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Soil horizon development and altitudinal-vegetational zonation decouple microbial biomass from bulk soil nutrient availability across a Himalayan treeline ecotone

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Abstract. Alpine treeline ecotones are environmentally sensitive transition zones where vegetation structure, soil development, and nutrient cycling interact over short spatial scales. Despite increasing attention to Himalayan treeline dynamics, the distribution of soil microbial biomass and its stoichiometric relationships across vegetation and soil horizon gradient remains poorly understood. We quantified microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP), together with soil organic carbon (SOC) and total nitrogen (TN), across four altitudinal–vegetational zones and four genetic soil horizons in the Rolwaling Himalayan treeline ecotone, Nepal. Linear mixed-effects models were used to evaluate the effects of soil horizon development, vegetation zonation, and slope aspect on microbial biomass concentrations and stoichiometric relationships. Soil horizon development was the dominant control on microbial biomass distribution. MBC, MBN, and MBP declined significantly with depth ($p < 0.001$), with the surface O-horizon accounting for 55.7 %, 62.0 %, and 62.8 % of the cumulative horizon-wise concentrations of MBC, MBN, and MBP, respectively. Altitudinal-vegetational zonation generated pronounced non-linear patterns. SOC and TN concentrations were highest in the lower krummholz zone dominated by Rhododendron campanulatum, whereas MBC and MBN concentrations peaked in lower dwarf shrub heath zone, demonstrating a clear decoupling between bulk soil nutrient concentrations and microbial biomass. Microbial phosphorus concentrations exceeded microbial nitrogen concentrations by 3.9-fold across the study area, yielding an overall microbial biomass stoichiometry of 3.4:0.3:1 (MBC:MBN:MBP), substantially different from globally synthesised microbial biomass ratios, indicating plasticity in microbial biomass stoichiometry in stress environments in treeline ecotones. Our results indicate that microbial biomass distribution across the Rolwaling treeline ecotone is shaped by both soil horizon differentiation and altitudinal-vegetational

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zonation. The contrasting spatial patterns of microbial biomass and bulk soil nutrient concentrations suggest that measurements of total soil nutrients alone may not fully capture biologically active nutrient dynamics in high-elevation ecosystems.

1 Introduction

Alpine treeline ecotones represent transitional environments between closed montane forests and treeless alpine vegetation and are characterized by abrupt changes in vegetation structure, ecosystem functioning, and environmental conditions over relatively short spatial distances (Körner & Paulsen, 2004; Körner, 1998). These ecosystems have attracted considerable scientific attention because they are highly sensitive to climate change and often serve as indicators of environmental responses to global warming (Seddon et al., 2016; Björk & Molau, 2007). Traditionally, treeline formation has been explained primarily through thermal limitation, including growth limitation, where low temperatures restrict meristematic activity, and carbon sink limitation, where tissue development is constrained despite adequate carbon assimilation (Kullman, 2021; Körner, 2003; Hoch et al., 2002). Globally, these mechanisms converge around a growing season mean soil temperature threshold of approximately 6.4 ± 0.7 °C (Körner, 1998).

The treeline ecotone of the Rolwaling Himal in Nepal presents an unusual exception to this general framework. Previous studies have reported growing season mean soil temperatures of approximately 7.6 °C, substantially exceeding the globally observed growing season mean soil temperature threshold (Müller et al., 2016). Despite these relatively warm conditions, upward treeline advance remains limited, and vegetation dynamics exhibit delayed responses to recent climate warming (Schickhoff et al., 2015, 2023). This apparent thermal decoupling suggests that factors beyond temperature alone contribute to ecosystem functioning and vegetation dynamics in the Rolwaling treeline ecotone. Globally, increasing evidence indicates that vegetation composition, topography, geomorphology, soil development, and moisture availability interact to regulate ecological processes across treeline ecotones (Schwab et al., 2022; Holtmeier & Broll, 2017; Sigdel et al., 2018). As a result, uncovering the belowground processes associated with these environmental gradients has emerged as a key research focus.

While aboveground vegetation patterns and treeline dynamics in Rolwaling have been increasingly documented (Schickhoff et al., 2023; Schwab et al., 2022, 2016), much less research effort has been directed towards understanding belowground biological processes. The traditional treelines models primarily emphasize aboveground controls. However, nutrient limitation is increasingly recognized as an important constraint on tree growth and seedling establishment in high-altitude treeline ecosystem (Wang et al., 2025; Davis et al., 2018; Sullivan et al., 2015). Among belowground components, soil microorganisms play a central role because they regulate the decomposition of soil organic matter (SOM), nutrient mineralization, and the transformation of nutrients into biologically available forms (Singh & Gupta, 2018; Van Der Heijden et al., 2008). Consequently, microbial biomass represents an important indicator of nutrient cycling and ecosystem functioning in terrestrial ecosystems.

In high-altitude treeline regions, low soil temperature and recalcitrant organic inputs slow down the decomposition of organic matter and the mineralization process (Rawat et al., 2021; Kammer et al., 2009; Loomis et al., 2006). Consequently, there often

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is a non-linear relationship between total soil nutrient concentrations and plant available nutrients (Van Sundert et al., 2018; Prager et al., 2017). Because soil microorganisms play a crucial role in SOM mineralization process, limited microbial activity can hinder seedling establishment, forest regeneration, tree population dynamics and soil-plant feedback that influence seedling survival and growth (Angulo et al., 2019; Moscatelli et al., 2017; Sveinbjörnsson et al., 1992). Furthermore, with increasing elevation, soil microbial communities shift from bacterial-dominated to fungal-dominated, with fungal communities favoring nitrogen immobilization over mineralization (Thébault et al., 2014). In this context, soil microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) and their stoichiometric ratios provide more sensitive indicators of nutrient limitation and carbon dynamics compared to total soil nutrient concentrations alone (Luo et al., 2022). This is because, soil microorganisms can provide immediate physiological response to climate warming, drought, increased ultra-violet (UV) light, decreased snow cover (Chen et al., 2021; Bing et al., 2016).

Globally, treeline ecotones regions are stress environment for life. For the micro-organisms also, to survive such stress environment, their community should exhibit strong physiological plasticity which directly impact their Carbon Use Efficiency (CUE) i.e. how effectively the microbial community convert carbon to biomass (Mooshammer et al., 2014). Under the extreme environmental stress, such as frequent and erratic freezing and thawing cycles, nutrient poor soil, high pH and severe drought conditions, microbes substantially reduce their CUE focusing on survival and maintaining their body over the biomass growth. Hence microbes actively decoupled their internal stoichiometry from global relatively fixed internal carbon-to-nitrogen-to-phosphorus (C:N:P) ratio (Cleveland & Liptzin, 2007), breaking down the generally strict homeostasis rule (Fan et al., 2021). The distribution of this critical microbial biomass is highly heterogeneous, governed simultaneously by horizontal ecotone shifts and vertical soil profile (Gupta et al., 2024; Lepcha & Devi, 2020; Eilers et al., 2012). This vertical differentiation is influenced by litter quality, soil moisture and soil nutrient availability (Zheng et al., 2018). Soil microbial biomass fractions are also influenced by soil properties such as soil reaction (pH), clay content, land degradation, soil parent material, soil depth and soil development (Gupta et al., 2024; Beugnon et al., 2023; Singh & Gupta, 2018; Van Sundert et al., 2018). Similarly, from the horizontal dimension, soil microbial pool is influenced by changing vegetation composition (Schwab et al., 2022; Bargali et al., 2018) and seasonal climate with temperature and moisture being the primary regulatory factors (Broadbent et al., 2024; Huang et al., 2023; Shen et al., 2021). Similar vegetation-mediated effects on soil microbial biomass are well documented as an ecotonal effect in Himalayan treeline ecosystem (Gupta et al., 2024). Yet, how these horizontal and vertical dimensions interact to shape soil microbial stoichiometry remains unexplored in Nepal Himalaya.

Within the Nepalese context and to the best of our knowledge, Gaudel et al. (2024) and King et al. (2011) investigated soil microbial biomass in high-altitude ecosystem. Specifically, Gaudel et al. (2024) demonstrated that microbial mechanisms are pivotal in regulating SOC mineralization under nitrogen-enriched conditions, particularly during upward treeline migration. In contrast, King et al. (2011) reported lowest microbial biomass concentrations at extreme altitudes (>5100 m) in Nepal's Annapurna region indicating soil water availability is the primary limiting factor for microbial life in these high-elevation soils.

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Given that the Rolwaling treeline appears decoupled from recent global warming, investigating microbial biomass and its stoichiometric ratios can help to better understand if nutrient immobilization or structural constraints within the soil microbiome act as a bottleneck for seedling recruitment and ecotone advance under climate change. To our knowledge, this is the first study to analyse soil MBC, MBN and MBP in the context of nutrient availability, biomass stoichiometric ratio and potential limitations to tree growth across treeline ecotone regions of Nepal. Hence, we hypothesize that:

H1: Soil microbial biomass concentrations (MBC, MBN, and MBP) are primarily structured by soil horizon development and therefore decline significantly with soil depth.

H2: Vegetational zonation across the treeline ecotone modifies microbial biomass distribution, generating non-linear patterns along the altitudinal gradient that differ from those observed in bulk soil nutrient concentrations.

H3: Microbial biomass stoichiometry (MBC: MBN: MBP) deviates from globally reported microbial elemental ratios, reflecting localized nutrient constraints and microbial adaptation within the Himalayan treeline ecotone.

To achieve this, we first quantified microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) across an altitudinal-vegetational and soil depth gradient. We then identified nutrient patterns by analysing the stoichiometric ratios of this microbial biomass and evaluating their variation across different depths (soil horizons) and altitudinal-vegetational zones. Finally, we assessed the ecological significance of these microbial biomass trends for treeline dynamics, focusing particularly on their relationship with vegetational structure such as open shrub heath versus a closed krummholz canopy and site exposures.

2 Materials and Methods

2.1 Study area and sampling design

The study area is located in Dolakha district of Bagmati province, within the Gaurishankar Conservation Area (GCA) in the northeast part of central Nepal, within the Rolwaling valley (N 27°53′, E86°22′), which is shaped by the Rolwaling river (Fig. 1).

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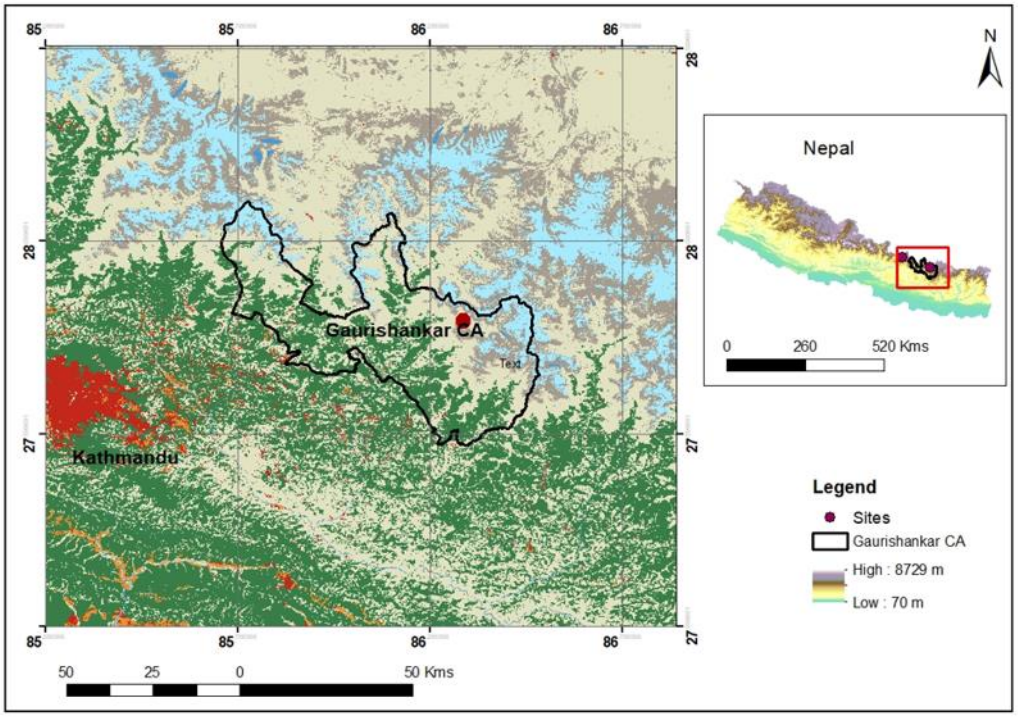

Figure 1. Map of Study Site and Sampling Slope Sectors (Rolwaling Beding).

This remote valley lies near the border of Nepal with Tibet and is known for its rugged terrain, high altitude and distinct ecological characteristics (Bürzle et al., 2017). Across the treeline ecotone, both the northeast (NE) and northwest (NW) slope sectors were divided into four altitudinal-vegetational zones (Fig. 2): LC (lower krummholz), UC (upper krummholz), LD (lower dwarf shrub heath) and UD (upper dwarf shrub heath). The characteristics of all four altitudinal-vegetational zones are provided in (Table 1). A stratified random sampling approach was used to collect the data. Within each zone of both NE and NW slope sectors, four randomly selected sample plots (20 m × 20 m) were established using a numbered grid map of the area to be sampled followed by a Microsoft Excel generated number table for the selection of the grids to collect data.

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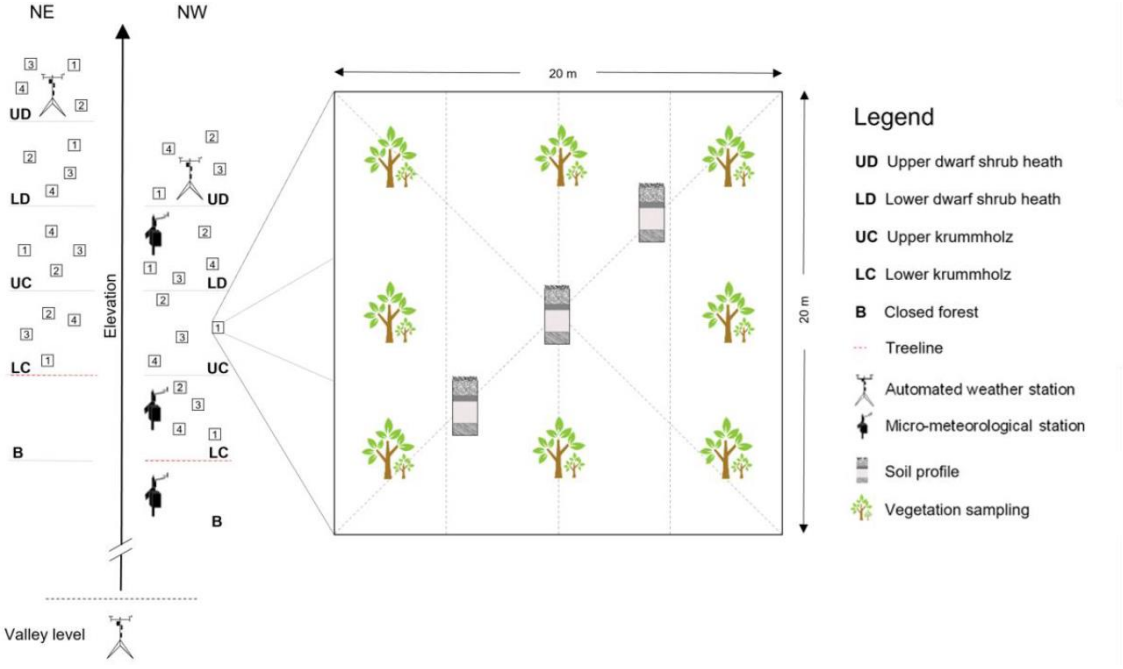

Figure 2. Experimental design and sampling framework across the elevational gradient. Sampling was conducted on Northeast (NE) and Northwest (NW) aspects spanning four altitudinal-vegetational zones, from Lower krummholz to upper dwarf shrub heath (UD). Replicated vegetation plots, soil profile pits, automated weather stations, and micro-meteorological stations were distributed along the gradient to assess vegetation, soil, and microclimatic variability. The inset shows the configuration of a 20 m × 20 m plot used for vegetation and soil sampling, including the location of vegetation sampling points and soil profile pits. Treeline boundaries on both aspect sectors are indicated by dashed red lines.

Table 1. Research site and altitudinal-vegetational zones characteristics in study area

Altitudinal/ Vegetation zones	Altitude (m a.m.s.l.) Northwest slope	Altitude (m a.m.s.l.) Northeast slope	Zone characteristics
Lower krummholz (LC)	3910 - 3990	4010 - 4065	Lower section of the krummholz forest, border to uppermost subalpine closed forest; dense and impenetrable cover of *Rhododendron campanulatum* thickets; shrubs plant almost absent (clean forest floor), presence of stunted individual of *Abies spectabilis* and *Betula utilis*, *R. campanulatum* stem are perpendicular to slope, deep rooted plants with deeper soil profiles
Upper krummholz (UC)	3990 - 4080	4065 - 4112	Upward extended section of krummholz forest, dense impenetrable krummholz mat turns into big patches of shorter *R. Campanulatum* thicket, occasional presence of *Rhododendron* shrubs species (*R. antopogon* and *R. setosum*), presence of few low-growing or *young A.* spectabilis, B. utilis and scatter Sorbus microphylla (dbh < 7 cm) presence

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Lower dwarf shrub heath (LD)	4080 - 4170	4112 - 4214	Alpine vegetation zone composed of dwarf shrub heaths *Rhododendron* dwarf thickets (compact growth) of *R. anthopogon, R. setosum, R. lepidotum*; presence of sparse, very short height (30- 50 individual *R. campanulatum*)
Upper dwarf shrub heath (UD)	4170 - 4260	4214 - 4247	Complete mat of *Rhododendron* shrubs species (R. *antopogon and R. setosum), R. lepidotum. R. antopogon is dominant among three Rhododendron species, shallow depth of soil profile limits the root depths, big boulders and rocks cover the dominant surface outcrops*

2.2 Climate, topography and vegetation

Climatologically, the study area is defined by a sharp moisture and temperature divide: winters are freezing and dry, whereas the summer months are temperate and moist due to the influx of the South-Asian monsoon. This monsoonal airflow (June–September) accounts for roughly 80 % of the local annual rainfall, totalling between 1,200 and 1,300 mm at treeline elevations (Karki et al., 2020; Weidinger et al., 2018). The mean annual air temperature measured in the study slopes ranges 2.8 °C to 3.6 °C (Müller et al., 2016). Due to its rugged topography, low population pressure, and remote character, the ecosystem on this north-facing slope persists in a near-natural state. Both slope sectors were highly inclined with a mean slope of 37 %. The geological parent material consists of pegmatitic rocks of granitic composition (Drollinger et al., 2017). The soils were classified as Podzols (World Reference Base, 2022) having a coarse-grained (sandy) texture and significant leaching of soil organic matter (SOM) and sesquioxide from the surface to sub-surface layers (podzolization). The soils exhibited generally low pH levels which slightly increased with depth, ranging from 2.5 to 4 (Müller et al., 2017). Soil profiles in both NE and NW slope sectors show O, Ah, E, and Bh horizons. Altitudinal-vegetational wise distinct ecological zones emerge: an upper subalpine mixed forest dominated by Abies spectabilis and Betula utilis, an intermediate krummholz belt composed mainly of Rhododendron campanulatum, and an alpine dwarf shrub heath network at the upper extreme dominates with shrub species of Rhododendron anthopogon, Rhododendron setosum and Rhododendron lepidotum (Schickhoff et al., 2023; Schwab et al., 2020).

2.3 Field sampling

Soil samples were collected in April and May 2023 from 32 sampling plots set up across four distinct altitudinal-vegetational zones on both the northeast and northwest slope sectors of the Rolwaling research site (Figs. 1 and 2). From each plot, composite samples were collected, with each sample representing the soil horizons of the three randomly selected soil profiles in the plot. In the LD and UD zones, trial-and error approaches were used to find suitable soil profile locations, as much of the plot area was obstructed by large stones and boulders, limiting the root zone and soil depth. In total, 103 soil samples were collected representing O-horizons, Ah-horizons, E-horizons and Bh-horizons. O, Ah and E-horizons were present in all 4 altitudinal-vegetational zones however Bh-horizon was absent in UD zone where soil profile was very shallow and mostly

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rocky. Samples were dried at 40 °C, sieved with 2 mm sieve and stored in the lab for further analysis. For soil samples of O-horizons, where SOM were the main soil constituent, were gently crushed by hand and plant residues (e.g. roots and twigs) were removed prior to analysis.

2.4 Laboratory analysis

Soil samples were analysed for MBC, MBN and MBP, alongside total carbon (TC) and total nitrogen (TN). Prior to analysis, samples were reactivated by adjusting to 60 % water-filled pore space (WFPS) and incubated at 20 °C for three weeks. Gravimetric water content was determined separately for each soil horizon by drying samples at 105 °C for 24 hours. MBC and MBN were determined via the chloroform fumigation-extraction method (Brookes, 2001; Vance et al., 1987). A total of 7.5 g of moist soil was fumigated with 80 ml of ethanol-free $CHCl_3$ in a vacuum desiccator for 24 hours. Both fumigated and non-fumigated (control) samples were extracted with 30 ml of 0.05 M K_2SO_4 and shaken for one hour. Extracts were filtered using demineralized water-flushed filter paper; the initial 2 ml of filtrate was discarded to prevent carbon contamination. Final extracts were analysed for C and N concentrations using a C/N analyser (Analytik Jena GmbH, Jena, Germany). MBP was estimated using the anion exchange membrane (AEM) method (Kouno et al., 1995) with hexanol substituted for chloroform to prevent membrane degradation (McLaughlin et al., 1986). This process involved three steps (a) resin preparation: AEM stripes were conditioned twice in 0.5 M $NaHCO_3$ for one hour and rinsed with de-ionised water, (b) absorption: AEM stripes were immersed in soil-water suspensions (non-fumigated, hexanol-fumigated, and blanks) and allowed for overnight (16 hours) shaking, and finally (c) desorption and measurement: Absorbed MBP was desorbed in 30 ml 0.5 NaCl/HCl solution for one hour. The concentrations of P were determined via inductively Coupled Plasma Optical Emission Spectroscopy (Analytik Jena PlasmaQuant 9100 Elite) to avoid the traditionally used hazardous reagent (Srivastava et al., 2004). Final biomass values were corrected for extraction efficiency using the following k-factors: for MBC - k_{EC} = 0.45 (Vance et al., 1987), for MBN- k_{EN} = 0.54 (Brookes et al., 1985), and for MBP- k_{EP} = 0.40 (Brookes et al., 1982).

Total carbon and nitrogen of soil samples were determined using an elemental analyser (vario EL III; Elementar, Germany). Dry and sieved soil samples representing soil horizons from each soil profiles were milled for 12 minutes to get pulverized samples. Then, 40 mg of the milled samples were weighted for elemental analysis of C and N in vario EL IIII. Carbonates were absent from the soils; therefore, total carbon was assumed equivalent to SOC.

The expression of bulk soil SOC and TN and microbial biomass parameters are discussed primarily as mass-based concentrations; mg g^{-1} soil for bulk soil properties e.g. SOC and TN and µg g^{-1} soil for microbial biomass fractions. The vertical profile contributions represented the relative apportionment of concentration intensity across genetic soil horizons rather than elemental stocks (g m^{-2}) of the soil properties.

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2.5 Statistical analysis

All statistical analyses were conducted in R version 4.4.2 (R Core Team, 2024) using the lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017), performance (Lüdecke et al., 2021), and emmeans (Lenth et al., 2026) packages. Prior to modelling, raw data were meticulously screened for chloroform fumigation extraction (CFE) anomalies. Observations yielding zero or negative flushes, where extractable nutrient concentration in non-fumigated controls were equal or exceeded those of fumigated samples, were classified as experimental artifacts of the CFE procedure and assigned as missing values (n = 3 for MBC, n= 5 for MBN and n= 1 for MBP). To satisfy the assumptions of normality and homoscedasticity required for linear modelling, concentrations of soil and microbial parameters (SOC, TN, MBC, MBN, MBP) and their respective stoichiometric ratios (C: N, C:P, N:P) were log10 transformed. Linear mixed-effects models (LMMs) were constructed separately for each response variable using the lmer() function from the lme4 package. The fixed effect's structure evaluated all main effects and their corresponding two-way interactions among three categorical predictors: altitudinal-vegetational zone, aspect, and soil horizon. To account for nested variation among sampling locations, "Plot ID" was included as a random intercept term in all models. The LMM for MBP was simplified to an additive model to resolve singularity issues. The full model interaction structure was defined as follows:

$$\log_{10}(Y_{ijk}) \sim (\text{Zone} + \text{Aspect} + \text{Soil horizon})^2 + (1 \mid \text{Plot ID})$$

Where, Yijk is the response variable (microbial biomass or nutrient concentration).

Model diagnostics were systematically verified using the check_model() function from the performance package (Lüdecke et al., 2021b) via visual inspection of residual tracking, Q-Q plots, and leverage diagnostics. Fixed effects were tested using Satterthwaite's method for degrees of freedom, as implemented in the lmerTest package. Significance was determined at $\alpha = 0.05$. For terms displaying significant interaction effects, post-hoc pairwise slices of the estimated marginal means (EMMs) were computed using the emmeans package with Tukey's Honestly Significant Difference (HSD) adjustment for multiple testing. All plots were generated using ggplot2. Soil microbial biomass stoichiometric ratios were calculated from the respective microbial biomass concentrations, and the soil microbial quotients (qMIC) and biomass nitrogen fraction were derived by dividing MBC with SOC and MBN with TN in percentage, respectively.

3 Results

3.1 Total soil organic carbon and nitrogen dynamics

Soil organic carbon concentrations varied significantly among soil horizons ($F_{(3,47.38)} = 128.60$, $p < 0.001$), altitudinal–vegetational zones ($F_{(3,25.28)} = 8.50$, $p < 0.001$), and slope aspects ($F_{(1,28.59)} = 4.57$, $p < 0.05$). Across all vegetation zones, SOC concentrations decreased markedly with depth, with the O-horizon containing the highest concentrations followed by the Ah-horizon (Fig. 3).

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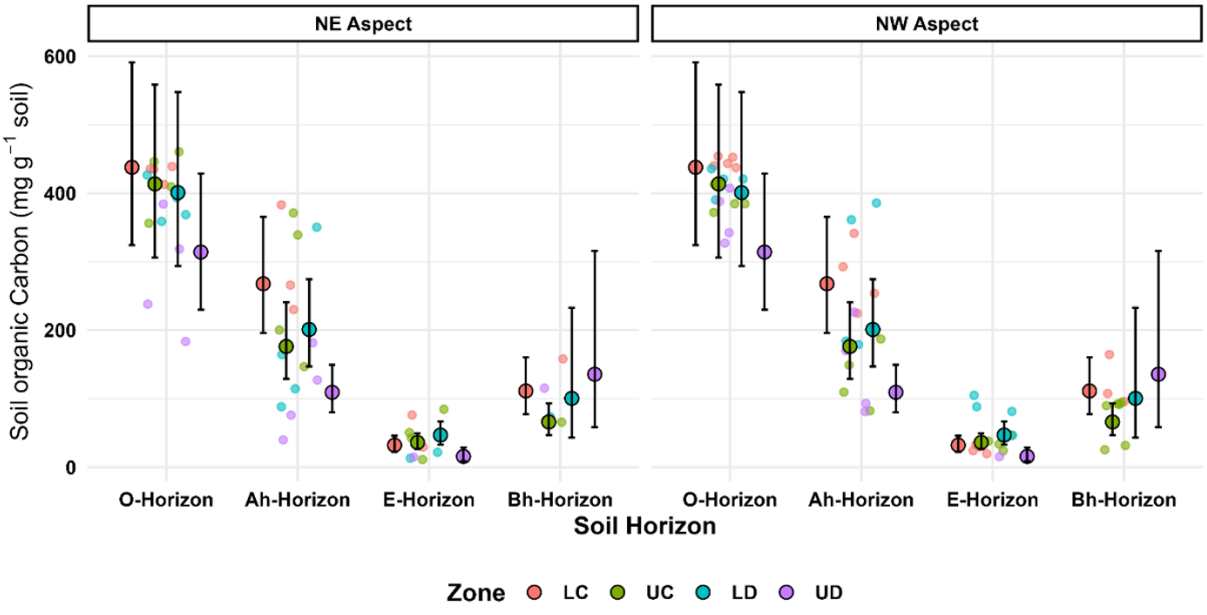

Figure 3. Soil organic carbon concentrations across different soil horizons and altitudinal vegetational zones under two slope aspects (NE and NW), four topographic zones: lower convex (LC), upper convex (UC), lower depression (LD), and upper depression (UD). Coloured dots represent individual observations for each zone, while the larger outlined circles indicate the mean SOC concentration for each group. Vertical error bars denote the variability around the mean.

Collectively, the O- and Ah-horizons accounted for 81.7 % of the cumulative horizon-wise SOC concentration measured across the soil profile and contained approximately 4.5 times higher SOC concentrations than the underlying E- and Bh-horizons. The decreasing trend with depth was interrupted by enrichment in the Bh-horizon, where SOC concentrations were 2.17 times higher than in the overlying E-horizon.

Across the altitudinal–vegetational gradient, SOC concentrations exhibited a distinct non-linear pattern. The highest mean SOC concentrations occurred in the lower krummholz zone (LC), followed by a 22 % decline in the upper krummholz zone (UC). SOC concentrations subsequently increased in the lower dwarf shrub heath (LD) before declining again in the upper dwarf shrub heath (UD) (Fig. 3). Northeast-facing slopes contained significantly higher SOC concentrations than northwest-facing slopes.

Total nitrogen (TN) concentrations displayed patterns similar to those observed for SOC. TN varied significantly among soil horizons ($F_{(3,49.41)} = 110.00$, $p < 0.001$) and altitudinal–vegetational zones ($F_{(3,27.86)} = 7.54$, $p < 0.001$). The highest TN concentrations occurred in the O-horizon followed by the Ah-horizon, with these two horizons collectively accounting for 81.5 % of the cumulative horizon-wise TN concentration across the soil profile. TN concentrations declined strongly from the O-horizon to the E-horizon, which contained the lowest concentrations, before increasing again within the Bh-horizon.

The bulk soil C: N ratio varied significantly among soil horizons ($F_{(3,42.39)} = 26.99$, $p < 0.001$) and slope aspects ($F_{(1,24.97)} = 9.64$, $p < 0.01$), but not among altitudinal–vegetational zones ($F_{(3,23.32)} = 2.59$, $p > 0.05$). C:N ratios declined from the O-

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245 horizon to the Ah- and E-horizons, where values were reduced by approximately 23–25 %, before increasing again in the Bh-horizon to values comparable with those observed in surface organic horizons. Despite substantial variation in SOC and TN concentrations among vegetation zones, soil C:N ratios remained comparatively stable across the treeline ecotones.

3.2 Quantification of microbial biomass carbon, nitrogen and phosphorus

3.2.1 Microbial biomass carbon

250 Despite substantial Microbial biomass carbon concentrations were strongly influenced by soil horizon development ($F_{(3,41.37)}$ = 46.63, p < 0.001), whereas neither aspect nor the main effect of altitudinal–vegetational zone significantly affected MBC concentrations (p > 0.05; Table 2). Across all vegetation zones, MBC concentrations declined markedly with soil depth. The O-horizon contained the highest mean MBC concentration (1247.3 µg C g^{-1} soil), accounting for 55.7 % of the cumulative horizon-wise MBC concentration measured across the soil profile. Relative to the O-horizon, mean MBC concentrations were
255 reduced by 50.6 % in the Ah-horizon, approximately 80 % in the E-horizon, and approximately 90 % in the Bh-horizon (Fig. 4).

Although the overall main effect of altitudinal–vegetational zone was not significant ($F_{(3,22.89)}$ = 1.43, p = 0.259), mean MBC concentrations displayed a distinct non-linear pattern across the treeline ecotone. The lowest mean concentrations occurred in the krummholz zones (LC and UC), whereas the highest mean concentration was recorded in the lower dwarf shrub heath (LD;
260 815.7 µg C g^{-1} soil). MBC concentrations subsequently declined slightly in the upper dwarf shrub heath (UD; 748.8 µg C g^{-1} soil), resulting in the overall pattern LD > UD > LC > UC (Fig. 4).

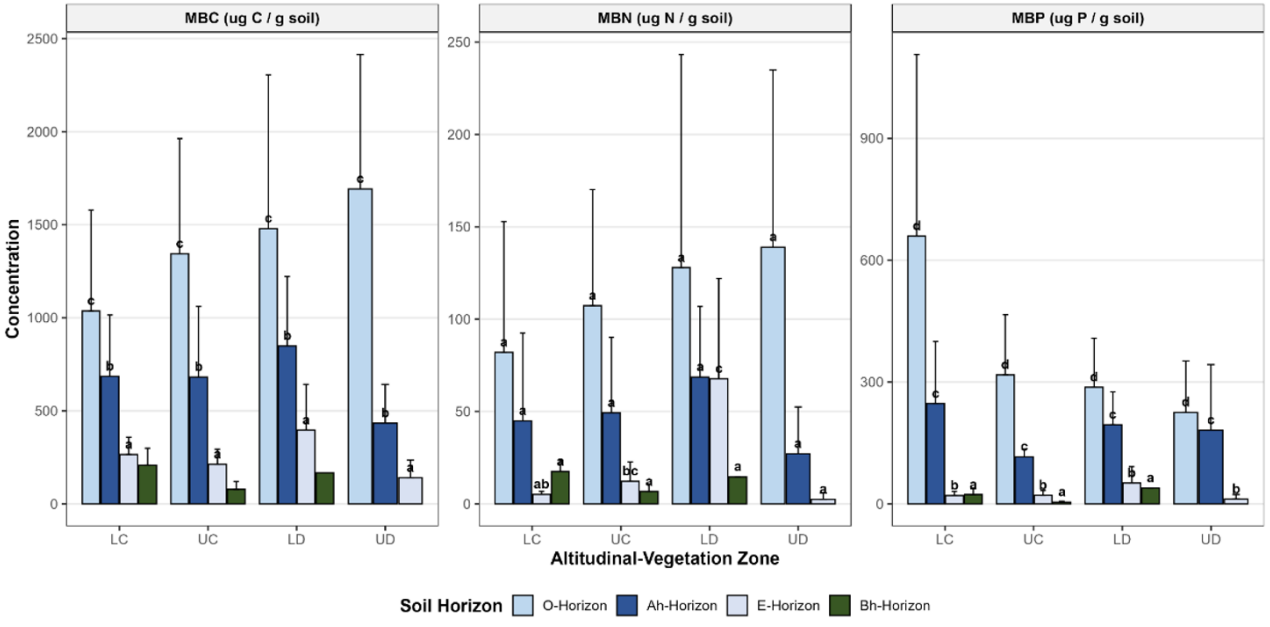

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Figure 4. MBC, MBN, and MBP concentrations across an altitudinal-vegetational ecotone gradient (LC: Lower krummholz, UC: Upper krummholz, LD: Lower dwarf shrub heath, UD: Upper dwarf shrub heath) stratified by soil horizons (O-, Ah-, E-, and Bh-horizons). Bars represent mean values and error bars denote standard deviation. Different lowercase letters indicate statistically significant differences ($p < 0.05$) based on linear mixed-effects models (LMMs). For MBN, where a significant Zone × Soil Horizon interaction was observed ($p < 0.05$), letters denote differences among vegetation zones sliced within each individual soil horizon using a Sidak-adjusted post-hoc comparison. For MBC and MBP, letters track the dominant, highly significant main effect of vertical depth stratification across soil horizons ($p < 0.001$). The missing data cluster for the Bh-horizon within the UD zone represents no Bh-horizon

Table 2. Descriptive statistics of bulk soil nutrient concentrations, microbial biomass fractions, microbial quotients, and microbial stoichiometric ratios across four altitudinal–vegetational zones and soil horizons in the Rolwaling Himalayan treeline ecotone.

Zone	Soil Horizon	SOC (mgg^{-1} soil)	Total Nitrogen (mgg^{-1} soil)	Soil C: N Ratio	MBC (µg C g^{-1} soil)	MBN (µg N g^{-1}soil)	MBP (µg P g^{-1} soil)	qMIC (%)	MBC: MBN	MBC: MBP	MBN: MBP	MBC:MBN :MBP
LC	O-Horizon	439.04± 11.94	15.72± 2.36	27.92	1036.5± 542.9	82± 70.8	659.3± 447.1	0.24	12.63	1.57	0.12	1.6: 0.1: 1
LC	Ah-Horizon	273.76± 62.59	13.37± 3.03	20.48	672.8± 307.1	39.3± 46.8	238.3± 143.9	0.25	17.11	2.82	0.16	2.8: 0.2: 1
LC	E-Horizon	35.3± 20.62	1.67± 0.97	21.12	267.3± 78.1	7.3± 8.3	18.3± 10.2	0.76	36.74	14.57	0.4	14.6: 0.4: 1
LC	Bh-Horizon	116.25± 37.6	4.22± 1.72	27.56	140.1± 126.5	11.7± 10.2	15.7± 15.4	0.12	12	8.93	0.74	8.9: 0.7: 1
UC	O-Horizon	405.03± 34.17	14.34± 1.96	28.25	1343.5± 619.9	107.3± 62.8	310.9± 139.8	0.33	12.52	4.32	0.35	4.3: 0.3: 1
UC	Ah-Horizon	198.25± 104.38	8.76± 4.9	22.63	585.6± 399.4	30.8± 40	111.2± 57.3	0.3	18.99	5.26	0.28	5.3: 0.3: 1
UC	E-Horizon	41.43± 21.59	1.82± 0.98	22.78	191± 96.4	10.7± 10.6	24.3± 14.1	0.46	17.82	7.86	0.44	7.9 : 0.4: 1
UC	Bh-Horizon	70.21± 30.12	2.42± 1	29.01	117± 75.3	6± 4.3	5.3± 5.4	0.17	19.6	22.13	1.13	22.1: 1.1 : 1
LD	O-Horizon	401.97± 28.38	13.16± 2.19	30.54	1312.6± 898.3	127.9± 115.5	272.5± 119.1	0.33	10.27	4.82	0.47	4.8: 0.5: 1

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LD	Ah-Horizon	228.46± 118.62	9.19± 4.11	24.85	750.8± 442.3	60.1± 42.9	177.7± 88.6	0.33	12.5	4.23	0.34	4.2: 0.3: 1
LD	E-Horizon	59.35± 37.78	2.58± 1.52	23.01	347.8± 249.3	56.4± 55.9	48.1± 37.1	0.59	6.16	7.23	1.17	7.2: 1.2: 1
LD	Bh-Horizon	73.51	4.28	17.18	166.2	14.6	39	0.23	11.39	4.26	0.37	4.3: 0.4 : 1
UD	O-Horizon	323.75± 77.62	12.17± 2.46	26.61	1333.5± 859.8	139± 96	293.2± 142.8	0.41	9.59	4.55	0.47	4.5: 0.5: 1
UD	Ah-Horizon	124.48± 63.33	5.82± 2.76	21.39	434.6± 211.6	17± 23.7	236.7± 196	0.35	25.59	1.84	0.07	1.8: 0.1: 1
UD	E-Horizon	15.06± 0.42	0.88± 0.13	17.21	140± 94.8	2.4± 3.4	11.6± 11	0.93	57.5	12.08	0.21	12.1: 0.2: 1
UD	Bh-Horizon	115.41	4.08	28.29	70.2	NA	2.5	0.06	NA	28.63	NA	NA

Values are presented as mean ± standard deviation.
SOC = soil organic carbon; TN = total nitrogen; MBC = microbial biomass carbon; MBN = microbial biomass nitrogen; MBP = microbial biomass phosphorus; qMIC = microbial quotient (MBC/SOC × 100).
Altitudinal–vegetational zones: LC = lower krummholz, UC = upper krummholz, LD = lower dwarf shrub heath, UD = upper dwarf shrub heath.
Values without standard deviations represent single observations where the corresponding soil horizon occurred in only one soil profile.
NA indicates insufficient observations to calculate the respective parameter.

A marginally significant interaction between altitudinal–vegetational zone and soil horizon was detected ($F_{(8,41.31)} = 2.10$, p = 0.058), indicating that the magnitude of vertical MBC stratification varied among vegetation zones. However, the general decline in MBC concentrations with increasing soil depth was observed consistently across all zones.

Linear regression analyses revealed positive relationships between SOC and MBC concentrations across the study area. However, the strength of these relationships varied among vegetation zones, with the strongest associations observed in the lower and upper krummholz zones (Fig. 5).

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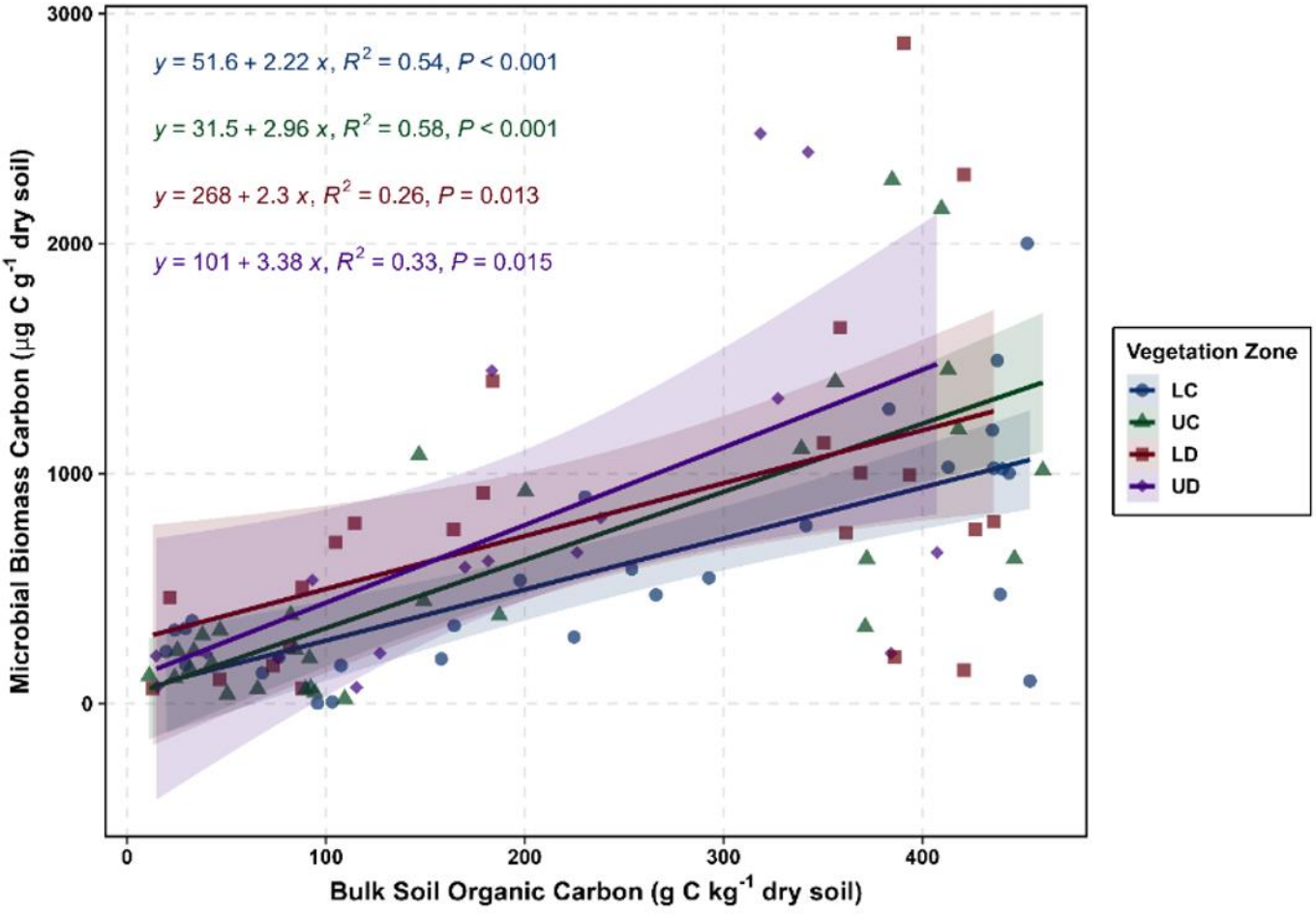

Figure 5. Linear regression analysis between Bulk Soil Organic Carbon (SOC; g C kg⁻¹ soil) and Microbial Biomass Carbon (MBC; µg C g⁻¹ soil) across the altitudinal-vegetational zones. Individual plot observations are differentiated by specific marker shapes and colours corresponding to each zone: Lower krummholz (LC), Upper krummholz (UC), Lower dwarf shrub heath (LD), and Upper dwarf shrub heath (UD). Solid lines represent the linear regression trends for each zone, accompanied by shaded 95 % confidence interval bands. The linear regression equation, coefficient of determination (R^2), and statistical significance value (p-value) for each respective zone are displayed in a line within the plot frame

3.2.2 Microbial biomass nitrogen

Microbial biomass nitrogen concentrations varied significantly among soil horizons ($F_{(3,41.84)} = 24.32$, $p < 0.001$) and altitudinal–vegetational zones ($F_{(3,22.19)} = 3.78$, $p < 0.05$). In addition, a significant interaction between vegetation zone and soil horizon was detected ($F_{(8,41.73)} = 3.19$, $p < 0.01$), indicating that the vertical distribution of MBN differed among vegetation zones.

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Across the soil profile, MBN concentrations declined markedly with depth. The O-horizon contained the highest mean MBN concentration and accounted for more than 62 % of the cumulative horizon-wise MBN concentration measured across the profile. Mean MBN concentrations decreased substantially in the underlying Ah-horizon and continued to decline within the E- and Bh-horizons (Fig. 5).

Altitudinal–vegetational zonation significantly influenced MBN distribution across the treeline ecotone. Mean MBN concentrations followed the sequence LD > UD > LC > UC, with the highest concentrations occurring in the lower dwarf shrub heath (LD) and the lowest concentrations in the upper krummholz zone (UC). The mean MBN concentration in LD was approximately twice that observed in the lower krummholz zone (LC).

The significant zone × horizon interaction reflected differences in the magnitude of vertical MBN stratification among vegetation zones. While MBN concentrations generally declined with depth in all zones, MBN concentrations in the Bh-horizon slightly exceeded those of the E-horizon in the LC zone, whereas the opposite pattern was observed in most other vegetation zones.

3.2.3 Microbial biomass phosphorus

Microbial biomass phosphorus concentrations varied significantly among soil horizons ($F_{(3,73)} = 67.78$, $p < 0.001$) and altitudinal–vegetational zones ($F_{(3,73)} = 4.57$, $p < 0.01$). Due to variance constraints at the plot level, MBP was analysed using an additive mixed-effects model. Neither the zone × horizon nor the aspect × horizon interaction terms were significant ($p > 0.05$).

MBP concentrations exhibited strong vertical stratification across the soil profile. The O-horizon contained the highest mean MBP concentration and accounted for 62.8 % of the cumulative horizon-wise MBP concentration measured across all samples. A marked decline in MBP concentration occurred in the Ah-horizon, and concentrations decreased further within the E- and Bh-horizons. Collectively, the O- and Ah-horizons contributed more than 93 % of the cumulative horizon-wise MBP concentration across the soil profile.

Altitudinal–vegetational zonation significantly influenced MBP concentrations. In contrast to MBC and MBN, which reached maximum concentrations in the lower dwarf shrub heath (LD), MBP concentrations were highest in the lower krummholz zone (LC). Mean MBP concentrations followed the sequence LC > LD > UD > UC (Fig. 4).

Across most samples, MBP concentrations exceeded corresponding MBN concentrations, with mean MBP concentrations approximately 3.9 times greater than MBN concentrations across the study area.

The distribution of microbial nutrients across the treeline ecotone was characterized by high spatial variability, with coefficients of variation increasing in the order MBC (94.7 %) < MBP (122.0 %) < MBN (131.5 %). MBC and MBN displayed similar spatial patterns, reaching highest concentrations in LD and following the sequence LD > UD > LC > UC. In contrast, MBP concentrations were highest in LC and followed the sequence LC > LD > UD > UC.

Across the altitudinal–vegetational gradient, microbial biomass and bulk soil nutrient concentrations displayed contrasting spatial distributions. Whereas SOC and TN concentrations were highest in the lower krummholz zone (LC), MBC and MBN

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335 concentrations reached maximum values in the lower dwarf shrub heath (LD). In contrast, MBP concentrations followed a pattern more closely aligned with bulk soil nutrient concentrations.

3.2.4 Multivariate relationships among microbial and soil properties

Principal component analysis (PCA) explained 57.5 % of the total variance, with PC1 and PC2 accounting for 39.0 % and 18.5 %, respectively (Fig. 6). PC1 represented a gradient associated primarily with SOC, TN, and MBP, whereas MBC and MBN
340 loaded positively along both PC1 and PC2. Samples from the lower krummholz zone (LC) clustered predominantly in the direction of SOC, TN, and MBP vectors, while samples from the lower dwarf shrub heath zone (LD) were more closely associated with MBC and MBN. The ordination therefore revealed a spatial separation between zones characterized by high bulk soil nutrient concentrations and those characterized by high microbial biomass concentrations. Elevation showed comparatively weak loading relative to biological and edaphic variables.

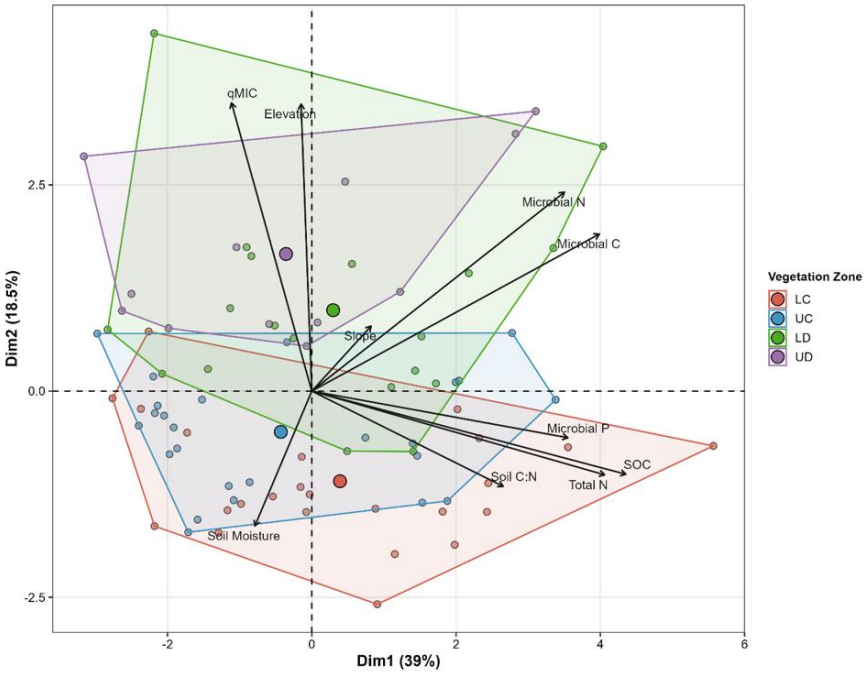

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Figure 6. Principal component analysis (PCA) of microbial biomass fractions, microbial stoichiometric indices, bulk soil nutrient concentrations, and environmental variables across four altitudinal–vegetational zones of the Rolwaling treeline ecotone. Polygons represent the distribution of samples within each vegetation zone. Vector length and direction indicate the relative contribution of individual variables to the ordination space.

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3.3 Stoichiometry ratios of microbial biomass and their variation

Microbial stoichiometric ratios exhibited distinct responses to soil horizon development and altitudinal–vegetational zonation (Table 2). The MBC:MBN ratio varied significantly among soil horizons ($F_{(3,46.9)} = 4.50$, $p < 0.01$) and vegetation zones ($F_{(3,23.9)} = 3.51$, $p < 0.05$). In contrast, variation in the MBC:MBP ratio was explained solely by soil horizon ($F_{(3,58.8)} = 9.77$, $p < 0.001$) and showed no significant response to vegetation zone or slope aspect ($p > 0.05$). The MBN: MBP ratio exhibited the strongest spatial sensitivity, with a significant vegetation zone × soil horizon interaction ($F_{(8,42.1)} = 2.22$, $p < 0.05$). Pronounced horizon-specific differences were observed for all microbial stoichiometric ratios. The MBC:MBN ratio showed exceptionally high variability within the E-horizon (CV = 396.9 %), where values were significantly higher than those observed in the O-horizon. Post-hoc comparisons indicated that the mean MBC:MBN ratio in the E-horizon exceeded that of the O-horizon by approximately 17.8-fold ($p < 0.01$). The MBC ratio also varied significantly with depth and displayed a progressive increase from the organic surface horizons toward the mineral soil profile. In contrast, the Bh-horizon represented the only soil environment in which microbial nitrogen exceeded microbial phosphorus, with an average MBN:MBP ratio greater than 1.

Altitudinal–vegetational zonation significantly influenced microbial stoichiometric relationships across the treeline ecotone. The highest mean MBC:MBN ratios occurred in the upper dwarf shrub heath (UD), whereas lower values were recorded in the krummholz zones (LC and UC) (Table 2). The significant zone × horizon interaction observed for MBN further indicates that the relative allocation of microbial nitrogen and phosphorus varied among vegetation zones and soil horizons.

Across the entire study area, the cumulative microbial biomass stoichiometry was approximately 3.4:0.3:1 (MBC: MBN: MBP). Mean MBP concentrations (197.45 μg P g^{-1} soil) exceeded corresponding MBN concentrations (50.65 μg N g^{-1} soil) by approximately 3.9-fold across all samples. Consequently, the observed microbial biomass stoichiometry differed substantially from globally reported microbial elemental ratio.

3.4 Soil microbial quotient (qMIC) and microbial nitrogen fraction (qN)

The microbial quotient (qMIC) varied significantly among soil horizons ($F_{(3,70.8)} = 7.70$, $p < 0.001$), whereas the microbial nitrogen fraction (qN) showed no significant response to soil horizon, altitudinal–vegetational zone, or slope aspect ($p > 0.05$; Table 3). Across the study area, mean qMIC and qN values were 0.40 % and 0.84 %, respectively (Fig. 7). The qMIC displayed pronounced vertical variation, with the highest values occurring in the E-horizon and the lowest values in the Bh-horizon. Mean qMIC in the Bh-horizon (0.19 %) was approximately 75 % lower than that observed in the E-horizon. Considerable spatial variability was evident for both indices, reflected by coefficients of variation of 86.3 % for qMIC and 165.7 % for qN. Although the effects of altitudinal–vegetational zone were not statistically significant, mean qMIC values were highest in the lower and upper dwarf shrub heath zones (LD and UD; 0.45 %). Similarly, mean qN values ranged from 0.47 % in the lower krummholz zone (LC) to 1.53 % in the lower dwarf shrub heath zone (LD), indicating substantial spatial heterogeneity across the treeline ecotone.

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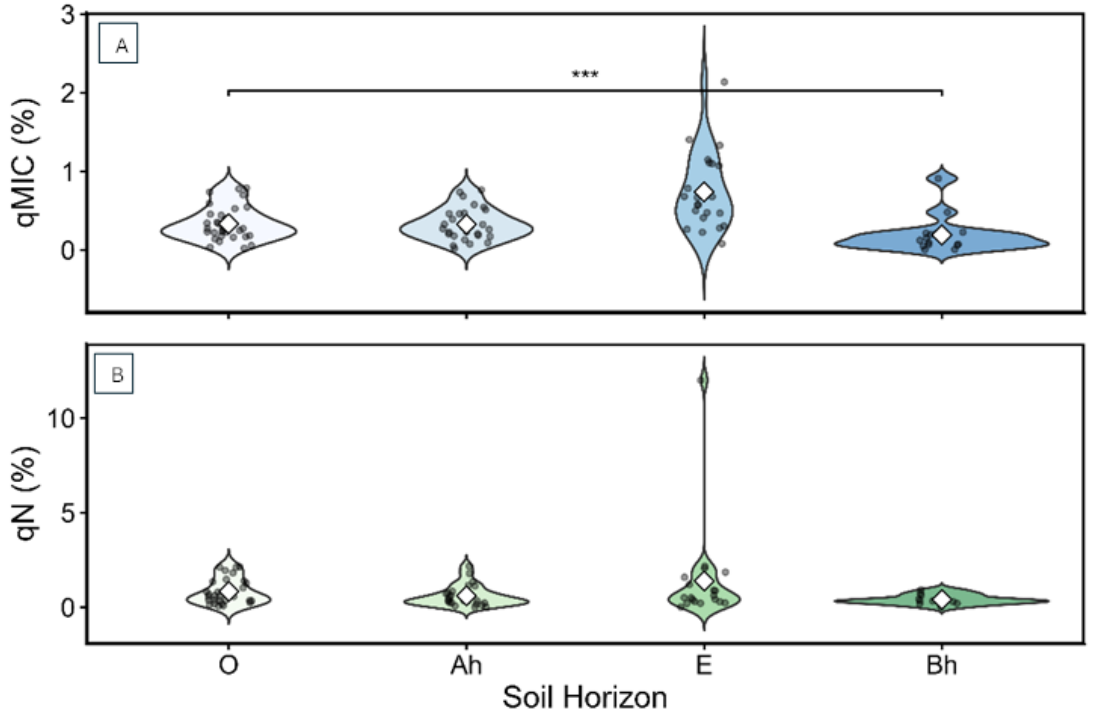

Figure 7: (A) Microbial quotient (qMIC; microbial biomass carbon as a percentage of total soil organic carbon) and (B) microbial nitrogen fraction (qN; microbial biomass nitrogen as a percentage of total soil nitrogen) across four distinct soil horizons (O, Ah, E, and Bh). Violin plots represent the kernel density distribution of the data; the white diamond indicates the arithmetic mean, and the overlaid jittered points represent individual plot observations. Significance levels were determined at the $\alpha = 0.05$ threshold

4 Discussion

4.1 Vertical pedogenic control of microbial biomass and nutrient concentrations

The strong vertical stratification of soil microbial biomass and bulk soil nutrients observed across the Rolwaling treeline ecotone confirms our first hypothesis that soil horizon development is the primary control on microbial biomass distribution. Across all altitudinal–vegetational zones, concentrations of SOC, TN, MBC, MBN and MBP declined significantly with depth, with the organic O-horizon functioning as the principal zone of biological activity. More than half of the cumulative horizon-wise microbial biomass concentrations occurred within the O-horizon, while microbial biomass concentrations in the Bh-horizon were reduced by 90–97 % relative to the surface layer. Similar depth-dependent declines have been reported across alpine treeline ecotone regions, where vertical gradients in substrate availability and soil development exert stronger control

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on microbial biomass than broader climatic gradients (Gao et al., 2024; Joshi et al., 2023; Wang et al., 2022; Chen et al., 2021; Donhauser & Frey, 2018; Bargali et al., 2018; Serna-Chavez et al., 2013).

The observed pattern is closely linked to the pedogenic characteristics of the Rolwaling soils. Previous investigations have classified these soils as strongly podzolized systems developed under acidic vegetation, coarse-textured granitic parent material and intense monsoonal precipitation exceeding 1300 mm yr^{-1} (Drollinger et al., 2017; Müller et al., 2017). Under these conditions, dissolved organic carbon and organo-metal complexes are continuously translocated from surface horizons into deeper mineral layers, producing pronounced vertical differentiation of soil properties. Consequently, the O- and Ah-horizons accumulate fresh organic inputs and biologically available nutrients, whereas the E-horizon experiences substantial nutrient depletion through eluviation. The marked increase in SOC and TN concentrations (Fig. 3) from the E- to Bh-horizon observed in this study reflects this classical podzolization process and confirms earlier findings from the same treeline ecotone (Drollinger et al., 2017; Müller et al., 2017).

Despite the accumulation of SOC and TN concentrations in Bh-horizons relative to E-horizons, microbial biomass concentrations remained low throughout the mineral soil profile. This observed divergence suggests that total nutrient concentrations may not fully reflect the microbial habitat quality in podzolic soils. The linear regression analysis between SOC and MBC (Fig. 5) clearly illustrate the weak correlation, specifically in UD and LD altitudinal-vegetational zones. A substantial fraction of carbon and nutrients accumulated in spodic horizons is likely associated with Fe- and Al-organic complexes or mineral surfaces, reducing accessibility for microbial uptake and growth (Kögel-Knabner et al., 2008) and become chemically immobilized through sorption and co-precipitation being nutrient-poor horizon (Petticord et al., 2025). Similar decoupling was observed by Min et al. (2024) in forest sites suggesting that high soil SOC-TN ratios accelerate necromass turnover and recycling by living organisms.

The steep decline of microbial biomass with depth also reflects changes in substrate quality. Surface organic horizons receive continuous inputs of litter, root exudates and partially decomposed organic matter that sustain active microbial communities. In contrast, deeper mineral horizons are dominated by older, more chemically recalcitrant compounds (Kögel-Knabner et al., 2008); this down-profile reduction explains the sharp decline in microbial biomass, as deep mineral horizons lack the labile resources necessary to sustain large microbial populations. This interpretation can further be explained by the energy limitation theory (Filimonenko & Kuzyakov, 2025; Manzoni & Porporato, 2009). Surface horizons accumulate high-molecular-weight organic compounds, but only a subset of these are accessible without specialized extracellular enzymes. In deeper horizons, many substrates are chemically bound to mineral surfaces or occluded within aggregates, increasing the energetic cost of extraction and suppressing microbial growth (Schmidt et al., 2011). Although microbial processes were not measured directly in this study, the observed reduction in microbial biomass concentrations with depth is consistent with increasing energetic and chemical constraints in mineral soil horizons.

In our study, O-horizons of all four altitudinal-vegetational zones have higher C:N ratio (Table 2) ranging from 26:1 in UD to 30:1 in LD compared to their corresponding sub surface soil horizons. Notably, the C: N ratio is highest in the O-horizon of the dwarf shrub heath zone, (LD) rather than in zones where thick R. campanulatum krummholz that contributes largely to the

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organic layer. The high C:N ratio of LD is probably caused by the limited amount of nitrogen in soil in this zone (Müller et al., 2017). These findings reflect nutrient limitation even when carbon is abundant but present in forms less favourable for microbial uptake as R. campanulatum leaves and litters contents high lignin-rich material (Semwal et al., 2003). Hence, the depth gradients of microbial biomass C, N and P are simultaneously controlled by an energy-limited microbial environment, where decomposition and mineralization are constrained both thermodynamically and chemically.

Notably, soil total nutrient concentrations displayed a characteristic bulge (Fig. 3) in the vertical soil profile. Concentrations in the Bh-horizon were over three times higher than in the E-horizon, indicating marked nutrient depletion in the eluvial layer and enrichment in the illuvial Bh-horizon. This vertical differentiation is diagnostic for active podzolization processes, where monsoon precipitation of approximately 1300mm yr-1facilitates leaching of soil nutrients, as described for high-altitude slopes in Himalaya (World Reference Base WRB, 2022; Müller et al., 2017; Drollinger et al., 2017; Bäumler, 2004). Furthermore, the higher concentration of MBN in the Bh-horizon of LC zone compared to upper leached E-horizon suggests that podzolization process is intense in LC zone. We also observed a significant altitudinal-vegetational zone and soil horizon interaction for MBN which clearly indicates that the vegetation composition of the study sites dictates the vertical distribution of microbial nitrogen.

4.2 Decoupling between microbial biomass and bulk soil nutrient concentrations

A particularly notable finding of this study was the spatial decoupling between bulk soil nutrient concentrations and microbial biomass concentrations across the treeline ecotone. While SOC and TN concentrations reached their maxima within the lower krummholz zone (LC), microbial biomass carbon and nitrogen concentrations were highest in the lower dwarf shrub heath (LD) zone. Consequently, zones characterized by the greatest accumulation of soil carbon and nitrogen did not coincide with zones supporting the highest microbial biomass concentrations.

This pattern contrasts with the commonly assumed positive relationship between total soil organic matter and microbial biomass (Gupta et al., 2024; Joshi & Garkoti, 2023; Manral et al., 2023) and suggests that microbial communities respond more strongly to nutrient accessibility and substrate quality than to total nutrient concentrations (Liddle et al., 2020; Webster et al., 2001). Although SOC and TN concentrations provide information on the quantity of accumulated organic matter, they do not necessarily reflect the fraction available for microbial assimilation and growth because only a relatively small and dynamic portion of total soil organic matter is readily accessible to microorganisms (Lehmann & Kleber, 2015; Kuzyakov & Xu, 2013). Previous studies from alpine and forest ecosystems have similarly reported weak or inconsistent relationships between total soil organic matter and microbial biomass where organic matter stabilization, organo-mineral interactions, and substrate recalcitrance limit microbial utilization despite substantial carbon accumulation (Donhauser & Frey, 2018; Van Sundert et al., 2018; Prager et al., 2017).

The Rolwaling treeline ecotone provides a particularly interesting example of this phenomenon. Drollinger et al. (2017) reported strong altitudinal declines in several plant-essential nutrients (Na, Mg, K and P) across the same ecotone, while Müller et al. (2017) documented highly acidic podzolic soils with intense nutrient leaching and organic matter translocation. Our

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findings extend these observations by demonstrating that microbial biomass concentrations do not mirror the spatial distribution of SOC and TN concentrations. Instead, microbial biomass appears to be regulated by the biological availability of nutrients within these organic matter pools. The PCA further demonstrated (Fig. 6) that bulk soil nutrient concentrations (SOC and TN) clustered with the lower krummholz zone, whereas MBC and MBN were associated with the dwarf shrub heath zones, reinforcing the observed decoupling between microbial biomass and total nutrient concentrations.

The lower krummholz (LC) zone represents the clear example of this decoupling. Despite exhibiting the highest SOC and TN concentrations, this zone maintained comparatively low MBC and MBN concentrations and among the lowest microbial biomass quotients (qMIC) and microbial biomass nitrogen fractions (qN) (Fig. 7). Such patterns suggest that a substantial fraction of organic matter accumulated within krummholz soils may be inaccessible to microbial communities (see 4.1 above). Similar observations have been reported from subalpine Rhododendron-dominated ecosystems where persistent organic matter accumulation occurs alongside relatively slow microbial turnover rates and reduced decomposition efficiency (Rawat et al., 2021; Bargali et al., 2018).

Conversely, the alpine dwarf shrub heath zones (LD and UD) supported substantially higher microbial biomass concentrations despite lower bulk nutrient concentrations. The elevated microbial quotient values observed in these zones indicate that a larger proportion of available carbon was incorporated into microbial biomass. This suggests more efficient microbial utilization of organic substrates and potentially faster nutrient cycling relative to the krummholz zone. As shown in (Table 1), the LD and UD zones were primarily dominated by mixed shrub species, whereas the LC and UC zones were characterised by dense krummholz stands of Rhododendron campanulatum. Shrub species occurring in the LD and UD zones generally produce smaller, thinner leaves that dry rapidly after senescence, resulting in litter that is more exposed to atmospheric conditions. In contrast, the dense R. campanulatum krummholz in the LC and UC zones forms a thick litter mat that can physically protect organic matter from direct solar radiation and wind exposure. Therefore, the relatively greater incorporation of organic substrates into mineral soil in the LD and UD zones may reflect the combined effects of litter quality, reduced surface litter retention, and increased exposure to wind and radiation (Barnes et al., 2015; Cleveland et al., 2014).

The divergence between bulk soil nutrient concentrations and microbial biomass concentrations therefore highlights a critical ecological distinction between nutrient quantity and nutrient availability because microbial biomass functions as a labile reservoir of potentially plant -available nutrients (Brookes, 2001; Anderson & Domsch, 1980). While the krummholz zone functions as a major zone of carbon and nitrogen accumulation, the alpine dwarf shrub heath appears to function as a zone of enhanced microbial processing. Consequently, microbial biomass concentrations provide complementary information to bulk soil chemistry and reveal functional differences in nutrient cycling that would remain undetected if only SOC and TN concentrations were considered. Hence, this explanation clearly supports our second hypothesis where microbial biomass showed the non-linear distribution along the altitudinal gradient followed the general sequence of LD > UD > LC > UC. These findings emphasize that biologically available nutrient fractions rather than total nutrient concentrations are likely to be the primary control of microbial abundance and activity within the Rolwaling treeline ecotone.

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4.3 Microbial stoichiometry and nutrient constraints in the treeline ecotone

Microbial biomass stoichiometry provides insight into how soil microorganisms allocate and retain nutrients under environmental constraints. Across the Rolwaling treeline ecotone, microbial stoichiometric ratios differed substantially from globally reported averages, supporting our third hypothesis that microbial communities exhibit localized stoichiometric responses to environmental conditions (Hall et al., 2011). The observed microbial biomass stoichiometry 3.4:0.3:1 (MBC: MBN: MBP) differed substantially from the globally averaged microbial biomass ratio of 60:7:1 reported by Cleveland and Liptzin (2007), indicating that microbial elemental composition in this Himalayan treeline ecosystem has pronounced departure from globally synthesised patterns. Although soil microorganisms are often considered relatively homeostatic compared with plants, growing evidence demonstrates that microbial communities can exhibit substantial stoichiometric flexibility or microbial stoichiometric plasticity in response to environmental stress and resource imbalances (Fan et al., 2021; Mooshammer et al., 2014). Fan et al. (2021) demonstrated that water availability, temperature, and soil nutrient status are the primary factors influencing the stoichiometric homeostasis of soil microbial biomass in alpine grasslands on the Tibetan Plateau. This finding is consistent with our results, which show substantial variation in soil stoichiometric ratios both along the soil profile and across altitudinal and vegetation zones, suggesting that microbial communities respond to local environmental and edaphic conditions. Furthermore, experimental evidence from Fanin et al. (2013) indicates that variations in microbial stoichiometry are linked to changes in microbial community structure, highlighting the capacity of microbial communities to adjust their elemental composition in response to environmental conditions. Similar departures from global stoichiometric expectations have been reported from cold alpine ecosystems of the Tibetan Plateau and Gongga Mountain, where microbial communities display elevated stoichiometric plasticity and strong spatial variability in nutrient allocation (Fan et al., 2021; Sun et al., 2013). Hence, in alpine ecosystems where low temperatures, nutrient limitations, and strong seasonal fluctuations impose physiological constraints on microbial growth. Consequently, microbial biomass stoichiometry often reflects local environmental conditions rather than a fixed global elemental composition.

Our results reveal a disproportionately high microbial phosphorus concentration, exceeding microbial nitrogen by approximately fourfold. This pattern may indicate enhanced microbial phosphorus retention or accumulation relative to nitrogen, a pattern typical of nutrient-limited ecosystems where phosphorus is prioritized for essential cellular functions (Elser et al., 2003; Manzoni & Porporato, 2009). In the Rolwaling Himal, treeline ecotones are defined by extreme conditions and our findings of low microbial biomass and near 1:1 MBN:MBP ratios align with other regional studies such as on Gongga Mountain, where MBP follows a parabolic relationship with altitude, often equalling or exceeding MBN in subalpine zones (Sun et al., 2013). In the central Himalaya, microbes act as nutrient sponges during the rainy season, rapidly immobilizing P and resulting in low N:P ratios (Manral et al., 2023). Similarly, on the Tibetan Plateau, microbes exhibit high stoichiometric plasticity, accumulating P as a strategic response to cold stress and scarcity (Fan et al., 2021). The high MBC:MBN ratios observed in our upper dwarf (UD) and E horizons suggest that nitrogen limitation is particularly severe. These likely forces microbial communities to divert carbon toward maintenance and nutrient scavenging rather than growth (Allison et al., 2010).

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This supports the N limitation hypothesis (Körner, 2003) in high-altitude soils, where low temperatures suppress nitrification and ammonification pathways, reducing nitrogen availability despite ongoing plant and microbial demand.

However, the consistently higher values of microbial biomass phosphorus compared to microbial biomass nitrogen highlight the need for further investigation into microbial dynamics within Himalayan treeline ecotones. Such patterns suggest that phosphorus may play a more critical role in regulating microbial processes at high altitudes than previously recognized. A comprehensive examination of microbial community structure and functional composition is therefore essential to understand the underlying mechanisms driving these imbalances. Detailed analyses of soil bacterial, fungal, and protistan communities could offer valuable insights into how these groups are assembled, how they interact, and how their ecological functions shift along environmental gradients. This, in turn, would enhance our understanding of nutrient cycling and microbial adaptation strategies in sensitive alpine ecosystems experiencing rapid climatic change.

4.4 Ecological implications for Himalayan treeline ecosystem functioning

The observed patterns of microbial biomass and stoichiometry have important implications for understanding nutrient cycling and ecosystem functioning within Himalayan treeline ecotones. While treeline research has traditionally focused on climatic controls such as temperature limitation, increasing evidence suggests that belowground processes may substantially influence ecosystem responses to environmental change (Mayor et al., 2017; Sullivan et al., 2015). Our results indicate that microbial biomass distribution across the Rolwaling treeline ecotone is not simply governed by elevation or bulk soil nutrient concentrations, but rather by the interaction between soil development and vegetation composition represented by four altitudinal-vegetational zones.

A key finding of our research was the spatial separation between nutrient accumulation and microbial biomass hotspots. Krummholz-dominated zones had the highest SOC and TN concentrations but relatively low microbial biomass, whereas alpine dwarf shrub heath supported higher microbial biomass despite lower nutrient concentrations. This suggests that nutrient availability and microbial utilization efficiency, rather than total nutrient stocks alone, are more important determinants of belowground ecosystem functioning. Similar distinctions between nutrient quantity and accessibility have been recognized as critical drivers of carbon and nutrient cycling in cold ecosystems (Van Sundert et al., 2018; Prager et al., 2017).

Traditional treeline theories such as the growth limitation hypothesis and the carbon balance hypothesis have historically focused on plant physiology (Körner & Paulsen, 2004). However, it is important to note that the study site in the Rolwaling Himal exhibited a GSMT significantly higher (7.6 ± 0.6 °C) than the global treeline average of 6.4 ± 0.7 °C (Körner, 2012; Müller et al., 2016). This suggests that the observed reductions in microbial biomass cannot be attributed solely to thermal energy constraints. Instead, they are more likely related to chemical constraints associated with the species-specific traits of Rhododendron campanulatum. In Himalayan treeline ecotone, species-specific or plant community composition role for treeline dynamics were previously reported (Schwab et al., 2020; Gaire et al., 2017). Similarly, research from the Rolwaling region indicates that Rhododendron campanulatum species may exert strong allelopathic effects (Bürzle et al., 2017; Schickhoff et al., 2015). This species, through the accumulation of lignin-rich litter, together with the production of secondary

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metabolites, such as polyphenols and terpenoids, in its leaves and roots observed in allelochemical chemical profiling have been reported in Himalaya that may contribute to inhibit the growth of neighbouring plant species and suppress microbial metabolic pathways. These allelopathic effects represent a conceptual example of plant-mediated legacy effects that persist in soil systems, as demonstrated by Heinen et al. (2020), who found that plant communities leave soil legacies that affect succeeding vegetation through fungal-mediated pathways. Recalcitrant litter inputs, modified soil–climate interactions, and potential allelopathic suppression of microbial communities may collectively explain the persistence of organic matter accumulation in the krummholz zone despite temperatures 1.2 °C above the global average. These processes may create positive feedback whereby the chemical legacy of *R. campanulatum* maintains conditions favourable to its continued dominance in the LC zone of the Rolwaling Himal treeline ecotone.

5 Conclusion

This study provides the first integrated assessment of microbial biomass carbon, nitrogen, phosphorus, and microbial stoichiometry across an altitudinal–vegetational and soil horizon gradient within a Himalayan treeline ecotone. Soil horizon development emerged as the dominant control on microbial biomass distribution, with MBC, MBN, and MBP concentrations declining sharply from the organic O-horizon to underlying mineral horizons. This strong vertical stratification reflects the influence of podzolization and the progressive reduction in biologically available substrates with depth.

Despite pronounced accumulation of soil organic carbon and total nitrogen within krummholz-dominated zones, microbial biomass concentrations were highest in alpine dwarf shrub heath communities. This divergence indicates that microbial biomass distribution does not necessarily mirror patterns of bulk soil nutrient concentrations and highlights the importance of nutrient accessibility rather than nutrient quantity in regulating belowground biological processes.

Microbial biomass stoichiometry deviated substantially from globally reported microbial elemental ratios, indicating localized nutrient constraints and considerable stoichiometric flexibility within the treeline ecotone. The strong variability of microbial stoichiometric relationships among soil horizons and vegetation zones further suggests that nutrient allocation by microbial communities is shaped by the combined influence of pedogenesis and vegetation composition.

Overall, our findings demonstrate that nutrient cycling in the Rolwaling treeline ecotone is governed by the interaction of vertical soil development and horizontal vegetation zonation. Integrating microbial biomass and stoichiometric indicators with conventional soil nutrient measurements therefore provides a more comprehensive understanding of ecosystem functioning in Himalayan alpine environments. Future research combining microbial community composition, extracellular enzyme activities, and nutrient transformation rates will be essential for identifying the mechanisms linking vegetation change and belowground ecosystem processes under ongoing climate change.

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Data availability

The datasets generated and/or analysed during the current study are not publicly available because they form part of an ongoing research project. They will be made publicly available upon completion of the project. In the meantime, the datasets are available from the corresponding author upon reasonable request.

Author contributions

RA Conceptualised and designed the study, developed the methodology, conducted the field sampling, performed the laboratory analyses, curated and analysed the data, prepared the figures and visualizations, interpreted the results, and wrote the original manuscript draft. **TS** contributed to the study conceptualization, developed the methodology, supervised the research, secured funding, provided resources, administered the project, and critically reviewed and edited the manuscript. **YO** contributed to supervise the research, interpreted the results, and critically reviewed and edited the manuscript. **US** and **JB** contributed to provided resources, administered the project, and critically reviewed and edited the manuscript. **JH** contributed to field sampling and laboratory analyses. **MP** contributed to field sampling, laboratory analyses, and manuscript review and editing. **AM, CG, SS, CKS, NS** and **RPC** contributed to the interpretation of the results and critically reviewed and edited the manuscript. All authors discussed the results, contributed to the final interpretation of the findings, and approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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