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Microbial carbon use efficiency along an altitudinal gradient

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ABSTRACT

Soil microbial carbon-use efficiency (CUE), described as the ratio of growth over total carbon (C) uptake, i.e. the sum of growth and respiration, is a key variable in all soil organic matter (SOM) models and critical to ecosystem C cycling. However, there is still a lack of consensus on microbial CUE when estimated using different methods. Furthermore, the significance of many fundamental drivers of CUE remains largely unknown and inconclusive, especially for tropical ecosystems. For these reasons, we determined CUE and microbial indicators of soil nutrient availability in seven tropical forest soils along an altitudinal gradient (circa 900–2200 m a.s.l) occurring at Taita Hills, Kenya. We used this gradient to study the soil nutrient (N and P) availability and its relation to microbial CUE estimates. For assessing the soil nutrient availability, we determined both the soil bulk stoichiometric nutrient ratios (soil C:N, C:P and N:P), as well as SOM degradation related enzyme activities. We estimated soil microbial CUE using two methods: substrate independent ^{18}O-water tracing and ^{13}C-glucose tracing method. Based on these two approaches, we estimated the microbial uptake efficiency of added glucose versus native SOM, with the latter defined by ^{18}O-water tracing method. Based on the bulk soil C:N stoichiometry, the studied soils did not reveal N limitation. However, soil bulk P limitation increased slightly with elevation. Additionally, based on extracellular enzyme activities, the SOM nutrient availability decreased with elevation. The ^{13}C-CUE did not change with altitude indicating that glucose was efficiently taken up and used by the microbes. On the other hand, ^{18}O-CUE, which reflects the growth efficiency of microbes growing on native SOM, clearly declined with increasing altitude and was associated with SOM nutrient availability indicators. Based on our results, microbes at higher elevations invested more energy to scavenge for nutrients and energy from complex SOM whereas at lower elevations the soil nutrients may have been more readily available.

1. Introduction

Soil microorganisms play an important role in regulating the global terrestrial carbon (C) cycle. This is largely attributed to their significant contribution to the decomposition of soil organic carbon (SOC) (Qiao et al., 2019). Soil microbes produce extracellular enzymes to decompose soil organic matter (SOM) into accessible and available forms of C, nitrogen (N) and phosphorus (P) (Hill et al., 2008). Extracellular enzymes

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play an important role in SOM decomposition to meet the nutrient and energy demands of microbial growth and functioning. In fact, the fate of C in soil, i.e. either respired as CO_2 or retained and possibly stabilized in SOM, is determined not only by the plant litter quality, but also by the soil microbes' different strategies to allocate energy obtained from SOM decomposition to both microbial growth and production of extracellular enzymes (Liang et al., 2017). Microbial carbon use efficiency (CUE), defined as the ratio of organic C allocated for growth to organic C taken up (Manzoni et al., 2012; Sinsabaugh et al., 2013), indicates how much of the C used by microbes is directed to anabolic reactions, and thus potentially remains in the soil (Manzoni et al., 2018). The amount of C taken up by microbial biomass is calculated as the sum of C allocated to growth and respired out (Sinsabaugh et al., 2013).

For CUE calculations, respiration can be measured as rates of total CO_2 produced, O_2 consumption, or the specific rate of $^{13}CO_2$ produced after addition of labeled substrates (e.g. glucose). The other component i.e. growth, can be estimated as rates of biomass increase, e.g. incorporation of labeled substrate to microbial biomass during microbial growth, or use of the labeled substrate for protein production or genomic DNA construction during cell division (Bååth, 1990, 1994; Sinsabaugh et al., 2016). Empirical estimates of microbial CUE range from near zero to over 0.88 (Saifuddin et al., 2019), with low values implying that little C is converted to microbial biomass relative to respiration and high values the opposite. Some part of the variations in reported microbial CUE may be due to abiotic and biotic factors such as temperature, pH, substrate quality and soil microbial community (Manzoni et al., 2012; Qiao et al., 2019; Pold et al., 2020), while some parts of the variability may be also due to methodological differences (Frey et al., 2013; Geyer et al., 2019).

Based on the *soil microbial C pump* concept by Liang et al. (2017), the C used for microbial growth – through the *in vivo* turnover pathway – is important for accumulation of stable SOM in the form of dead microbial remains (Kallenbach et al., 2016; Liang et al., 2017; Angst et al., 2019). Several studies have demonstrated the ability of microorganisms to produce compounds, which are more persistent against decomposition and accumulate in soils contributing to the stable C pools (Kindler et al., 2006; Grandy and Neff, 2008; Yao and Shi, 2010). Consequently, higher CUE could also lead to increased formation of stable C and higher soil C stocks in terrestrial environments (Liang et al., 2017; Soares and Rousk, 2019). On the other hand, previous studies have demonstrated that higher CUE with climate warming accelerates SOM decomposition and loss of soil C (e.g. Nottingham et al., 2019). However, other studies have reported constant or decreased CUE after long-term warming (Frey et al., 2013). Thus, the dominant microbial response to warming i.e. either decrease in CUE or acclimation of CUE could have potentially important consequences for SOC storage (Allison et al., 2010; Walker et al., 2018). Currently there is no agreement on the magnitude of such a feedback to climate warming.

CUE is a key variable in SOM models (Schimel, 2013) and there is a need to better understand the microbial CUE variability in the soil (Qiao et al., 2019). This remains critical for improving the accuracy of models and better understanding of future trajectories in soil-climate feedbacks (Geyer et al., 2016). Soil temperature has been widely recognized as a principal factor controlling microbial physiology, notably growth and respiration (Zheng et al., 2019). Thus, changes in sensitivity to temperature of the two components results in differences in CUE as a function of climate (Ye et al., 2019). The assumption that maintenance respiration increases more than growth in response to temperature has led to the expectation that microbial CUE could decline with climate warming (Allison et al., 2010; Dijkstra et al., 2011). Also, increased microbial turnover at higher temperatures has been found to reduce CUE (Kirschbaum, 2004; Hagerty et al., 2014; Li et al., 2019). On the contrary, other studies have also demonstrated that the CUE of glucose metabolism is rather insensitive to *in situ* temperature (e.g. Dijkstra et al., 2011; Qiao et al., 2019). Thus, understanding climatic and environmental controls of microbial CUE is especially important for tropical soils, which are important C storages and may contain significant reservoirs of labile-C (Drake et al., 2019; Nottingham et al., 2020; Zimmermann et al., 2012; Sayer et al., 2019).

In stoichiometric theory, nutrient-limited growth occurs when the availability of an essential element, e.g. N or P relative to C (nutrient: C ratios), falls below the critical ratio or threshold element ratio required for optimum growth (Manzoni et al., 2012). To acquire energy and nutrients from SOM, microbes produce various SOM degradation related enzymes to break down the complex polymeric plant and microbial derived substrate (Baldrian, 2017). Low soil nutrient availability leads to changes in nutrient acquisition strategies, changes in microbial growth and respiration rates, and possibly changes in CUE (Keiblinger et al., 2010; Sinsabaugh et al., 2013). Microorganisms growing on high C:N ratio substrates have excess C compared to N, which has been suggested to decrease CUE, through increased overflow respiration or exudation of C in form of proteins and other metabolic compounds (Manzoni et al., 2012; Sinsabaugh et al., 2016). Microbial CUE in nutrient limited conditions is also decreased because microbes can adapt to nutrient limitations by changing their metabolism and investing more energy to production of nutrient-acquisition related enzymes or taking up more C than required and respiring the excess C through overflow respiration (Manzoni et al., 2012, 2017; Geyer et al., 2016; Mehnaz et al., 2019).

Feedback mechanisms between C:N:P ratios of different terrestrial ecosystems under varying environmental conditions e.g. nutrient availability affecting microbial CUE, will affect geochemical cycling of elements. Thus, it is important to explore microbial physiological responses to varying eco-climatic conditions to better understand soil microbial C metabolism. Only a limited number of studies have investigated microbial CUE in tropical forest soils, especially those occurring along an altitudinal gradient (e.g. Nottingham et al., 2019). Previous studies have demonstrated that N limitation increases with altitude in moist montane tropical forests (e.g. Kaspari et al., 2008; Fisher et al., 2013; Nottingham et al., 2018). However, to our knowledge, there are no studies estimating microbial CUE in African tropical forests occurring along an altitudinal gradient. For these reasons, we took advantage of the elevation and temperature gradient occurring in Taita Hills, Kenya to study the legacy effect of *in situ* temperature to soil nutrient availability and its relation to microbial CUE estimates. Based on previous studies from other tropical mountain gradients (e.g. Kaspari et al., 2008; Fisher et al., 2013; Nottingham et al., 2018), we hypothesized that 1) soil nutrient availability will decrease with elevation because of lower mean annual temperatures limiting decomposition. Following this, we expected that 2) soil microbial CUE will decrease with elevation because microbes need to invest more energy to production of nutrient acquisition related enzymes and less C to their growth. For assessing nutrient availability, we utilized two aspects: stoichiometric nutrient ratios (soil bulk C, N and P concentrations), and SOM degradation related extracellular enzyme activities to estimate how easily available the nutrients were in the SOM. We determined microbial CUE and microbial growth using both a substrate-independent method with ^{18}O-water (Spohn et al., 2016a), and substrate-dependent method with ^{13}C-glucose. Utilization of these two techniques allowed us to estimate the microbial uptake efficiency of easily utilizable glucose versus more complex native SOM, which was defined with the ^{18}O-water tracing method.

2. Materials and methods

2.1. Study area

Taita Hills are located in Southeastern Kenya (03° 25' S, 38° 20' E) constituting the northernmost part of the Precambrian Eastern Arc mountain range known for its rich biodiversity (Platts et al., 2011). The general elevation range is 500–1000 m above sea level (a.s.l.) on the lower plains, to between 1300 and 1800 m a.s.l. At higher elevations of the hills, with the highest peak Vuria reaching 2208 m a.s.l. Rainfall is

bimodal with long rains in March–May and short rains in October–December. January and February are the hottest and driest months (mean annual temperature (MAT) 19.6 °C for the highlands and 26.9 °C for the lowlands in 2016), while from June to September it is dry and cool (MAT 16.9 °C for the highlands and 23.2 °C for the lowlands in 2016). The MAT in the highlands is measured by a weather station at 1599 m a.s.l. managed by the Taita Research Station of the University of Helsinki, and the MAT from the lowlands is measured at Voi airport at 612 m a.s.l. from Kenya Meteorological Department (KMD). The hills above 1500 m are often covered by fog providing additional precipitation as fog deposition (Räsänen et al., 2020). The fog deposition can provide up to 17% addition to annual precipitation based on studies done at the top of Vuria at 2200 m (Helle, 2016).

2.2. Site selection and soil sampling

Soils were sampled from seven different forest sites: Vuria (2200 m a.s.l), Ngangao (1800 m a.s.l), Yale (1850 m a.s.l), Fururu (1700 m a.s.l), Macha (1600 m a.s.l), Dembwa (1000 m a.s.l) and Semba in the middle of Taita Sisal Estate (900 m a.s.l), located along an altitudinal gradient to the top of Taita Hills (Fig. 1). The five sites at higher elevations (Vuria, Ngangao, Yale, Fururu and Macha) are located within the moist montane forest with an average annual rainfall of 800 mm and Macha at mid-elevation with average rainfall of 600 mm. The two lower sites (Dembwa and Semba) are drier forests with an average annual rainfall of 700 mm as estimated from the measurements of the closest weather stations. The MATs for the sites in the highlands are 14.0–20.3 °C (2200-1600 m a.s.l.) and for the sites in lowlands 22.0–23.6 °C (900–1000 m a.s.l.). The temperature and rainfall values are from weather stations of the Taita Research Station. Soils in the study area are diverse, but soils at Semba and Dembwa are mainly Rhodic Ferralsols (FAO), at Macha, Fururu, Yale and Ngangao are humic Cambisols (FAO), and at Vuria are Umbrisols (FAO) (FURP, 1987; Omoro et al., 2013; Njeru et al., 2017).

Three sampling plots, each with an area of 100 m^2, 10 m × 10 m and located ca. 30 m apart horizontally, were selected to represent each forest site. The slope was similar in all sites (ca. 20%) and the aspect was north-east. Soil temperature at each site was measured during sampling using Tinytag Data Loggers (Tinytag Plus 2 –TGP-4017, Internal Temperature −40 °C - +85 °C). Twenty soil cores of 0–10 cm depth were randomly sampled from each plot using a 3 cm diameter soil auger and mixed together to form one composite sample per plot (n = 3 per site). The sampled 0–10 cm of soil consisted of organic layer at the five sites located in the moist montane rain forest, except that the lowest of them (Macha) had some mineral particles at the bottoms of some sampled cores. At the two low elevation sites the 0–10 cm top soil was a mixture of organic and mineral soil. Soil sampling was conducted between 22nd to October 31, 2018, at the start of the October–December rain period. Immediately after sampling, visible plant debris and roots were removed and soil was sieved through a 4.0 mm sieve (standard size for sieving organic soils) and stored under moist field conditions at 5 °C for maximum 10 days before analysis.

2.3. Soil physical and chemical analyses

Soil dry weights were determined by weighing soil samples before and after drying at 105 °C for 24h (3 analytical replicates). Water holding capacity (WHC) was determined by saturating the soil for 2 h in a funnel immersed in water, and thereafter let to drain for 24 h through a Whatman® Grade 42, Ashless Filter Paper. Water-saturated weight (at 100% WHC) of the soil was determined gravimetrically after drying at 105 °C for 24 h.

Dried soil (oven dried at 40 °C) was used for all chemical analyses. Soil pH was determined in Milli-Q water (ratio of 1:2.5) using a pH meter (WTW InoLab pH Level 1 ba12217e). Aliquots of air-dried soil were ground (mortar and pestle) and used for total C, N and P analyses. Total organic C (TOC) and total N (TN) were measured using a varioMax

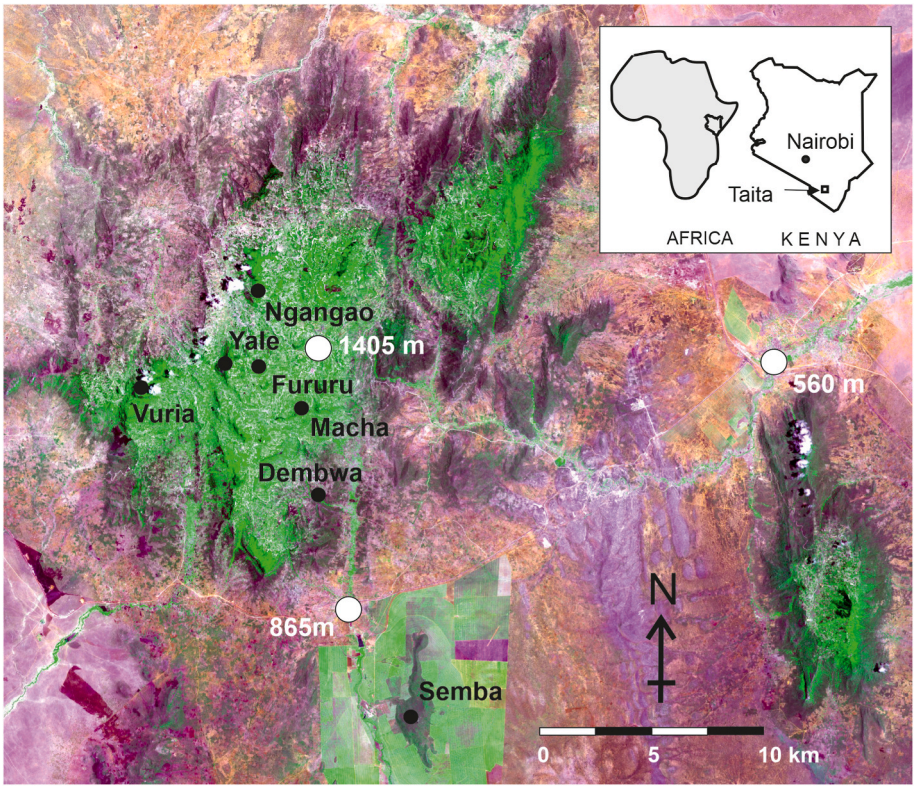

Fig. 1. Forest sites in the Taita Hills in south-eastern Kenya. Green colour in the Sentinel satellite image represents humid forested and vegetated areas, while reddish and blueish areas represent drier areas with sparse dry vegetation. The green area in the lowlands around Semba forest is the sisal plantation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

CN Elementar Analyzer (Hanau, Germany). Total P (TP) was determined by ICP-OES (Spectroflame, Spectro) after digestion in concentrated nitric acid (HNO_3) (Huang and Schulte, 1985) and heating in a microwave oven. All the results were standardized per gram of soil (dw) for further data analyses.

2.4. Carbon use efficiency (CUE): ^{18}O-water and ^{13}C-glucose tracing methods

Two methods, 1) ^{18}O-water and 2) ^{13}C-glucose tracing were used to determine CUE. For both methods, a temperature of 15 °C and a 24 h incubation period were used (Geyer et al., 2019). A short incubation period (24 h) was chosen to avoid confounding effects of changes in microbial community composition or acclimation of microbial physiology that may occur in longer incubation periods (Bradford, 2013; Geyer et al., 2019).

2.4.1. ^{18}O-water tracing method and calculations

Microbial ^{18}O-CUE (and the turnover time of soil microbial biomass) were determined based on incorporation of ^{18}O- into microbial DNA (Spohn et al., 2016a). For this purpose, soil samples were first pre-incubated for 2 weeks at 15 °C and at 45% of WHC. After pre-incubation, two aliquots of 200 mg of each sample were weighed into 2 ml Eppendorf cups®. The open Eppendorf cups containing the amended soils were immediately transferred into 20 ml incubation vials and sealed with a crimp cap. One of them was labeled with ^{18}O–H_2O (97.0 at%, Sigma Aldrich, St Louis, MO, US) with a syringe (Hamilton, Reno, NV, US) to reach 20.0 at% of ^{18}O in the final soil water and 60% of WHC. The other sample was amended with the same volume of non-labeled deionized water to serve as natural abundance control. After labeling, the vials were evacuated and filled with a standard gas of known CO_2 concentration to a pressure to of 1.3 bar. The incubation vials were incubated for 24 h at 15 °C. After 24 h of incubation, a 15 ml gas sample was taken from each vial with a gas syringe (SGE Syringe, Trajan Scientific and Medical, Victoria, Australia), and was transferred to an evacuated 5 ml vial. The CO_2 concentration in each sample was determined using gas chromatography (Agilent 7890A GC, Agilent Technologies, Santa Clara, USA). Directly after the gas sample was taken, the Eppendorf cups were taken out, closed, and put into liquid N_2 in order to freeze the soils. Subsequently, the soil samples were stored at −80 °C until DNA extraction.

DNA was extracted from the entire soil sample of the labeled and the non-labeled soil using a DNA extraction kit (FastDNA™ SPIN Kit for Soil, MP Biomedicals). Manufacturer instructions were followed with the following two exceptions. First, the first centrifugation time was increased to 15 min in order to remove a larger proportion of the cell debris from the supernatant. Second, the entire matrix containing the DNA was loaded on the filter and not just a third of it as suggested by the provider. The weight of the DNA extract was determined, and the DNA concentration in each extract was determined fluorometrically from a 5 μL aliquot by Picogreen assay (Sandaa et al., 1998) using a kit (Quant-iT™ PicoGreen® dsDNA Reagent, Invitrogen). The remainder of the DNA extract was dried in a silver capsule at 60 °C overnight to remove any water. Subsequently, the ^{18}O abundance and the total O content were measured using a Thermochemical elemental analyzer (TC/EA Thermo Fisher) coupled via a Conflo III open split system (Thermo Fisher) to an Isotope Ratio Mass Spectrometer (Delta V Advantage, Thermo Fisher).

Soil microbial biomass C and N (MBC and MBN, respectively) were determined by chloroform fumigation extraction (CFE) (Vance et al., 1987). Ethanol free chloroform ($CHCl_3$) was used to fumigate 3 g of moist soil for 24 h in a desiccator at room temperature in the dark. Soluble C and N from fumigated and non-fumigated soil samples were extracted with 40 ml of 0.05 M K_2SO_4 via agitation on an orbital shaker (30 min, 200 rotations min^{-1}) and filtered using Whatman® Grade 42, Ashless Filter Paper and subsequently through a 0.45 μm syringe filter (Sartorius, Minisart High Flow, PES). Thereafter, the extracts were analyzed for total organic C and total dissolved N (mineral and organic) using the TOC-VCPH Shimadzu (Japan). MBC and MBN were calculated by subtracting total organic C and total dissolved N values of the non-fumigated sample from the fumigated sample, respectively. Since the entire microbial C and N can be extracted by K_2SO_4, a kEC and kEN factors of 0.45 (Jörgensen, 1996) and 0.54 (Brookes et al., 1985) were used to convert microbial C and N flush into MBC and MBN, respectively.

Soil respiration flux was determined using the amount of CO_2–C produced during the 24h incubation period (Equation (1)). The DNA produced during the incubation period (Equation (2)) of each labeled sample was calculated based on the abundance of ^{18}O in (i) labeled DNA, (ii) non-labeled DNA (natural abundance), and (iii) soil water of the labeled sample (Spohn et al., 2016a). The amount of DNA produced during the incubation was transformed into the amount of MBC produced during the incubation by using a sample specific Cmic/DNA ratio (fDNA) (Poeplau et al., 2019).The flux of C allocated to biomass production was calculated by dividing the amount of MBC produced during the incubation by the incubation period (Equation (3)).

$$C_{respiration} = \frac{p \times V}{R \times T} \times M \times \Delta CO_2 \times \frac{1}{g\ soil\ \times t} \quad (1)$$

$$DNA_{produced} = O_{Total} \times \frac{^{18}O_{DNA}}{^{18}sol} \times \frac{100}{31.21} \quad (2)$$

$$C_{growth} = \frac{DNA_{produced} \times fDNA}{g\ soil \times t} \quad (3)$$

In equation (1), p is the pressure (kPa) in the vial, V is the volume (l) of the vial headspace, R is the universal gas constant (8314 J mol^{-1} K^{-1}), T is the temperature (K) at which the standard gas is injected into the vial, M is the molecular mass of carbon (12.01 g mol^{-1}), and ΔCO_2 is the increase in CO_2 concentration (ppm) during the incubation time t (h). In equation (2), O_{Total} (μg) is the total amount of O in the DNA eluate, $^{18}O_{DNA}$ (at% excess) is the difference in at% ^{18}O between the labeled and the non-labeled natural abundance control samples, and ^{18}sol is enrichment of the final soil solution, which is adjusted to 20 at% ^{18}O. The average % w/w of O in DNA is 31.21.

The amount of C taken up by the microbial biomass (C_{Uptake}) during the incubation time was calculated as

$$C_{Uptake} = C_{growth} \times t + C_{respiration} \times t \quad (4)$$

where C_{Growth} is the flux of C allocated to biomass production (growth), $C_{Respiration}$ is the flux of C allocated to the production of CO_2 (respiration), and t the incubation period of 24 h.

Subsequently, microbial ^{18}O-CUE was calculated using equation (5) (Manzoni et al., 2012; Sinsabaugh et al., 2013).

$$\text{Microbial CUE} = \frac{C_{Growth}}{C_{Growth} + C_{Respiration}} \quad (5)$$

The turnover time of microbial biomass was calculated using equation (6) below.

$$\text{Turnover time} = \frac{MBC}{C_{Growth}} \quad (6)$$

2.4.2. ^{13}C-glucose tracing method and calculations

Microbial ^{13}C-CUE was determined by tracing uptake and mineralization of ^{13}C enriched glucose. First, 20 g fresh soil was pre-incubated in aluminum cups covered with a thin layer of plastic film to minimize evaporation for 48 h at 15 °C at 45% WHC. At the end of the pre-incubation period, soils received a glucose amendment of 0.05 mg glucose-C g^{-1} dry soil dissolved in Milli-Q water, so that the soils reached 60% WHC. This glucose concentration represents a typical low substrate amendment rate commonly used in previous tracer studies

(Frey et al., 2013; Geyer et al., 2019). The used glucose concentration is within the range that is commonly found in soils and was chosen so that it would have minimum to no effect on microbial metabolism (Dijkstra et al., 2011), would not cause significant net growth of microbial biomass (Geyer et al., 2019), and would give CUE values that are as representative of unamended soil. Universally labeled ^{13}C-glucose was diluted with unlabeled glucose to achieve a total glucose enrichment of 5 at%. Control samples received only Milli-Q water.

After glucose addition, soils were mixed with a spatula, and transferred into 500 ml incubation jars. The jars were closed with rubber septa, flushed with CO_2 free air, and moved to Panasonic MIR-154-PA incubators set at 15 °C. Increases in CO_2 concentrations inside the jars during the 24 h incubation were monitored four times (time 0, 6, 12 and 24 h after glucose addition), by sampling the head space with a syringe and needle (100 μl at a time). The CO_2 concentration was determined using gas chromatography (Hewlett-Packard 6890). Cumulative CO_2–C accumulated into the jars during the 24 h incubation, and its ^{13}C content were used to calculate the cumulative respiration derived from added glucose (^{13}R; μg $^{13}CO_2$–C g^{-1} soil) using the two-pool mixing model (Equations (7)–(10)) (Karhu et al., 2022).

$$f_{glucose} = \frac{^{13}CO_2 - ^{13}SOM}{^{13}C_{glucose} - ^{13}SOM} \quad (7)$$

$$f_{SOM} = 1 - f_{glucose} \quad (8)$$

$$R_{SOM} = R_{total} \times f_{SOM} \quad (9)$$

$$^{13}R = R_{total} - R_{SOM} \quad (10)$$

Where $f_{glucose}$ and f_{SOM} represent the fraction of CO_2 derived from respiration of glucose and SOM, respectively, and $^{13}C_{glucose}$ and ^{13}SOM represent the ^{13}C-atom % in glucose and in SOM, respectively, and R_{total} represent the total respiration measured. The variable ^{13}R is needed in the calculation of CUE (see Equation (14) below).

At 24 h after glucose addition, a 20 ml CO_2 gas sample was taken from each incubation jar through the septum, and injected into He-flushed and evacuated glass vials (12 ml Exetainer®, Labco Limited, UK) using a syringe and needle. Thereafter, the δ^{13}C of the CO_2 was determined using a DeltaPlusXL (Thermo Finnigan) continuous flow isotope-ratio mass spectrometer with references NBS-19, NBS-18 and L-SVEC to normalize raw isotope data. The analytical precision was ±0.15‰ (1σ).

Growth is estimated from ^{13}C incorporation into microbial biomass (Frey et al., 2013). Soil was immediately extracted (i.e. right after the final gas sampling) for microbial biomass C and N by the chloroform fumigation extraction (CFE) method (Vance et al., 1987) as described above in section 2.4.1. The K_2SO_4 extracts were freeze-dried before ^{13}C measurement. The δ^{13}C of microbial biomass was analyzed by a continuous-flow isotope ratio mass spectrometer (IRMS; Thermo Finnigan DELTA XPPlus, Bremen, Germany) interfaced with an elemental analyzer (Flash EA 1112 Series, Thermo Finnigan, Bremen, Germany) via the open split interface (Conflow III, Thermo Finnigan, Bremen, Germany). External precision was ≤±0.16‰ for the stable isotopic analysis and ≤±0.9% for elemental analysis (Biasi et al., 2008). Total microbial growth (^{13}MBC; μg C g^{-1} soil) was calculated as the product of total MBC (μg C g^{-1} soil) and the percent of total microbial biomass labeled (% ^{13}MBC) using the equations below as described by Geyer et al. (2019).

$$^{13}MBC_x = \frac{^{13}DOC_F \text{ x } DOC_F - ^{13}DOC_{NF} \text{ x } DOC_{NF}}{DOC_F - DOC_{NF}} \quad (11)$$

$$^{13}MBC\% = \frac{^{13}MBC_t - ^{13}MBC_c}{^{13}sol - ^{13}MBC_c} \text{ x } 100 \quad (12)$$

$$^{13}MBC = (DOC_F - DOC_{NF}) \times \frac{^{13}MBC\%}{100} \quad (13)$$

Microbial ^{13}C-CUE was then calculated by the following equation (14) (Geyer et al., 2019).

$$\text{Microbial CUE} = \frac{13_{MBC}}{13_{MBC} + 13_R} \quad (14)$$

Where $^{13}DOC_F$, DOC_F, $^{13}DOC_{NF}$, and DOC_{NF} represent the atom % and total C concentrations (μg C g^{-1} soil) of fumigated (F) and non-fumigated (NF) K_2SO_4 extracts, respectively. $^{13}MBC_t$ and $^{13}MBC_c$ (denoted as $^{13}MBC_x$ in Eq. 11) are the atom % of sample treatments and natural abundance controls, and ^{13}sol is the atom % of amendment solution (5 at%). ^{13}MBC is total microbial growth (μg C g^{-1} soil) and ^{13}R is the cumulative respiration derived from added glucose (μg $^{13}CO_2$–C g^{-1} soil) during the 24 h incubation period (see Equation (10)).

2.5. Extracellular enzyme activity assays

Activities of cellobiosidase (EC 3.2.1.91), β-glucosidase (EC 3.2.1.21), chitinase (EC 3.2.1.14), leucine amino-peptidase (EC 3.4.11.1), acid phosphatase (EC 3.1.3.2), β-xylosidase (EC 3.2.1.37) were measured from fresh soil samples 10 days after sampling (stored at +5 °C) using fluorometric substrates according to (Bell et al., 2013). Soil suspensions were prepared by adding 2 g soil to 100 ml of 100 mM, pH 5.5, sodium acetate buffer and homogenizing for 1 min in a mortar. The resulting suspensions were continuously stirred using a magnetic stir plate. 200 μl of soil suspension was added to microplates (96-well) and mixed with 50 μl of substrate; and for blanks 200 μl of buffer and 50 μl of the respective substrate was added per well. All substrates were obtained from Sigma-Aldrich: 4-methylumbelliferyl β-D-cellobioside (substrate for cellobiosidase), 4-methylumbelliferyl D-glucopyranoside (β-glucosidase), 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide (chitinase), leucine-aminomethylcoumarin (leucine amino-peptidase), 4-methylumbelliferyl phosphate acid (acid phosphatase), 4-methylumbelliferyl-B-D-xyloside (β-xylosidase). The plates were incubated in room temperature (20 °C) for 140min and reaction in the well was stopped using 10 μm 1M NaOH, except for leucine-aminopeptidase. Fluorescence was measured with a plate reader (BMGLabtech, ClarioStar) using excitation at 360 nm and emission at 460 nm. Quenched standard curves were built to each sample separately on 4-methylumbelliferone (MU), or 7-amino-4-methylcoumarin (AMC) for leucine amino-peptidase, by adding the same volume of soil slurry to each standard as was used in the enzyme activity assay. Enzymatic activities were expressed as nmol of MU/AMC product per 1g of soil dry mass per hour.

Oxidative enzymes (phenol oxidase EC 1.14.18.1 and peroxidase EC 1.11.1.x) were measured according to Marx et al. (2001). One ml of soil suspension (prepared as above) was mixed with 1 ml of 20 mM DOPA solution (L-3,4-dihydroxyphenylalanin) in sodium acetate buffer (100 mM, pH 5.5). As negative control 1 ml of sodium acetate buffer and 1 ml of 20 mM DOPA solution were prepared; blanks contained 1 ml of sodium acetate buffer and 1 ml of soil suspension. Samples and blanks were shaken 10 min and centrifuged (5 min, 2000g). Then, 250 μl aliquots were dispensed into 96-well microplates. For peroxidase activity, samples of soil received 10 μl of 0.3% H_2O_2. The absorption was measured at 450 nm (starting point) and plates were incubated in darkness for 20 h at 20 °C and after that time the absorption was measured again (ending point). Enzymatic activities were expressed as nmol of DOPA per 1g of soil dry mass per hour.

2.6. Data processing, calculations and statistical analysis

Critical C:N ratio for microbes was calculated by following the formula where the microbial biomass C:N ratio is multiplied with the ratio

of nitrogen use efficiency (NUE) over ^{13}C-CUE (Mooshammer et al., 2014) (Eq. (15)).

$$critical\ C:N = MBC:MBN \times \frac{NUE}{CUE} \quad (15)$$

For this calculation, a universal NUE value of 0.9 was used (Soong et al., 2020). Furthermore, we chose to use ^{13}C-CUE values instead of ^{18}O-CUE values because we assume that ^{13}C-CUE values are more substrate specific and would correspond with the universal NUE value used. The critical C:N ratio for microbes was calculated, and compared against soil bulk C:N ratio to assess the C and N limitation of microbes in terms of total bulk elements. Similarly, we compared the critical microbial N:P ratio against soil bulk N:P ratio using an estimate of 6.3–10 for critical microbial N:P from a metastudy by Čapek et al. (2018). When the soil C:N or N:P ratios exceeded the estimated critical C:N or N:P ratios, the growth of microbes was considered to be limited by the latter element.

In addition to critical C:N and N:P ratios versus bulk SOM, enzymatic ratios of ln (cellobiosidase)/ln (chitinase) and ln (cellobiosidase)/ln (acid phosphatase) representing C:N and C:P acquisition ratios, respectively, were also calculated because they represent SOM nutrient availability and microbial nutrient acquisition strategies in soils (Waring et al., 2014). The ratio of ln (cellobiosidase)/ln (chitinase + phosphatase) represents the C:NP acquisition ratio and was used as an indicator for labile C availability versus the need of microbes to invest in nutrient acquisition. Mineralization quotient (qM) was expressed as the fraction of total organic C mineralized throughout the ^{18}O-water tracing method's incubation time (Moscatelli et al., 2005).

Linear Pearson correlations between elevation, soil temperature, microbial CUE, qM and other soil properties were calculated in R program (R Core Team, 2019) with rcorr-function of the Hmisc package (Harrell and Dupont, 2017) and visualized using corrplot package (Wei and Simko, 2017). Correlations with statistical significance ($p \leq 0.05$) were marked with asterisks.

We partitioned the variance observed in ^{18}O-growth and ^{18}O-respiration, as well as in ^{13}C-growth and ^{13}C-respiration by soil stoichiometric controls (soil C:N and N:P), enzyme activities indicating labile-C degradation (cellulose, β-glucosidase, and β-xylanase), nutrient acquisition (chitinase and phosphatase), and lignin degradation (peroxidase and phenoloxidase). The variance partitioning of substrate uptake reflecting growth and respiration was conducted with varpart-function from vegan package (Oksanen et al., 2022) by having both measured variables as response data in the model. The statistical significance of variance partitioning analysis and fractions of interest from variance partitioning were assessed with rda-function combined with anova.cca-function from vegan package. All non-significant explanatory groups were excluded from the final model. We further partitioned separately the variance observed in growth and respiration with commonality analysis. The unique and total contribution of soil C:N and N:P, as well as labile-C degradation and nutrient acquisition related enzyme activities, to individual variation of ^{18}O-growth, ^{18}O-respiration, ^{13}C-growth and ^{13}C-respiration was conducted with commonalityCoefficients-function from yhat package (Nimon et al., 2008). To determine the statistical significance of each explanatory variable, regression analysis was performed with lm-function from stats package (R Core Team, 2019), and the level of statistical significance was set to $p \leq 0.05$.

3. Results

3.1. Soil properties along the altitudinal gradient

Soil chemical and biological properties of tropical forest soils occurring at Taita Hills varied significantly along its altitudinal gradient (Fig. 2, Table 1). The measured soil *in situ* temperatures decreased along the altitudinal gradient ($R = -0.97$, $P < 0.001$). Total soil C and total soil N concentrations increased with elevation ($P < 0.01$ for both, $R = 0.89$ for soil C and $R = 0.89$ for soil N). Conversely, total soil P concentration did not change along the altitudinal gradient. Soil C:N and N:P ratios increased with elevation ($R = 0.86$, $P < 0.001$ for soil C:N and $R = 0.64$, $P < 0.01$ for soil N:P). Similar to total soil C and N concentrations per gram of dry soil, MBC and MBN and the microbial C:N ratio also tented to increase with elevation. However, of these only MBC ($R = 0.42$, $P < 0.05$) and the MBC:MBN ratio ($R = 0.47$, $P < 0.05$) were significantly correlated to elevation. Microbial critical C:N varied from 6.2 to 15.38 and was in a similar range with the soil bulk C:N ratios (Table 1). However, the soil N:P at higher elevations (1705–2184 m a.sl.) varied between 13.3 and 24.7 and was over the universal critical N:P estimate of 6.3–10 (see Čapek et al., 2018), which indicates P limitation of microbial growth. In lower elevation sites the soil N:P value was under or equal to the universal critical N:P (937–1580 m a.s.l) (Table 1).

Elevation displayed a positive correlation with chitinase ($R = 0.59$, $P < 0.01$) and phosphatase ($R = 0.51$, $P < 0.05$) activities (Fig. 2, Table 2). Conversely, cellobiosidase and ratios of C:N, C:P and C:NP acquisition related enzyme activities displayed a negative correlation with elevation ($R = -0.56$, $P < 0.01$ for cellobiosidase, and $R = -0.79$, $P < 0.001$ for C:N, $R = -0.67$, $P < 0.001$ for C:P, and $R = -0.69$, $P < 0.001$ for C:NP acquisition related enzyme activity ratio, Fig. 2). Soil respiration measured during ^{18}O-CUE incubation increased with altitude ($R = 0.73$, $P < 0.001$) and also correlated positively with soil MBC ($R = 0.74$, $P < 0.001$) and soil C:N ratio ($R = 0.53$, $P < 0.05$) (**Supplementary Fig. 1**, **Supplementary Fig. 2**, **Supplementary Table 1**).

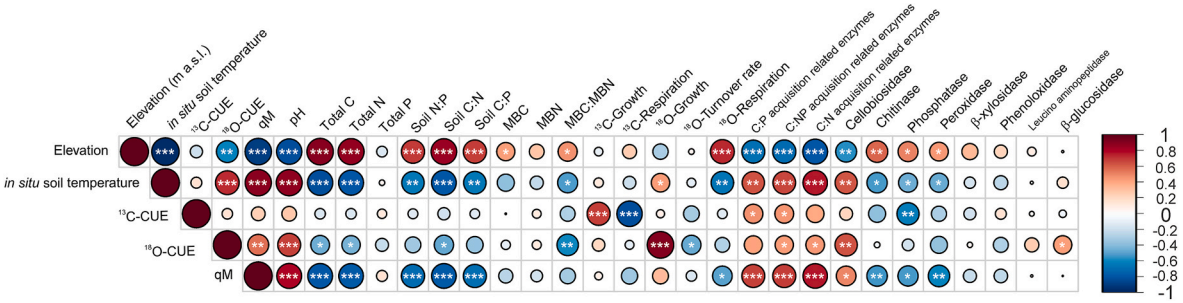

Fig. 2. Correlogram presenting the Pearson correlations between elevation, microbial CUE estimates and soil properties and soil enzymatic activies. Correlations with statistical significance are marked with asterisks; * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$. All statistically significant correlations are visualized in Supplementary Fig. 1, and individual R-values and p-values are presented in Supplementary Table 1. The microbial C:N acquisition ratio was calculated from enzyme activities as ln (cellobiosidase)/ln (chitinase), C:P acquisition ratio was calculated from enzyme activities as ln (cellobiosidase)/ln (phosphatase), and C:NP acquisition ratio was calculated from enzyme activities as ln (cellobiosidase)/ln (chitinase + phosphatase). MBC: microbial biomass C; MBN: microbial biomass N; ^{18}O-respiration, ^{18}O-growth and ^{18}O-turnover rate: soil respiration, growth and turnover in ^{18}O-method; ^{13}C-respiration and ^{13}C-growth: soil respiration and growth in ^{13}C-method; qM: mineralization quotient.

Table 1
Soil properties in each forest studied. Values are averages of three replicate sites ± standard deviation. Microbial biomass C (MBC), microbial biomass N (MBN), soil respiration and growth in ^{18}O-method (^{18}O-respiration and ^{18}O-growth), cumulative soil respiration and growth in ^{13}C-method (^{13}C-respiration and ^{13}C-growth) as well as soil P have been calculated per gram of dry soil. Critical C:N for microbes was calculated as soil MBC:MBN multiplied by NUE/^{13}C-CUE, where NUE is estimated to be 0.9 according to Soong et al. (2020).

	Vuria	Ngangao	Yale	Fururu	Macha	Dembwa	Semba
Elevation (m a.s.l.)	2184	1853	1873	1705	1580	1058	937
in situ soil temperature (°C)	12.98	14.5	14.74	15.99	16.37	19.98	23.92
MBC (mg C g^{-1})	2.28 ± 0.66	0.55 ± 0.18	0.82 ± 0.14	1.62 ± 0.50	0.93 ± 0.32	1.08 ± 0.12	0.73 ± 0.03
MBN (mg N g^{-1})	0.32 ± 0.07	0.09 ± 0.03	0.11 ± 0.02	0.29 ± 0.10	0.14 ± 0.03	0.17 ± 0.02	0.15 ± 0.02
MBC:MBN	7.1 ± 0.4	6.7 ± 0.7	7.4 ± 0.5	5.7 ± 0.6	6.4 ± 0.4	6.5 ± 0.1	5.0 ± 0.4
^{18}O-Respiration (μg g^{-1} h^{-1})	3.45 ± 0.32	1.91 ± 0.13	2.26 ± 0.15	2.72 ± 0.20	1.87 ± 0.01	1.62 ± 0.02	1.55 ± 0.02
^{18}O-Growth (μg g^{-1} h^{-1})	0.24 ± 0.03	0.08 ± 0.01	0.27 ± 0.02	0.38 ± 0.07	0.10 ± 0.06	0.20 ± 0.3	0.47 ± 0.07
^{18}O-CUE	0.06 ± 0.002	0.04 ± 0.005	0.11 ± 0.010	0.12 ± 0.023	0.05 ± 0.028	0.11 ± 0.014	0.23 ± 0.028
^{13}C-CUE	0.67 ± 0.14	0.69 ± 0.03	0.57 ± 0.04	0.66 ± 0.03	0.57 ± 0.07	0.74 ± 0.06	0.65 ± 0.09
Cumulative ^{13}C-Growth (μg ^{13}C g^{-1})	26.6 ± 7.16	25.6 ± 2.14	24.0 ± 1.46	20.4 ± 2.06	23.0 ± 4.78	26.7 ± 4.01	26.3 ± 11.72
Cumulative ^{13}C-Respiration (μg ^{13}C g^{-1})	12.87 ± 4.95	11.28 ± 1.48	18.33 ± 3.23	10.55 ± 0.59	16.72 ± 1.86	8.92 ± 1.41	12.86 ± 0.87
Soil P (mg g^{-1})	1.09 ± 0.008	0.34 ± 0.011	0.46 ± 0.111	0.75 ± 0.072	1.24 ± 0.095	1.27 ± 0.282	0.57 ± 0.065
Soil pH	4.1 ± 0.12	3.8 ± 0.04	3.1 ± 0.10	4.6 ± 0.19	4.1 ± 0.06	5.4 ± 0.07	6.2 ± 0.08
Total C (%)	18.6 ± 0.95	10.1 ± 0.96	9.6 ± 0.40	10.3 ± 0.82	8.6 ± 0.62	3.7 ± 0.04	3.6 ± 0.28
Total N (%)	1.59 ± 0.06	0.83 ± 0.07	0.82 ± 0.02	0.99 ± 0.07	0.87 ± 0.09	0.42 ± 0.01	0.39 ± 0.02
Soil C:N ratio	11.7 ± 0.18	12.2 ± 0.28	11.6 ± 0.33	10.4 ± 0.15	10.0 ± 0.28	8.9 ± 0.13	9.4 ± 0.15
Soil N:P ratio	14.56 ± 1.16	24.69 ± 4.85	18.59 ± 2.64	13.28 ± 2.64	6.99 ± 0.89	3.37 ± 0.58	6.86 ± 1.39
Critical C:N	11.17 ± 3.71	9.70 ± 1.95	13.04 ± 1.77	8.62 ± 2.15	11.26 ± 2.63	8.72 ± 0.76	7.93 ± 2.64

Table 2
Soil enzyme activities in different forests studied. Values are average of three replicate sites ± standard deviation. Enzymatic activities are expressed as nmol of MU/AMC/DOPA product per gram of soil dry weight per hour.

Forest Site	Elevation (m a.s.l.)	Leucine aminopeptidase (nmol g^{-1} h^{-1})	Chitinase (nmol g^{-1} h^{-1})	β-glucosidase (nmol g^{-1} h^{-1})	Cellobiosidase (nmol g^{-1} h^{-1})	Acid phosphatase (nmol g^{-1} h^{-1})	Peroxidase (nmol g^{-1} h^{-1})
Vuria	2184	326.1 ± 30.9	335.4 ± 55.2	284.8 ± 42.4	32.0 ± 8.1	1201.2 ± 170.8	2.3 ± 0.80
Ngangao	1853	77.3 ± 8.5	264.8 ± 33.1	141.5 ± 17.9	34.0 ± 3.3	1320.4 ± 22.6	3.4 ± 1.53
Yale	1873	93.8 ± 8.2	491.1 ± 69.3	134.8 ± 19.2	11.9 ± 1.2	2884.3 ± 196.4	2.7 ± 0.43
Fururu	1705	650.0 ± 98.6	480.7 ± 59.4	216.8 ± 36.1	68.2 ± 8.1	1232.9 ± 106.5	3.6 ± 1.02
Macha	1580	111.8 ± 3.4	195.2 ± 30.0	159.9 ± 8.5	25.3 ± 3.1	1600.9 ± 156.5	3.9 ± 0.55
Dembwa	1058	93.2 ± 12.3	76.4 ± 12.2	148.0 ± 24.9	50.8 ± 9.2	224.5 ± 15.3	0.3 ± 0.02
Semba	937	303.8 ± 32.6	169.8 ± 50.2	274.3 ± 36.3	82.5 ± 11.3	982.5 ± 99.8	0.9 ± 0.63

3.2. CUE and its determining factors along the gradient

The two methods used for estimating microbial CUE provided contradictory answers to our main question and hypothesis on the relationship between CUE and the elevation, and thus SOM nutrient availability (Fig. 2, Supplementary Figs. 1–3, Supplementary Table 1).

Microbial CUE determined by the ^{13}C-glucose tracing method did not display significant correlation with elevation or other soil properties such as nutrient availability, other than acid phosphatase activity ($R = -0.63$, $P < 0.01$) and ratios of C:P ($R = 0.47$, $P < 0.05$) and C:NP ($R = 0.45$, $P < 0.05$) acquisition related enzymes (Fig. 2).

The ^{18}O-CUE decreased with increasing elevation ($R = -0.60$, $P < 0.01$), and thus displayed a positive correlation with *in situ* soil temperature ($R = 0.72$, $P < 0.001$) and soil pH ($R = 0.67$, $P < 0.001$) (Fig. 2). Microbial ^{18}O-CUE also correlated with variables, which indicate better SOM nutrient availability, correlating positively with mineralization quotient qM ($R = 0.55$, $P < 0.01$), cellobiosidase activity ($R = 0.63$, $P < 0.01$), and C:N and C:NP acquisition ratios ($R = 0.4$, $P < 0.05$ for both, weak correlation), and negatively correlating with soil C:N ($R = -0.47$, $P < 0.05$) and microbial C:N ($R = -0.59$, $P < 0.01$) ratios (Fig. 2).

3.3. Persistence of SOM along the gradient

Higher altitude soils, with high C and N concentrations and low pH, also had low qM values indicating more persistent SOM (Fig. 2, Supplementary Fig. 1, Supplementary Table 1). The qM index describing organic C mineralization correlated positively with *in situ* soil temperature ($R = 0.88$, $P < 0.001$), pH ($R = 0.76$, $P < 0.001$) and cellobiohydrolase activity ($R = 0.49$, $P < 0.05$). On the other hand, qM correlated negatively with elevation ($R = -0.89$, $P < 0.001$), total soil C and N concentrations ($R = -0.8$, $P < 0.001$ for both), soil C:N and N:P ratios ($R = -0.78$, $P < 0.001$ for soil C:N and $R = -0.67$, $P < 0.001$ for soil N:P), and acid phosphatase ($R = 0.52$, $P < 0.05$) and peroxidase ($R = 0.59$, $P < 0.01$) activities.

3.4. Linkage between soil properties and CUE along the gradient

For partitioning the variation in measured ^{18}O-growth and ^{18}O-respiration, and ^{13}C-growth and ^{13}C-respiration, we utilized four categories of variables: stoichiometric controls, easily utilizable C degradation related enzyme activities, nutrient acquisition related enzyme activities, and lignin degradation related enzyme activities. In the case of ^{18}O-uptake related variables, ^{18}O-growth and ^{18}O-respiration, in total 66% of the variation could be explained with variables from these four categories. From these, stoichiometric controls alone explained only 2% of the observed variation, whereas easily utilizable C degradation related enzyme activities alone explained 4% of the variation (Fig. 3). The interactive effect of stoichiometric controls and easily utilizable C degradation related enzymes explained 27% of the variation with statistical significance ($P < 0.01$). The enzyme activities related to nutrient acquisition from SOM alone explained 25% of the variation ($P < 0.01$). Lignin degradation related enzyme activities did not explain the observed variation with statistical significance and were omitted from the model. In case of ^{13}C-uptake related variables, ^{13}C-growth and ^{13}C-respiration, the variation could not be explained with statistical significance by the explanatory variables from the four selected categories, and thus the results of the model are not shown here.

When analyzing the effect of soil stoichiometry and C degradation

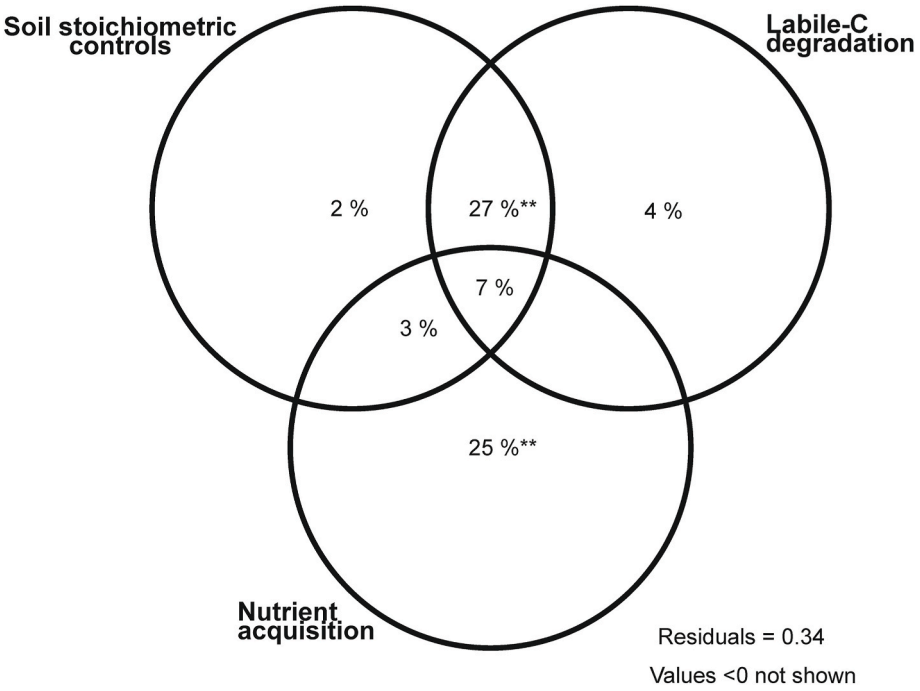

Fig. 3. Visualized variance partitioning of ^{18}O-growth and ^{18}O-respiration, where three categories of variables are utilized: stoichiometric controls (soil C:N, soil N:P), labile C degradation related enzyme activities (β-glucosidase, cellobiosidase, β-xylosidase) and nutrient acquisition related enzyme activities (chitinase, phosphatase). Statistical significance of the explained variance is marked with asterisks (**$p < 0.01$).

and nutrient acquisition related enzyme activities to independent variance of ^{18}O and ^{13}C respiration and growth, the selected variables explained 77.8% of the observed variation in ^{18}O-respiration, 61.4% in ^{18}O-growth, 65.7% in ^{13}C-respiration, and 25.0% in ^{13}C-growth (Table 3, Supplementary Table 2). The variance in ^{18}O-respiration was mainly explained by enzyme activities related to nutrient acquisition from SOM (i.e. chitinase and phosphatase), which had unique contributions of 27.7% and 15.0% to the explained variance, respectively ($p < 0.01$ for chitinase and $p < 0.05$ for phosphatase with regression analysis). Soil stoichiometric controls and easily utilizable C degradation related β-glucosidase and β-xylosidase contributed to 14.2–45.2% of the explained variance of ^{18}O-respiration, although their unique contributions were small and statistically insignificant. Although, all the selected variables had small unique effect on the variance in ^{18}O-growth, and were statistically insignificant, the total contribution of easily utilizable C degradation related enzymes, β-glucosidase and cellobiosidase, contributed of 55.5% and 65.3% of the explained variance. Soil C:N ratio contributed the highest unique proportion of 9.3% of the explained variance in ^{18}O-growth. The variance in ^{13}C-respiration was mainly explained by phosphatase activity, which contributed to 78.1% of the explained variance ($p < 0.01$ with regression analysis).

4. Discussion

To our knowledge, there are a limited number of studies that have investigated how soil chemistry affects microbial CUE in tropical forest soils (Kaspari et al., 2008; Fisher et al., 2013; Nottingham et al., 2018). Here, we studied how soil nutrient availability changes in tropical

Table 3
Variance partitioning analysis (commonality analysis) output representing commonality coefficients, unique, common and total contribution of each predictor variable to the regression effect. Total contribution of the predictor variable to observed variation in 18O-respiration, 18O-growth, 13C-respiration, and 13C-growth was calculated as sum of unique and common coefficients. The proportion of variance explained by the predictor uniquely and in total is presented in parenthesis as % of R2, which was calculated as Unique/R2*100 and Total/R2*100, respectively. Output presenting the partitioning of the regression effects in detail is presented in Supplementary Table 2.

		R^2	Soil C:N	Soil N:P	β-glucosidase	Cellobiosidase	β-xylosidase	Chitinase	Phosphatase
^{18}O-Respiration		0.7783							
	Unique		0.0355 (4.6%)	0.0468 (6.0%)	0.0007 (0.1%)	0.0229 (2.9%)	0.0395 (5.1%)	0.2156 (27.7%)	0.1165 (15.0%)
	Common		0.2472	0.0643	0.1854	−0.0134	0.3124	0.1307	−0.0752
	Total		0.2827 (36.3%)	0.1111 (14.3%)	0.1861 (23.9%)	0.0095 (1.2%)	0.3519 (45.2%)	0.3463 (44.5%)	0.0413 (5.3%)
^{18}O-Growth		0.6144							
	Unique		0.0569 (9.3%)	0.0228 (3.7%)	0.0472 (7.7%)	0.0055 (0.9%)	0.0014 (0.2%)	0.0261 (4.2%)	0.0075 (1.2%)
	Common		0.0141	0.0019	0.2937	0.396	0.026	0.0518	−0.0043
	Total		0.071 (11.6%)	0.0247 (4.0%)	0.3409 (55.5%)	0.4015 (65.3%)	0.0274 (4.5%)	0.0779 (12.7%)	0.0032 (0.5%)
^{13}C-Respiration		0.6586							
	Unique		0.0101 (1.5%)	0.0034 (0.5%)	0.039 (5.9%)	0.0279 (4.2%)	0.0007 (0.1%)	0.028 (4.3%)	0.2617 (39.7%)
	Common		0.0846	0.0389	−0.0384	0.1604	0.0425	0.0662	0.2527
	Total		0.0947 (14.4%)	0.0423 (6.4%)	0.0006 (0.1%)	0.1883 (28.6%)	0.0432 (6.6%)	0.0942 (14.3%)	0.5144 (78.1%)
^{13}C-Growth		0.2499							
	Unique		0.0015 (0.6%)	0.0199 (8.0%)	0.0671 (26.9%)	0.0434 (17.4%)	0.0211 (8.4%)	0.0779 (31.2%)	0.0091 (3.6%)
	Common		−0.0013	−0.0192	−0.067	−0.0329	−0.021	0.0409	0.0448
	Total		0.0002 (0.1%)	0.0007 (0.3%)	0.0001 (0.04%)	0.0105 (4.2%)	0.0001 (0.04%)	0.1188 (47.5%)	0.0539 (21.6%)

forests along a temperature and altitudinal gradient in the Taita Hills, Kenya, and how this affects microbial CUE estimates. In our gradient, *in situ* soil temperatures declined with altitude. We assume that the different *in situ* soil temperatures have shaped the soil chemistry, microbial community functionality and other soil properties, which can be seen as a legacy effect in our CUE incubations.

4.1. Nutrient availability along Kenyan Taita Hills gradient

We hypothesized that the soil nutrient availability would decrease with elevation because of the colder climate in higher elevations. For assessing the soil nutrient availability, we utilized soil stoichiometric controls, i.e. soil C:N, soil C:P and soil N:P ratios, as well as nutrient and C acquisition related enzyme activities and their ratios. The soil bulk chemistry indicated that the studied soils were not significantly limited by the bulk N availability. In fact, the soil C:N in comparison to critical C:N suggests the microbes may have been primarily C limited along the gradient (Soong et al., 2020). Soil bulk P limitation seems to increase with elevation based on comparison of our soil N:P ratios to the critical N:P estimate by Čapek et al. (2018). This finding is similar to previous studies from tropical elevation gradients from the Peruvian Andes (Nottingham et al., 2015) but contrary to the general assumption that tropical montane forests are N-limited and lowland forests P-limited (Fisher et al., 2013).

Activities of the extracellular enzymes chitinase and phosphatase, involved in the N and P cycling, respectively, increased with elevation. Furthermore, the enzymatic C:N and C:P acquisition ratios decreased with elevation. These results suggest that even though the bulk soil stoichiometry did not indicate significant nutrient limitation along our gradient, microbes might be experiencing higher C:nutrient ratios than simple bulk chemistry indicates with soil nutrients being stored in more chemically complex forms at higher elevations. Similarly, mineralization quotient, which represents the fraction of total organic C mineralized throughout the incubation time, decreased with elevation and soil bulk C:N and N:P ratios in our gradient. The mineralization quotient can be used as an indicator of SOM persistence against microbial degradation activity (Moscatelli et al., 2005). Together the results from enzymatic C:N and C:P acquisition ratios and mineralization quotient suggest that the quality of SOM in Taita Hills forest soils occurring at higher elevations is characterized by lower availability of nutrients than those located at lower elevations. Nonetheless, the microbes were likely primarily C limited across the whole gradient and were only secondarily limited by the availability of P and N (Soong et al., 2020).

4.2. Microbial CUE along altitudinal gradient

Numerous studies have demonstrated that microbial CUE declines with decreasing nutrient availability (e.g. Manzoni et al., 2012; Blagodatskaya et al., 2014; Zheng et al., 2019). According to the theory of ecological stoichiometry, microbial CUE strongly depends on the relative availability of C and nutrients, particularly N and P (Sterner and Elser, 2003; Manzoni et al., 2012, 2017), and is expected to increase along gradients where soil C:N ratio decreases and nutrient availability increases (Takriti et al., 2018). Since build-up of new biomass requires synthesis of N and P containing structural molecules, such as proteins, DNA and RNA (Soong et al., 2020), we suspect that this secondary limitation in nutrient availability at higher elevations might cause an imbalance between supply and microbial demand and may have lowered microbial growth in our gradient.

Along our gradient, microbial CUE as estimated by the ^{18}O-tracing method displayed a clear decrease with elevation, but CUE as estimated by the ^{13}C-method did not change with elevation. In fact, in the variance partitioning, ^{18}O-growth and ^{18}O-respiration could be explained relatively well with soil properties, but ^{13}C-respiration was explained mainly by phosphatase activity and ^{13}C-growth was not significantly affected by soil properties. There is a possibility that the different pre-incubation times in ^{13}C and ^{18}O methods might contribute to the differences between the ^{13}C-CUE and ^{18}O-CUE patterns observed in this study, because the two-week pre-incubation prior to ^{18}O-CUE analysis could have depleted the amount of labile-C in the soil and changed the soil microbial community from its original composition. However, based on the method evaluation paper by Schroeder et al. (2021), the effect of two-week pre-incubation most likely had a similar effect on all the studied soils and did not contribute significantly to the different patterns of ^{13}C-CUE and ^{18}O-CUE of this study. Although the concepts for estimating CUE using the ^{13}C and ^{18}O methods are similar, there are some critical differences discussed by Geyer et al. (2019) and Hu et al. (2022), which most likely contributed to the different ^{13}C-CUE and ^{18}O-CUE patterns in our study.

We observed high ^{13}C-glucose CUE values, which indicate balanced microbial growth (Dijkstra et al., 2015). Typically, glucose is easily taken up by microbes and does not require extracellular enzymes for biodegradation, leading to incorporation of ^{13}C-label to microbial biomass within a short duration, and favoring fast growing *r*-strategists over slow growing *K*-strategists. In our gradient the soil microbial populations were most likely diverse with both fast growing *r*-strategists, which are able to allocate the excess C to their growth, as well as slow growing *K*-strategists which typically allocate the excess C to the production of SOM degradation related enzymes (Manzoni et al., 2012; Bonner et al., 2018). Thus, since glucose is easily utilized by microbes, the results of the ^{13}C-glucose method in our gradient might reflect CUE of the soil *r*-strategists rather than the total community involved in native SOM decomposition. Still, weak positive correlation between ^{13}C-CUE and C:P acquisition related enzymes, and negative correlation with phosphatase activity suggest that limitation in P availability might have shaped the ^{13}C-CUE values of glucose along our gradient.

The microbial ^{18}O-CUE was correlated significantly with soil properties, such as C:N ratio, pH and nutrient availability along our gradient, corroborating the results of previous studies (Silva-Sánchez et al., 2019; Soares and Rousk, 2019). Especially, SOM nutrient availability is found to regulate variation of microbial CUE, and soils containing high proportions of easily degradable carbohydrates and proteins in the SOM are proposed to have higher microbial CUE than those with more recalcitrant compounds (Manzoni et al., 2012; Geyer et al., 2019; Qiao et al., 2019), since poor substrate quality associated with recalcitrant compounds require microbes to invest more energy to enzyme production (Manzoni, 2017). Additionally, Soares and Rousk (2019), found that when the recalcitrance of the available substrates increases (i.e. soil C quality decreases) and the abiotic factors change (pH, soil temperature, soil moisture), the overall microbial community structure might change significantly. Thus, lower microbial ^{18}O-CUE in higher elevations of our gradient might indicate a shift between fast and slow growing microbes due to the legacy effect of different *in situ* temperatures or by the different litter qualities between the high and low elevation sites (Keiblinger et al., 2010; Wetterstedt and Ågren, 2011).

We found that along our gradient SOM nutrient availability (as indicated by extracellular enzyme activity and qM) and soil pH were more strongly correlated with microbial ^{18}O-CUE than soil stoichiometric controls. This finding was similar to a study from a wide latitudinal gradient in western Siberia by Takriti et al. (2018). There, the range of soil C:N ratios was wider than in our study, but still SOM quality and pH were stronger determinants for substrate use efficiency than stoichiometry. Other studies have also demonstrated a strong link between SOM quality and microbial CUE (Manzoni, 2017; Soares and Rousk, 2019). These results support the view that soil C:N ratios alone are not always good predictors of microbial CUE (Sinsabaugh et al., 2016). However, other studies have demonstrated that the effect of N-availability becomes more evident in soils with comparable SOM properties (e.g. Silva-Sánchez et al., 2019; Soares and Rousk, 2019).

Based on our variance partitioning analyses, labile-C degradation related enzyme activities were more associated to microbial growth on SOM, whereas nutrient availability of the substrate, as reflected by

chitinase and phosphatase activities, was positively associated to microbial ^{18}O-respiration. Soil C:N ratio correlated negatively with ^{18}O-CUE and positively with ^{18}O-respiration along the altitude gradient. Soil C:N ratio contributed to explaining the observed variation both in microbial respiration and growth. A recent meta-analysis by Hu et al. (2022) indicates that N addition causes increases in microbial ^{18}O-growth rate, and thus increases in ^{18}O-CUE estimates, which may have been the case in also our gradient. Our results from variance partitioning also support the *microbial carbon pump* theory where labile C is easily taken up by the microbes and transformed into microbial biomass through microbial growth, whereas modification of recalcitrant lignin compounds and scavenging SOM for nutrients causes CO_2 emissions from soil (Liang et al., 2017). However, some of these CO_2 fluxes can also originate from extracellular enzyme activity, and thus may not be directly correlated with microbial growth and microbial CUE. We suggest that the low ^{18}O-CUE in higher elevations of our gradient indicates greater need of microbes to allocate energy for nutrient acquisition related enzyme production than for growth compared to microbes at low elevations.

5. Conclusions

Based on the soil bulk chemistry, the critical C:N ratio did not differ from the soil C:N ratio along the Kenyan Taita Hills gradient, but based on the soil N:P ratio, soil bulk P availability decreased slightly with elevation. Additionally, the gradient in extracellular enzyme activities reveal that microbes at higher elevations need to invest more energy to scavenging nutrients and energy from complex SOM than at lower elevations. We assume that the different *in situ* soil temperatures along our gradient have shaped the soil chemical properties and microbial community's functionality, which could be seen as a legacy effect in our CUE incubations. Microbial CUE estimates were variable between the two methods used (^{18}O and ^{13}C-glucose tracing), but within the ranges reported in previously published research. The ^{13}C-CUE did not change between studied soils, likely because glucose was efficiently taken up and used by fast growing microbes. On the other hand, ^{18}O-CUE, which reflects the growth efficiency of microbes growing on native SOM, clearly declined with increasing altitude and negatively correlated with complex SOM degradation related extracellular enzyme activities. We suggest that low ^{18}O-CUE at higher elevations of our gradient indicates greater need for modification of SOM for nutrients and energy, whereas at lower elevations soil nutrients might have been more readily available. Our results highlight the need to investigate a wide variety of high-altitude tropical soils in order to better understand and predict how the changing climate will affect C and nutrient cycling.

Declaration of competing interest

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

Data availability

The data is published now via Zenodo (doi:10.5281/zenodo.6940625).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2022.108799.

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