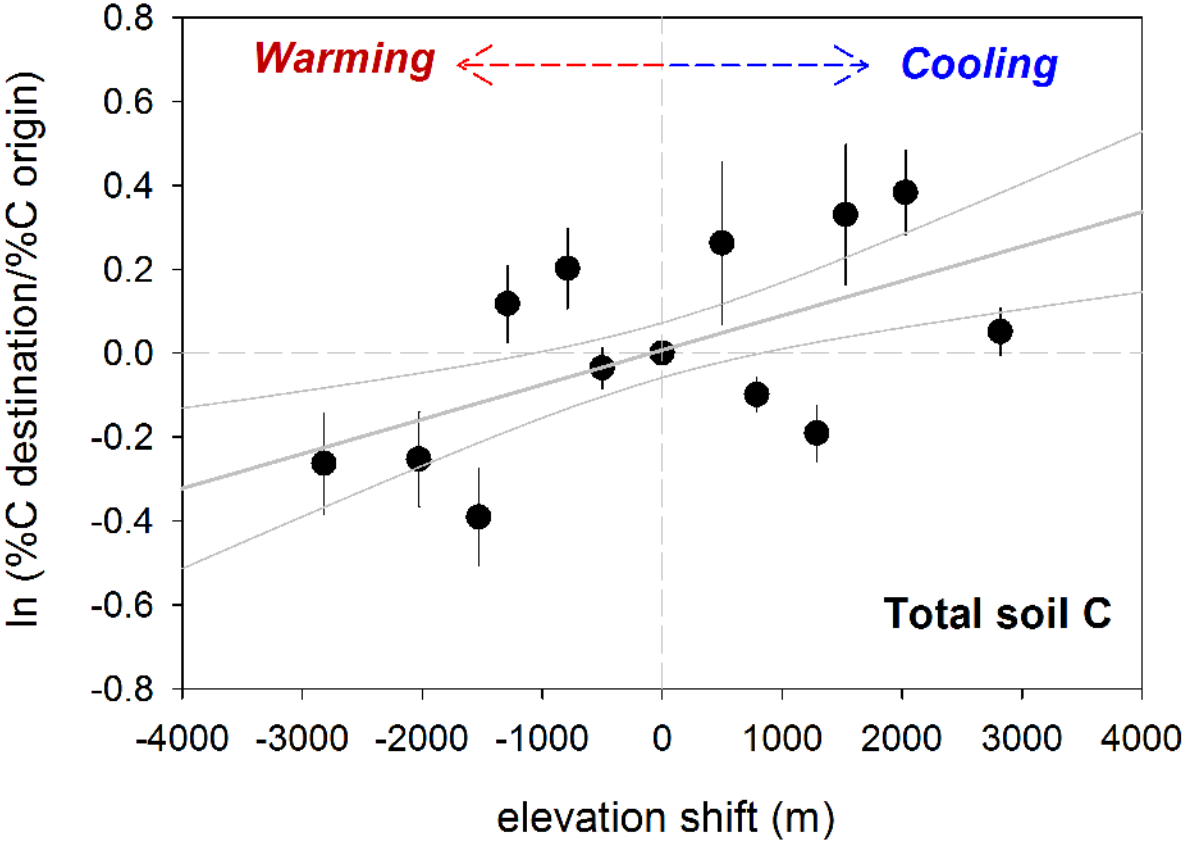
723

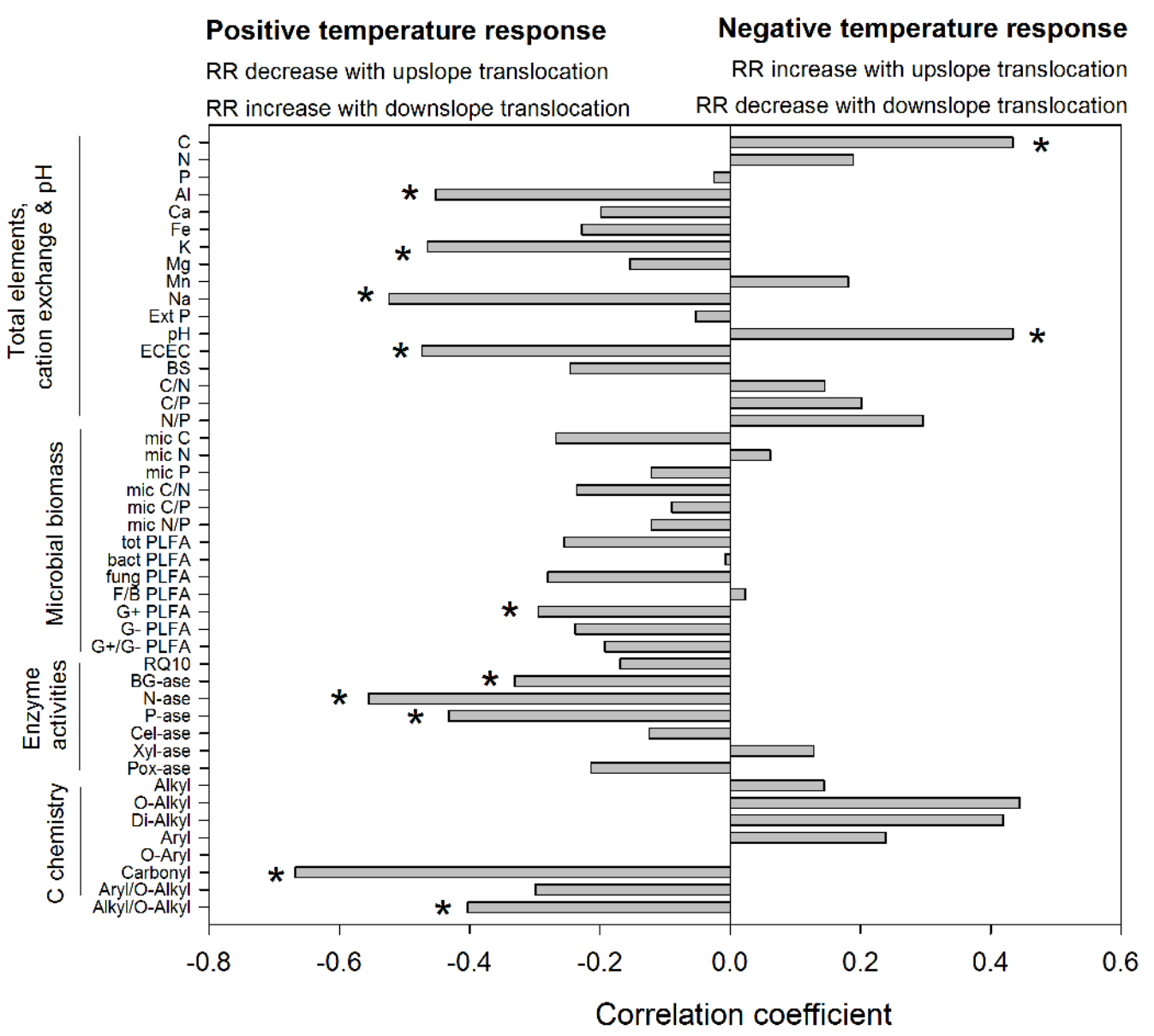
Site name	Elevation (m asl)	Lat	Long	Mean annual temp (°C)	Mean annual precipitation (mm yr^{-1})	Parent material	Soil classification
Explorer's Inn plot 3 (TP3)	210	-12.830	-69.271	26	3199	Pleistocene alluvial terrace	Inceptisol
Tono	1000	-12.866	-71.401	21	3100	Paleozoic shales-slates	Inceptisol
San 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

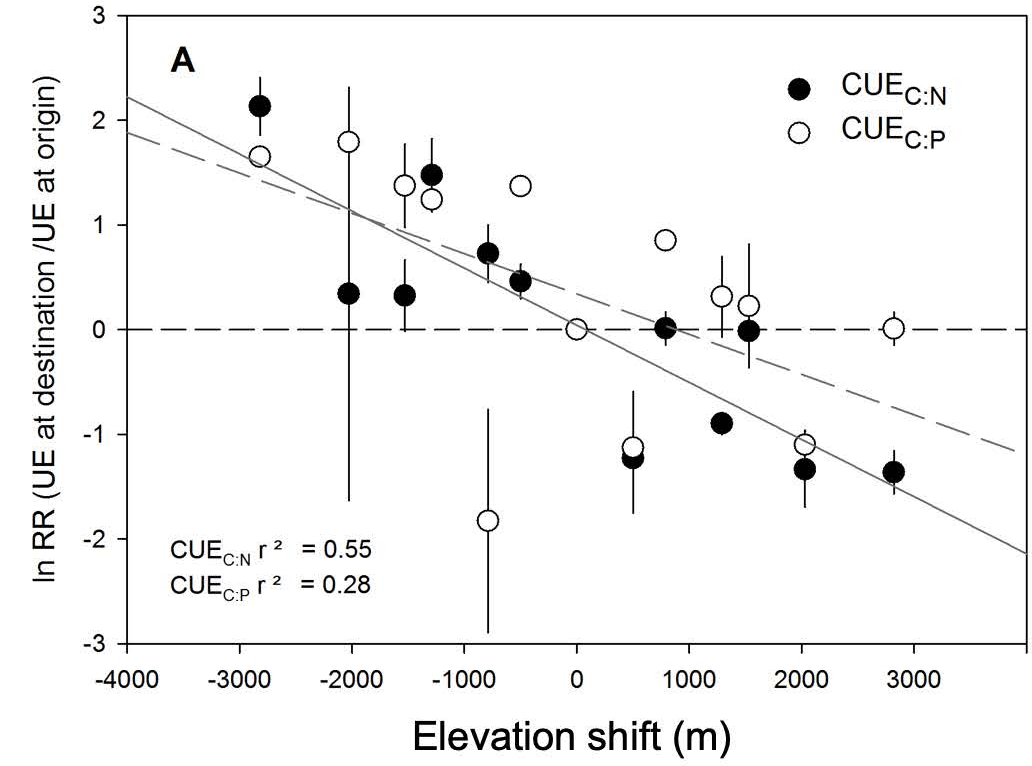
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741

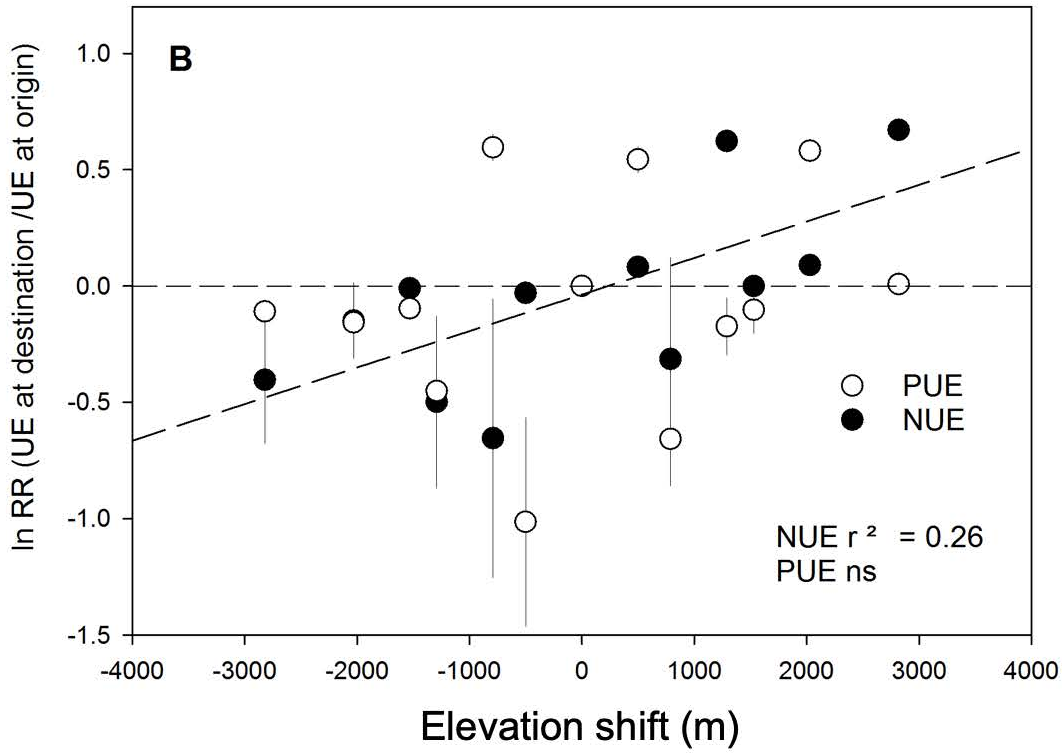
Table 2. The effect of soil and environmental properties on the relative response of total soil C (A) and on the instantaneous temperature sensitivity of microbial respiration (B). Mixed-effects models were fitted using maximum likelihood, by beginning with full model (70 variables) and step-wise parameter 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.

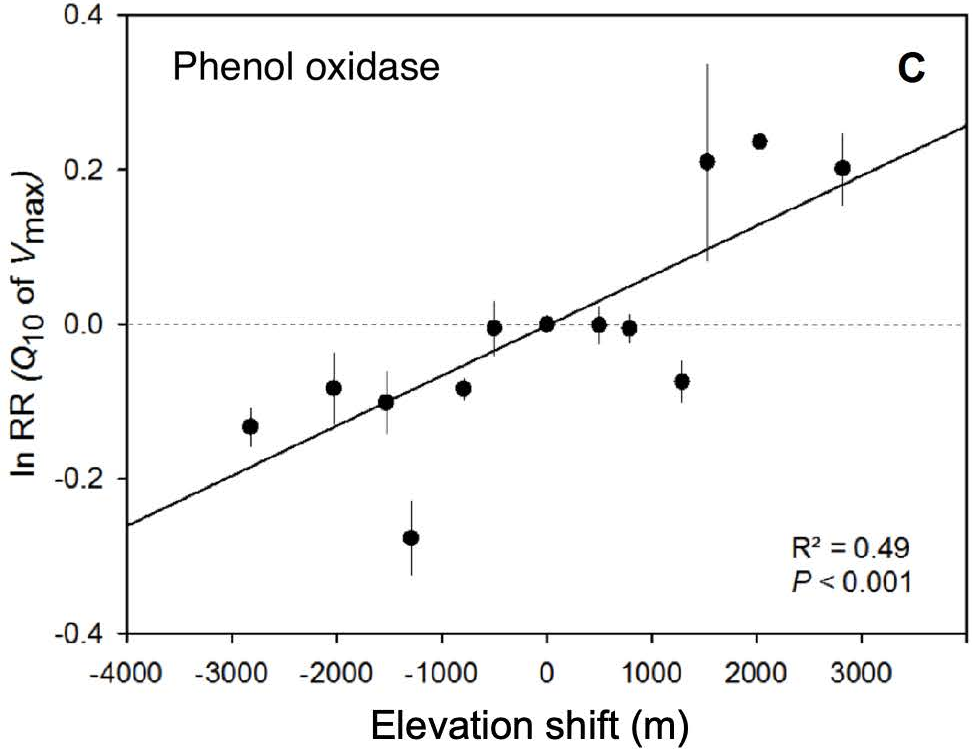
A) Relative response of total soil C				
	Parameter	SE	P-value	X^2 test
Fixed effects				
Total PLFA	0.00498	0.00264	0.0680	0.0311 *
Alkyl:*O*-Alkyl	-0.69858	0.30904	0.0311	0.0323 *
Random effects				
Soil Origin	0.40469	0.27731	0.1545	
AIC value				11
R^2				0.631
B) Relative response of RQ_{10}			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

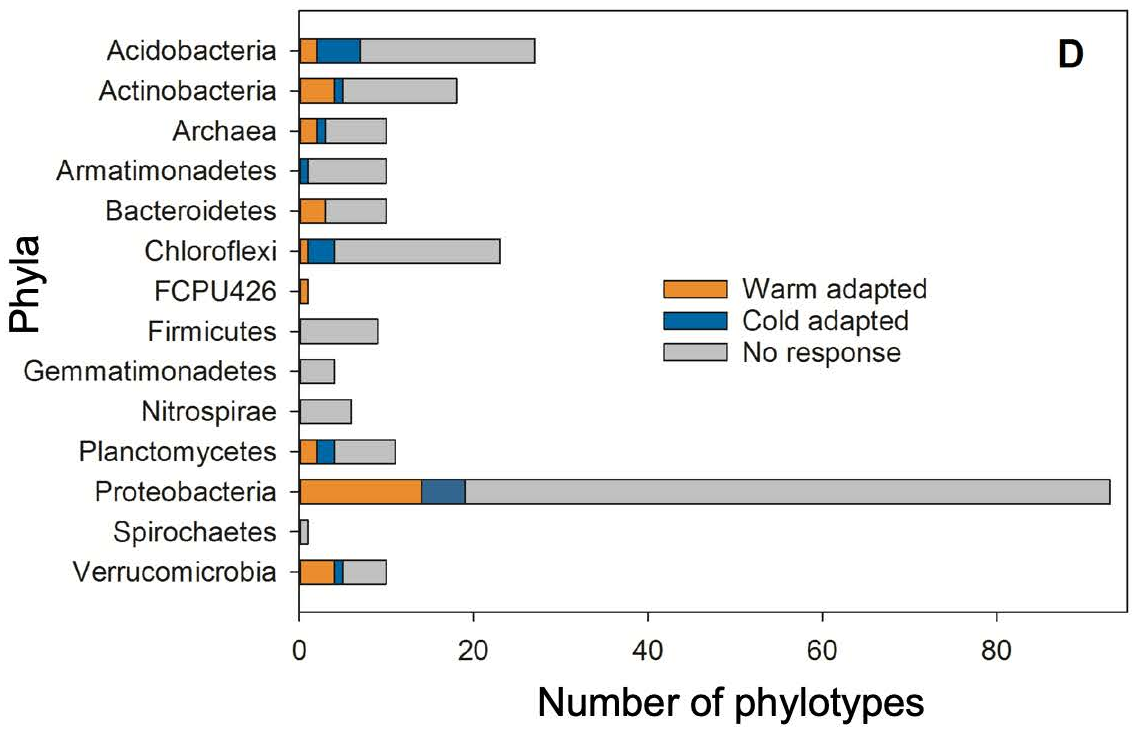

Warming
Cooling
Total soil C
ln (%C destination/%C origin)
elevation shift (m)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-4000
-3000
-2000
-1000
0
1000
2000
3000
4000


Positive temperature response
RR decrease with upslope translocation
RR increase with downslope translocation
Negative temperature response
RR increase with upslope translocation
RR decrease with downslope translocation
Total elements, cation exchange & pH
Microbial biomass
Enzyme activities
C chemistry
C
N
P
Al
Ca
Fe
K
Mg
Mn
Na
Ext P
pH
ECEC
BS
C/N
C/P
N/P
mic C
mic N
mic P
mic C/N
mic C/P
mic N/P
tot PLFA
bact PLFA
fung PLFA
F/B PLFA
G+ PLFA
G- PLFA
G+/G- PLFA
RQ10
BG-ase
N-ase
P-ase
Cel-ase
Xyl-ase
Pox-ase
Alkyl
O-Alkyl
Di-Alkyl
Aryl
O-Aryl
Carbonyl
Aryl/O-Alkyl
Alkyl/O-Alkyl
-0.8
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
Correlation coefficient


A
CUE$_{C:N}$
CUE$_{C:P}$
CUE$_{C:N}$ r ² = 0.55
CUE$_{C:P}$ r ² = 0.28
ln RR (UE at destination /UE at origin)
Elevation shift (m)


B
PUE
NUE
NUE r ² = 0.26
PUE ns
ln RR (UE at destination /UE at origin)
Elevation shift (m)


Phenol oxidase
C
R² = 0.49
P < 0.001
ln RR (Q10 of Vmax)
Elevation shift (m)


D
Acidobacteria
Actinobacteria
Archaea
Armatimonadetes
Bacteroidetes
Chloroflexi
FCPU426
Firmicutes
Gemmatimonadetes
Nitrospirae
Planctomycetes
Proteobacteria
Spirochaetes
Verrucomicrobia
Phyla
Warm adapted
Cold adapted
No response
Number of phylotypes

Supplementary information for

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

Andrew T. Nottingham[1, 2], Jeanette Whitaker[3], Nick J. Ostle[4], Richard D. Bardgett[5], Niall P. McNamara[3], Noah Fierer[6], Norma Salinas[7], Adan J. Q. Ccahuana[8], Benjamin L. Turner[2] & Patrick Meir[1,9]

Correspondence to: Andrew Nottingham, School of Geosciences, University of Edinburgh, Drummond Street, Edinburgh EH8 9XP, UK. email: andrew.nottingham@ed.ac.uk. Tel: +44 (0) 131 651 4314 ; Fax: +44 (0) 131 650 2524

This PDF file includes:

Appendix 1

Supplementary Materials and Methods

Soil analyses

Total C and N were determined for dried, ground soil samples using a TruSpec CN Elemental Determinator (LECO, USA). Total P was determined by ignition (550°C, 1 h) followed by extraction in 1 M H_2SO_4, with phosphate detection in neutralised extracts at 880 nm by automated molybdate colorimetry using a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO, USA). Mineral N and P availability were determined using ion exchange resins (Nottingham *et al.* 2015). Other organic and inorganic phosphorus fractions were determined using a modification of Hedley sequential extraction (in 1M NaOH, 1M HCl) (Hedley *et al.* 1982) and exchangeable cations were extracted in 0.1 M BaCl (Hendershot & Duquette 1986). Amorphous metal oxide concentrations (Al, Fe, Mn) were determined by extraction in ammonium oxalate (pH 3), with detection by ICP–OES (Courchesne and Turmel, 2008). Soil pH was determined in H_2O (soil solution, 1:2.5 w:v). Gravimetric moisture content, bulk density (dried for 24 h at 105 ^oC) and water holding capacity (the amount of water remaining in the soil after being saturated and left to drain for 12 h) were calculated for composite soil samples for each site. Soil microbial biomass C and N were measured by fumigation-extraction (Brookes *et al.* 1985; Vance *et al.* 1987), using ethanol-free chloroform as the fumigant followed by extraction with potassium sulphate (K_2SO_4). Soil microbial biomass C and N were measured by fumigation-extraction (Brookes *et al.* 1985; Vance *et al.* 1987), using ethanol-free chloroform as the fumigant followed by extraction with potassium sulphate (K_2SO_4). Extracts of fumigated and unfumigated soil were

47 analyzed for extractable organic C using a Shimadzu 5000A TOC analyzer (Shimadzu, Milton
48 Keynes, UK). The extracts were analysed for microbial biomass N by colorimetry on a
49 continuous flow stream autoanalyzer (Bran and Luebbe, Northampton, UK), following oxidation
50 with potassium persulphate ($K_2S_2O_8$), by mixing 1.5 ml filtrate with 4.5 ml of 0.165 M $K_2S_2O_8$
51 then autoclaving for 30 min at 121 ºC (Ross 1992). Microbial C and N were calculated as the
52 difference in the respective nutrient between fumigated and unfumigated extracts, and corrected
53 for unrecovered biomass using a *k* factor of 0.45 (Jenkinson *et al.* 2004). Microbial biomass P
54 was determined by hexanol fumigation and extraction with anion-exchange membranes (Kouno
55 *et al.* 1995). Phosphate was recovered from anion-exchange membranes by shaking for 1 h in 50
56 ml of 0.25 M H_2SO_4, with detection in the acid solution by automated molybdate colorimetry
57 using a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO, USA). Extractable P was determined
58 on unfumigated samples and microbial P was calculated as the difference between the fumigated
59 and unfumigated samples, with correction for unrecovered biomass using a k_p factor of 0.4
60 (Jenkinson *et al.* 2004). The C composition of soils was analysed by solid-state cross polarization
61 magic angle spinning (CP/MAS) ^{13}C NMR spectroscopy. The spectra were recorded at the
62 University of Durham, UK, using a Varian VNMRS spectrometer operating at 100.56 MHz with
63 a 4 mm rotor MAS probe. The spectra were plotted in the chemical shift range from 0 to 200
64 ppm, and the integrated total signal intensity apportioned among different compound classes in
65 the samples. The relative contributions of the different signal regions were corrected for spinning
66 sidebands at 111 ppm. Chemical shift regions for C were identified as follows: alkyl (0-46 ppm),
67 *O*-alkyl (46-92 ppm), di-*O*-alkyl (92-110 ppm), aryl (110-140 ppm), *O*-aryl (140-165 ppm) and
68 carbonyl (165-190 ppm) (Alarcon-Gutierrez *et al.* 2008).

Phospholipids were extracted from 1.5 g soil fresh weight. Identification of individual PLFAs was carried out using gas chromatography mass spectrometry (GC-MS) using an Agilent Technologies 5973 Mass Selective Detector coupled to an Agilent Technologies 6890 GC. Concentrations were calculated for all identifiable PLFAs via an internal standard (C19FAME, Sigma-Aldrich). Gram-positive (GP) bacteria were identified by the terminal and mid-chain branched fatty acids (15:0i,15:0a, 16:0i, 17:0i,17:0a) and cyclopropyl saturated and mono unsaturated fatty acids (16:1ω7, 7,cy-17:0,18:1ω7, 7,8cy-19:0) were considered indicative of gram-negative (GN) bacteria (Rinnan & Bååth 2009). The fatty acids 18:2ω6,9 and 18:1ω9 were considered to represent saprotrophic and ectomycorrhizal (SP/ECM) fungi (Kaiser *et al.* 2010). Total PLFA concentration (μg g^{-1} soil dwt) was calculated from all identified PLFAs (15:0,14:0,16:0, 16:1, 16:1ω5, 16:0,17:1ω8, 7Me-17:0, br17:0, br18:0, 18:1ω5, 18:0, 19:1; plus those listed above). The ratio of fungal to bacterial (F:B) PLFAs and GP to gram-negative bacteria (GP:GN) PLFAs were taken to represent the relative abundance of these microbial functional groups.

Calculations

Temperature response of soil carbon and other soil properties. We evaluated the effect of translocation on soil properties across all soil types using 2-way ANOVA with 'origin site' and 'destination' and their interaction as factors. We used 1-way ANOVA to determine the effect of translocation on each specific soil property for specific soil types, with significant pairwise differences determined by Tukey HD tests (data log-transformed; significant at $P < 0.05$). To determine the magnitude and direction of the translocation effect on soil properties, we determined relative response ratios (RR) for each soil property. The relationship between the RR

for each metric and elevation-shift treatment was determined by using linear regression between RR(metric) and translocation distance as a continuous variable. This approach allowed determination of the relative effect of translocation (warming or cooling) on each property independently to soil type. Therefore, we quantified the responses of individual soil properties to temperature manipulation, irrespective of soil type. We used 'elevation-shift' as our continuous variable and when reporting results because this was our imposed treatment, however elevation-shift is highly correlated to temperature-difference (R^2 =0.99) and we assume that temperature was the principle environmental change as a result of translocation.

Temperature response of soil microbial taxa. To examine the temperature response of specific taxa we grouped bacteria and fungi assigned to phylotypes (operational taxonomic units, OTUs, at 97% similarity). The temperature responses of phylotypes were defined by the differences in relative abundances between the translocated soil (at destination elevation) and the control soil (re-inserted at the site of origin). For each genus, we calculated relative response ratios (RR) of relative abundances. Because some phylotypes were not present in all soil treatments (origin x destination), we only retained phylotypes that yielded > 3 RR among treatments to enable determination of the regression of RR by elevational shift; resulting in 289 phylotypes in the final analysis. To determine the effect of elevation shift (temperature change) on relative abundance of phylotypes, regressions of RR against elevational shift were determined, where elevational shift was either upslope or downslope, from -2820 m (15^{o}C warming) to +2820 m (-15^{o}C cooling). Significant positive relationships indicated an increased relative abundance with increased elevation ('cold adapted'), while significant negative relationships indicated increased relative abundance with decreased elevation ('warm adapted') (where $p < 0.05$).

115 ***Temperature response of substrate use efficiency (SUE).*** We determined parameters
116 $CUE_{C:N}$, $CUE_{C:P}$, NUE and PUE for each experimental replicate (n = 3), and evaluated the effect
117 of soil origin (i.e. 'soil type response') and destination (i.e. 'temperature manipulation response')
118 for each parameter using 1-way ANOVA (Tukey HD differences among elevations). We further
119 investigated patterns by calculating the RR of SUE and determined regressions of RR against
120 elevation shift where positive relationships indicated a 'cooling' response and negative
121 relationships a 'warming' response (where $p < 0.05$).
122 ***Mixed effects models to show the effect of temperature perturbation (translocation) on***
123 ***soil carbon and on RQ_{10}.*** To determine which soil or environmental property best explained the
124 effect of translocation on i) soil carbon and ii) the temperature sensitivity of microbial respiration
125 (RQ_{10}), linear mixed effects models were used (R; lme4). Random effects of 'soil origin' and
126 'soil destination' were included. Fixed terms were 75 environmental (temperature, rainfall,
127 moisture) and soil properties including total soil nutrients, cations, microbial nutrients, activities
128 and Q_{10} responses of 7 extracellular enzymes, stoichiometric ratios of elements in soil microbes
129 and enzymes, PLFA and functional groups and their ratios (total PLFA, bacterial, fungal,
130 Bacterial:fungal ratios), NMR spectra components (alkyl, O-alkyl and alkyl:O-alkyl ratios). All
131 terms included in models are known to affect soil carbon cycling and therefore may determine
132 the overall effect of translocation on soil carbon and temperature sensitivity of RQ_{10}.
133 To normalise the translocation effect across all soil types, we used log-transformed
134 relative response ratios (RR) as model parameters for all variables. Therefore, we evaluated the
135 effect of soil and environmental properties (75 in total) on the relative response of total soil C
136 (RR of total C) and the relative response of the temperature sensitivity of microbial respiration
137 (RR of RQ_{10}). In all cases we began with full models and removed terms which improved the

model fit. Akaikes Information Criterion (AIC) was used to guide model selection, where a lower AIC represented a better model fit to the data for the given number of included parameters, with full and reduced models (fitted by maximum likelihood) compared using AIC likelihood ratio tests to test the statistical significance of individual fixed effects (Zuur *et al.* 2009). To avoid co-linearity, we used correlation matrices to identify pairs of correlated terms (greater than 0.6 or less than -0.6), and removed the least significant of the correlated pair from the model. Random effects of soil destination and soil origin were included, which provided a powerful indication of the resilience (soil origin significant) or plasticity (soil destination significant) of each soil property to the temperature perturbation.

The final parsimonious model was fitted by restricted maximum likelihood, validated for normal distribution of residuals and homogeneity of variance, and summarised by values for conditional R^2 (variance explained by fixed + random factors) and marginal R^2 (variance explained by fixed effects only) (Nakagawa & Schielzeth 2013). To assess the relative contribution of each fixed effect to the model, null models (excluding one fixed effect term in turn) were compared to the final full model, to estimate % variance explained by each fixed effect term separately (by subtraction of marginal R^2 for full model - null model). This approach allowed identification of the fixed effects which explained most of the observed variance in the data, and therefore the relative importance of each parameter for describing RR of total C and RR of RQ_{10}.

Translocation experiment rationale

Our estimate of tropical forest soil C loss under warming is based on the average response of soil -in the absence of plants- to temperature manipulation across a gradient of

161 lowland to montane tropical forest. Given the nature of a translocation experiment, our results
162 are based on predominant warming effects on soil from upper-elevations and cooling effects on
163 soil from lower-elevations. Despite this, we can infer the response of soil C cycling to warming
164 based on its response to cooling, if we assume no substrate limitation to growth under short-term
165 warming; a proven experimental approach (Karhu *et al.* 2014). This inference is possible because
166 we know that the temperature response of microbial growth and respiration in these lowland
167 forest soils follows the square root model across the range 0ºC - 35ºC (Bååth 2018; Nottingham
168 *et al.* 2019). However, our study does not address longer-term responses of lowland forests to
169 warming, including changes in nutrient cycling and associated plant-soil feedbacks (Melillo *et al.*
170 2011), and whether the physiological adaptations we observed (Figure 3) would eventually
171 ameliorate soil C losses, as shown in a 26 year warming experiment in temperate forest (Melillo
172 *et al.* 2017). These longer-term and plant-soil effects are important questions for future tropical
173 forest research and require *in situ* experimentation to address (Cavaleri *et al.* 2015).
174 The translocation method comes with a further caveat that it does not entirely restrict the
175 migration of microbial communities into the translocated soil. However, our methodology and
176 results together suggest that this was not a significant component of the change in microbial
177 community composition. First, the soil cores were as large as possible given the logistical
178 constraints to transporting the cores between the remote locations, 10 cm diameter x 50 cm depth
179 = 4000 cm^3. The soil cores were translocated with a soil collar and a funnel to adjust input of
180 rainfall (the collar extending 20 cm above the soil surface), which helped to isolate the
181 translocated soil from the surrounding soil. Thus, immigration of microbial communities would
182 have only been possible by airborne and precipitation routes. Second, studies showing large
183 spatial heterogeneity of soil microbial communities in soils (e.g. rhizosphere soils; or soils

associated with leaf-litters of different tree species; Fanin *et al.* (2014)), point to a greater role of environmental conditions, substrate availability and soil physico-chemical structure in shaping microbial communities, rather than immigration through dispersal (the latter would result in increased homogeneity of communities in soils, which is not observed). Third, the high consistency of changes in microbial communities in our study and in a study of global temperature gradients (Oliverio *et al.* 2017), suggests that the majority of changes in community composition we observed were the result of the temperature manipulation. Given these points, migration was likely to only have contributed a very small, if any significant, component of the change in microbial communities.

Method for determination of CUE and implications

Microbial carbon use efficiency (CUE), is a parameter that quantifies the proportion of carbon stabilized against carbon respired by the soil microbial biomass and this definition is represented in recent widely-cited models that have been used to predict the effect of climate change on the soil carbon cycle (Wieder *et al.* 2013; Hagerty *et al.* 2018). However, it can be difficult to compare CUE values across studies because CUE is an emergent property of multiple processes, including C-assimilation, respiration and resource allocation for enzyme synthesis; and there are several different methods used for its estimation. The first and most commonly used approach is the use of isotopic tracer methods. These methods determine the net response of ^{13}C-uptake, immobilization and respiratory release but results can be difficult to contextualize with the wider literature, and especially that of measurements made in natural ecosystems, because they are dependent on the specific substrate added and its concentration (Hagerty *et al.* 2018). To overcome the problem of estimating CUE under 'natural' substrate availability, ^{18}O

tracer methods have recently been developed to quantify C-cycling processes without requiring amendment of the soil with additional C-substrates (Spohn *et al.* 2016). An alternative approach is to determine the multiple emergent properties that are used in model parameterization of CUE, including microbial biomass, available substrate pools and enzyme activity. This approach has been recommended as being more useful to help test and develop CUE-climate models(Hagerty *et al.* 2018). Here we followed a similar approach, the stoichiometric method (Sinsabaugh *et al.* 2016), whereby CUE was determined relative to resource acquisition for N (microbial C and available C pools relative to available soil N, microbial N and C and N-degrading enzymes) and P. This stoichiometric method requires the assumptions that: (i) the analysis characterizes all enzymatic activity associated with N or P acquisition; and (ii) that the activities of these enzymes are proportional to microbial investment. The N and P- degrading enzymes we measured have been shown to be correlated with N and P- availability (Olander & Vitousek 2000). We suggest that, with careful consideration of these assumptions, this method of quantifying CUE provides an intuitive and informative metric which is of relevance to modelers and that can be understood in terms of its constituent parameters.

References in Supplementary Information

1.
Alarcon-Gutierrez, E., Floch, C., Ziarelli, F., Albrecht, R., Le Petit, J., Augur, C. *et al.* (2008). Characterization of a Mediterranean litter by (13)C CPMAS NMR: relationships between litter depth, enzyme activities and temperature. *Eur J Soil Sci*, 59, 486-495.
2.
Bååth, E. (2018). Temperature sensitivity of soil microbial activity modeled by the square root equation as a unifying model to differentiate between direct temperature effects and microbial community adaptation. *Global Change Biol*, 24, 2850-2861.
3.

Brookes, P.C., Landman, A., Pruden, G. & Jenkinson, D.S. (1985). Chloroform fumigation and the release of soil-nitrogen - a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol Biochem*, 17, 837-842.
4.
Cavaleri, M.A., Reed, S.C., Smith, W.K. & Wood, T.E. (2015). Urgent need for warming experiments in tropical forests. *Global Change Biol*, 21, 2111-2121.
5.
Fanin, N., Hattenschwiler, S. & Fromin, N. (2014). Litter fingerprint on microbial biomass, activity, and community structure in the underlying soil. *Plant Soil*, 379, 79-91.
6.
Hagerty, S.B., Allison, S.D. & Schimel, J.P. (2018). Evaluating soil microbial carbon use efficiency explicitly as a function of cellular processes: implications for measurements and models. *Biogeochemistry*, 140, 269-283.
7.
Hedley, M.J., Stewart, J.W.B. & Chauhan, B.S. (1982). Changes in Inorganic and Organic Soil-Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations. *Soil Sci Soc Am J*, 46, 970-976.
8.
Hendershot, W.H. & Duquette, M. (1986). A Simple Barium-Chloride Method for Determining Cation-Exchange Capacity and Exchangeable Cations. *Soil Sci Soc Am J*, 50, 605-608.
9.
Jenkinson, D.S., Brookes, P.C. & Powlson, D.S. (2004). Measuring soil microbial biomass. *Soil Biol Biochem*, 36, 5-7.
10.
Kaiser, C., Koranda, M., Kitzler, B., Fuchslueger, L., Schnecker, J., Schweiger, P. *et al.* (2010). Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. *The New phytologist*, 187, 843-858.
11.
Karhu, K., Auffret, M.D., Dungait, J.A.J., Hopkins, D.W., Prosser, J.I., Singh, B.K. *et al.* (2014). Temperature sensitivity of soil respiration rates enhanced by microbial community response. *Nature*, 513, 81-84.
12.
Kouno, K., Tuchiya, Y. & Ando, T. (1995). Measurement of soil microbial biomass phosphorus by an anion-exchange membrane method. *Soil Biol Biochem*, 27, 1353-1357.
13.
Melillo, J.M., Butler, S., Johnson, J., Mohan, J., Steudler, P., Lux, H. *et al.* (2011). Soil warming, carbon-nitrogen interactions, and forest carbon budgets. *P Natl Acad Sci USA*, 108, 9508-9512.
14.
Melillo, J.M., Frey, S.D., DeAngelis, K.M., Werner, W.J., Bernard, M.J., Bowles, F.P. *et al.* (2017). Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. *Science*, 358, 101-104.
15.
Nakagawa, S. & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4, 133-142.

16.
Nottingham, A.T., Bååth, E., Reischke, S., Salinas, N. & Meir, P. (2019). Adaptation of soil microbial growth to temperature: using a tropical elevation gradient to predict future changes. *Global Change Biol*.
17.
Nottingham, A.T., Turner, B.L., Whitaker, J., Ostle, N., McNamara, N.P., Bardgett, R.D. *et al.* (2015). Soil microbial nutrient constraints along a tropical forest elevation gradient: a belowground test of a biogeochemical paradigm. *Biogeosciences*, 12, 6489-6523.
18.
Olander, L.P. & Vitousek, P.M. (2000). Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry*, 49, 175-190.
19.
Oliverio, A.M., Bradford, M.A. & Fierer, N. (2017). Identifying the microbial taxa that consistently respond to soil warming across time and space. *Global Change Biol*, 23, 2117-2129.
20.
Rinnan, R. & Bååth, E. (2009). Differential Utilization of Carbon Substrates by Bacteria and Fungi in Tundra Soil. *Appl Environ Microb*, 75, 3611-3620.
21.
Ross, D.J. (1992). Influence of sieve mesh size on estimates of microbial carbon and nitrogen by fumigation extraction procedures in soils under pasture. *Soil Biol Biochem*, 24, 343-350.
22.
Sinsabaugh, R.L., Turner, B.L., Talbot, J.M., Waring, B.G., Powers, J.S., Kuske, C.R. *et al.* (2016). Stoichiometry of microbial carbon use efficiency in soils. *Ecological Monographs*, 86, 172-189.
23.
Spohn, M., Potsch, E.M., Eichorst, S.A., Woebken, D., Wanek, W. & Richter, A. (2016). Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. *Soil Biol Biochem*, 97, 168-175.
24.
Vance, E.D., Brookes, P.C. & Jenkinson, D.S. (1987). An extraction method for measuring soil microbial biomass-C. *Soil Biol Biochem*, 19, 703-707.
25.
Wieder, W.R., Bonan, G.B. & Allison, S.D. (2013). Global soil carbon projections are improved by modelling microbial processes. *Nat Clim Change*, 3, 909-912.
26.
Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. (2009). *Mixed effects models and extensions in ecology with R*. Springer Science & Business Media, New York, USA.

Appendix 2: Figures S1 to S6

Figure S1. Sites and reciprocal experimental design, The Kosñipata valley, Peru. Soil cores were reciprocally translocated among 4 sites with a 2820 m and 15ºC mean annual temperature difference, as mean annual temperature (MAT) is determined by elevation (R^2= 0.99). The reciprocal design therefore resulted in an elevation shift treatment of 2820, 2030, 1530, 1290, 790, 500, 0, -500, -790, -1290, -1530, -2030, -2820 m, which was equivalent to a temperature treatment of -15.3, -9.6, -9, -6.3, -5.7, -3.3, 0, 3.3, 5.7, 6.3, 9, 9.6, 15.3 ºC. We used this single axis of 'elevation shift' to determine the relative responses of soil and microbial properties to translocation; thereby identifying the common temperature responses of properties in soils from a different origin.

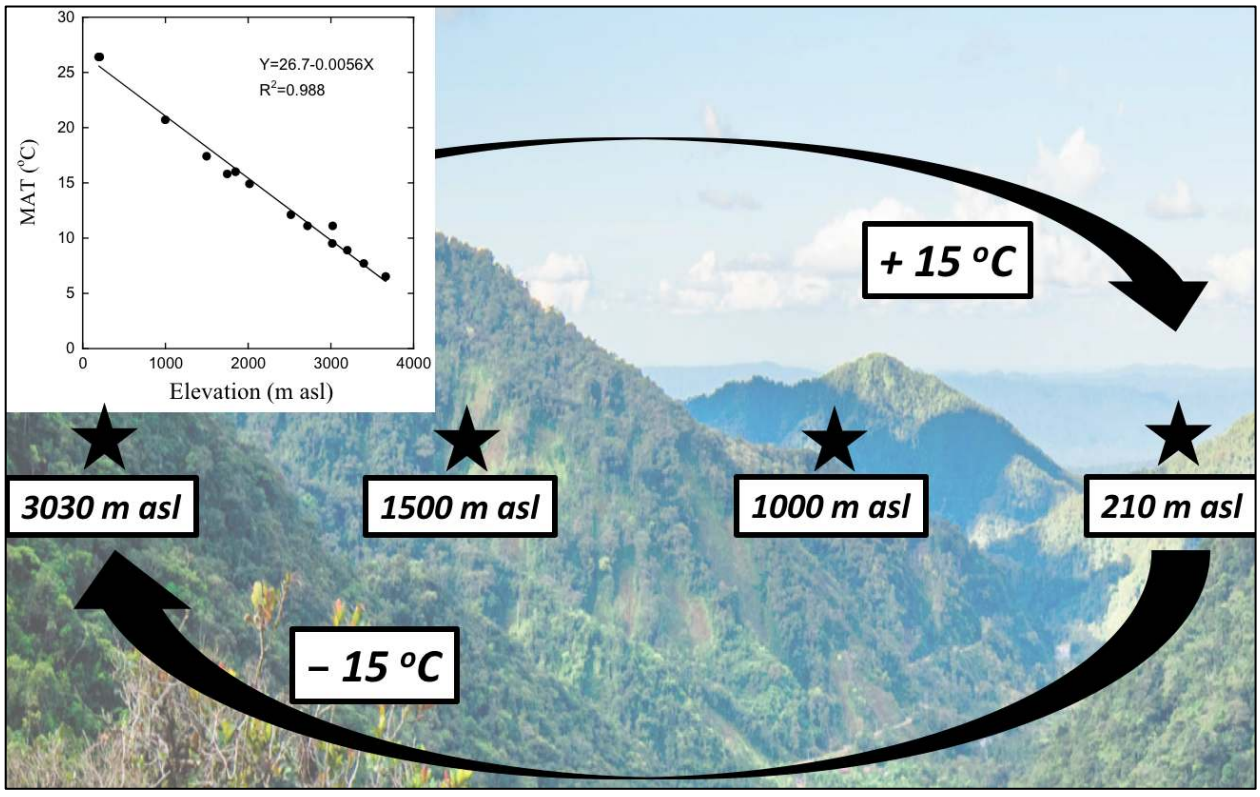


Figure S2. The log response ratios (RR) of 5 'cold adapted' and 5 'warm adapted' microbial phylotypes against elevation shift (warming/cooling) following 5 years of translocation. Here we show a subset of relationships for 10 microbial phylotypes, of the 48 significant relationships identified in Figure S3. The total abundance of identified phylotypes pooled by phyla are shown in Figure 4, with phylotypes grouped by 'cold adapted', 'warm adapted' and 'no response'.

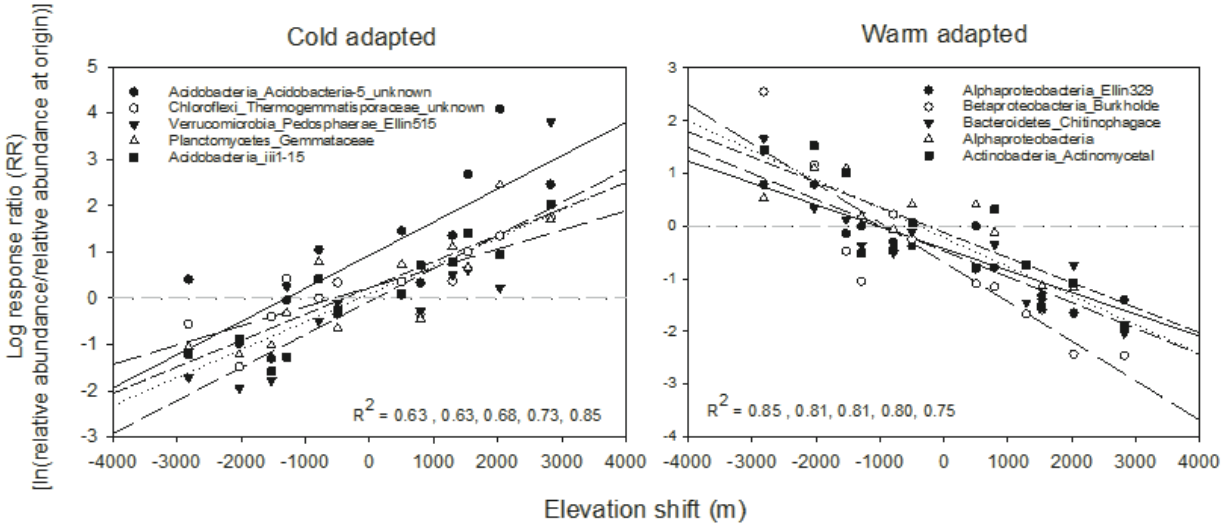


Figure S3. The effects of elevation shift (warming/cooling) on the log response ratios (RR) of the relative abundance of microbial phylotypes following 5 years of translocation. RR values were calculated [ln (relative abundance of phylotype at origin/ relative abundance of phylotype at destination)] and linear regressions between RR value and elevation shift (m) were determined (e.g. Fig. S2). A negative relationship ('warm adapted phylotypes') represents an increase in RR with warming/decrease in RR with cooling, and a positive relationship ('cold adapted phylotypes') represents a decrease in RR with warming/increase in RR with cooling. Of 289 detected phylotypes, we identified 30 warm-adapted (26 bacterial, 2 archaea and 2 fungal), 18 cold adapted phylotypes (16 bacteria, 2 fungal) and 241 that did not respond to translocation.

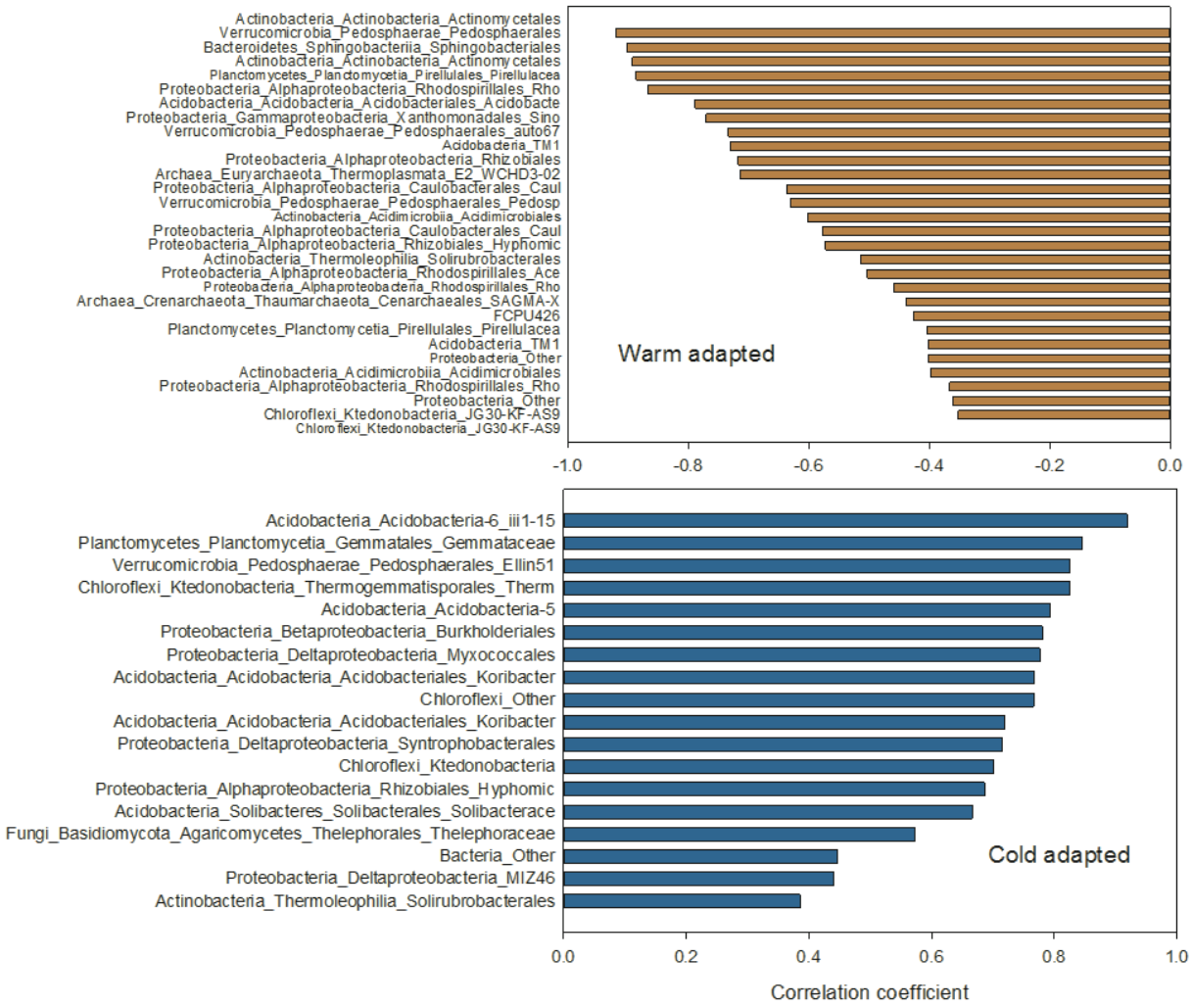


Figure S4. The log response of enzymatic Q_{10} of V_{max} to warming and cooling (5 years of reciprocal translocation). We calculated the Q_{10} of V_{max} by determining V_{max} at 2ºC, 10ºC, 20ºC, 30ºC, 40ºC and fitting a Q_{10} function (equations 1-2). The temperature response was estimated using linear regression of relative response ratio [ln (Q_{10} of V_{max} at destination/ Q_{10} of V_{max} at origin)] against the elevation shift ($p < 0.05$).

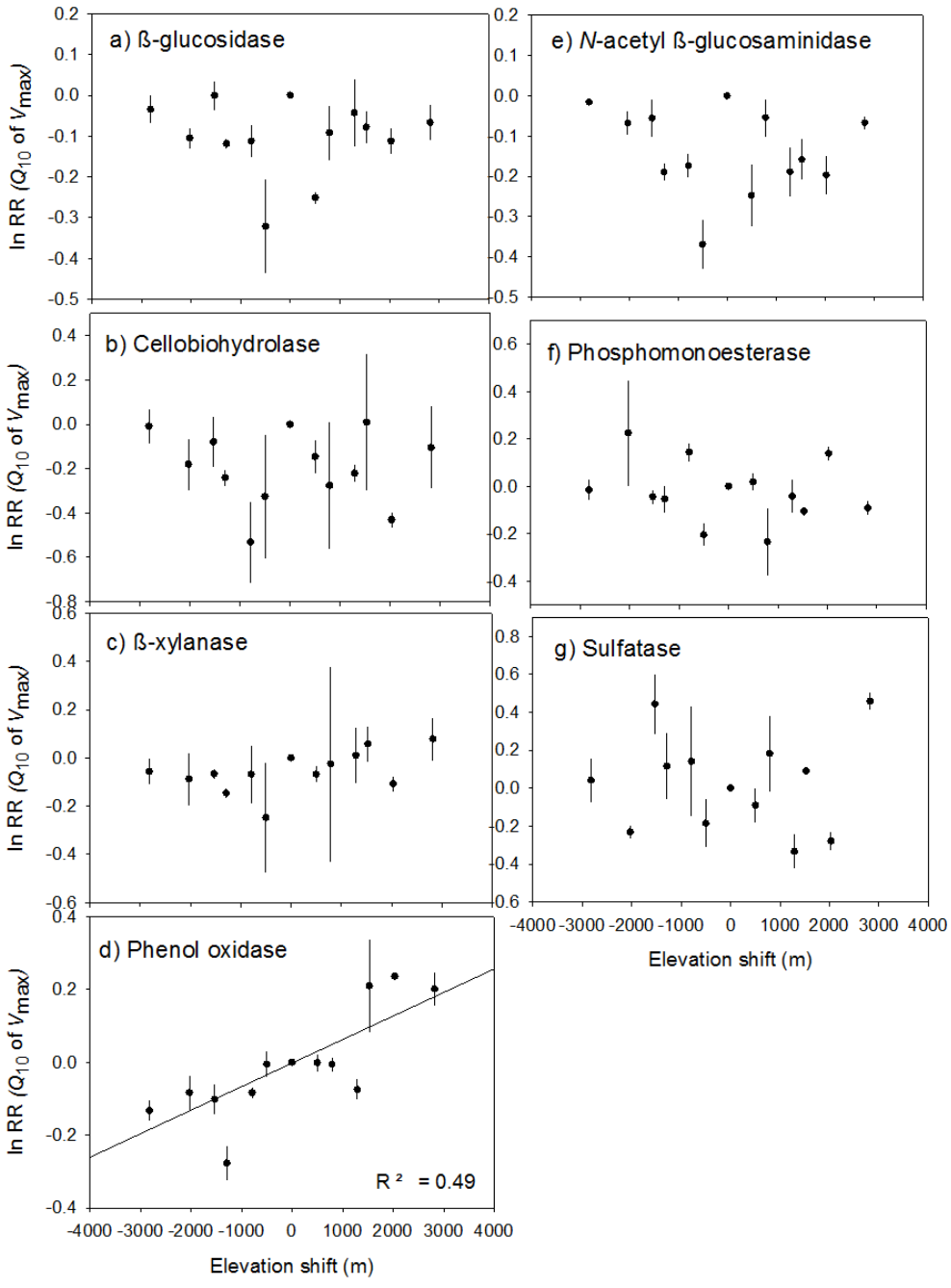


Figure S5. The average Q_{10} across all soils for instantaneous microbial respiration (RQ_{10}) and of V_{max} for seven different enzymes. Significant differences between enzyme classes are shown by different lower-case letters (1-way ANOVA; Tukey HD; $P < 0.05$)

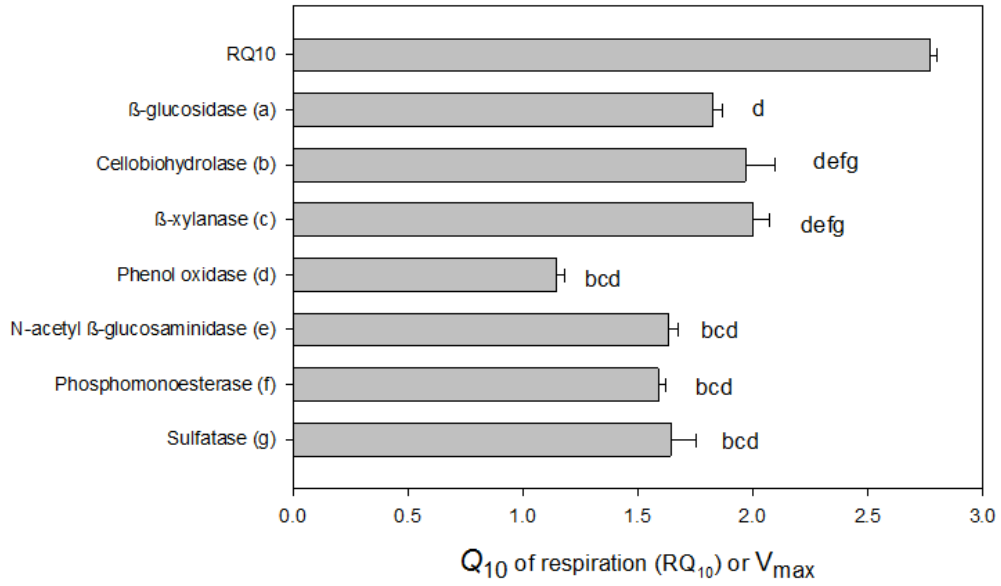
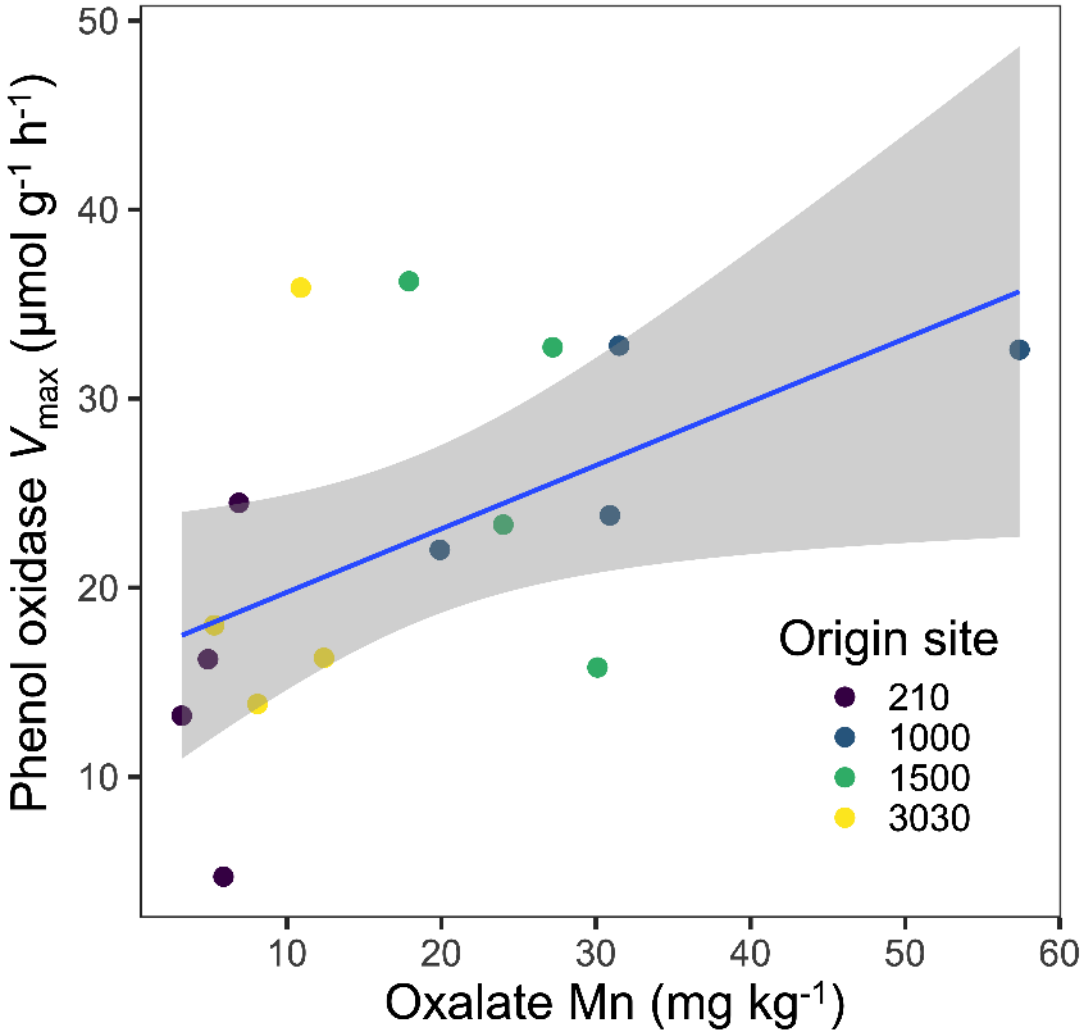


Figure S6. The relationship between phenol oxidase activity (V_{max}) and amorphous Manganese (Mn) oxidase concentration. The points are grouped by site of origin (m a.s.l). The relationship was determined by linear regression, $R^2 = 0.28$, $F=6.8_{14}$, $P = 0.02$. There was no relationship between the temperature sensitivity of phenol oxidase activity (Q_{10} of V_{max}) and amorphous Mn oxidase ($R^2 = 0.05$, df = 14, F = 0.25, $P = 0.62$).



Appendix 3: Tables S1 to S3

Table S1: Soil properties for soil cores following five years of reciprocal translocation. Soil cores were reciprocally translocated among sites at 210, 1000, 1500, 3030 m elevation, which represented temperature manipulations ('T difference') of -15.3, -9.6, -6.3, -3.3, 0, 3.3, 6.3, 9.6 and 15.3ºC. Enzyme activities are reported for standard assays performed at 30ºC, enzymatic Q_{10} values were calculated by the determining enzyme activities at temperatures 0, 10, 22, 30 and 40ºC and fitting a Q_{10} function (equations 1-2). All values are averages of three experimental replicates (n = 3), with 1 SE in parentheses.

Sample code	AA1	AB1	AC1	AD1	BA1	BB1	BC1	BD1	CA1	CB1	CC1	CD1	DA1	DB1	DC1	DD1
Origin elevation (m asl)	3030	1500	1000	210	3030	1500	1000	210	3030	1500	1000	210	3030	1500	1000	210
Destination elevation (m asl)	3030	3030	3030	3030	1500	1500	1500	1500	1000	1000	1000	1000	210	210	210	210
Translocation (m)	0	1530	2030	2820	-1530	0	500	1290	-2030	-500	0	790	-2820	-1290	-790	0
T origin (ºC)	11.1	17.4	20.7	26.4	11.1	17.4	20.7	26.4	11.1	17.4	20.7	26.4	11.1	17.4	20.7	26.4
T destination (ºC)	11.1	11.1	11.1	11.1	17.4	17.4	17.4	17.4	20.7	20.7	20.7	20.7	26.4	26.4	26.4	26.4
T difference (ºC)	0	-6.3	-9.6	-15.3	6.3	0	-3.3	-9	9.6	3.3	0	-5.7	15.3	9	5.7	0
pH	3.3	3.5	3.7	3.6	3.4	3.7	3.8	3.6	3.4	3.7	3.8	3.7	3.2	3.5	3.6	3.5
	(0.01)	(0.04)	(0.04)	(0.04)	(0.10)	(0.11)	(0.03)	(0.09)	(0.02)	(0.04)	(0.10)	(0.14)	(0.02)	(0.02)	(0.00)	(0.02)
Total C	18.7	14.8	6.9	1.4	13.7	9.0	6.4	1.1	15.2	8.7	4.2	1.2	15.1	10.4	5.3	1.4
(%)	(2.0)	(4.1)	(1.0)	(0.1)	(1.0)	(1.1)	(1.9)	(0.1)	(1.3)	(0.4)	(0.4)	(0.0)	(1.5)	(1.0)	(0.6)	(0.2)
Total N	1.05	1.01	0.62	0.24	0.88	0.67	0.62	0.26	0.94	0.60	0.47	0.22	0.97	0.79	0.57	0.26
(%)	(0.07)	(0.25)	(0.04)	(0.01)	(0.05)	(0.09)	(0.10)	(0.01)	(0.06)	(0.01)	(0.03)	(0.01)	(0.06)	(0.08)	(0.05)	(0.03)
Total P	1342	1353	983	253	1259	1254	950	233	1325	1354	861	243	1190	1280	623	258
(mg kg-1)	(11)	(14)	(14)	(5)	(47)	(30)	(96)	(9)	(68)	(24)	(33)	(1)	(72)	(51)	(189)	(4)
Total CN ratio	18	14	11	6	16	13	10	4	16	15	9	6	16	13	9	5
	(0.7)	(0.5)	(0.9)	(0.4)	(0.4)	(0.3)	(1.2)	(0.2)	(0.5)	(0.5)	(0.4)	(0.1)	(0.6)	(0.3)	(0.4)	(0.3)
Total CP ratio	139	109	70	57	108	71	65	49	115	65	48	51	127	81	125	53
	(16.3)	(29.8)	(10.2)	(2.6)	(4.3)	(7.7)	(13.0)	(4.2)	(9.0)	(4.1)	(3.5)	(1.8)	(10.8)	(8.4)	(64.8)	(8.3)
Total NP ratio	8	7	6	9	7	5	6	11	7	5	5	9	8	6	13	10
	(0.6)	(1.8)	(0.4)	(0.4)	(0.2)	(0.7)	(0.5)	(0.8)	(0.5)	(0.1)	(0.2)	(0.2)	(0.5)	(0.6)	(6.7)	(1.0)
resin-extractable P	65.71	5.90	2.81	2.05	70.97	2.31	4.36	1.78	18.78	3.96	3.21	1.67	12.07	11.37	10.50	4.10
(mg kg^{-1})	(11.48)	(2.29)	(0.87)	(0.80)	(2.86)	(1.16)	(0.72)	(0.49)	(1.96)	(1.29)	(1.21)	(0.76)	(1.00)	(2.97)	(0.76)	(1.29)
NaOH - Pi	228	153	151	43	229	163	186	43	NA	NA	NA	NA	59	190	122	NA
(mg kg^{-1})	(39)	(23)	(23)	(3)	(17)	(14)	(26)	(10)	NA	NA	NA	NA	(9)	(3)	(1)	NA
NaOH – Po	856	531	289	78	890	447	404	66	NA	NA	NA	NA	807	601	261	NA
(mg kg^{-1})	(14)	(30)	(27)	(3)	(19)	(34)	(81)	(6)	NA	NA	NA	NA	(64)	(10)	(22)	NA
Al	27.4	15.7	7.1	6.2	37.3	40.7	5.0	6.5	16.4	11.4	3.7	11.0	22.2	26.4	5.8	12.2
(mg kg^{-1})	(0.7)	(1.1)	(0.8)	(0.4)	(4.5)	(7.4)	(0.1)	(0.3)	(0.6)	(1.6)	(0.3)	(0.8)	(2.0)	(0.4)	(0.9)	(1.3)
Ca	0.50	1.47	0.73	0.24	1.96	4.44	1.01	0.58	0.70	0.96	0.51	0.92	0.52	0.91	0.39	0.58
(mg kg^{-1})	(0.02)	(0.10)	(0.12)	(0.03)	(0.27)	(0.78)	(0.02)	(0.03)	(0.01)	(0.07)	(0.04)	(0.01)	(0.03)	(0.01)	(0.01)	(0.05)
Fe	2.49	1.19	0.54	0.31	3.68	1.31	0.33	0.32	2.03	0.49	0.26	0.76	1.18	1.32	0.27	0.44
(mg kg^{-1})	(0.06)	(0.11)	(0.07)	(0.02)	(0.44)	(0.36)	(0.04)	(0.02)	(0.07)	(0.11)	(0.04)	(0.12)	(0.10)	(0.22)	(0.03)	(0.14)
K	0.70	0.41	0.30	0.17	0.75	1.31	0.24	0.21	0.26	0.39	0.21	0.30	0.32	0.54	0.28	0.22
(mg kg^{-1})	(0.01)	(0.03)	(0.05)	(0.02)	(0.11)	(0.24)	(0.00)	(0.00)	(0.01)	(0.05)	(0.02)	(0.02)	(0.04)	(0.02)	(0.05)	(0.03)
Mg	0.58	0.75	0.16	0.09	0.75	0.79	0.21	0.03	0.43	0.39	0.05	0.08	0.22	0.21	0.07	0.17
(mg kg^{-1})	(0.03)	(0.07)	(0.08)	(0.02)	(0.11)	(0.11)	(0.01)	(0.01)	(0.01)	(0.07)	(0.02)	(0.00)	(0.05)	(0.00)	(0.04)	(0.03)
Mn	0.06	0.16	0.10	0.03	0.15	0.19	0.08	0.05	0.10	0.15	0.02	0.08	0.08	0.05	0.04	0.00

(mg kg^{-1})	(0.01)	(0.01)	(0.02)	(0.00)	(0.02)	(0.04)	(0.00)	(0.00)	(0.00)	(0.02)	(0.00)	(0.01)	(0.01)	(0.00)	(0.01)	(0.00)
Na	0.20	0.46	0.15	0.11	0.25	0.43	0.17	0.39	0.13	0.13	0.42	0.32	0.45	0.22	0.10	0.18
(mg kg^{-1})	(0.01)	(0.33)	(0.03)	(0.02)	(0.04)	(0.05)	(0.03)	(0.22)	(0.01)	(0.04)	(0.29)	(0.05)	(0.35)	(0.01)	(0.00)	(0.05)
ECEC	31.9	20.1	9.1	7.2	44.8	49.2	7.1	8.1	20.1	13.9	5.2	13.5	25.0	29.7	7.0	13.8
($cmol_c$ kg^{-1})	(0.56)	(1.29)	(1.16)	(0.49)	(5.48)	(8.94)	(0.15)	(0.26)	(0.71)	(1.86)	(0.21)	(0.87)	(2.43)	(0.24)	(1.10)	(1.53)
Base Saturation	6.33	15.33	14.33	8.33	8.33	14.33	23.00	14.67	7.67	13.33	23.00	12.00	6.00	6.00	12.00	8.67
(%)	(0.33)	(1.86)	(1.33)	(0.33)	(0.33)	(0.33)	(0.58)	(2.19)	(0.33)	(0.88)	(4.51)	(1.00)	(1.53)	(0.00)	(0.58)	(0.33)
microbial P	68.6	84.4	59.5	21.7	46.2	38.1	47.2	20.4	7.2	14.7	31.8	11.3	55.9	38.7	90.7	74.6
(mg P kg^{-1})	(5.7)	(44.3)	(9.9)	(8.4)	(13.6)	(10.1)	(11.1)	(7.3)	(2.5)	(4.8)	(14.0)	(5.4)	(11.9)	(19.1)	(29.2)	(20.4)
microbial C	93.0	369.3	195.0	51.9	261.5	218.8	315.4	65.5	104.1	253.7	450.7	103.5	544.4	227.4	211.8	171.1
(mg C kg^{-1})	(27.3)	(79.3)	(15.3)	(14.2)	(70.8)	(79.7)	(91.2)	(32.7)	(36.5)	(15.8)	(94.7)	(7.4)	(15.2)	(17.7)	(15.2)	(38.3)
microbial N	106.6	130.5	130.2	33.0	124.2	109.9	110.8	31.6	73.6	64.2	321.2	11.9	47.9	19.7	29.2	17.4
(mg N kg^{-1})	(22.4)	(19.2)	(2.4)	(8.0)	(13.7)	(5.3)	(20.1)	(9.0)	(47.0)	(32.7)	(200.1)	(8.3)	(22.0)	(13.5)	(14.3)	(9.3)
microbial CN ratio	1.0	2.8	1.5	1.7	2.2	2.0	3.3	2.0	NA	6.7	2.4	23.4	32.3	NA	54.9	13.7
	(0.4)	(0.4)	(0.1)	(0.5)	(0.8)	(0.8)	(1.3)	(0.5)	NA	(2.9)	(0.8)	(12.7)	(24.2)	NA	(49.7)	(4.5)
microbial CP ratio	1.4	6.0	3.5	2.6	6.6	NA	8.6	4.0	15.1	20.2	19.4	18.7	10.6	7.7	NA	2.5
	(0.5)	(1.7)	(0.7)	(0.4)	(1.9)	NA	(4.1)	(1.6)	(5.4)	(4.7)	(7.5)	(11.4)	(2.2)	(3.9)	NA	(0.7)
microbial NP ratio	1.6	2.3	2.3	2.0	3.6	NA	2.9	2.4	12.2	4.0	15.1	1.6	0.8	0.7	NA	0.2
	(0.4)	(0.7)	(0.3)	(0.8)	(1.7)	NA	(1.2)	(1.2)	(11.5)	(1.1)	(11.2)	(0.8)	(0.3)	(0.6)	NA	(0.1)
Total PLFA	50.2	53.0	31.0	9.1	49.3	45.2	38.5	6.9	29.6	26.1	26.4	7.0	41.1	39.5	30.5	10.7
(μg g^{-1})	(8.1)	(20.1)	(4.6)	(0.9)	(3.5)	(5.7)	(13.9)	(0.2)	(5.6)	(4.1)	(4.2)	(0.9)	(1.7)	(6.4)	(4.9)	(0.4)
Bacterial PLFA	4.7	2.4	2.3	0.5	4.0	2.4	2.4	0.3	2.1	1.3	1.8	0.3	3.2	2.7	1.8	0.5
(μg g^{-1})	(1.4)	(0.6)	(0.3)	(0.1)	(0.5)	(0.4)	(1.0)	(0.1)	(0.4)	(0.3)	(0.1)	(0.1)	(0.2)	(0.7)	(0.4)	(0.1)
Fungal PLFA	24.9	24.5	15.9	5.1	26.7	24.8	20.5	4.0	15.1	14.7	13.7	3.9	21.9	21.6	16.9	6.3
(μg g^{-1})	(2.8)	(7.2)	(2.4)	(0.3)	(1.0)	(3.0)	(7.6)	(0.1)	(2.6)	(2.0)	(2.8)	(0.6)	(1.1)	(2.9)	(3.0)	(0.2)
Fungal:Bacterial ratio	0.18	0.11	0.15	0.10	0.15	0.10	0.11	0.07	0.14	0.08	0.15	0.07	0.15	0.12	0.11	0.08
	(0.03)	(0.04)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.04)	(0.01)	(0.01)	(0.02)	(0.03)	(0.01)
Gram-positive bacteria	12.6	14.6	7.4	2.6	10.8	11.5	9.9	2.0	7.6	7.0	7.1	2.1	11.7	11.7	9.5	3.7
(μg g^{-1})	(2.2)	(6.8)	(1.2)	(0.3)	(0.5)	(1.7)	(3.3)	(0.0)	(1.6)	(0.8)	(1.5)	(0.3)	(0.6)	(1.5)	(1.7)	(0.2)
Gram-negative bacteria	11.8	9.2	8.3	2.5	15.5	12.9	10.3	1.9	7.1	7.5	6.3	1.8	9.8	9.2	7.0	2.5
(μg g^{-1})	(0.5)	(0.3)	(1.1)	(0.1)	(0.4)	(1.3)	(4.2)	(0.1)	(0.9)	(1.1)	(1.3)	(0.2)	(0.7)	(1.3)	(1.2)	(0.0)
Gram-positive:Gram-negative ratio	1.06	1.55	0.88	1.06	0.70	0.89	1.01	1.06	1.05	0.93	1.13	1.19	1.20	1.27	1.38	1.49
	(0.15)	(0.69)	(0.04)	(0.13)	(0.02)	(0.06)	(0.07)	(0.10)	(0.10)	(0.04)	(0.10)	(0.04)	(0.07)	(0.07)	(0.13)	(0.10)
RQ_{10}	2.94	2.84	2.64	2.68	2.91	2.66	2.70	2.93	2.65	2.78	2.86	2.76	2.88	2.77	2.60	2.71
	(0.11)	(0.03)	(0.05)	(0.12)	(0.04)	(0.09)	(0.05)	(0.08)	(0.10)	(0.08)	(0.01)	(0.14)	(0.00)	(0.12)	(0.08)	(0.06)
Phosphomonoesterase	15.15	14.99	13.09	9.02	13.27	25.71	6.36	2.40	9.96	15.12	16.35	10.77	9.46	13.36	17.31	8.42
(nmol MU g^{-1} min^{-1})	(2.82)	(3.82)	(0.67)	(2.04)	(2.48)	(0.59)	(1.59)	(0.32)	(2.22)	(4.05)	(5.19)	(2.24)	(2.99)	(3.69)	(2.73)	(0.53)
N-acetyl β-glucosaminidase	2.57	2.19	1.08	0.81	1.44	5.05	0.68	0.20	2.58	0.51	1.52	1.29	1.72	1.56	3.49	1.64
(nmol MU g^{-1} min^{-1})	(0.18)	(1.39)	(0.15)	(0.33)	(0.41)	(0.03)	(0.25)	(0.11)	(0.65)	(0.12)	(0.31)	(0.17)	(0.51)	(0.85)	(0.15)	(0.36)
Sulfatase	0.64	0.38	0.60	0.19	0.11	0.15	0.27	0.05	0.53	0.36	0.57	0.15	0.19	0.08	0.25	0.06
(nmol MU g^{-1} min^{-1})	(0.00)	(0.12)	(0.07)	(0.11)	(0.04)	(0.05)	(0.05)	(0.02)	(0.10)	(0.18)	(0.06)	(0.12)	(0.08)	(0.03)	(0.09)	(0.03)
β-glucosidase	4.37	6.08	4.13	1.56	4.25	9.21	3.47	0.26	3.28	1.44	3.24	1.14	2.68	1.80	5.19	1.67
(nmol MU g^{-1} min^{-1})	(0.98)	(4.26)	(1.04)	(0.47)	(1.57)	(0.09)	(0.66)	(0.02)	(0.46)	(0.59)	(1.22)	(0.11)	(0.46)	(0.67)	(0.58)	(0.40)
Cellobiohydrolase	0.78	0.43	1.25	0.05	0.35	0.29	0.15	0.16	0.44	0.37	0.60	0.13	0.75	0.64	0.68	0.13
(nmol MU g^{-1} min^{-1})	(0.17)	(0.19)	(0.56)	(0.03)	(0.05)	(0.12)	(0.07)	(0.12)	(0.11)	(0.11)	(0.22)	(0.08)	(0.14)	(0.05)	(0.26)	(0.07)
β-xylanase	2.59	1.25	1.54	0.37	1.89	0.50	0.45	0.21	2.57	0.90	0.78	0.34	3.44	2.88	1.46	0.65
(nmol MU g^{-1} min^{-1})	(0.40)	(0.54)	(0.44)	(0.12)	(0.20)	(0.06)	(0.22)	(0.05)	(0.63)	(0.35)	(0.21)	(0.22)	(0.35)	(0.43)	(0.53)	(0.33)
Phenol oxidase	18.0	23.3	22.0	16.2	13.8	36.2	32.8	4.7	16.3	32.7	23.8	24.5	35.9	15.8	32.6	13.2
(μmol g^{-1} h^{-1}))	(2.9)	(1.5)	(2.5)	(1.4)	(0.6)	(2.9)	(4.3)	(0.8)	(3.2)	(3.0)	(3.1)	(1.3)	(2.9)	(2.8)	(1.3)	(1.3)
Phosphomonoesterase Q_{10}	1.75	1.55	1.62	1.56	1.65	1.72	1.44	1.65	1.65	1.40	1.41	1.38	1.68	1.63	1.63	1.71
	(0.06)	(0.03)	(0.05)	(0.05)	(0.02)	(0.03)	(0.05)	(0.12)	(0.04)	(0.07)	(0.21)	(0.21)	(0.05)	(0.10)	(0.06)	(0.09)

N-acetyl β-glucosaminidase Q_{10}	1.66	1.54	1.60	1.72	1.70	1.80	1.53	1.53	1.49	1.25	1.94	1.74	1.65	1.49	1.63	1.84
	(0.04)	(0.08)	(0.08)	(0.03)	(0.11)	(0.03)	(0.11)	(0.09)	(0.05)	(0.08)	(0.09)	(0.08)	(0.02)	(0.03)	(0.05)	(0.10)
Sulfatase Q_{10}	1.43	1.58	1.65	1.97	1.91	1.44	2.01	0.90	1.17	1.22	2.18	1.56	1.67	1.66	2.70	1.24
	(0.04)	(0.02)	(0.08)	(0.09)	(0.37)	(0.05)	(0.18)	(0.08)	(0.04)	(0.15)	(0.04)	(0.32)	(0.30)	(0.28)	(0.68)	(0.37)
β-glucosidase Q_{10}	1.92	1.79	1.86	1.85	2.05	1.94	1.62	1.90	1.67	1.42	2.08	1.81	1.76	1.72	1.86	1.97
	(0.08)	(0.07)	(0.06)	(0.08)	(0.08)	(0.02)	(0.02)	(0.15)	(0.03)	(0.15)	(0.06)	(0.12)	(0.05)	(0.02)	(0.08)	(0.06)
Cellobiohydrolase Q_{10}	2.02	2.50	2.06	1.54	2.11	2.28	2.75	1.33	1.48	1.77	3.16	1.35	1.78	1.79	1.92	1.65
	(0.19)	(0.64)	(0.07)	(0.26)	(0.27)	(0.43)	(0.20)	(0.05)	(0.25)	(0.47)	(0.61)	(0.35)	(0.10)	(0.07)	(0.32)	(0.67)
β-xylanase Q_{10}	1.83	2.15	2.00	2.37	1.78	2.02	2.08	2.23	1.59	1.66	2.23	2.45	1.60	1.75	2.11	2.18
	(0.02)	(0.15)	(0.07)	(0.22)	(0.03)	(0.23)	(0.07)	(0.24)	(0.31)	(0.33)	(0.28)	(0.83)	(0.05)	(0.03)	(0.27)	(0.11)
Phenol oxidase Q_{10}	1.21	1.47	1.40	1.37	1.04	1.17	1.10	1.04	1.07	1.17	1.10	1.11	1.03	0.89	1.01	1.12
	(0.07)	(0.20)	(0.01)	(0.06)	(0.07)	(0.03)	(0.03)	(0.03)	(0.10)	(0.04)	(0.02)	(0.02)	(0.03)	(0.04)	(0.02)	(0.06)
Carbonyl	18.66	13.35	6.18	1.15	10.95	8.99	6.41	0.92	12.14	8.72	4.57	1.24	12.09	9.34	5.33	1.36
(g C kg^{-1})	(2.03)	(3.70)	(0.87)	(0.07)	(0.78)	(1.08)	(1.93)	(0.05)	(1.03)	(0.40)	(0.49)	(0.05)	(1.20)	(0.88)	(0.60)	(0.22)
O-Aryl	13.06	13.35	6.18	1.01	8.21	7.19	5.13	0.57	9.11	7.84	3.33	0.99	10.58	8.30	4.79	0.81
(g C kg^{-1})	(1.42)	(3.70)	(0.87)	(0.06)	(0.59)	(0.87)	(1.55)	(0.03)	(0.77)	(0.36)	(0.36)	(0.04)	(1.05)	(0.78)	(0.54)	(0.13)
Aryl	22.39	22.25	8.93	1.58	13.69	12.58	7.70	1.15	16.70	13.07	4.99	1.61	16.63	13.49	6.92	1.49
(g C kg^{-1})	(2.44)	(6.16)	(1.25)	(0.09)	(0.98)	(1.52)	(2.32)	(0.06)	(1.42)	(0.60)	(0.54)	(0.06)	(1.65)	(1.27)	(0.78)	(0.24)
di-O-Alkyl	16.79	14.84	6.87	1.58	13.69	8.99	6.41	1.03	15.18	7.84	4.16	1.24	16.63	10.38	5.86	1.36
(g C kg^{-1})	(1.83)	(4.11)	(0.96)	(0.09)	(0.98)	(1.08)	(1.93)	(0.06)	(1.29)	(0.36)	(0.45)	(0.05)	(1.65)	(0.97)	(0.66)	(0.22)
O-Alkyl	65.30	53.41	24.73	5.61	53.40	30.55	21.80	3.90	59.20	26.15	13.72	4.33	58.95	34.24	17.58	4.47
(g C kg^{-1})	(7.12)	(14.79)	(3.47)	(0.34)	(3.82)	(3.68)	(6.57)	(0.21)	(5.03)	(1.20)	(1.48)	(0.16)	(5.86)	(3.22)	(1.99)	(0.73)
Alkyl	50.38	31.15	15.80	3.31	36.97	21.57	17.31	4.01	37.95	23.53	11.22	2.97	36.27	26.98	12.25	4.07
(g C kg^{-1})	(5.49)	(8.63)	(2.22)	(0.20)	(2.64)	(2.60)	(5.22)	(0.22)	(3.22)	(1.08)	(1.21)	(0.11)	(3.61)	(2.53)	(1.39)	(0.66)

437
438

Table S2: The effects of soil destination (translocation) and soil origin on soil properties. 2-way ANOVA results for soil response variables: soil elements and ratios (C, N, P, C:N, C:P, N:P), microbial biomass and ratios (mic C, mic N, mic P, mic C:N, mic C:P, mic N:P), phosphorus fractions (resin P, Po, Pi), cations (Al, K, Mg, Mn, Ca, Na), soil pH, cation exchange capacity (ECEC), base saturation (BS), soil enzymes V_{max} determined at 30°C and their temperature sensitivity determined over 2 - 40°C (Q_{10}): β-glucosidase (β-ase), cellobiohydrolase (Cel), *N*-acetyl β-glucosaminidase (*N*-ase), phosphomonoesterase (P-ase), sulfatase (S-ase), β-xylanase (Xyl) and phenol oxidase (Pox); and the temperature sensitivity of CO_2 efflux determined over 5 - 33°C (RQ_{10}).

Response	Response	Df	SS	MS	F	P	sig
C	orig	1	34.3	34.3	138.8	3.357e-15	***
	dest	1	0.41	0.41	1.6511	0.2055	
	orig:dest	1	0.004	0.004	0.0152	0.9024	
	Residuals	44	10.9	0.25			
N	orig	1	10.1	10.1	109.6	1.598e-13	***
	dest	1	0.10	0.10	1.05	0.310	
	orig:dest	1	0.006	0.006	0.06	0.800	
	Residuals	44	4.1	0.09			
P	orig	1	13.9	13.9	62.2	5.87e-10	***
	dest	1	0.17	0.17	0.76	0.388	
	orig:dest	1	0.001	0.001	0.004	0.953	
	Residuals	44	44	9.80			
C:N	orig	1	7.12	7.12	142.5	2.172e-15	***
	dest	1	0.107	0.107	2.13	0.152	
	orig:dest	1	0.0003	0.0003	0.006	0.939	
	Residuals	44	2.214	0.05			
C:P	orig	1	4.55	4.55	50.63	7.74e-09	***
	dest	1	0.051	0.051	0.572	0.453	
	orig:dest	1	0.008	0.008	0.089	0.767	
	Residuals	44	3.950	0.090			
N:P	orig	1	0.292	0.292	2.748	0.105	
	dest	1	0.018	0.018	0.173	0.679	
	orig:dest	1	0.011	0.011	0.103	0.750	
	Residuals	44	4.562	0.106			
Al	orig	1	10.795	10.795	32.949	8.121e-07	***
	dest	1	0.042	0.042	0.127	0.724	
	orig:dest	1	0.579	0.579	1.77	0.191	
	Residuals	44	14.415	0.328			
Ca	orig	1	1.118	1.118	2.440	1.126	
	dest	1	0.000	0.000	0.000	0.998	
	orig:dest	1	0.382	0.382	0.835	0.366	
	Residuals	44	20.155	0.458			
Fe	orig	1	22.106	22.106	76.642	3.42e-11	***
	dest	1	0.619	0.619	2.144	0.150	
	orig:dest	1	0.641	0.641	2.222	0.143	
	Residuals	44	12.691	0.288			
K	orig	1	3.908	3.908	17.723	1.24e-04	***

	dest	1	0.119	0.119	0.540	0.467	
	orig:dest	1	1.343	1.343	6.093	0.018	*
	Residuals	44	9.700	0.220			
Mg	orig	1	18.859	18.859	26.311	6.99e-04	***
	dest	1	1.883	1.883	2.627	0.113	
	orig:dest	1	1.448	1.448	2.020	0.163	
	Residuals	44	30.104	0.717			
Mn	orig	1	5.433	5.433	8.960	4.61e-03	**
	dest	1	2.433	2.433	4.012	0.052	
	orig:dest	1	1.159	1.159	1.911	0.174	
	Residuals	44	25.469	0.606			
Na	orig	1	0.0003	0.0003	0.0005	0.982	
	dest	1	0.0001	0.0001	0.0003	0.987	
	orig:dest	1	0.247	0.247	0.493	0.487	
	Residuals	44	22.046	0.501			
ECEC	orig	1	10.177	10.177	35.517	3.87e-07	***
	dest	1	0.01	0.01	0.035	0.852	
	orig:dest	1	0.603	0.603	2.104	0.154	
	Residuals	44	12.607	0.287			
BS	orig	1	2.200	2.200	13.704	5.93e-04	***
	dest	1	0.228	0.228	1.423	0.239	
	orig:dest	1	0.024	0.024	0.145	0.702	
	Residuals	44	7.065	0.161			
pH	orig	1	0.06	0.06	29.0	3.05e-06	***
	dest	1	0.001	0.001	0.70	0.408	
	orig:dest	1	0.00003	0.00003	0.015	0.903	
	Residuals	44	0.083	0.002			
Resin P	orig	1	50.253	50.253	100.123	1.10e-12	***
	dest	1	0.009	0.009	0.017	0.896	
	orig:dest	1	5.168	5.168	10.297	0.003	**
	Residuals	44	21.08	0.502			
Pi	orig	1	2.778	2.778	10.347	0.00346	**
	dest	1	0.391	0.391	1.455	0.239	
	orig:dest	1	2.909	2.909	10.840	0.0029	**
	Residuals	26	6.977	0.268			
Po	orig	1	18.917	18.917	94.277	3.90e-10	***
	dest	1	0.021	0.021	0.102	0.752	
	orig:dest	1	0.222	0.222	1.108	0.302	
	Residuals	26	5.217	0.201			
Carbonyl	orig	1	6.0222	6.0222	76.6815	3.395e-11	***
	dest	1	0.0468	0.0468	0.5963	0.4441	
	orig:dest	1	0.1905	0.1905	2.4260	0.1265	
	Residuals	44	3.4556	0.0785			
O-aryl	orig	1	6.4236	6.0222	43.6266	4.346e-08	***
	dest	1	0.2234	0.0468	1.5172	0.2246	
	orig:dest	1	0.0015	0.1905	0.0101	0.9203	
	Residuals	44	6.4786	0.1472			
aryl	orig	1	6.6035	6.6035	57.8958	1.479e-09	***
	dest	1	0.1883	0.1883	1.6511	0.2055	
	orig:dest	1	0.0117	0.0117	0.1023	0.7506	
	Residuals	44	5.0186	0.1141			
di-O-aryl	orig	1	7.0503	7.0503	133.1919	6.712e-15	***
	dest	1	0.0569	0.0569	1.0743	0.3056	
	orig:dest	1	0.0643	0.0643	1.2142	0.2765	
	Residuals	44	2.3291	0.0529			

O-alkyl	orig	1	8.4117	8.4117	230.3520	<2e-16	***
	dest	1	0.2425	0.2425	6.6399	0.0134	*
	orig:dest	1	0.0737	0.0737	2.0173	0.1626	
	Residuals	44	1.6067	0.0365			
alkyl	orig	1	6.4990	6.4990	175.7298	<2e-16	***
	dest	1	0.0083	0.0083	0.2251	0.6375	
	orig:dest	1	0.0667	0.0667	1.8025	0.1863	
	Residuals	44	1.6273	0.0370			
Alkyl:	orig	1	0.10513	0.10513	6.5091	0.0143	*
O-alkyl	dest	1	0.16718	0.16718	10.3510	0.0024	**
	orig:dest	1	0.31436	0.31436	19.4639	6.539e-05	***
	Residuals	44	0.71063	0.01615			
Mic C	orig	1	1.843	1.843	3.085	0.086	
	dest	1	2.493	2.493	4.172	0.047	*
	orig:dest	1	0.204	0.204	0.342	0.562	
	Residuals	44	25.692	0.597			
Mic N	orig	1	7.293	7.293	5.591	0.023	*
	dest	1	14.233	14.233	10.910	0.002	**
	orig:dest	1	0.006	0.006	0.005	0.945	
	Residuals	44	54.798	1.304			
Mic P	orig	1	0.362	0.362	0.420	0.521	
	dest	1	0.664	0.664	0.770	0.385	
	orig:dest	1	2.08	2.08	2.413	0.128	
	Residuals	44	36.185	0.862			
Mic CN	orig	1	1.629	1.629	1.294	0.262	
	dest	1	29.009	29.009	23.034	2.04e-05	***
	orig:dest	1	0.186	0.186	0.148	0.702	
	Residuals	44	52.896	1.259			
Mic CP	orig	1	0.610	0.610	0.810	0.373	
	dest	1	6.380	6.380	8.465	5.82e-03	**
	orig:dest	1	3.666	3.666	4.864	0.033	*
	Residuals	44	30.900	0.754			
Mic NP	orig	1	2.907	2.907	1.478	0.231	
	dest	1	8.996	8.996	4.573	0.039	*
	orig:dest	1	2.032	2.032	1.033	0.316	
	Residuals	44	78.698	1.967			
Tot PLFA	orig	1	10.865	10.865	38.993	1.77e-07	***
	dest	1	0.160	0.160	0.574	0.453	
	orig:dest	1	0.114	0.114	0.408	0.527	
	Residuals	44	11.703	0.279			
bact PLFA	orig	1	20.055	20.055	53.617	5.05e-09	***
	dest	1	0.473	0.473	1.266	0.267	
	orig:dest	1	0.043	0.043	0.114	0.738	
	Residuals	44	15.710	0.374			
fung PLFA	orig	1	9.653	9.653	37.985	2.31e-07	***
	dest	1	0.054	0.054	0.214	0.646	
	orig:dest	1	0.101	0.101	0.398	0.532	
	Residuals	26	10.673	0.254			
Fung:Bact	orig	1	1.991	1.991	22.837	2.18e-05	***
	dest	1	0.207	0.207	2.514	0.120	
	orig:dest	1	0.012	0.012	0.151	0.699	
	Residuals	44	3.459	0.082			
Gram-pos	orig	1	8.525	8.525	30.969	1.67e-06	***
	dest	1	0.001	0.001	0.003	0.959	
	orig:dest	1	0.180	0.180	0.655	0.423	

	Residuals	44	11.562	0.275			
Gram-neg	orig	1	10.850	10.850	43.309	5.80e-08	***
	dest	1	0.216	0.216	0.862	0.359	
	orig:dest	1	0.033	0.033	0.131	0.719	
	Residuals	44	10.522	0.251			
β-ase	orig	1	6.848	6.848	9.189	0.004	**
	dest	1	0.569	0.569	0.763	0.387	
	orig:dest	1	0.344	0.344	0.461	0.501	
	Residuals	44	32.789	0.745			
P-ase	orig	1	1.2817	1.2817	3.5021	0.06795	
	dest	1	0.0369	0.0369	0.1008	0.75236	
	orig:dest	1	0.4659	0.4659	1.2730	0.26531	
	Residuals	44	16.103	0.3660			
N-ase	orig	1	5.558	5.5579	6.8913	0.01187	*
	dest	1	0.494	0.4939	0.6124	0.43809	
	orig:dest	1	1.858	1.8576	2.3032	0.13626	
	Residuals	44	35.486	0.8065			
Cel	orig	1	18.292	18.2921	17.2702	14.71e-04	***
	dest	1	0.145	0.1453	0.1372	0.7128370	
	orig:dest	1	0.483	0.4829	0.4559	0.5030673	
	Residuals	44	46.604	1.0592			
Xyl	orig	1	28.2914	28.2914	44.7950	3.228e-08	***
	dest	1	0.3494	0.3494	0.5532	0.4610	
	orig:dest	1	0.0478	0.0478	0.0756	0.7846	
	Residuals	44	27.7894	0.6316			
Pox	orig	1	0.4633	0.46329	1.5679	0.2171	
	dest	1	0.1586	0.15860	0.5368	0.4677	
	orig:dest	1	0.1909	0.19087	0.6460	0.4259	
	Residuals	44	13.0012	0.29548			
RQ_{10}	orig	1	0.007792	0.0077920	2.3250	0.1345	
	dest	1	0.000890	0.0008904	0.2657	0.6088	
	orig:dest	1	0.003751	0.0037509	1.1192	0.2959	
	Residuals	44	0.147458	0.0033513			
β-ase Q_{10}	orig	1	0.00206	0.00206	0.1496	0.7008	
	dest	1	0.00701	0.00701	0.5102	0.4788	
	orig:dest	1	0.02438	0.02438	1.7747	0.1897	
	Residuals	44	0.60454	0.0137396			
P-ase Q_{10}	orig	1	0.05050	0.05050	3.6122	0.06392	
	dest	1	0.00099	0.000986	0.0705	0.79181	
	orig:dest	1	0.003751	.003639	0.2603	0.61246	
	Residuals	44	0.61509	0.013979			
N-ase Q_{10}	orig	1	0.01473	0.01473	0.9732	0.3293	
	dest	1	0.00001	0.00001	0.0006	0.9801	
	orig:dest	1	0.01201	0.01201	0.7935	0.3779	
	Residuals	44	0.66578	0.01513			
S-ase Q_{10}	orig	1	0.0015	0.00152	0.0125	0.9116	
	dest	1	0.0049	0.00488	0.0398	0.8428	
	orig:dest	1	0.0934	0.09343	0.7620	0.3874	
	Residuals	44	5.3949	0.12261			
Pox Q_{10}	orig	1	0.0302	0.0302	2.9646	0.0921	
	dest	1	0.5175	0.5175	50.833	7.381e-09	***
	orig:dest	1	0.0059	0.0059	0.5821	0.4496	
	Residuals	44	0.4479	0.0102			
Xyl Q_{10}	orig	1	0.51228	0.51228	10.0043	0.002829	**
	dest	1	0.08152	0.08152	1.5920	0.213686	

	orig:dest	1	0.01329	0.01329	0.2595	0.613039
	Residuals	44	2.25306	0.05121		
Cel Q_{10}	orig	1	0.1425	0.1425	0.9702	0.3300
	dest	1	0.1273	0.1273	0.8670	0.3569
	orig:dest	1	0.0195	0.0195	0.1326	0.7175
	Residuals	44	6.4618	0.1469		

449
450

Table S3: The effects of soil destination (translocation) and soil origin on substrate use efficiencies. Values for CUE_{CN}, CUE_{CP}, NUE and PUE were calculated according to equations 3-4 (means with 1 SE in parenthesis, where n = 3). The origin effects and destination effects on substrate use efficiencies were evaluated using 1-way ANOVA. There were no origin effects: CUE_{CN} (SS = 0.35, df = 15, F = 0.98, $p = 0.44$); CUE_{CP} (SS = 0.30, df = 15, F = 0.78, $p = 0.44$); NUE (SS = 0.55, df = 15, F = 0.92, $p = 0.46$); PUE (SS = 0.51, df = 15, F = 0.87, $p = 0.49$). In contrast, destination effects were significant for all parameters: CUE_{CN} (SS = 0.35, df = 15, F = 9.56, $p = 0.002$); CUE_{CP} (SS = 0.30, df = 15, F = 4.78 ,$p = 0.02$); NUE (SS = 0.55, df = 15, F = 6.88, $p = 0.006$); PUE (SS = 0.51, df = 15, F = 5.63 ,$p = 0.012$); pairwise differences by Tukey HD tests are shown by lower case letters where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Code	Origin elev. (m asl)	Dest. elev. (m asl)	Temp diff. (oC)	Elev. diff. (m)	CUE_{CN}			CUE_{CP}			NUE			PUE		
AA1	3030	3030	0	0	0.04	(0.01)		0.04	(0.01)		0.98	(0.01)		0.98	(0.01)	
AB1	1500	3030	-6.3	1530	0.09	(0.02)		0.21	(0.10)		0.96	(0.01)		0.86	(0.08)	
AC1	1000	3030	-9.6	2030	0.05	(0.02)		0.15	(0.02)		0.98	(0.01)		0.92	(0.02)	
AD1	210	3030	-15.3	2820	0.12	(0.03)		0.21	(0.04)		0.94	(0.01)		0.88	(0.03)	
BA1	3030	1500	6.3	-1530	0.06	(0.02)		0.18	(0.06)		0.97	(0.01)		0.89	(0.04)	
BB1	1500	1500	0	0	0.08	(0.03)		0.13	(0.01)		0.96	(0.02)		0.94	(0.01)	
BC1	1000	1500	-3.3	500	0.06	(0.03)		0.18	(0.07)		0.97	(0.02)		0.89	(0.05)	
BD1	210	1500	-9	1290	0.19	(0.02)		0.32	(0.10)		0.90	(0.01)		0.74	(0.10)	
CA1	3030	1000	9.6	-2030	0.19	(0.14)		0.25	(0.05)		0.86	(0.11)		0.84	(0.04)	
CB1	1500	1000	3.3	-500	0.13	(0.02)		0.50	(0.04)		0.93	(0.01)		0.40	(0.14)	
CC1	1000	1000	0	0	0.16	(0.10)		0.45	(0.07)		0.90	(0.07)		0.52	(0.14)	
CD1	210	1000	-5.7	790	0.48	(0.07)		0.48	(0.04)		0.42	(0.17)		0.47	(0.10)	
DA1	3030	210	15.3	-2820	0.33	(0.10)		0.21	(0.02)		0.70	(0.16)		0.88	(0.01)	
DB1	1500	210	9	-1290	0.39	(0.10)		0.44	(0.01)		0.62	(0.18)		0.60	(0.02)	
DC1	1000	210	5.7	-790	0.36	(0.11)		0.12	(0.08)		0.61	(0.24)		0.94	(0.04)	
DD1	210	210	0	0	0.46	(0.06)		0.20	(0.07)		0.48	(0.17)		0.87	(0.07)	
Origin effects (average value by origin)																
	3030	(a)			0.15	(0.07)		0.17	(0.05)		0.88	(0.07)		0.90	(0.03)	
	1500	(b)			0.17	(0.07)		0.32	(0.09)		0.87	(0.08)		0.70	(0.12)	
	1000	(c)			0.16	(0.07)		0.23	(0.08)		0.86	(0.09)		0.82	(0.10)	
	210	(d)			0.32	(0.09)		0.30	(0.07)		0.68	(0.14)		0.74	(0.10)	
Destination effects (average value by destination)																
		3030	(a)		0.07	(0.02)	d**	0.15	(0.04)	a*	0.97	(0.01)	a**	0.91	(0.03)	c*
		1500	(b)		0.10	(0.02)	d**	0.20	(0.01)		0.95	(0.01)		0.87	(0.01)	c*
		1000	(c)		0.24	(0.02)		0.42	(0.02)	c*	0.78	(0.01)	a*	0.56	(0.01)	b*a*
		210	(d)		0.39	(0.02)	a**b**	0.24	(0.02)		0.60	(0.01)	b*a**	0.82	(0.01)	