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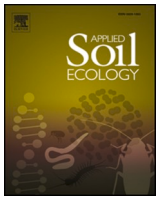

Microbial carbon use efficiency and soil organic carbon stocks across an elevational gradient in the Peruvian Andes

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ABSTRACT

Soils of mountain ecosystems are one of the most vulnerable ecosystems to climate change, while the ecosystem services they produce are significant and currently at risk. High altitude soils contain high C stocks, but due to difficult access to sites these areas are understudied. Moreover, how the C and N cycling is changing in response to climate change in these ecosystems, is still unclear. Microbial carbon use efficiency (CUE) and its dependency on the environmental constraints along the altitudinal gradients is one important unknown factor. Here we present results from an altitudinal gradient study (3500 to 4500 m a.s.l.) from a *Polylepis* forest in the Peruvian Andes. We measured the soil organic carbon (SOC) stocks and microbial metabolic CUE by ^{13}C glucose tracing and microbial resource use efficiency ($CUE_{C:N}$) based on enzyme activity measurements. We expected to find an increase in SOC stock, microbial nutrient limitations, and lower CUE with elevation. SOC stocks depended on soil development and followed a unimodal curve that peaks at 4000 m in two of the three studied valleys. Neither ^{13}CUE nor $CUE_{C:N}$ changed significantly with altitude. Soil C:N ratio, β-glucosidase, chitinase, and phosphatase enzyme activities increased with elevation, but peroxidase activity decreased with elevation. We suggest that more labile organic matter left at high elevation could compensate for the increasing nutrient limitation at high elevation, resulting in no noticeable change in CUE with elevation.

1. Introduction

Soils constitute the largest stock of terrestrial organic carbon (C) with 1500 Pg C in the top meter (Scharlemann et al., 2014). Tropical forest encompasses 42 % of land area in the world and stores 34 to 55 % of global ecosystem C stocks (Pan et al., 2011; Beer et al., 2010). Tropical forest soils store 151 Pg C corresponding to 10 % of global soil C stocks (Pan et al., 2011). Increasing temperatures have already disproportionately affected cold areas (Wan et al., 2015). These high altitude, artic and temperate regions are predicted to experience higher than average warming due to climate change (Hoegh-Guldberg et al., 2018; Vuille et al., 2015). Low temperatures contribute to slow soil organic matter (SOM) decomposition rates and accumulation of relatively labile C in the organic soil layers (Simon et al., 2018). Thus, soils at these cold areas may be more vulnerable to C losses because of increased warming, and because the temperature sensitivity of SOM decomposition has been shown to be even higher in colder soils (Karhu et al., 2014; Frey et al., 2013). Although, the magnitude of warming in high latitude and high-altitude areas are comparable (Vuille et al., 2008), much less research is conducted on high altitude soils, such as the Andes.

South American Andes have two distinctive landscapes, a cold high western side facing the Pacific Ocean, called Puna, and the eastern slope that includes high cloudy and low rainy Amazon Forest. Soils in the Amazon are strongly weathered, while the western Puna Andean soils

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are poorly developed (Portes et al., 2016; Girardin et al., 2013). In Peru, C inventory studies have mainly focused on the eastern Amazon region, while the western Puna region of the Andes has received much less attention (Vásquez et al., 2014), even though these ecosystems can also contain large C stocks (Gibbon et al., 2010; Rolando et al., 2017; Vásquez et al., 2014; Wilcox, 1984). By the end of the century, projections based on SRES A2-IPCC indicate that the mean annual air temperature in the tropical Andes may increase by 4.5–5 °C (Vuille et al., 2008). Therefore, it is important to estimate C stocks and vulnerability of the Andean soils, especially because global projections of C models are not applicable to these ecosystems with complex topography and scarce data (Vuille et al., 2008).

Polylepis is an autochthonous plant genus of the Andes adapted to grow at higher and colder conditions (Kessler et al., 2014) reaching up to 5000 m a.s.l. (Lipton, 2008). These forests have been threatened by land use and climate warming (Caballero-Villalobos et al., 2021; Kessler et al., 2014; Toivonen et al., 2017), and <10 % of its original area remains in Peru and Bolivia (Fjeldså & Kessler 1996 cited by Zutta et al., 2012). *Polylepis* forests are important for improving water catchment, infiltration, and storage (Kessler et al., 2014) and harbour a great biodiversity of endemic fauna and flora. The magnitude and importance of the soil organic carbon (SOC) stocks in these forest sites have been recognized (Caballero-Villalobos et al., 2021; Hertel and Wesche, 2008; Vásquez et al., 2014). Despite their ecological importance, it remains unclear how SOC stocks change along the elevation gradient in these ecosystems (Kessler et al., 2014; Sevillano-Ríos and Rodewald, 2017; Vásquez et al., 2014). Furthermore, the vulnerability of SOC stocks to climate warming is uncertain. In this context, a gradient study can contribute to understanding how soil C decomposition responds to global warming (Niu et al., 2019; Simon et al., 2018).

The microbial metabolism has received special attention recently (Liang et al., 2017; Manzoni et al., 2012; Six et al., 2006), but microbial controls on the formation and decomposition of SOM remain unclear (Schimel and Schaeffer, 2012). Carbon use efficiency (CUE) is the amount of C taken up by microbes to build up new microbial biomass relative to the C taken up and it is often regulated by substrate availability, environmental and ecological factors (Sinsabaugh et al., 2017). CUE is found to be correlated to nutrient availability (declining with increasing SOM C:N and C:P ratios). It could be expected that, since soils at high altitudes are relatively more nutrient-limited, CUE could be decreasing with altitude because microbes at high altitudes need to invest more energy to scavenge nutrients and energy compared to low altitudes (Mganga et al., 2022; Zheng et al., 2018; Liang et al., 2017; Manzoni et al., 2012). However, this may be site specific and dependent on the main nutrient limiting microbial growth. Low P availability is more common in recently weathered western Andean soils at high altitudes (Girardin et al., 2013) compared to the Amazonian high lands. In the Amazonian eastern slope of the Andes, soil microbial communities at high elevations (above 3000 m a.s.l.) showed higher CUE and were more N-limited than lowlands (200 m a.s.l.), which are prone to P limitations and lower CUE (Whitaker et al., 2014a).

To calculate CUE, microbial growth and respiration are often measured by microbial response to added substrates. These microbial indicators are obtained by laboratory soil incubations, monitoring of microbial respiration, extraction of soil microbial biomass, and isotope tracing of ^{13}C labelled substrates or ^{18}O-water tracing. Similarly, these indicators can also be estimated by ecological stoichiometry theory (Geyer et al., 2019; Sinsabaugh et al., 2016). Microbial stoichiometry depends on soil C, N and P contents. The stoichiometric imbalance occurs when the substrate has lower N or P contents or higher C:N or C:P ratios than the microbial critical ratio or threshold elemental ratio (Mooshammer et al., 2014; Sinsabaugh et al., 2016). It can either inhibit energy investment and nutrient acquisition without affecting the CUE, or CUE could decline because microorganisms will produce more extracellular enzymes to make nutrients available from SOM with an energy cost and overflow respiration (Manzoni et al., 2012). Therefore, the stoichiometric approach can contribute to the understanding of the interactions of SOM C:N:P ratios and the soil microbial CUE (Cleveland and Liptzin, 2007; Sinsabaugh et al., 2016).

The aim of this study was to estimate SOC stocks of the organic soil layer along an altitudinal gradient on the western side of Andes and determine whether there is a link between microbial CUE and SOC stocks. We also aimed to identify key soil variables that best explain the variability in microbial CUE along the gradient. We investigated SOC stocks in *Polylepis* forest patches along an elevational gradient (3500 to 4500 m asl) in three valleys located in the north of the *Huascarán* National Park: Paron, Llanganuco and Ulta. We determined CUE by ^{13}C-glucose tracing method and by the stoichiometric approach, total concentrations of C, N, P as indicators of C:N:P stoichiometry of the soil, and enzyme activities as indicators of microbial availability of C and nutrients. Specifically, we hypothesized that: (1) Soils at high elevations have larger SOC stocks, (2) soil N and P availability decreases with elevation, and (3) CUE decreases with elevation since we expect that nutrient availability is controlling CUE along this gradient.

2. Materials and methods

2.1. Study sites and field sampling

Soils were obtained from *Huascarán* National Park (8.7°–10.0°S, 77.1°–77.8°W) (SERNANPE, 2010) in May 2019, between the dry (May–September) and wet season (October–April). MAP ranges from 600 to 1000 mm increasing with elevation (INRENA, 2003). These soils are poorly developed and classified, according to WRB, as Leptosols (Gardi et al., 2015) below 4000 m and Umbrisols above 4000 m (Portes et al., 2016). In general, Andean highlands experience high diurnal temperature variation and low thermal seasonality. MAT is strongly related to elevation, from 8 °C at 3500–4000 m a.s.l. to 3 °C at 4800 m a.s.l. (Mateo et al., 2022; Caballero-Villalobos et al., 2021; Portes et al., 2016). Since meteorological data is very limited in the studied sites, we used elevation as a proxy for MAT.

Soil samples were collected in three valleys: Paron, Llanganuco and Ulta (Fig. 1). In each valley, five elevations at 3500, 3750, 4000, 4250 and 4500 m a.s.l. were selected. At each elevation, three plots of ca. 0.01 ha were established within a selected *Polylepis* forest according to their similar Southwest facing aspect using Satellite imagery of 30 m resolution (courtesy of the U.S. Geological Survey) processed with QGIS 3.4.1 & Grass GIS 7.4.2. Distance between each *Polylepis* forest patch was 30 m. We focused on the soil organic horizons because they contain higher values of organic C and were present across all elevations (Portes et al., 2016).

At each plot, 20 soil cores were collected using simple random sampling representing the organic layer. Sampling depth was limited by presence of stones and boulders (Portes et al., 2016). Average depth recordings were used in the calculation of SOC stocks. Bulk density (Mg m^{-3}) was determined from two Kopecky cores as described in Hao et al. (2008). In situ soil temperature was measured during sampling at 5 cm depth using Tinytag Data Loggers (Plus 2 –TGP-4017).

After sampling, soils were sieved through a 4.7 -mm mesh, and stored at 5 °C in re-sealable plastic bags until analysis.

2.2. Abiotic soil properties

Total soil C and N were determined (dried at 60 °C ground soils) using a CN828 Elemental Analyzer (LECO, USA). We measured, Total Phosphorus (P) from 0.2 g of dried at 60 °C and ground soils, by nitric acidic digestion (EPA 3051A Method 6020), followed by ICP-OES spectroscopy (Thermo Scientic iCap 6000), where aluminium (Al) and calcium (Ca) were also obtained. Soil mineral nitrogen was extracted from fresh soils (soil to 1 M KCl, 1 to 10 ratio, v:v), as in Kalu et al. (2021). Finally, SOC stock (Mg ha^{-1}) was the total soil C content per hectare in the average sampled depth, calculated by multiplying total C

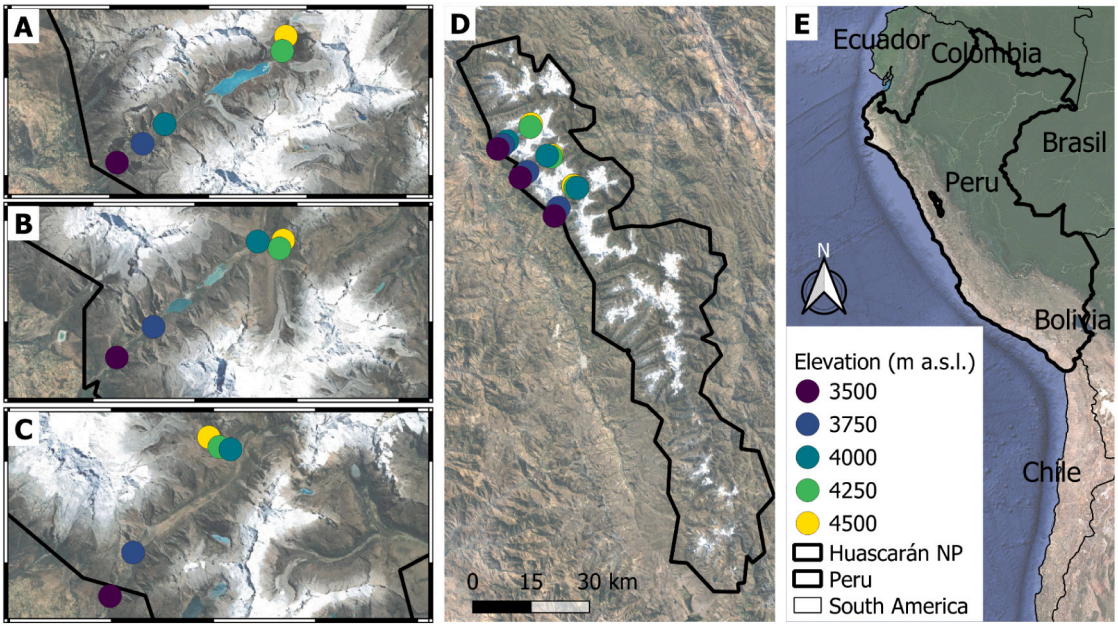

Fig. 1. Study sites and elevations in the *Huascarán* national park. A. Paron, B. Llanganuco, C. Ulta valleys. A, B and C map frames are at 5 km intervals. D. *Parque Nacional Huascarán* (PNH) border and E. Perú border. Map Data: © 2022 NASA/TerraMetrics, © 2022 Google.

(%), area (10,000 m^2), bulk density (Mg m^{-3}) and soil depth (m). Gravimetric moisture content and water holding capacity (WHC) were determined similar to Whitaker et al. (2014a). Soil pH (soil: H_2O, 1:5 v: v) was measured with a glass electrode (Sentix® 81, INOLab pH Level 1, WTW Germany).

2.3. Potential soil enzyme activities

Enzymatic activities were measured from 2 g fresh soil homogenised in a slurry with 100 mM sodium acetate buffer pH 5.5. The activities of phenol oxidase (EC 1.11.1.7) and peroxidase (EC 1.14.18.1) were measured spectrophotometrically (Marx et al., 2001). The absorption was measured (BMGLabtech, ClarioStar) 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 1 g of dry mass per hour.

The activities of acid phosphatase (EC 3.1.3.2), chitinase (EC 3.2.1.14), β-glucosidase (EC 3.2.1.21), β-xylosidase (EC 3.2.1.37), cellobiohydrolase (EC 3.2.1.91) were measured using fluorometric substrates (Sigma) (Bell et al., 2013). Fluorescence was measured with a ClarioStar (BMGLabtech, Germany) plate reader using excitation at 355 nm and emission at 460 nm. The standard curve was based on 4-methylumbelliferone (MU) or 7-amino-4-methylcoumarin (AMC). Enzymatic activities were expressed as nmol of MU or AMC per 1 g of dry mass per hour.

Finally, we calculated the enzymatic activity ratios to acquire C, N and P nutrients as indicators for shortage of N and P as in Nottingham et al. (2015a) and Sinsabaugh et al. (2009).

2.4. Microbial biomass

Microbial biomass carbon (MBC) and nitrogen (MBN) and dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) were measured using the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987) using 3 g of fresh soil. Soils were fumigated with chloroform (stabilised with amylene, Sigma) for 24 h. Thereafter, both control and fumigated soils were shaken for 30 min with 40 ml of 0.05 M K_2SO_4 solution and filtered through 0.45 µm Minisart© PES filters (Sartorious). DOC and total N in the solution were measured with Shimadzu (TOC-VCPH and TNM-1). MBC and MBN was then calculated as the difference between fumigated and control samples. No correction factor for extraction was applied (Leckie et al., 2004).

2.5. Microbial respiration and microbial CUE

We used the ^{13}C-glucose tracing method to determine soil microbial carbon use efficiency (CUE) (Geyer et al., 2019). Soils corresponding to 15 g dry weight were rewetted to 55 % of WHC and preincubated for 24 h in 100 ml glass bottles at 15 °C inside a dark incubator (MIR-154-PE, Panasonic). Perforated plastic caps were placed on top of each glass bottle to allow ventilation. Thereafter, 0.05 mg ^{13}C-glucose- per g soil dry weight was used (Frey et al., 2013; Geyer et al., 2019). The ^{13}C labelled glucose ($^{13}C_6$ glucose, 99 %, Cambridge Isotope Laboratories, Inc.) was mixed with unlabelled glucose to obtain a 5 atom % solution. Then, 2 ml of this solution was added to each soil sample, after which the bottles were tightly sealed with butyl rubber caps and aluminium crimp caps and flushed with CO_2- free air followed by the 24 h incubation at 15 °C. Control treatment received 2 ml of Milli-Q® water.

At the end of the 24 h incubation, 100 µl of the headspace was injected directly into a gas chromatograph (HP 6890 GC System, Agilent Technologies Inc.) for determination of CO_2 concentrations. To determine $^{13}CO_2$ production, a 20 ml gas sample was taken using a syringe and needle then injected into 12 ml He-flushed and evacuated exetainers (Labco, UK). These samples were shipped to Centre for Stable Isotope Research and Analysis (KOSI) lab at Göttingen University for the $^{13}CO_2$ measurements with an IRMS (Delta plus XP, Conflo III, Thermo Fisher, Germany) coupled with a GC (GC-Box, Thermo fisher, Germany and Poraplot Q, Combi-PAL autosampler, Zwingen, Switzerland).

From the CO_2 samples measured by IRMS, we obtained the delta ^{13}C values for both control and glucose addition treatment, which were transformed into atom% values for the purpose of mixing model calculations.

We traced the fraction of respired CO_2 from the labelled substrate as in Eq. (1):

$$^{13}C\ Respiration = C_{total}\ Respiration \times \frac{atom\%_{sample} - atom\%_{control}}{atom\%_{glucose} - atom\%_{control}} \quad (1)$$

$$SOM\ Respiration = C_{total}\ Respiration - {}^{13}C\ Respiration \quad (2)$$

where ^{13}C Respiration is the CO_2 originated from glucose decomposition, SOM respiration is the CO_2 originated from SOM decomposition and C_{total} Respiration is the total soil microbial respiration, all the units are in μg CO_2-C g^{-1} soil.

Concentrations of MBC and DOC were measured as described above (see Section 2.4), and their ^{13}C contents were analysed after freeze-drying of the K_2SO_4 extracts (Christ, Gamma2-16 LSC) with IRMS (Delta plus XP, Conflo III, Thermo fisher, Germany) in Goettingen University in the Centre for Stable Isotope Research and Analysis in Germany.

2.6. Microbial CUE calculations

Microbial CUE was calculated from the added amounts of ^{13}C labelled glucose as the ratio of ^{13}C incorporated in the biomass (^{13}MBC) and C uptake (sensu ^{13}MBC and $^{13}CO_2$ respired). The C-labelled-fractions were estimated using the calculations of the ^{13}C-glucose tracing method presented by Geyer et al. (2019).

$$atom\%\mathrm{MBC} = \frac{[(atom\%F\ DOC\ \mathrm{x}\ F\ DOC) - (atom\%NF\ DOC\ \mathrm{x}\ NF\ DOC)]}{(F\ DOC - NF\ DOC)} \quad (3)$$

$$\%^{13}MBC = \frac{(atom\%MBC_t - atom\%MBC_c)}{(atom\%sol - atom\%MBC_c)} \times 100 \quad (4)$$

$$^{13}MBC = (F\ DOC - NF\ DOC) \times \%^{13}MBC \div 100 \quad (5)$$

$$Microbial\ CUE = \frac{^{13}MBC}{^{13}MBC + {}^{13}C\ Respiration} \quad (6)$$

where $atom\%F\ DOC$, $F\ DOC$, $atom\%NF\ DOC$ and $NF\ DOC$ represent the atom % and total C concentrations (μg C g^{-1} soil) of fumigated (F) and non-fumigated (NF) K_2SO_4 extracts, respectively. $atom\%MBC_t$ and $atom\%MBC_c$ are the atom % of sample treatments and natural abundance controls, and $atom\%sol$ is the atom % of amendment solution (5 atom %). ^{13}MBC is the ^{13}C present in soil microbial biomass (μg C g^{-1} soil). $^{13}C\ Respiration$ is the cumulative respiration derived from added glucose (μg $^{13}CO_2$-C g^{-1} soil) after the 24 h incubation.

In addition to the ^{13}C glucose CUE, we also calculated $CUE_{C:N}$ based on stoichiometric modelling (Sinsabaugh and Follstad Shah, 2012; Sinsabaugh et al., 2016; Geyer et al., 2019), also called *Carbon-use Efficiency from Stoichiometry Theory (CUE_{ST})* by Schimel et al. (2022), and for overall clarity we refer to it as Stoichiometric CUE ($CUE_{C:N}$) throughout in this paper. The $CUE_{C:N}$ method is based on the enzyme activity measurements, where β-glucosidase (BG) is used as an indicator enzyme for C acquisition and the sum of chitinase (CHI) and leucine aminopeptidase (LAP) is used as an indicator for N acquisition (Sinsabaugh et al., 2016; Geyer et al., 2019):

$$CUE_{C:N} = CUE_{max}[S_{C:N}/(S_{C:N} + K)] \quad (7)$$

where $CUE_{C:N}$ is the stoichiometric CUE, $CUE_{max} = 0.6$ (Roels, 1980), $S_{C:N}$ is the scalar ratio and the half-saturation constant is $K = 0.5$ as in Michaelis-Menten model (Geyer et al., 2019)

$$\text{and } S_{C:N} = ((1/EEA_{C:N})/(MB_{C:N}/L_{C:N})) \quad (8)$$

where $EEA_{C:N}$ is the C:N ratio of the ecoenzymatic activities calculated as $BG/(CHI + LAP)$, $MB_{C:N}$ is the C:N ratio of microbial biomass, and $L_{C:N}$ is the C:N ratio of labile SOM (Sinsabaugh et al., 2016). For labile SOM C:N ratio we used the DOC and dissolved total N extracted from non-fumigated control samples, as in Geyer et al. (2019).

2.7. Microbial threshold elemental ratio (TER) calculations

The threshold C:N ratio ($TER_{C:N}$) was calculated as shown in Eq. (9), using NUE of 0.83 for organic soil horizons (Mooshammer et al., 2014). If SOM C:N is lower than $TER_{C:N}$ microbes are C or energy limited, and if SOM C:N is higher than $TER_{C:N}$, then N will limit microbial growth (Mooshammer et al., 2014).

$$TER_{C:N} = MB_{C:N} \times \frac{NUE}{CUE} \quad (9)$$

We further compared bulk soil C:P and N:P ratios to microbial critical C:P and N:P ratios from literature to estimate the relative importance of P or N limitation to microbial growth across the gradient. We can expect microbial phosphorus limitation in soil with C:P ratios >186 (Sinsabaugh et al., 2009) and in soil with N:P ratios >6.3 (Čapek et al., 2018).

2.8. Statistical analysis

All statistical analysis was performed using R software (R Core Team, 2021). Prior to ANOVA, each variable was checked individually with Shapiro test for normal distribution ($p < 0.05$) with shapiro.test function (R Core Team, 2021) and visually with ggplot2 package (Wickham, 2016).

To evaluate the importance of elevation, factorial ANOVA was performed from a linear model with elevation as a fixed factor and valleys as a random factor. The post hoc test was carried out using Tukey's Honestly Significant Difference (HSD) test with a significant level of $p < 0.05$ with agricolae package (de Mendiburu, 2021). The analysis was performed separately for each valley ($n = 15$) and through all the data set ($n = 44$). The relationship between elevation and soil C, N or P concentrations were tested and visualized with scatter plots and Pearson's values of significance $p < 0.05$. Furthermore, we calculated a Pearson correlation matrix between measured variables with Hmisc package (Harrell and Dupont, 2018). The analysis was performed over all the data set ($n = 44$). Correlations were visualized with correlogram constructed with corrplot package (Wei and Simko, 2021). A multiple linear regression was calculated to predict SOC stocks and CUE based on environmental variables.

3. Results

3.1. Soil carbon stocks

Thickness of the soil organic layers varied significantly across elevations and valleys, being on average between 3 and 10 cm (Table 1). Bulk density decreased with elevation, with values of 0.16 g cm^{-3} above 4000 m (Table 1). The SOC stocks were not linearly related to elevation between 3500 and 4500 m (Fig. 2). In fact, the soil organic layer at mid elevation (4000 m) was thicker and thus the soil SOC stocks at this altitude were markedly higher than those at the lowest and the highest elevations (Table 2). This was especially clear for the Ulta valley at 4000 m, where we observed the highest SOC stocks of 154.2 Mg C ha^{-1} (Table 2). Moreover, a multiple linear regression was calculated to predict SOC stocks based on environmental variables: aluminium, elevation, water content and pH. The overall regression was statistically significant (R^2 of 0.18, $F(4, 39) = 3.31$, $p < 0.05$) and total aluminium was the only significant variable ($\beta = 3.8$, $p < 0.05$).

3.2. Soil abiotic properties and elemental composition

Soils above 4000 m were significantly moister than the ones at lower elevation at the time of sampling (Table 1). Soil pH did not show a trend with elevation, although soils at 4500 m were significantly more acidic than soils at 3500 m. In situ soil temperature at the time of sampling decreased with elevation, from 11 °C at 3500 m to 5 °C at 4500 m ($r = -0.92$, $p < 0.001$) (Table 1).

Table 1
Soil abiotic properties of the organic layer across the *Huascarán* National Park gradient: pH, in situ soil temperature and water content, organic layer depth and bulk density. Average values per elevation ± SD are presented in bold and statistically significant differences between different elevations are denoted by bold capital letters (n = 9). Average values ± SD per valley are compared between different elevations across the gradient (n = 3) separately for each valley, and statistically significant differences are denoted by small letters formatted as follows: Llanganuco valley is in italics, Paron valley is underlined and Ulta valley is in regular form (Tukey HSD significant level of 0.05).

Elevation (m a.s.l.)	Valley	pH				In situ soil temperature (°C)				Water content (g H_2O g^{-1} soil dw)				Organic layer depth (cm)				Soil bulk density (g cm^{-3})			
3500		**5.9**	**±**	**0.5**	**B**	**10.8**	**±**	**0.7**	**D**	**0.9**	**±**	**0.4**	**A**	**3.0**	**±**	**1.5**	**A**	**0.5**	**±**	**0.3**	**B**
	Llanganuco	6.0	±	0.7	*a*	10.9	±	0.6	*d*	0.6	±	0.1	*a*	3.3	±	2.7	*a*	0.5	±	0.0	*b*
	Paron	6.0	±	0.5	a	10.5	±	0.1	e	1.4	±	0.0	ab	2.8	±	1.1	a	0.1	±	0.0	ab
	Ulta	5.8	±	0.3	ab	10.9	±	1.1	b	0.9	±	0.3	a	2.8	±	0.3	a	0.6	±	0.4	ab
3750		**5.6**	**±**	**0.5**	**AB**	**9.2**	**±**	**1.2**	**C**	**1.0**	**±**	**0.4**	**A**	**4.3**	**±**	**1.8**	**A**	**0.6**	**±**	**0.4**	**B**
	Llanganuco	5.4	±	0.8	*a*	9.9	±	0.8	*cd*	1.2	±	0.3	*b*	3.9	±	2.6	*a*	0.5	±	0.3	*ab*
	Paron	5.8	±	0.4	a	9.1	±	0.4	d	1.3	±	0.3	ab	3.3	±	0.9	a	0.1	±	0.0	ab
	Ulta	5.6	±	0.2	ab	8.6	±	1.9	b	0.6	±	0.2	a	5.7	±	0.7	a	1.1	±	0.1	b
4000		**5.7**	**±**	**0.3**	**AB**	**8.5**	**±**	**0.4**	**C**	**1.1**	**±**	**0.4**	**A**	**10.9**	**±**	**6.9**	**B**	**0.4**	**±**	**0.2**	**AB**
	Llanganuco	5.5	±	0.2	*a*	8.7	±	0.6	*c*	1.5	±	0.1	*cb*	5.6	±	1.6	*a*	0.2	±	0.1	*a*
	Paron	5.8	±	0.2	a	8.2	±	0.2	c	0.9	±	0.2	a	7.5	±	1.6	b	0.3	±	0.1	b
	Ulta	5.8	±	0.2	b	8.5	±	0.4	b	0.9	±	0.3	a	19.6	±	3.8	b	0.7	±	0.2	ab
4250		**5.8**	**±**	**0.2**	**AB**	**6.8**	**±**	**0.8**	**B**	**1.9**	**±**	**0.7**	**B**	**6.7**	**±**	**5.3**	**AB**	**0.2**	**±**	**0.1**	**A**
	Llanganuco	5.7	±	0.2	*a*	7.0	±	0.7	*b*	1.4	±	0.2	*cb*	11.4	±	7.6	*a*	0.2	±	0.1	*ab*
	Paron	5.9	±	0.3	a	7.4	±	0.3	b	1.8	±	0.4	b	5.5	±	0.8	ab	0.1	±	0.0	a
	Ulta	5.8	±	0.2	b	5.8	±	0.0	a	2.7	±	0.5	b	3.1	±	1.1	a	0.1	±	0.1	a
4500		**5.4**	**±**	**0.5**	**A**	**5.2**	**±**	**0.4**	**A**	**2.2**	**±**	**0.6**	**B**	**4.9**	**±**	**1.8**	**A**	**0.2**	**±**	**0.0**	**A**
	Llanganuco	5.8	±	0.6	*a*	5.1	±	0.4	*a*	1.9	±	0.3	*c*	3.6	±	0.5	*a*	0.2	±	0.0	*a*
	Paron	5.3	±	0.1	a	5.6	±	0.1	a	1.9	±	0.3	b	6.7	±	0.9	b	0.2	±	0.0	ab
	Ulta	5.1	±	0.4	a	4.8	±	0.1	a	2.9	±	0.5	b	4.5	±	2.1	a	0.2	±	0.0	a

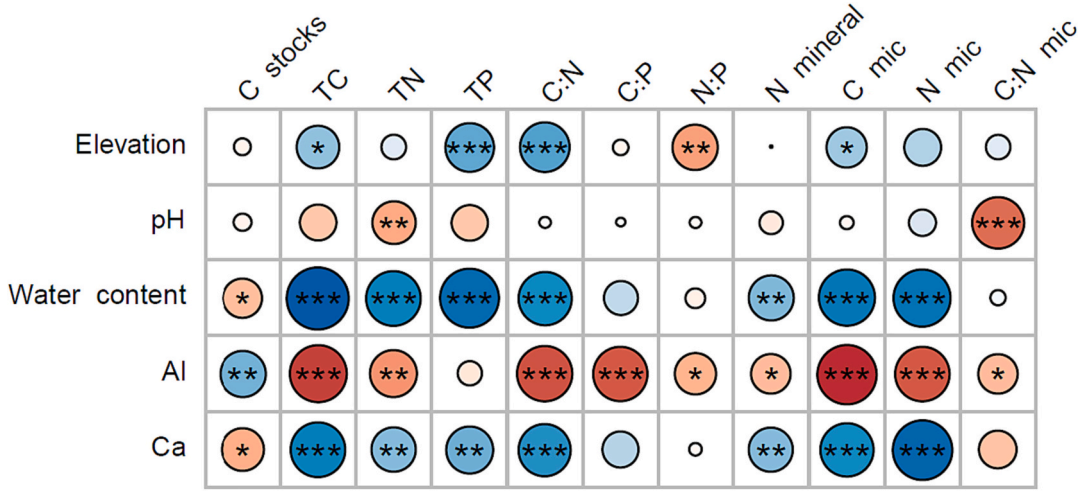

Fig. 2. Correlations of soil properties and elements: soil C stock (Mg C ha^{-1}), TC, TN and TP (%), soil C:N, C:P and N:P ratios, mineral N content (mg g^{-1} soil), microbial C and N (mg g^{-1} soil), and microbial C:N ratio, elevation (m a.s.l.), pH, water content (g water g^{-1} soil), Al and Ca contents (mg g^{-1} soil). Statistical significances are marked with asterisks of $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$(***). Correlation strength is represented by circle size and chroma, and its type denoted by colour i.e., positive (in blue) or negative (in red), (n = 44). Individual r and p values are presented in Supplementary Tables S1 and S2, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Generally, soils above 4000 m tended to have greater contents of total C, N and P. When looking at C content in each valley separately (Table 2), C content increased significantly with elevation only in the Ulta valley ($r = 0.65$, $p < 0.05$). Neither soil total N nor mineral N contents varied with elevation. Soil P content increased with elevation ($r = 0.5$, $p < 0.001$, in the combined data, n = 44), and this increase was significant in the Llanganuco ($r = 0.65$, $p < 0.01$) and Ulta ($r = 0.62$, $p < 0.05$) valleys (Table 2), but not in Paron. Furthermore, SOM C:N ratios increased with elevation ($r = 0.51$, $p < 0.001$), but C:P ratios did not and N:P ratios tended to decrease with elevation, but the correlation was not strong ($r = -0.43$, $p < 0.01$) (Table 2, Fig. 2).

We did not find statistically significant correlations between elevation and Al or Ca, but Al and Ca were correlated negatively with each other ($r = -0.55$, $p < 0.001$). The Al content correlated negatively with soil C and N contents ($r = -0.68$ for TC and $r = -0.47$ for TN, $p < 0.001$ for both), while Ca content correlated positively with soil C and N contents (Fig. 2).

3.3. Soil microbial biomass, stoichiometry, and enzyme activities

Soil microbial biomass did not change across elevations. The average MBC was 3.61 ± 1.5 mg C g^{-1}soil dw and MBN was 0.26 ± 1.1 mg N g^{-1}soil dw and, average MBC:MBN ratio was 13.87 ± 2.2 (average ± SD) (Table 3). Soil microbial biomass was higher in soils with higher moisture and Ca contents ($p < 0.001$) (Fig. 2), as well as in soils with higher bulk C, N and P contents ($p < 0.01$) (Fig. 2). But it decreased with higher soil Al and SOC stocks ($p < 0.001$ for both) (Fig. 2). In all the studied soils, N:P ratios were over 6.3 and C:P ratios were over 186

Table 2
Soil major nutrients (C, N and P) contents and soil C stocks and C:N, C:P and N:P ratios in soils along three valleys of the *Huascarán* National Park across the elevations studied. Average values ± SD per elevation are presented in bold and statistically significant differences denoted by bold capital letters (n = 9). The values presented are average ± SD in each valley is compared across the gradient (n = 3), and statistically significant differences are denoted by lowercase letters formatted as follows: Llanganuco valley is in italics, Paron valley is underlined and Ulta valley is in regular form (Tukey HSD significant level of 0.05).

Elevation	Valley	Total C			Total N			Total P			C stocks			C:N			C:P			N:P		
		%			%			%			(Mg C ha^{-1})											
3500		**23.4**	±	**6.6**	**AB**	**1.4**	±	**0.3**	**A**	**0.06**	±	**0.011**	**A**	**26.8**	±	**14.2**	**A**	**16.4**	±	**2.3**	**AB**	**393.4**	±	**138.4**	**A**	**23.5**	±	**5.9**	**B**
	Llanganuco	20.5	±	7.5	*a*	1.2	±	0.3	*a*	0.05	±	0.008	*a*	28.7	±	10.6	*a*	17.1	±	3.9	*a*	399.9	±	189.2	*a*	22.5	±	6.4	*a*
	Paron	30.4	±	0.4	a	1.8	±	0.0	a	0.06	±	0.000	a	11.9	±	4.7	a	17.1	±	0.2	a	520.6	±	2.1	b	30.5	±	0.2	c
	Ulta	21.8	±	5.4	a	1.4	±	0.3	ab	0.07	±	0.011	ab	34.8	±	16.0	ab	15.3	±	0.8	a	302.1	±	50.4	a	19.8	±	3.2	a
3750		**26.6**	±	**10.5**	**AB**	**1.6**	±	**0.7**	**A**	**0.07**	±	**0.014**	**AB**	**53.2**	±	**35.1**	**A**	**16.1**	±	**2.4**	**AB**	**362.6**	±	**117.1**	**A**	**22.4**	±	**6.8**	**AB**
	Llanganuco	37.5	±	4.4	*b*	2.3	±	0.7	*b*	0.09	±	0.004	*b*	56.2	±	12.9	*a*	17.2	±	3.0	*a*	427.2	±	64.6	*a*	25.9	±	9.2	*a*
	Paron	27.1	±	5.4	a	1.5	±	0.3	a	0.06	±	0.008	a	12.5	±	2.4	a	17.5	±	0.7	a	444.6	±	38.4	ab	25.4	±	1.5	bc
	Ulta	15.1	±	4.3	a	1.1	±	0.3	a	0.07	±	0.013	a	90.9	±	11.5	cb	13.7	±	0.2	a	216.1	±	25.4	a	15.8	±	1.7	a
4000		**21.4**	±	**8.9**	**A**	**1.3**	±	**0.4**	**A**	**0.07**	±	**0.020**	**AB**	**76.9**	±	**71.8**	**A**	**15.6**	±	**3.0**	**A**	**294.1**	±	**76.2**	**A**	**18.7**	±	**3.2**	**AB**
	Llanganuco	31.6	±	2.7	*ab*	1.7	±	0.0	*ab*	0.09	±	0.004	*b*	32.6	±	14.1	*a*	18.2	±	1.9	*a*	332.5	±	41.7	*a*	18.2	±	0.5	*a*
	Paron	20.6	±	2.2	a	1.3	±	0.2	a	0.06	±	0.010	a	44.2	±	14.0	b	16.4	±	1.3	a	331.7	±	28.2	a	20.2	±	0.4	a
	Ulta	11.8	±	3.8	a	0.9	±	0.2	a	0.06	±	0.008	a	154.2	±	81.9	c	12.1	±	1.2	a	217.9	±	87.6	a	17.7	±	5.8	a
4250		**32.1**	±	**9.8**	**AB**	**1.7**	±	**0.4**	**A**	**0.09**	±	**0.028**	**C**	**32.9**	±	**31.19**	**A**	**19.3**	±	**3.4**	**CB**	**349.7**	±	**118.7**	**A**	**17.7**	±	**3.3**	**A**
	Llanganuco	23.4	±	0.5	*a*	1.5	±	0.3	*ab*	0.10	±	0.022	*b*	58.2	±	48.0	*a*	15.7	±	2.2	*a*	230.7	±	41.8	*a*	14.7	±	1.4	*a*
	Paron	30.0	±	8.9	a	1.4	±	0.3	a	0.07	±	0.019	a	22.1	±	7.6	ab	21.9	±	2.2	b	461.7	±	118.7	ab	20.8	±	3.4	ab
	Ulta	43.0	±	2.2	b	2.1	±	0.1	c	0.12	±	0.006	c	18.5	±	8.8	a	20.3	±	2.0	b	356.7	±	19.9	a	17.6	±	0.9	a
4500		**33.7**	±	**5.3**	**B**	**1.6**	±	**0.3**	**A**	**0.09**	±	**0.021**	**BC**	**26.2**	±	**10.2**	**A**	**20.8**	±	**1.9**	**C**	**376.91**	±	**77.581**	**A**	**18.1**	±	**2.7**	**AB**
	Llanganuco	28.2	±	5.2	*a*	1.5	±	0.3	*ab*	0.09	±	0.002	*b*	16.8	±	5.7	*a*	19.4	±	0.7	*a*	312.4	±	54.7	*a*	16.2	±	3.2	*a*
	Paron	34.5	±	2.4	a	1.5	±	0.0	a	0.08	±	0.005	a	35.6	±	4.5	b	23.1	±	1.2	b	463.1	±	12.4	ab	20.0	±	1.1	a
	Ulta	38.5	±	3.2	b	1.9	±	0.2	bc	0.11	±	0.027	cb	26.2	±	9.7	a	19.8	±	0.9	b	355.2	±	53.3	a	17.9	±	2.4	a

(Table 2).

SOM C:N ratios varied between 10.7 and 24.5 and were correlated positively with increasing cellobiosidase, β-glucosidase, chitinase and phosphatase enzyme activities (r = 0.48 to 0.63, p < 0.001), but negatively with peroxidase activity (r = −0.53, p < 0.001) (Table 4, Fig. 3). We found a strong correlation between chitinase and β-glucosidase activities (r = 0.82, p < 0.001) across all the gradients. At high elevation, soils had a higher ratio of β-glucosidase-to-peroxidase enzyme activity (r = 0.51, p < 0. 001) (Table 4). The ratio of peroxidase and chitinase activities correlated negatively with SOM C:N ratio (r = 0.48, p < 0.001) (Fig. 3). SOM respiration tended to increase with elevation, since it was correlated with SOM C:N ratio (r = 0.7, p < 0.001) (Fig. 4). We found an increasing SOM respiration rate with higher activities of cellulases (r = 0.45, p < 0.01 for cellobiosidase and r = 0.54, p < 0.001 for β-glucosidase), but less SOM respiration with increasing peroxidase activity (r = −0.48, p < 0.001) (Fig. 4).

3.4. Carbon use efficiency (CUE)

On average, microbial ^{13}CUE was 0.65 and did not correlate with elevation (Table 5, Fig. 4). Thus, we found no trend between biochemically defined microbial ^{13}CUE and in situ soil moisture content, pH, organic layer thickness or bulk density, which all changed along the elevation (Table 1, Fig. 2). The multiple linear regression analysis to predict CUE based on abiotic environmental variables was not significant (results not shown).

The amount of ^{13}C glucose dedicated to growth correlated positively with microbial ^{13}CUE (r = 0.95, p < 0.001) (Fig. 4). Thus, microbial ^{13}C-growth correlated with soil properties in a similar way as microbial ^{13}CUE. In contrast, ^{13}C - glucose respired correlated negatively ^{13}CUE (r = −0.55, p < 0.001 for ^{13}CUE) and the ^{13}C respiration from glucose was higher in soils with lower microbial C:N ratio (r = −0.5, p < 0.001) (Fig. 4).

The stoichiometric $CUE_{C:N}$ (on average 0.65) did not correlate with ^{13}CUE (on average 0.32). Neither of them correlated statistically significantly with altitude. Only soils above 4000 m a.s.l. differed from the lower altitude soils (3500–4000 m a.s.l) when these two groups were compared. The calculated stoichiometric $CUE_{C:N}$ decreased with increasing bulk SOM C:N ratios (Fig. 4).

4. Discussion

We studied SOC stocks in highland forests of *Polylepis* from 3500 to 4500 m a.s.l. in the *Huascarán* National Park. Our main finding was that SOC stocks did not increase with elevation, contrary to our first hypothesis. Generally, SOM N:P ratios over 6.3 and C:P ratios over 186 in all the studied soils indicated that P, rather than N, was probably overall the more important limiting nutrient to microbial growth, after C, which is usually always considered the main limiting factor to microbial growth (Demoling et al., 2008; Soong et al., 2020). Although C:P ratios did not change with elevation, increasing phosphatase activity suggests a lower P availability to microbes with increasing elevation. The increasing SOM C:N ratios with altitude, which were also correlated with chitinase activity, indicate decreasing N-availability to microbes, thus also partly supporting our hypothesis 2 that N availability to microbes decreases with elevation. Contrary to our third hypothesis on the nutrient limitation controlling CUE along our gradient, both ^{13}CUE and $CUE_{C:N}$, seemed to be also related to the quality or decomposability of SOM along the gradient (Karhu et al., 2010; Walker et al., 2018).

4.1. Do SOC stocks increase with elevation?

The climate and topographic gradient in the Andes (Mateo et al., 2022; Caballero-Villalobos et al., 2021; Portes et al., 2016) and the strong linear relationship between elevation and MAT (R^2 = 0.98) in Llanganuco valley (Mateo et al., 2022) led us to hypothesise that low soil

Table 3
Soil microbial C, microbial N, microbial C:N ratio, dissolved organic C (DOC) and dissolved total N (DTN) along three valleys of the *Huascarán* National Park across the elevations studied. Average values ± SD per elevation are presented in bold and statistically significant differences denoted by bold capital letters (n = 9). The values presented are average ± SD in each valley are compared across the gradient (n = 3), and statistically significant differences are denoted by lowercase letters formatted as follows: Llanganuco valley is in italics, Paron valley is underlined and Ulta valley is in regular form (Tukey HSD significant level of 0.05).

Elevation	Valley	Microbial C				Microbial N				Microbial C:N				DOC				DTN			
(m a.s.l.)		(mg C g^{-1}soil dw)				(mg N g^{-1}soil dw)								(mg C g^{-1}soil dw)				(mg C g^{-1}soil dw)			
3500		**3.2**	**±**	**1.5**	**AB**	**0.3**	**±**	**0.1**	**AB**	**12.8**	**±**	**0.5**	**A**	**1.7**	**±**	**0.6**	**AB**	**0.09**	**±**	**0.04**	**A**
	Llanganuco	2.3	±	0.8	*a*	0.2	±	0.1	*a*	12.9	±	0.2	*ab*	1.7	±	0.9	*a*	0.07	±	0.05	*ab*
	Paron	4.2	±	1.1	ab	0.3	±	0.1	a	13.1	±	0.7	a	2.3	±	0.1	a	0.13	±	0.01	c
	Ulta	3.4	±	2.1	ab	0.3	±	0.2	ab	12.6	±	0.6	a	1.5	±	0.4	a	0.09	±	0.03	a
3750		**3.5**	**±**	**1.1**	**AB**	**0.3**	**±**	**0.1**	**AB**	**14.9**	**±**	**2.7**	**A**	**1.4**	**±**	**0.6**	**A**	**0.08**	**±**	**0.04**	**A**
	Llanganuco	4.1	±	0.9	*b*	0.3	±	0.1	*a*	17.1	±	3.7	*b*	1.6	±	0.4	*a*	0.11	±	0.03	*ab*
	Paron	4.1	±	0.3	ab	0.3	±	0.0	a	13.9	±	1.7	ab	1.9	±	0.5	a	0.10	±	0.04	cb
	Ulta	2.4	±	0.8	ab	0.2	±	0.1	a	13.8	±	0.9	a	0.8	±	0.2	a	0.04	±	0.02	a
4000		**2.5**	**±**	**1.3**	**A**	**0.2**	**±**	**0.1**	**A**	**13.4**	**±**	**1.1**	**A**	**1.7**	**±**	**1.0**	**AB**	**0.08**	**±**	**0.06**	**A**
	Llanganuco	3.8	±	0.4	*ab*	0.3	±	0.0	*a*	13.2	±	0.8	*ab*	2.5	±	1.1	*a*	0.16	±	0.04	*b*
	Paron	2.8	±	0.9	a	0.2	±	0.1	a	14.5	±	0.8	ab	1.7	±	0.8	a	0.07	±	0.02	abc
	Ulta	0.9	±	0.2	a	0.1	±	0.0	a	12.5	±	0.7	a	0.8	±	0.3	a	0.03	±	0.01	a
4250		**4.4**	**±**	**1.6**	**B**	**0.3**	**±**	**0.1**	**B**	**13.4**	**±**	**2.1**	**A**	**3.1**	**±**	**1.8**	**B**	**0.10**	**±**	**0.10**	**A**
	Llanganuco	2.7	±	0.6	*ab*	0.2	±	0.1	*a*	13.2	±	1.2	*ab*	1.4	±	0.2	*a*	0.04	±	0.01	*a*
	Paron	4.9	±	1.1	ab	0.3	±	0.1	a	15.5	±	1.3	ab	2.6	±	0.4	a	0.04	±	0.01	ab
	Ulta	5.5	±	1.3	b	0.5	±	0.1	b	11.4	±	1.3	a	5.4	±	0.9	b	0.21	±	0.11	a
4500		**4.5**	**±**	**1.2**	**B**	**0.3**	**±**	**0.1**	**AB**	**14.7**	**±**	**3.2**	**A**	**2.6**	**±**	**1.2**	**AB**	**0.11**	**±**	**0.09**	**A**
	Llanganuco	3.4	±	0.3	*ab*	0.3	±	0.1	*a*	11.7	±	1.5	*a*	2.1	±	0.1	*a*	0.10	±	0.03	*ab*
	Paron	5.3	±	0.9	b	0.3	±	0.0	a	17.2	±	1.5	b	2.4	±	0.2	a	0.03	±	0.01	a
	Ulta	4.7	±	1.3	b	0.3	±	0.1	ab	15.1	±	3.6	a	3.4	±	2.0	ab	0.19	±	0.11	a

temperatures at high altitudes would slow down SOM decomposition rates and allow SOC to accumulate. But, contrary to our first hypothesis, SOC stocks peaked in the middle of our gradient at 4000 m a.s.l. Similarly, Zimmermann et al. (2010) detected highest SOC stocks in the middle of their gradient (at 3030 m a.s.l. in a gradient stretching between 2994 and 3825 m) on the eastern slope of the Andes. Likewise, Simon et al. (2018) detected C stocks peaking at 3300 m a.s.l. on their Himalayan gradient, ranging from 2600 to 4000 m a.s.l. Their results were partly explained by the unimodal curve of the organic layer thickness that increases from lowest to mid elevation and decreases at the highest elevation (Simon et al., 2018; Zimmermann et al., 2010), similarly as in our study. In our gradient, organic layer thickness increased from 3000 to 4000 m a.s.l. and decreased from 4000 to 4500 m a.s.l., in Paron and Ulta valley (Table 1), thus in these valleys the SOC stock of the organic layer was larger at 4000 m a.s.l. but not in Llanganuco (Table 2). Apart from that peak, organic soil thickness did not vary across the gradient and SOC stocks did not change significantly between low and high elevation. Even though bulk density decreased with elevation, the calculated SOC stocks did not decrease because they were counterbalanced by the increasing C contents with elevation.

It has been shown in literature that tree height decreases towards the treeline while belowground biomass increases, and this switch in C allocation contributes to soil C stocks at high elevations (Hertel and Schöling, 2011). Accordingly, in a Bolivian *Polylepis* forest (*P. lanata* and *P. pepei*) at 1380 km southeast from *Huascarán* National Park, tree height decreased with elevation (from 3650 to 4050 m a.s.l.), while root density and area increased significantly with elevation, which confirms the switch in the belowground C allocation (Hertel and Wesche, 2008). On the contrary, for *P. incana* forest at 4200 m a.s.l., located 380 km southeast from *Huascarán* National Park, root biomass below 15 cm was scarce, but still these soils contain above 90 % of the ecosystem C stocks (Vásquez et al., 2014). Both the above-mentioned studies and our results converged in finding the highest SOC stocks at around 4000 m a.s.l. In our gradient, trees above 4000 m were reported to be taller and thicker, resulting in larger tree biomass compared to the trees at low elevation, which are smaller and fewer (Morales et al., 2018; Sevillano-Ríos and Rodewald, 2017). Nevertheless, our calculated SOC stocks decreased above 4000 m a.s.l., without significant differences between low (3500 m a.s.l.) and high elevations (4500 m a.s.l.).

Moreover, *P. albicans* is gradually displaced by *P. weberbaueri* above 4000 m a.s.l. (Morales et al., 2018; Sevillano-Ríos and Rodewald, 2017). This shift of *Polylepis* species is accompanied by a decrease in grass coverage and increase of moss and bare soil with elevation. Interestingly, at 4000 m a.s.l. *Polylepis* forest was the least dense forest and grasses were more abundant than at low and high elevation (Sevillano-Ríos and Rodewald, 2017). The abundancy of grasses at 4000 m a.s.l. could have influenced soil organic layer thickness due to the root structure of grasses contributing to soil profile development and the contribution of the rhizosphere to the stabilization of SOC (Sokol and Bradford, 2019). SOC stocks increased with the grass coverage reported by Sevillano-Ríos and Rodewald (2017), especially in Ulta ($r = 0.87$, $p < 0.001$) and Paron ($r = 0.63$, $p < 0.05$). Thus, grass coverage might be one reason for the peak in our unimodal SOC stocks at 4000 m a.s.l. in Paron and Ulta valleys. In contrast to Llanganuco, where no trends were found and can result from the lithological discontinuities as found by Portes et al. (2016) in a gradient in Llanganuco ranging from 3482 to 4363 m a.s.l. The aluminium content was the only significant abiotic environmental variable correlated with SOC stocks, which may reflect the importance of Al-SOM complexation in SOC stabilization in these soils as suggested by Portes et al. (2016). Particularly, higher Al contents in Ulta at 4000 m a.s.l and Llanganuco at 4250 m a.s.l. coincide with the highest C stocks in these valleys.

4.2. Does nutrient availability decrease with elevation?

When looking at the stoichiometric ratios of C:P and N:P, these clearly indicate that P, rather than N, was the secondary limiting nutrient for microbial growth across the whole elevational gradient, after C which is considered the main limiting factor (Soong et al., 2020). According to Sinsabaugh et al. (2009), SOM C:P ratios >186 indicate phosphorus limitation, and the C:P ratios of our soils were even higher i.e., 354.47 ± 107.9 (average ± SD). Based on the soil N:P ratios, soil microorganisms are considered P-limited at N:P ratios above 6.3 (Čapek et al., 2018), in our gradient, the N:P ratios were 19.99 ± 5.02, thus indicating clear P limitation. However, bulk SOM stoichiometric ratios of C:P, N:P and C:N may not describe well what is the nutrient availability to microbes (Jørgensen et al., 2013; Ostrowska and Porębska, 2015). Bulk SOM C:P ratio did not vary along the gradient, which would indicate that the magnitude of P limitation remained similar across the gradient. However, we observed higher phosphatase activities at higher

Table 4
Enzyme activities, and C:N, C:P, and N:P enzyme activity ratios. The values presented are averages of all the valleys (± SD, n = 9). Significant differences across the gradient are denoted by lowercase letters (Tukey HSD significant level of 0.05). The group of labile-C acquiring enzyme activities encompass the sum of β-xylosidase, cellobiohydrolase, β-glucosidase (nmol activity g soil dw^{-1} h^{-1}), lignin-C degradation associated enzymes is the sum of phenol oxidase and peroxidase (nmol DOPA g soil dw^{-1} h^{-1}), N acquiring enzyme activity is presented by Chitinase chitinase. P acquiring enzyme activity presented by phosphatase (nmol activity g soil dw^{-1} h^{-1}).

Elevation (m a.s.l.)	3500			3750			4000			4250			4500		
C labile	**402.9**	±	**191.0**	**a**	**613.6**	±	**303.0**	**a**	**587.0**	±	**651.0**	**a**	**672.9**	±	**442.0**	**a**	**871.8**	±	**480.0**	**a**
β-Xylosidase	34.9	±	39.2	a	83.4	±	60.2	a	73.2	±	139.8	a	39.7	±	37.2	a	96.3	±	115.4	a
Cellobiohydrolase	150.5	±	96.2	a	196.8	±	112.0	a	224.6	±	231.9	a	266.5	±	235.6	a	320.5	±	217.2	a
β-Glucosidase	217.5	±	104.5	a	333.4	±	176.6	a	289.2	±	286.6	a	366.7	±	200.6	a	455.0	±	160.5	a
C lignin	**1.5**	±	**0.8**	**ab**	**2.7**	±	**1.1**	**c**	**2.6**	±	**0.6**	**bc**	**1.7**	±	**1.0**	**abc**	**1.2**	±	**0.8**	**a**
Peroxidase	1.1	±	0.5	a	1.9	±	0.6	b	2.3	±	0.6	b	1.2	±	0.9	a	0.7	±	0.6	a
Phenoloxidase	0.4	±	0.3	a	0.7	±	0.8	a	0.4	±	0.3	a	0.5	±	0.3	a	0.3	±	0.4	a
Chitinase	196.7	±	114.0	a	379.7	±	224.6	a	313.1	±	260.5	a	416.6	±	267.8	a	491.9	±	256.9	a
Phosphatase	1586.8	±	561.2	a	3498.9	±	1912.0	ab	2874.6	±	1815.0	ab	4512.4	±	2823.0	b	4742.8	±	2801.0	b
Enzyme activity ratios																				
β-Glucosidase:chitinase (C:N)	1.30	±	0.76	a	1.12	±	0.94	a	1.11	±	0.73	a	1.84	±	3.15	a	1.09	±	0.44	a
Peroxidase:chitinase (C:N)	0.01	±	0.006	a	0.009	±	0.012	a	0.014	±	0.014	a	0.007	±	0.010	a	0.002	±	0.002	a
β-Glucosidase:phosphatase (C:P)	0.14	±	0.07	a	0.11	±	0.06	a	0.12	±	0.07	a	0.09	±	0.04	a	0.15	±	0.12	a
Chitinase:phosphatase (N:P)	0.13	±	0.08	a	0.12	±	0.07	a	0.13	±	0.10	a	0.11	±	0.07	a	0.16	±	0.13	a
β-Glucosidase:peroxidase (C:C)	273.2	±	233.7	a	186.5	±	116.4	a	135.1	±	126.4	a	584.0	±	566.9	ab	1046.7	±	807.5	b

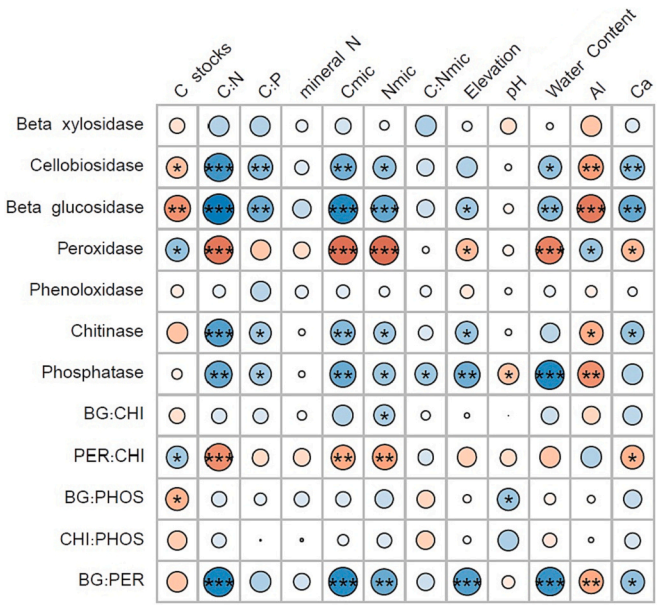

Fig. 3. Correlations of soil enzyme activities and soil properties: β-xylosidase (nmol activity g^{-1} soil h^{-1}), Cellobiohydrolase (nmol activity g^{-1} soil h^{-1}), β-glucosidase (nmol activity g^{-1} soil h^{-1}), Peroxidase (n mol DOPA g^{-1} soil h^{-1}), Phenoloxidase (n mol DOPA g^{-1} soil h^{-1}), Chitinase (nmol activity g^{-1} soil h^{-1}), Phosphatase (nmol activity g^{-1} soil h^{-1}). Enzyme activity ratios: β-glucosidase: chitinase (BG:CHI), Peroxidase: chitinase (PER:CHI), β-glucosidase: phosphatase (BG:PHOS), Chitinase: phosphatase (CHI:PHOS), β-glucosidase: peroxidase (BG:PER). Soil C stock (Mg C ha^{-1}), C:N, C:P and N:P ratios in soil, mineral N content (mg g^{-1} soil), microbial C and N (mg g^{-1} soil), and microbial C:N ratio, elevation (m a.s.l.), pH, water content (g water g^{-1} soil), Al and Ca contents (mg g^{-1} soil). Statistical significances are marked with asterisks of $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$(***). Correlation strength is represented by circle size and chroma, and its type denoted by colour i.e., positive (in blue) or negative (in red), (n = 44). Individual r and p values are presented in Supplementary Tables S3 and S4, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

elevations, which may indicate that the microbes experienced lower P availability, possibly due to more of the P being bound in organic matter, or otherwise in inaccessible forms. This partially supports our second hypothesis of decreasing nutrient availability with elevation. We also observed higher chitinase activities in soils at higher altitudes and with higher C:N ratios. Since the chitinase and phosphatase enzyme activities, N and P acquisition related enzymes, increased with elevation, it can be suggested that microbes experienced both decreasing N and P availability at higher elevations.

$TER_{C:N}$ denotes the threshold of the C:N ratio at which microbial metabolisms switches from C limitation to N limitation (Mooshammer et al., 2014). Our $TER_{C:N}$ calculated based on ^{13}CUE measurements was 18.6 ± 5.4, which is slightly lower than the $TER_{C:N}$ empirical estimates of 20 and 27 (Cui et al., 2018; Mooshammer et al., 2014; Sinsabaugh et al., 2009). In our gradient, in soils below 4000 m, soil microbes were mainly limited by energy and C, rather than N, because the average SOM C:N ratio of 16 ± 2.5 (n = 26) was lower than the $TER_{C:N}$. In contrast, soils above 4000 m, where SOM C:N ratio was 20.03 ± 2.8 (n = 18) (greater than TER) suggested relatively increasing N limitations for the microbial communities at high elevation. These findings support our second hypothesis that N could have become increasingly limiting to microbes at higher altitudes (Fisher et al., 2013; Hicks et al., 2019; Whitaker et al., 2014a, 2014b).

In the Peruvian tropical Amazon forests, soil microbes are N-limited at high elevations, and P-limited at low elevations (Fisher et al., 2013; Girardin et al., 2013). Nottingham et al. (2015a, 2015c) found that

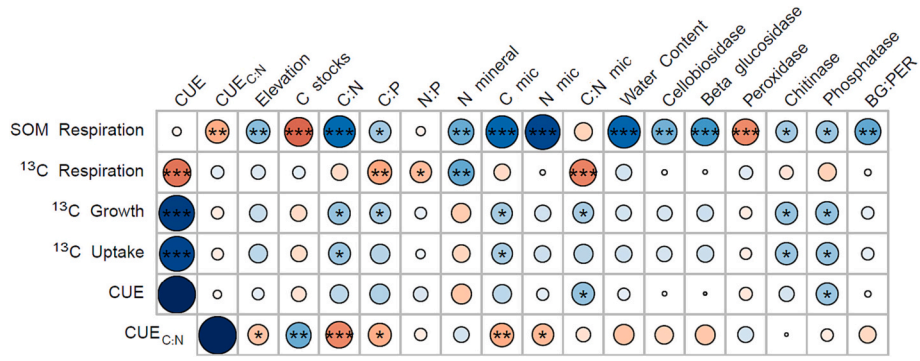

Fig. 4. Correlations of SOM respiration and glucose CUE and its components, $CUE_{C:N}$, soil properties and soil enzyme activities: SOM respiration (μg C g^{-1}soil), ^{13}C Respiration (μg ^{13}C g^{-1}soil), ^{13}C growth (μg ^{13}C g^{-1}soil), ^{13}C uptake (μg ^{13}C g^{-1} soil) and CUE. Soil properties: Elevation (m a.s.l.), soil C stock (Mg C ha^{-1}), C:N, C:P and N:P ratios in soil, mineral N content (mg g^{-1} soil), microbial C and N (mg g^{-1} soil), and microbial C:N ratio, pH, water content (g water g^{-1} soil dw), Cellobiosidase (nmol activity g^{-1} soil h^{-1}), β-glucosidase (nmol activity g^{-1} soil h^{-1}), Peroxidase (n mol DOPA g^{-1} soil h^{-1}), Chitinase (nmol activity g^{-1} soil h^{-1}), Phosphatase (nmol activity g^{-1} soil h^{-1}), β-glucosidase: peroxidase (BG:PER). Statistical significances are marked with asterisks of $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$(***). Correlation strength is represented by circle size and chroma, and its type denoted by colour i.e., positive (in blue) or negative (in red), (n = 44). Individual r and p values are presented in Supplementary Tables S5 and S6, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

β-glucosidase, chitinase and phosphomonoestarase activities decreased with elevation, in the tropical Amazon rainforests. This is because the weathered lowlands have more relatively recalcitrant C and less available P, and since the N:P enzymatic activity ratio increased with elevation, highlands have low N availability which constrains microbial metabolism over P (Nottingham et al., 2015a, 2015b). Contrary to this, it seems that on the Puna side, P was overall the main limiting nutrient, according to the high phosphatase activity measured compared to the highest values found by Notthingham et al. (2015a) and the stoichiometric soil C:P ratios over 186 and N:P ratios over 6.3 along the gradient (Sinsabaugh et al., 2009; Čapek et al., 2018).

In addition to being used as an indicator for the relative microbial C and N availability, C:N ratio has been extensively used as an indicator of SOM quality. One reason is that early litter decomposition rates can be predicted based on litter C:N ratios, low SOM C:N ratios could indicate initial high decomposition rate (Berg and McClaugherty, 2020). Another reason is that during the decomposition process, C is consumed, CO_2 is released and thus, the C:N ratio of the material being decomposed decreases (Berg, 2014). Assuming that the tree litter C:N ratios are rather similar across the gradient (assumed to be similar for the different *Polylepis* species), the higher SOM C:N ratios at higher altitudes led us to infer that at high elevations SOM could have a lower humification degree (Berg, 2014; Bu et al., 2012; Martins et al., 2011). The low bulk density values observed at high elevation could also be associated with a less advanced decomposition stage of SOM (Bu et al., 2012; Martins et al., 2011; Simon et al., 2018).

Much research has suggested that low temperature at high elevations reduces SOM decomposition rate which in turn, contributes to the accumulation of soil C, even the labile fractions (Simon et al., 2018; Nottingham et al., 2015a; Vásquez et al., 2014; Hagedorn et al., 2010). Accordingly, our enzyme activity measurements indicate that there is more labile C left in the soil at higher elevations. In our gradient, the β-glucosidase to peroxidase ratio increased with elevation. The ratio of β-glucosidase to peroxidase has been also used as proxy of SOM complexity, with larger values of β-glucosidase to peroxidase ratio meaning more easily decomposable C sources available, relative to lignin compounds (Takriti et al., 2018) suggesting that there might be a higher proportion of easily available C fractions in soils at high elevation and more recalcitrant forms of C at lower elevations (Jasso-Flores et al., 2020).

4.3. Does CUE decrease with elevation?

We hypothesized that CUE would decrease with elevation, because of lower nutrient availability at high elevations. The average ^{13}CUE across the whole gradient was 0.65. These ^{13}CUE values are very similar to the ones measured by Geyer et al., 2019 and Qiao et al., 2019. The stoichiometric $CUE_{C:N}$ values, on average 0.32 were also similar compared to other studies using this method (Sinsabaugh et al., 2016; Geyer et al., 2019). However, neither ^{13}C glucose CUE nor $CUE_{C:N}$ changed significantly with elevation. Moreover, we found positive correlations between ^{13}CUE and bulk SOM C:N and C:P stoichiometric ratios, from which C:N increased with elevation. When comparing the SOM C:N and $TER_{C:N}$ across all elevations, their ratio (SOM C:N over $TER_{C:N}$) averaged exactly 1.0. This indicates a balanced C:N availability for microbes, which could explain the rather high ^{13}CUE values, ranging on average from 0.58 to 0.76.

However, there is still support for increasing N limitation to microbes with altitude. The ratio of SOM C:N to $TER_{C:N}$ increased with altitude, the aforementioned ratio values <1 indicate C limitation, and values above 1 indicate N limitation. The average value for the two highest altitudes at 4250 and 4500 m was 1.23 (±0.32, n = 18), indicating that at these higher elevations, microbes could have been N limited. Whereas, at lower altitudes (from 3500 to 4000 m) the C:N to $TER_{C:N}$ ratio averaged 0.85 (±0.24, n = 26), indicating C limitation. The stoichiometric $CUE_{C:N}$ under N limitations above 4000 m was on average 0.29, while under C limitations from 4000 m downwards the stoichiometric $CUE_{C:N}$ was on average 0.35, so this trend aligns with our third hypothesis, suggesting that limiting N and P availability decreases CUE, however this alteration in CUE was not significant. Our results indicate that bulk SOM stoichiometric ratios may not be best explanatory factors for the relative C, N or P availability that microbes experience. At higher altitudes, the better decomposability of SOM may balance out the high SOM C:N ratio so that the N requirements of microbes are met, even though the bulk SOM C:N ratio indicates N limitation. This was supported by the positive correlation between glucosidase and chitinase activities: the available energy from labile C decomposition could be used for synthesis of chitinase, i.e. for N-acquisition (Li et al., 2019; Mori, 2020).

4.3.1. ^{13}C glucose tracing

The ^{13}C glucose tracing method applied in this study followed by a 24 h incubation might also reflect the ^{13}C enriched biomass of fast-growing r-strategist rather than the K-strategist, thus microbial

Table 5
Microbial respiration (CO_2) from soil organic matter (SOM) and from the added ^{13}C glucose, carbon use efficiency (CUE), calculated based on the total ^{13}C uptake, the proportion of C used for respiration and growth, and the stoichiometric $CUE_{C:N}$. The values presented are averages of all the valleys (n = 9 ± SD). Significant differences across the gradient are denoted by lowercase letters (Tukey HSD significant level of 0.05).

Elevation	SOM respiration			^{13}C glucose CUE			^{13}C uptake			^{13}C glucose respiration			^{13}C growth			$CUE_{C:N}$		
(m a.s.l)	(μg C g^{-1}soil)						(μg C g^{-1}soil)			(μg C g^{-1}soil)			(μg C g^{-1}soil)					
3500	196.8	±	83.9	ab	0.63	±	0.09	a	20.1	±	5.9	a	7.1	±	0.7	ab	13.1	±	5.6	ab	0.32	±	0.08	a
3750	191.2	±	106.7	ab	0.70	±	0.13	a	25.1	±	8.8	a	6.6	±	0.7	a	18.5	±	8.5	ab	0.38	±	0.07	a
4000	154.6	±	102.9	a	0.56	±	0.10	a	18.9	±	3.5	a	7.9	±	0.6	b	10.9	±	3.8	a	0.35	±	0.12	a
4250	325.8	±	147.6	b	0.68	±	0.19	a	28.7	±	11.7	a	7.4	±	0.9	ab	21.4	±	12.3	b	0.29	±	0.08	a
4500	317.3	±	121.1	b	0.67	±	0.15	a	24.3	±	5.6	a	7.3	±	1.3	ab	17.0	±	6.5	ab	0.29	±	0.07	a

community composition could play a role on ^{13}CUE values (Soares and Rousk, 2019). Since we might expect differences at the community level composition across the gradient, the differences in nutrient availability could be masked by the ^{13}C glucose additions followed by the 24 h incubation at 15 °C. In our gradient, in soils at high altitude, we could expect naturally more abundance of r-strategist since those soils might contain more labile C, and those microbes could be more efficient in using that labile C (such as glucose), which could balance out the lower N-availability. This is in line with the higher ^{13}C-Growth above 4000 m of 19.6 ± 9 on average, and a lower ^{13}C-Growth from 4000 m downwards of 14.2 ± 6.

Similarly, on the Amazon side, the ^{13}CUE of the labile C substrates added increased with elevation and correlated positively with bulk SOM C:N ratio (Hicks et al., 2019; Whitaker et al., 2014a). This may explain the high ^{13}CUE value of 0.68 found above 4000 m under less decomposed SOM. While at lower altitudes with more decomposed SOM, it is expected that bigger K-strategists community abundance (slow microbes growers) overrun r-strategists (Wei et al., 2020). Thus, at low elevation, under C-limitations, glucose addition might have stimulated only the r-strategists part of the microbial community, and probably therefore ^{13}CUE was not statistically different to ^{13}CUE at high elevation. Moreover, glucose is a readily available C source that can be used without the need of extracellular enzymes. So, it is expected that nutrient limitation on glucose use is not as forthcoming, as for more recalcitrant native SOM with varying C:N ratios.

4.3.2. Stoichiometric CUE

SOM respiration increased with the stoichiometric imbalance of C:N over $TER_{C:N}$, which could indicate higher overflow respiration with higher SOM C:N ratios (Schimel et al., 2022). There was a negative correlation between stoichiometric CUE_{CN} and SOM respiration (even when SOM respiration was normalised per soil C content), and a positive correlation between CUE_{CN} and soil C stock. We also observe that SOM respiration increases with increasing labile C- and N-acquiring enzyme activities. Following this reasoning, the stoichiometric $CUE_{C:N}$ and particularly SOM respiration corroborates the need of microbial homeostasis with the elemental composition of SOM (Cleveland and Liptzin, 2007; Cui et al., 2021).

At high elevations, soils with high C:N contents, microbes will need to acquire N from SOM in order to match their microbial mass elemental ratio similar to the ratio of the substrate (Sinsabaugh et al., 2013, 2016; Takriti et al., 2018), causing a relatively lower $CUE_{C:N}$ of 0.29 (±0.07, n = 18) compared to the 0.35 (±0.09, n = 26) found at low elevations.

5. Conclusions

In the *Polylepis* forest of the *Huascarán* National Park the highest SOC stocks occurred at mid altitudes around 4000 m a.s.l. Microbial communities were relatively more limited by decreasing N and P availability with elevation, since SOM C:N ratios, and chitinase and phosphatase enzyme activities increased with elevation. There were no statistically significant differences in ^{13}CUE nor stoichiometric $CUE_{C:N}$ across our gradient (3500–4500 m), probably because changes in lability of SOM counterbalanced the effects of low nutrient availability. However, $CUE_{C:N}$ showed a better response to SOM nutrient availability, reflecting a shift in the use of resources under mainly C versus increasingly N limited conditions. Neither of the CUE estimates measured at control temperature was related to elevation. For a quantitative estimate of potential C losses from these soils with climate warming, future studies in the *Huascarán* National Park should more directly measure the quality of C, and its decomposability at different temperatures in long-term incubation studies. Furthermore, the potential impact of climate warming on increasing presence of *P. albicans* with altitude, changes in the understory vegetation and thus possible changes in the quality and quantity of litter inputs remains unknown.

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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 that support the findings of this study are available from Dryad Digital Repository at https://doi.org/10.5061/dryad.h70rxwdqs.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.105228.

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