

Ecoenzymatic C: N: P stoichiometry reveals microbial nutrient limitations across seasonal, depth, and disturbance gradients in *Shorea robusta* forest soils of the Western Himalaya, India

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Research Article

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Posted Date: July 31st, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7100501/v1>

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1 **Title:** Ecoenzymatic C: N: P stoichiometry reveals microbial nutrient limitations across seasonal, depth, and disturbance
2 gradients in Shorea robusta forest soils of the Western Himalaya, India

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14 **ABSTRACT**

15 **Background and aims** Logging threatens nutrient (C, N, P) retention in *Shorea robusta* forests, disrupting biogeochemical
16 cycles by altering vegetation regeneration and soil biochemical feedbacks. Yet, combined effects of disturbance and
17 seasonality on microbial biomass, extracellular enzyme activities (EEAs), and stoichiometry remain underexplored,
18 particularly under nutrient-limited, climate-sensitive conditions.

19 **Methods** This study investigated how seasonal shifts, soil depths, and four disturbance regimes (no disturbance (ND): <
20 5% basal area removal, low disturbance (LD): < 20%, moderate disturbance (MD): < 50%, and high disturbance (HD): >
21 50%) shape microbial biomass and C-, N-, and P-cycling enzymes (cellobiosidase, β -glucosidase, β -acetyl-
22 glycosaminidase, urease, acid/alkaline phosphatase). It also explored relationships with understory vegetation (Shannon
23 diversity (H'), fine root biomass (FRB)) and soil physicochemical properties.

24 **Results** Microbial biomass and enzyme activities peaked in MD stands during monsoon. LD and HD stands exhibited
25 reduced C: N and C: P EEAs ratios, suggesting elevated P limitation. Depth-wise EEAs stoichiometry indicated increased
26 constraints on C and P in deeper layers. Vector analysis identified C and P as limiting elements, with partial alleviation at
27 HD. Structural equation modeling revealed stronger P than N limitation, driven by understory H' , FRB, microbial traits,
28 and soil nutrients. Positive correlations between microbial biomass, EEAs, and nutrient limitation indices suggest
29 microbial activity is shaped more by nutrient availability than by strict stoichiometric homeostasis.

30 **Conclusions** Overall, this study illustrates seasonality, depth, and disturbance interactively govern microbial nutrient
31 dynamics in sal forests, revealing microbial adaptability under shifting environmental constraints and implications for
32 nutrient cycling and ecosystem resilience.

33 **Keywords:** Disturbance gradients; Microbial resource limitation; Soil enzymes activity; Soil nutrients; Sub-tropical
34 ecosystems; Understory vegetation

35 **Highlights**

- 36 1. Effects of disturbance regimes and season changes on microbial and enzymatic activity are asymmetric, peaking in
37 moderate disturbance (MD).
- 38 2. Soil extracellular enzymes C: N: P stoichiometry reveals the microbial C and P limitation in sal forests.
- 39 3. Microbial C and P limitations are more pronounced in high disturbance (HD) stands compared to the control (ND)
40 stands.
- 41 4. Soil moisture, understory diversity, and fine root biomass were the key factors driving microbial C and N/P
42 limitations.
- 43 5. Vegetation attributes and soil nutrients influence microbial resource limitations more than soil properties.

44 **Introduction**

45 Tropical forest soils store a substantial proportion of global carbon (C) and contribute significantly to gross primary
46 productivity and C sequestration (Riutta et al. 2021; Shankar et al. 2025; Sandra et al. 2025). They also play a crucial role
47 in the global N and P cycles (Waring et al. 2014; James et al. 2021; Xinli et al. 2021). In recent decades, growing concerns
48 over climate change and disturbance-driven degradation have intensified efforts to conserve biodiversity (Fornwalt et al.
49 2018; Zhou et al. 2020; Sandra et al. 2025) and implement sustainable management practices to support multiple
50 ecosystem functions and long-term soil fertility (Lucas-Borja and Delgado-Baquerizo 2019; Jiang et al. 2023). Sal (*Shorea*
51 *robusta* Gaertn f.) forests hold transregional importance, spanning tropical, sub-tropical zones to the Himalayan foothills.
52 They are widely distributed across India, Bangladesh, and Nepal, with occurrences also reported in Bhutan, South China,
53 and Malaysia (Gautam and Mandal 2016; Riutta et al. 2021; Shankar and Garkoti 2025). However, these forests are
54 increasingly threatened by anthropogenic pressures such as logging, wildfires, agricultural expansion, and the invasion of
55 non-native species (Riutta et al. 2021; Kumar et al. 2021). These disturbances are significantly undermining nutrient
56 storage capacity, ecosystem services, and long-term ecological sustainability (Kim et al. 2019; Raj and Jhariya 2021;
57 Pandey et al. 2024). For example, disturbances caused by frequent logging and thinning (common silvicultural practices)

are widely employed to regulate stand biomass C composition, tree density, and promote understory vegetation diversity (Cao et al. 2021; Li et al. 2023; Shankar et al. 2025). These practices modify the forest microclimate by increasing light availability, temperature, and soil moisture, thereby influencing nutrient cycling and ecosystem development. Despite their ecological significance, below-ground systems, particularly soil health and microbial dynamics, are highly sensitive to such disturbances changes in microbial community structure and functions play a pivotal role in regulating nutrient availability and soil biochemical processes (Qui et al. 2021; Xue et al. 2023). Quantifying and understanding the mechanisms by which soil microbes acquire and allocate nutrients under disturbance regimes remains a major scientific challenge (He et al. 2020; Zeng et al. 2023). This involves uncovering microbial strategies by examining relative shifts in soil enzyme activities, highlighting their role in reallocating internal reserves to optimize growth, sustain population abundance, and uphold nutrient cycling amid changing environmental conditions.

Forest disturbance not only affects the above-ground systems but also significantly impact below-ground components (Wang et al. 2019; Riutta et al. 2021; Jiang et al. 2023). A recent study in *S. robusta* forest ecosystems (Shankar et al. 2025) reported significant increases in above-ground biomass C and soil organic C stocks in disturbed stands. These increases were primarily attributed to enhanced growth of a diverse tree community and understory vegetation stimulated by canopy opening following disturbance. In this context, microorganisms are vital components of both managed and natural forest soil ecosystems (Lull et al. 2020; Joshi et al. 2023; Shankar et al. 2024). They play a central role in soil nutrient dynamics and diverse biogeochemical processes, including humus formation, and the transformation and decomposition of soil organic matter (SOM) (Waring et al. 2014; Acosta et al. 2018; Wang et al. 2023). Microbes synthesize and release specific extracellular enzymes that maintain elemental homeostasis, particularly carbon (C), nitrogen (N), and phosphorus (P), which reflect the balance between resource availability and microbial demand for growth and reproduction (He et al. 2020; Xu et al. 2022; Zhen et al. 2022). In sal forests, microbial communities frequently encounter an imbalance in resource availability, leading to nutrient limitations (Raj and Jhariya 2021; Shankar and Garkoti 2025). In response, microbes adjust the relative allocation of extracellular enzyme activities (EEAs) to

81 optimize nutrient acquisition. These adjustments can directly or indirectly influence C and nutrients (N and P) storage and
82 cycling (Cenini et al. 2016; Chen et al. 2022; Jiang et al. 2023). As such, EEA serves as an important indicator of
83 microbial nutrient limitations and has been widely used to assess ecosystem nutrient constraints. The factors underlying
84 these limitations are often stand specific and temporally dynamic (Cao et al. 2021; Qui et al. 2021; Xue et al. 2023).
85 Therefore, a systematic analysis is essential to elucidate the ecological drivers that govern microbial nutrient acquisition
86 strategies, which are fundamental to understanding nutrient cycling and ecosystem functioning.

87 Many studies have investigated the effect of forest disturbances on soil microbial biomass and enzyme activity (Kim et al.
88 2019; Shankar and Garkoti 2023; Zeng et al. 2023). A meta-analysis of 49 studies across forest types identified
89 disturbance as a key driver of changes in soil microbial structure, biomass, and EEAs (Zhou et al. 2020). However, the
90 effects of disturbance magnitude on microbial metabolism and nutrient dynamics are complex, as reflected in contrasting
91 results from different forest ecosystems (Xue et al. 2023; Zeng et al. 2023). Moderate disturbance is often associated with
92 improved structural diversity, soil properties, and microbial activity, which collectively enhance vegetation growth and
93 microbial functions (Kim et al. 2019; Qui et al. 2021). In contrast, excessive disturbance can lead to degradation of both
94 above- and below-ground components, including loss of dominant species, such as *S. robusta*, disrupting plant-microbes
95 and soil-microbes interactions (Shankar et al. 2025). However, other studies have reported a significant reduction in
96 microbial diversity and EEAs at any level of disturbance, primarily due to a reduction in litterfall production, root
97 biomass, and nutrients (Jiang et al. 2023; Xue et al. 2023).

98 Emerging evidence suggests that soil EEAs and eco-enzymatic stoichiometry (EES) vary seasonally due to changes in
99 temperature, moisture, litter biomass influx rate, which influence microbial metabolism and enzyme production (Wan et
100 al. 2019; Cao et al. 2021). In tropical dry deciduous broadleaf forests (e.g., sal), seasonal variations in moisture and
101 nutrient availability strongly affect microbial activity and stoichiometry (Lull et al. 2020; Srivastava et al. 2020; Shankar
102 et al. 2025). Typically, EEAs are higher during summer and monsoon seasons than in winter, largely due to increased soil
103 temperature and moisture (Singh et al. 2017; Cao et al. 2021; Qui et al. 2021). However, most studies on microbial

ecosystem C: N: P stoichiometry have been limited to a single season, with a few investigating seasonal variations across different disturbance regimes (Jiang et al. 2023; Zeng et al. 2023; Qui et al. 2021). Increasing evidence suggests that the direction and intensity of disturbance interact with seasonal factors to influence microbial biomass, EEAs, and EES (Kim et al. 2019; Shankar and Garkoti 2023; Wan et al. 2019; Xue et al. 2023).

The choice to use EEAs to evaluate soil fertility and nutrient C: N: P stoichiometry depends on the availability of resources and the relative production of specific enzymes (Zhou et al. 2020; Xue et al. 2023). This approach acknowledges that each enzyme catalyzes a distinct biochemical reaction and, therefore, cannot represent the overall soil microbial activity, which encompasses a broad spectrum of enzymatic processes (Waring et al. 2014; Nannipieri et al. 2018). Moreover, because enzymes are substrate-specific, no single enzyme can comprehensively indicate the nutrient status of the soil (Mori et al. 2023). Thus, the most typical soil EEAs include C-acquiring (β -1,4-cellobiosidase (CBH), β -1,4-glucosidase (BG)), N-acquiring (β -1,4-N-acetyl-glucosaminidase (NAG)), and P-acquiring (alkaline phosphatase (ALP) or acid phosphatase (AcP)) (Cenini et al. 2016; Qui et al. 2021; Wei et al. 2024). These eco-enzymes are widely distributed in soils and are synthesized by microorganisms in response to the decomposition of organic matter or variations in C: N: P availability, which represents an important direct or indirect link between microbial ecological metabolism and nutrient stoichiometry theory (Jiang et al. 2023; Gao et al. 2024; Huang et al. 2024). Other soil enzymes include dehydrogenase (DHA) and urease (U) that can be used as soil biological indicators in forest ecosystems, globally (Lull et al. 2020; Srivastava et al. 2020; Shankar and Garkoti 2023). The balance between microbial growth and nutrient availability is often reflected in the nutrients required and the utilization efficiency of microbes that are widely studied by evaluating the C-N-P acquiring eco-enzymes and their stoichiometry ratios (Waring et al. 2014; Acosta et al. 2018; Wan et al. 2019; Wang et al. 2023). Recent research across various forest and grassland ecosystems has shown that EEAs and EES ratios are strongly influenced by SOM content, soil physicochemical properties (such as pH, moisture, and bulk density), and microbial characteristics (He et al. 2020; Hernández-Cáceres et al. 2022; Xu et al. 2022). Typically, soil EES ratios are negatively correlated with the stoichiometry of available soil nutrients (C: N: P) (Waring et al. 2014; Zhu et al. 2020; Qui

et al. 2021). However, in some forest ecosystems, studies have reported positive correlations (Cenini et al. 2016; Kim et al. 2019). In addition, disturbance-induced changes in tree structure and composition promote the growth of understory vegetation, significantly altering the above-ground litter influx rate and root biomass density (e.g., fine root biomass (FRB)), which may affect the soil microbial biomass and EEAs (Lull et al. 2020; Xu et al. 2022; Xue et al. 2023). For example, a recent study by Kim et al. (2019) reported significant increases in microbial biomass for EEAs from 11.1 to 57.7%, along with a 9.6 to 10.4% increase in soil organic carbon (SOC), under heavy disturbance intensity (30-44% basal area reduction) compared to undisturbed conditions in oak and larch forest ecosystems. This inconsistent relationship between soil EEAs, EES, and soil nutrient status and microbial properties may result from differences in stand characteristics, vegetation structural diversity, forest types, study region, and intensity of ecological disturbance (e.g., logging) (Qui et al. 2021; Jiang et al. 2023; Wang et al. 2023). This complexity highlights the importance of investigating the relative influences of understory vegetation diversity, fine root biomass (FRB), and soil physicochemical properties on EEAs and EES ratios, particularly in response to disturbances and seasonal dynamics in Southeast Asian environments, especially within sal (*S. robusta*) forest ecosystems.

To improve our understanding of soil microbial metabolism in dry deciduous broadleaf forest ecosystems, we designed to examine the interactive effect of the disturbance regimes and seasons on soil EEAs and EES ratios in sal forests in the western Himalaya. We also assessed how vegetation functional traits (e.g., FRB), structural diversity, soil physicochemical, and microbial properties influence EEA, EES, and nutrient limitations. Specifically, the present research aims to: (1) investigate seasonal changes in content and stoichiometry characteristics of C, N, and P in soil, microbial biomass, and EEAs across two soil depths under varying disturbance regimes; (2) quantify microbial investment strategies in C-, N-, and P-acquiring EEAs to infer the relative magnitudes of nutrient limitation throughout the seasons induced by disturbances; and (3) reveal key ecological constraints that relatively influences the microbial biomass, EEAs, and their nutrient limitations throughout seasons under varying disturbance regimes (see more in Fig. 1). We hypothesize that: (H1) moderate disturbance (MD) would increase EEAs compared to non-disturbed (ND) stands by promoting understory

growth and enhancing soil organic matter and improved soil conditions (Kim et al. 2019; Jiang et al. 2023); (H2) EEAs and their stoichiometry would increase during growing seasons following disturbances due to increasing nutrient competition between soil microbes and understory vegetation; (H3) expected higher microbial N and P limitations in our P-poor soils because microbial growth is frequently described to be P limited in tropical forests (Waring et al. 2014); (H4) disturbance-induced increases in understory vegetation biomass and diversity would strongly influence EEAs and EES through changes in soil nutrient and microbial biomass stoichiometry rather than soil physicochemical conditions (Qui et al. 2021; Zeng et al. 2023). The findings of this study are expected to enhance understanding of soil ecological stoichiometry and its underlying drivers, thereby contributing to the sustainable productivity and ecological resilience of sal (*S. robusta*) forests.

Material and methods

Study area description

The study sites were situated in the Baghani Forest Division (30°29'30.23" - 30°31'36.04" N, 77°42'11.16" - 77°44'30.80" E) in the Sirmour district of Himachal Pradesh, India, at an elevation of 450-700 m (Fig. 2). The region experiences a subtropical climate with three distinct seasons: monsoon (mid-June to September), winter (December to February), and a warm, dry summer (April-June). It experiences an average annual temperature of 21.2 °C and annual rainfall of 142.5 mm with 70-75% of the rainfall occurring between June and August (Fig. 2d). The soils are shallow (0-45 cm) and young, derived naturally through weathered processes of metamorphic rocks, which predominantly composed of feldspar, mica, illite, and vermiculite (Raina and Gupta 2009). According to the USDA soil taxonomy, the predominant soil types are Alfisols and Inceptisols, characterized by a stony loam texture (Shankar and Garkoti 2023). Soil organic carbon (SOC) levels range from 0.4% to 3.1%, with soils ranging from acidic to neutral in pH, and deficient in nitrogen and phosphorus, influenced by the surrounding vegetation (Pandey et al. 2024; Shankar et al. 2025).

171 In undisturbed forests, *Shorea robusta* Gaertn. f. (Sal) dominates the canopy. However, in disturbed forests, the dominance
 172 of *S. robusta* is shared coexisting with other tree species like *Acacia catechu*, *Murraya koenigii*, *Mallotus philippensis*,
 173 and *Terminalia tomentosa*. This shift results in a significant loss of basal area, approximately 70%, in disturbed stands. For
 174 a detailed description of canopy tree species composition, see Shankar and Garkoti (2023). The under-canopy vegetation is
 175 characterized by dense layers of shrubs, including *Lantana camara*, *Adhatoda vasica*, and *Flemingia strobilifera*,
 176 alongside herbs such as *Ageratum houstonianum* and *Lepidagathis incurva* (Shankar et al. 2025). Anthropogenic
 177 disturbances are prevalent in these forests with disturbance levels, ranging from 51% to 91%, key activities include
 178 logging, livestock grazing, and firewood collection (Rawat et al. 2020; Pandey et al. 2024). These disturbances are driven
 179 by the heavy reliance of the local community on sal and its associated species for timber, fuelwood, fodder, and medicinal
 180 resources, which are vital for their livelihoods (Gautam and Mandal 2016; Shankar and Garkoti 2025).

181 Shankar and Garkoti (2023) delineated the forest areas into distinct disturbance-affected stands based on the intensity of
 182 disturbance and the extent of canopy coverage (CC, %). The disturbance index (DI, %) was calculated at the stand level
 183 using the proportion of basal area removed from trees based on a formula adapted from Rao et al. (1990). The
 184 mathematical equation for DI estimation:

$$185 \quad DI = \left(\frac{\sum_{i=1}^{i=10} Basal\ area_{dead}}{\sum_{i=1}^{i=10} (Basal\ area_{live} + Basal\ area_{dead})} \right) \times 100 \quad (1)$$

186 These disturbance-affected stands were classified as follows: no disturbance (ND); $DI \leq 5$ and $CC > 70\%$, low disturbance
 187 (LD); $5 < DI \leq 20$ and $CC > 50\%$, moderate disturbance (MD); $20 < DI \leq 50$ and $CC < 50\%$, and high disturbance (HD);
 188 $DI > 50$ and $CC < 30\%$ (Fig. 1 (b)).

189 **Soil and vegetation sampling design**

190 To investigate the effects of disturbance regimes, seasons, and depths on microbial biomass, element-acquiring EEAs, and
 191 their stoichiometry, we selected 15 disturbance-affected stands (4 ND + 4 LD + 4 MD + 3 HD) across three adjacent forest

192 sites with similar geological features, climatic conditions, and vegetation compositions (Fig. 2). A stratified random
193 sampling method was employed to evaluate belowground ecological processes across disturbance-affected stands. To
194 minimize edge effects, two sampling blocks (20 m × 20 m; >10m buffer area) were located at random within each
195 disturbance-affected stand (Xue et al. 2023; Shankar and Garkoti 2024). From each block of a predefined disturbance-
196 affected stand, four soil cores were randomly collected at 1 to 1.5 meters from the base of dominant species tree stumps
197 using a stainless-steel cylindrical corer (15 cm × 5 cm × 5 cm) at soil depths of 0-15 cm and 15-30 cm (Pandey et al.
198 2024). These respective soil depths were then homogenized using the quartering and conning method to create composite
199 samples at the stand level. To evaluate the seasonal influence on belowground element-acquiring EEAs, stoichiometry, and
200 microbial nutrient acquisition pattern, sampling was repeated during three measurement seasons in each selected
201 disturbance affected stands: summer/early stage (April-May), monsoon/middle stage (June-August), and winter/dormant
202 stage (December-January), coinciding with the regional climatic pattern and the growth stages of the dominant
203 tree genus (*Shorea*). A total of 90 composite soil samples were obtained from different disturbance-affected stands (4 ND
204 + 4 LD + 4 MD + 3 HD) across seasons (summer, monsoon, and winter) in both surface (0-15 cm) and the depth soil (15-
205 30 cm). The freshly collected soil samples were placed in bags and stored at 4 °C before transportation to the laboratory.
206 The moist samples were sieved through a ≤ 2 mm mesh to remove visible stones, pebbles, plant roots, and debris, and then
207 homogenized. Each homogenized composite sample was divided into two portions: one was stored at 4 °C for soil
208 microbial biomass and enzyme activity analysis, while the other was air-dried for assessing soil physicochemical
209 properties.

210 In each disturbance-affected stand, five random quadrats of 5 × 5 m² were established for shrubs, and 1 × 1 m² quadrats
211 (with four points at the corners and one at the diagonal intersection) were used for herbs to assess cumulative understory
212 diversity. The Shannon-Wiener diversity index (H') was calculated for species diversity, while the Margalef index was
213 used to measure species richness (SR), following the method outlined by Kaushal and Baishya (2021). Shannon's diversity
214 and SR indices were subsequently computed for the understory layers using the formula:

$$H' = \sum_i^S P_i \times \ln P_i \quad (2)$$

where P_i represents the proportion of species ‘i’ in each forest stand, and ‘S’ denotes the total number of species. At the same time, fine roots (FRs) were collected approximately 1 to 1.5 m away from tagged dominant and co-dominant tree stumps within the pre-defined disturbance-affected stand, following the methodology outlined by Pandey et al. (2024). Seasonal and depth-wise extraction of fine roots (FRs) was collected using a cylindrical corer (dimensions: 5 cm × 5 cm × 15 cm), and the cores were refilled with the excavated soil after collecting the FRs. Each core was labeled, placed in a separate zip bag, and transported to the laboratory for biomass calculation. Fine root biomass (FRB) was determined by measuring the difference between fresh weights and the weight of representative samples after oven-drying at 64 °C for 48 h (Joshi et al. 2023).

Determination of soil physicochemical properties, microbial biomass, and enzyme activity

Soil bulk density (BD, g cm⁻³) was estimated on core samples collected using the cylinder corer method. A total of 90 independent soil cores were used, as described above. The fresh, moist, and dry soil weight was taken after oven drying at 104 °C for 48 h to calculate both moisture and BD. The BD was calculated following Joshi and Garkoti (2023):

$$BD \text{ (g cm}^{-3}\text{)} = \frac{\text{Oven dry weight of soil (<2 mm) in g}}{\text{Core volume(cm}^{-3}\text{)} - \left(\frac{\text{weight of coarse particles (>2 mm) in g}}{\text{particle density (2.65 g cm}^{-3}\text{)}} \right)} \quad (3)$$

Soil pH (1:5 w/v) was analyzed using the glass electrode (model 5 star, Thermo Orion, New Delhi, India) method described by Black et al. (1965). Soil organic carbon (SOC) and nitrogen (N) were determined using wet oxidation and the micro Kjeldahl digestion method described by Kumar et al. (2021). Soil available phosphorus (P) was determined using the molybdate antimony extractant solution and was analyzed using a spectrophotometer (UV-1800; Shimadzu, Mumbai, India) as detailed outlined by Shankar and Garkoti (2023), followed by Gao et al. (2024). Active inorganic nitrogen (N- NH₄⁺ and N-NO₃⁻) concentrations in the soils were extracted using 50 ml of 2M KCl solution, and the extracts were subsequently measured spectrophotometrically as described by Liu et al. (2014).

Soil microbial biomass was measured using the fumigation extraction method (Kim et al. 2019; Jiang et al. 2023). In brief, soil microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN) were extracted using the 0.5 N K₂SO₄ solution with 1: 4 soil: water ratio, while microbial biomass phosphorus (MBP) was extracted using 0.5 N molybdate antimony solution 1:10 soil: water ratio (Brookes et al. 1982). Two portions of 10 g of fresh soil samples were taken, with one portion fumigated with chloroform for 48 h, while the other soil samples were extracted directly without fumigation. The final MBC, MBN, and MBP were calculated based on the difference between fumigated and non-fumigated samples, applying conversion factors of 0.45, 0.54, and 0.40 (Vance et al. 1987), respectively. Soil microbial quotients (qMB) were calculated to estimate the microbial efficiency of utilizing soil SOC, N, and P. These ratios serve as proxies for microbial elements (SOC, N, and P) use efficiency, offering valuable information about the metabolic state of soil microorganisms and their role in nutrient cycling within ecosystems. The qMB (%) was calculated using the following formulae (Eq.(4)).

$$\text{Soil microbial quotients (qMB)} = \frac{\text{MBC (or MBN or MBP)}}{\text{SOC (or N or P)}} \times 100 \quad (4)$$

We quantified the potential activities of enzymes associated with microbial surfaces and/or extracellular enzyme activities (EEAs) involved in dominant microbial C, N, and P cycling using a modified colorimetric method, as previously described (Tabatabai and Bremner 1969; Waring et al. 2014). These major EEAs included C-acquiring enzyme: β -1,4-cellobiosidase (CBH), β -1,4-glucosidase (BG); N-acquiring enzyme: β -1,4-N-acetyl-glucosaminidase (NAG); and P-acquiring enzymes: alkaline phosphatase (ALP) and acid phosphatase (AcP) (Xu et al. 2021; Zeng et al. 2023). The activities of BG, CBH, NAG, ACP, and ALP were determined using p-nitrophenols (pNPs) as an end product, as described in detail by Acosta et al. (2018) and Jiang et al. (2023). In brief, 1 g of fresh soil was mixed with modified universal buffer (MUB, pH = 6.5) standard and incubated in a dark room at 37 °C for 1-2 h, with 1 ml of 4-nitrophenyl- β -D-cellobioside (pNPC), 4-nitrophenyl- β -D-glucopyranoside (pNPG) as substrates for CBH and BG EEAs activity at optimum conditions, respectively (Wan et al. 2019; Xu et al. 2022). For N-acquiring EEA activity, 1 g of fresh soil was mixed with acetate

258 buffer (pH =5.5) and incubated in a dark room for 1 h at 37 °C with 1 ml of 4-nitrophenyl N-acetyl-β-D-glucosaminide
 259 (pNNPG) as a substrate. A fresh 1g soil sample was incubated in the dark at 37 °C for 1 to 2 h with 4 ml of MUB at pH 6
 260 and 11, along with 1 ml of 4-nitrophenyl phosphate (pNPP) as the substrate for assessing AcP and ALP EEAs, respectively.
 261 Final C-N-P acquiring EEAs were calculated based on the buffer standard, soil dry weight, and incubation time after
 262 stopping the reaction by treating with calcium chloride (1 ml, 0.5N) and sodium hydroxide (4 ml, 0.5N) solution, and
 263 expressed as µg product g⁻¹ dry soil h⁻¹ (Kim et al. 2019; Shankar and Garkoti 2023; Xue et al. 2023). We also determined
 264 the dehydrogenase activity (DHA) to assess the microbial oxidative activity, which is often related to microbial respiration
 265 activity in soil (Zhao et al. 2018), and believe DHA is regarded as a potential bio-indicator of soil quality due to its
 266 integral role in microbial metabolic processes. Briefly, 1 g of fresh soil sample was incubated for 24 h at 28 °C with 0.2 ml
 267 of 3% triphenyl tetrazolium chloride (TTC). Triphenyl formazan (TPF), the end product, was quantified after a 6 h
 268 incubation with 10 ml of methanol, following the procedure outlined by Srivastava et al. (2020). Urease forms a
 269 significant part of the soil matrix and plays a role in the mineralization of organic matter N content in the soil (Mori et al.,
 270 2023). Urease activity (U, µg NH₃-N g⁻¹ soil h⁻¹) was estimated using urea as a substrate, and 5g of fresh soil was
 271 incubated for 5 h at 37 °C with diacetyl monoxime (DAM) in the presence of Thiosemicarbazide, boric acid, and sulphuric
 272 acid as outlined by Cao et al. (2021) and Shankar and Garkoti (2023).

273 The values of C-, N-, and P-acquiring EEAs were used to calculate the microbial C: N, N: P, and C: P acquisition ratios as
 274 expressed: ln(CBH + BG): ln(NAG), ln(CBH + BG): ln(ALP + AcP), and ln(NAG): ln(ALP + AcP) as followed by Waring
 275 et al. (2014) and Qui et al. (2021). The cumulative effects of disturbances, seasons, and depths on soil EEAs and their
 276 stoichiometry were expressed as follows (Eq.(5)):

$$277 \text{ Disturbance effect}_{i,j,k}(\%) = \frac{(E_{i,j,k} - E_{\text{control}})}{(E_{\text{control},j,k})} \times 100 \quad (5)$$

Where, i indicates the soil with disturbance regimes (LD, MD, and HD), j indicates a measured value of each enzyme activity and stoichiometry, k indicates the soil depths (0-15 and 15-30 cm), and ND indicates the variables in undisturbed stands soil as same as other disturb stands was as the control or reference.

The vector analysis Gao et al. (2024) and the scatter of enzyme C: N: P stoichiometry (horizontal and vertical) from Huang et al. (2024) were employed to assess soil microbial nutrient limitations across different disturbance regimes. A larger vector length (Vector L) signifies a greater microbial carbon (C) limitation, while a larger vector angle (Vector A) greater than 45° indicates phosphorus (P) limitation; angles below 45° suggest nitrogen (N) limitation. Vector L and Vector A were computed using equations (6 and 7), as outlined by Wang et al. (2023) and Huang et al. (2024).

$$\text{Vector L (unitless)} = \sqrt{[X^2 + Y^2]} \quad (6)$$

$$\text{Vector A (degree)} = \text{Degrees}[\text{Atan2}(X, Y)] \quad (7)$$

In the equations, $X = \ln(\text{CBH} + \text{BG}): \ln(\text{CBH} + \text{BG} + \text{NAG})$ and $Y = \ln(\text{CBH} + \text{BG}): \ln(\text{CBH} + \text{BG} + \text{AlP} + \text{AcP})$ denote the relative microbial demand for C, N, and P, respectively.

Statistical analysis

All statistical analyses and visualization were performed using R software (version: 4.3.0) (R Core Team 2023) with the aid of the RStudio interface (version: 2023.6.0.421). Soil microbial biomass and EEAs were natural-log transformed to improve the assumption of normality and heteroscedasticity performed by the Shapiro-Wilk and Levene's test, respectively (Chen et al. 2024). Three-factor analysis of variance (ANOVA) was performed to assess the main effect and interaction of different disturbance regimes, seasons, and soil depths on vegetation attributes, soil properties, microbial biomass, EEAs, and their stoichiometry at a significant level of $p < 0.05$. When the result of ANOVA was significant repeated Tuckey-HSD test was used for multiple comparisons across four disturbance regimes and two soil depths. Bonferroni's correction

298 was applied to minimize the type-1 error rate in the test family by adjusting for the increased number of paired
299 comparisons (Jiang et al. 2023; Shankar et al. 2025).

300 To understand overall relationships between belowground microbial resource acquisition patterns and forest variables, we
301 used a double-level approach. At first, we assessed the correlation matrix to determine the potential driving ecological
302 factors based on the strength and direction of linear association influencing soil microbial biomass and EEAs
303 stoichiometry ratios using the ‘Corrr’ package. Second, we used redundancy analysis (RDA) to determine the explanatory
304 power of vegetation (diversity and fine root biomass) and soil physicochemical properties as ecological factors on the
305 variance of soil microbial biomass and EEAs stoichiometry ratios (Kim et al. 2019; Xue et al. 2023). The RDA analysis
306 was performed using a ‘vegan’ package (Lepš and Šmilauer 2003). To identify the significant driving factors of soil
307 microbial and enzyme C: N: P stoichiometry, we adopted the forward selection and variance inflation factor ($VIF < 10$)
308 method to eliminate the variables to avoid multicollinearity among the factors (Xu et al. 2021; Shankar and Garkoti 2023).

309 We initially developed a priori model grounded in background information and established conceptual frameworks linking
310 microbial biomass, EEAs, and environmental variables (Fig. 1). This model was employed to examine the hypothesized
311 effects of ecological factors on belowground microbial resource acquisition strategies. In cases where the conceptual
312 model demonstrated poor fit, a simplified putative model was constructed by excluding specific pathways. The decision to
313 remove these pathways was informed by model performance metrics and the statistical significance (P-value) associated
314 with each pathway. Key variables that significantly influenced soil microbial and EEAs C: N: P stoichiometry, identified
315 through correlation and RDA, were subsequently incorporated into the structural equation modeling (SEM) to enhance
316 model precision and interpretability (Sandra et al. 2025). The final SEM model was selected based on the lowest Akaike
317 information criterion (AIC) value and goodness of fit statistics: ($\chi^2/df < 5$), Bentler’s Comparative Fit Index (CFI) > 0.95 ,
318 non-significant p-values ($p > 0.05$), and a Tucker-Lewis Index (TLI) close to the CFI value (Chen et al. 2024; Gao et al.
319 2024; Shankar and Garkoti 2025). The putative direct and indirect effects of SEM was performed using the ‘Lavaan’
320 package within R environment to evaluate the role of vegetation and soil environmental factors that divided into latent

321 factors such as soil physicochemical properties (pH and moisture), soil nutrients (N, P), and vegetation attributes (FRB,
322 H') on soil microbial properties and resource limitations (Rosseel 2012).

323 **Results**

324 **Variation in soil physicochemical properties and nutrient stoichiometry**

325 A three-factor analysis of variance revealed significant effects of disturbance regimes, seasons, soil depths, and their
326 interactions on soil physicochemical properties (Table S2). Soil bulk density (BD) varied significantly across seasons and
327 depths ($p < 0.001$), with the lowest values recorded during the monsoon season, being 9.9% and 18.4% lower than in
328 summer and winter, respectively. BD was also significantly ($p < 0.001$) lower in MD stands, followed by ND, LD, and
329 HD stands. In comparison among disturbance regimes, soil moisture significantly decreased with increasing soil depths (p
330 < 0.05) and was highest in MD stands, exceeding ND, LD, and HD stands by 4.8%, 12.2%, and 14.1%, respectively
331 (Table S2). Soil pH significantly ($p < 0.001$) increased with soil depths across the seasons and disturbance regimes ($p <$
332 0.001), except in ND stands during summer and winter, where it decreased with depths. Overall, soil pH ranged from 6.65
333 ± 0.17 to 7.28 ± 0.27 and followed the order: MD (7.2%) > HD (4.3%) > LD (2.8%) compared to ND stands. Similar
334 trends were observed for $\text{NH}_4^+\text{-N}$ and $\text{NO}_3\text{-N}$, which were significantly reduced with soil depths, and their seasonal
335 pattern were significantly ($p < 0.001$) higher in monsoon by 25.2% and 35.4%, and 19.5% and 58.5% than in summer and
336 winter seasons. Moreover, the $\text{NH}_4^+\text{-N}$ and $\text{NO}_3\text{-N}$ were highest in MD stands, which were higher than ND, LD, and HD
337 stands by 12.8% and 13.3%, 13.5% and 13.3%, and 15.3% and 13.3%, respectively (Table S2).

338 The seasonal variation in SOC and nitrogen (N) differed significantly across disturbance regimes, and the pattern of
339 seasonal variation also showed a significant difference between depths. Similarly to soil moisture, both SOC and N were
340 higher observed at the surface layer (0-15 cm) than the depth soil (15-30 cm) by 59.2% and 65.1%, respectively.
341 Specifically, SOC and N in MD stands were significantly higher than in ND, LD, and HD stands by 1.02% and 17.3%,
342 5.9% and 17.8%, and 14% and 18.1%, respectively (Table S3). Soil phosphorus (P) also decreased in depth, with a

343 significant 30% reduction in P content under MD stands with increasing soil depths. In comparison to ND stands, soil P
344 was significantly higher in MD (2.2%) and lower in HD (15%) across seasons. Soil C: N ratio was significantly ($p <$
345 0.001) lower in MD and HD stands than in ND and LD stands by 2.4% and 29.8% and 7.6% and 34.5%, respectively,
346 across seasons, except in winter seasons ($p \geq 0.05$). Soil N: P and C: P ratios were significantly lower in HD stands than in
347 ND, LD, and MD stands by 14.5% and 68.1%, 22.4% and 47.3%, and 28.1% and 48.4%, respectively (Table S3).

348 **Variation in content and stoichiometry ratios of microbial biomass C, N, and P in soils**

349 Microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) contents decreased significantly with
350 increasing soil depth and varied seasonally ($p < 0.001$) across disturbance regimes, being highest during the monsoon and
351 lowest in winter (Table 1). At the surface layer, MBC content was highest across all seasons and disturbance regimes
352 following the order: MD (299.6 ± 19.2 mg/kg) > ND (274 ± 29.2 mg/kg) > LD (251 ± 15.3 mg/kg) > HD (232.7 ± 18.6
353 mg/kg). Similarly, MBN and MBP contents mirrored this trend, being significantly higher in MD stands than ND, LD, and
354 HD stands by 7.2% and 0.5%, 23.8% and 20%, and 23.8% and 24.1%, respectively, across the soil depths and seasons.

355 The microbial biomass C: N ratio peaked under the HD stand and decreased with soil depth, except in LD and HD stands,
356 where the trend was inconsistent. Seasonal variations were significant ($p < 0.001$) across disturbance regimes, except
357 between monsoon and winter in MD and HD stands ($p \geq 0.05$, Table 1). The microbial biomass C: N ratio decreased
358 during the monsoon compared to summer and winter, with the highest decrease in LD stands (27.4%), followed by HD
359 (22.4%), ND (14.7%), and MD (9.4%) stands. Microbial biomass C:P and N:P ratios also significantly declined with
360 depths in ND and MD stands ($p < 0.05$), and their seasonal patterns varied significantly across disturbance regimes ($p <$
361 0.001) (Table 1). The qMBC was highest in surface soils of MD stands, while qMBN and qMBP generally increased with
362 depth in ND and MD stands across seasons (Table 1). In winter, qMBN and qMBP were significantly lower than in the
363 summer and monsoon by 29.8% and 5.2%, and 58.9% and 23.3%, respectively.

364 **Cumulative effect of disturbance, depth, and season on EEA and associated stoichiometry in soils**

Soil DHA, urease, C-N-P acquiring EEA, and EES ratios were significantly influenced by disturbance regimes, soil depths, seasons, and their interactions (Table 2, Fig. 3 and 4). Soil DHA activity was significantly ($p < 0.001$) higher under MD stands by 45.9%, 58.3%, and 32.8% compared to ND, LD, and HD stands, respectively. Moreover, soil DHA activity was significantly increased under monsoon and summer seasons by 58.6% and 55.6% compared to the winter season, and significant differences were observed between summer and monsoon seasons (Table 2). Similar to DHA, urease activity was highest in the monsoon season, which was higher by 7.6% and 23.7% compared to summer and winter seasons, respectively. Soil urease activity was highest in MD stands, which were significantly ($p < 0.001$) higher by 7.8%, 13.6%, and 13.6% than in ND, LD, and HD stands in all depths, except between LD and HD stands (Table 2, $p \geq 0.05$).

Compared to ND stands, C-cycling EEAs (CBH + BG) were significantly higher under MD stands and lower under LD and HD stands by 66.7%, 80%, and 84%, respectively. A significant ($p < 0.05$) seasonal effect on cumulative (BG + CBH) activity was observed across different disturbance regimes, with the highest activity observed during the monsoon, followed by summer, and the lowest during winter (Fig. 3 (a)). Moreover, the cumulative effect of (BG + CBH) activity decreased significantly with depths across the disturbance regimes, with the highest difference observed under MD stands, followed by ND, LD, and lowest in HD stands (Fig. 3 (a)). Similar to C-cycling EEAs, N- and P-cycling EEAs like NAG and alkaline (AIP) and acid phosphatase (AcP) also showed higher activity in MD stands but varied across depths and seasons, peaking during monsoon (Fig. 3 (b, c), Table 2). Enzyme C: N ratios mirrored BG activity, with maximum values in MD stands across seasons in all soil depths. Seasonal variations were observed with enzyme C: N, N: P, and C: P ratios being highest in surface soils during summer and monsoon by 27.3%, 87%, and 9.5% and 34.3%, 18.9%, and 86.2%, respectively, but inconsistent in winter, particularly in LD and HD stands (Fig. 4).

Characterization of microbial resource limitation

Vector analysis showed that vector length (L) was significantly lower in surface soil than in the subsurface soil (Fig. 5 (a)). Interestingly, in surface soil vector L was highest in HD stands, followed by LD, MD, and lowest in ND stands. Similar to

vector L, vector angle (A) exceeded $> 45^\circ$ across the disturbance regimes and depths, except for ND and MD stands of surface soil depth (Fig. 5 (b)). Overall, vector A increased significantly ($p < 0.05$) in LD (4.02%), HD (3.43%), and MD (1.23%) stands compared to ND stands. Both vector analysis and scatter points were distributed over the diagonal line, which indicated more microbial P limitation than N limitation, while some MD stands also showed N limitation (Fig. 5 (c, d)).

Environmental factors relationships with soil microbial biomass, EEAs, and associated stoichiometric ratio

Soil DHA, urease, EEAs (CBH, BG, NAG, AcP, and AIP), microbial biomass (MBC, MBN, and MBP), and EES (C: N, N: P, and C: P) were positively correlated with vegetation attributes (FRB and H'), soil nutrients (N, P, $N-NH_4$, and $N-NO_3$), and moisture but negatively correlated with soil BD and pH (Fig. 6). Additionally, most microbial quotients showed positive correlations with soil BD, SOC, moisture content, and FRB, but were negatively associated with soil N and P availability. In contrast, microbial biomass stoichiometry (C: N, N: P, and C: P ratios) and vector angle exhibited strong, significant ($p < 0.001$) negative correlations with vegetation attributes and the majority of soil physicochemical properties.

The RDA analysis revealed significant relationships among soil microbial biomass, EEAs, and stoichiometry with environmental variables (Fig. 7). The first two RDA axes explained over 93% of the total variance ($p < 0.05$). Soil moisture contributed more than 60% of the explained variation, followed by vector length (L), understory vegetation diversity (H'), vector angle (A), soil N, P, FRB, BD, and pH (Fig. 7 (a)). Soil BG, CBH, NAG, AcP, MBN, and MBC exhibited a positive correlation with soil moisture and N. In addition, soil AIP activity and MBP exhibited a positive correlation with understory H' and soil P availability, while displaying a negative correlation with soil BD, pH, and vector A (Fig. 7 (a)).

For soil microbial biomass and enzyme stoichiometry, RDA explained 67% of the variation, with the first two axes accounting for 99.9 % and 0.1% of the total variation, respectively (Fig. 7 (b)). Notably, understory H' was a dominant environmental factor with the highest explained variance of 46.1%, whereas vector A, soil moisture, FRB,

vector A, and P explained 22.2%, 20.3%, 4.5%, 3.6%, and 3.1%, respectively. Both the enzyme C: N: P stoichiometry ratios exhibited a positive correlation with H', FRB, soil moisture, P, and vector L, while the microbial biomass C: N: P stoichiometry ratio displayed a negative correlation with soil moisture, P, H' and FRB (Fig. 7 (b)).

Interconnection of soil microbial metabolic resource limitation with environmental factors

SEM revealed complex interconnections among changes in soil properties, nutrients, and FRBs, induced by different disturbances and seasons, which significantly influenced forest understory vegetation compositions. These factors, in turns, affect the microbial biomass and EEAs stoichiometry ratios patterns (Fig. 8). The combined direct and/or indirect putative effect of soil nutrient, properties, and vegetation attributes on microbial biomass, microbial C limitation, and microbial N or/ P limitation account for 72%, 87%, and 93% of the variance, respectively. Soil physicochemical properties significantly and positively affected microbial biomass (path coefficient, $\beta = 0.74$) and soil nutrients ($\beta = 0.42$). Microbial C limitation was positive and directly influenced by vegetation attributes ($\beta = 0.58$) and soil nutrients ($\beta = 0.38$), while depth ($\beta = -0.17$) and disturbance ($\beta = -0.27$) had negative impacts (Fig. 8 (a)). Conversely, microbial N or/ P limitation was positively and directly influenced by disturbance ($\beta = 0.37$) and vegetation attributes ($\beta = 0.58$) but negatively influenced by soil nutrients ($\beta = -0.58$) and depth ($\beta = -0.22$) (Fig. 8 (b)). The cumulative standardized effect (direct + indirect) of each predictor on microbial C limitation ranked as follows: vegetation attributes > soil nutrients > soil physicochemical properties > depth > disturbance. For microbial N or/ P limitation, the cumulative effect ranked in decreasing order as disturbance > vegetation attributes > depth > soil physicochemical properties > soil nutrients (Fig. 8 (c)).

Discussion

Variation in content and stoichiometric ratios of microbial biomass C, N, and P in soils

Supporting our first hypothesis, this study reveals a significant decline in soil microbial biomass under LD and HD regimes, while MD enhances microbial biomass across seasons. These patterns are primarily driven by disturbance-

431 induced changes in forest stand characteristics and microbial distribution, highlighting the mismatch between microbial
432 nutrient demand and substrate availability at the soil scale (Zhou et al. 2020; Jiang et al. 2023). Our findings align with
433 previous studies in temperate oak and *L. principis-rupprechtii* forests, which report that moderate disturbance increases
434 microbial biomass C by 22- 54 % compared to no disturbance and heavy disturbance (Kim et al. 2019; Qui et al. 2021).
435 These differences are driven by vertical differentiation in canopy structure and understory diversity, contributing to
436 heterogeneous distribution of substrates (e.g., leaves, roots) (Jiang et al. 2023; Zhang et al. 2023). Enhanced substrate
437 availability alters microbial community structure and promotes spatial heterogeneity in soil microbial biomass (Joshi et al.
438 2023; Shankar and Garkoti 2024). Disturbance regimes also influence litter input quality and quantity (e.g., above- and
439 below-ground), which directly affects microbial growth and abundance (Wang et al. 2019; Qui et al. 2021; Xue et al.
440 2023). In HD stands, soil microbial biomass decreases more rapidly with soil depth than in ND and MD stands- likely due
441 to reduced FRB, soil moisture, nutrients (N, P), and N availability (NH₄-N and NO₃-N), all of which are positively
442 correlated with microbial biomass. These findings are consistent with multiple previous studies reporting that microbial
443 biomass is positively influenced by changes in SOC, moisture, and nutrient availability after disturbance (Kim et al. 2019;
444 Cao et al. 2021; Jiang et al. 2023; Zeng et al. 2023). Seasonality is another major factor influencing below-ground
445 ecological processes, especially in tropical dry-deciduous forests where microbial biomass C and nutrient (N, P) dynamics
446 are largely governed by moisture and understory vegetation (Wan et al. 2019; Lull et al. 2020; Riutta et al. 2021). Our
447 study showed a peak attained in soil microbial biomass during the monsoon season in all disturbance regimes, coinciding
448 with higher understory vegetation diversity and abundance. This indicates that microbial biomass response to disturbance
449 is modulated by seasonal moisture and nutrient levels (Li et al. 2021; Manral et al. 2023). Vegetation structure also
450 influences soil moisture through its effect on infiltration and percolation of precipitation (Xue et al. 2023; Zeng et al.
451 2023). High soil moisture generally promotes microbial biomass, as reported in several previous studies (Kumar et al.
452 2021; Joshi et al. 2023; Shankar and Garkoti 2024). For instance, Qui et al. (2021) found that microclimatic factors, such

453 as moisture, explained 19.1% of the seasonal variation in microbial biomass among disturbance regimes in *L. principis-*
454 *rupprechtii* forests.

455 We observed significant variation in the stoichiometry of microbial biomass across different disturbance regimes and
456 seasons, emphasizing the diverse soil nutrient limitations faced by soil microorganisms. Soil microbial biomass
457 stoichiometry serves as a crucial indicator of the various energy sources available to microbes, playing a key role in
458 driving below-ground ecosystem processes (Jiang et al. 2021; Wang et al. 2023) and provides valuable insights into the
459 balance of microbial nutrient stocks and microbial stability (Joshi et al. 2023; Shankar and Garkoti 2024). In this study, the
460 observed imbalance in soil microbial stoichiometry partially reflects the adaptability of soil microorganisms to shifts in
461 soil physical structure and nutrient availability, in which adaptability plays a synergistic role in regulating microbial
462 nutrient dynamics within forest ecosystems (Cao et al. 2021; Gao et al. 2024). Notably, the microbial biomass N: P (2.1–
463 14.8) and C: P (15.9–60.6) ratios recorded in this study were substantially higher compared to previously reported values
464 for forest soils in the Himalaya region {1.7–2.4 and 10.8–20.4 (Joshi and Garkoti 2023); 1.9–3.1 and 11.9–27.7 (Manral et
465 al. 2023)} and other terrestrial ecosystems {0.54–7.6 and 2.8–57.1 (Gao et al. 2024)}. These elevated microbial biomass
466 N: P and C: P ratios suggest that soil microorganisms in forest soils allocate more resources to immobilizing N and C
467 relative to P, consistent with resource allocation strategies (Gao et al. 2024; Huang et al. 2024).

468 Consistent with our first hypothesis, the highest qMBC was recorded in MD stands, indicating that the SOC composition
469 in these stands is more chemically mutable. Moreover, the marked increase in qMBC during the monsoon season in MD
470 underscores the benefits of moderate disturbance in promoting microbial biomass accumulation. In contrast, the decline in
471 qMBC in high disturbance (HD) stands may result from reduced supply of resources and substrate availability (e.g.,
472 carbon inputs) due to excess loss of tree density biomass in HD stands (Shankar et al. 2025), along with alleviating
473 competition for resource-limited environments between understory and below-ground microbes indicates more energy
474 invested towards maintenance and stress tolerance rather than growth and reproduction. Previous studies have shown that
475 higher qMBC values are correlated with greater nutrient availability in the soil, allowing microbes to prioritize nutrient

allocation strategies that support growth and reproduction (Kim et al. 2019; Kumar et al. 2021; Xu et al. 2021). In our study, the higher qMBN and qMBP values observed in ND and MD stands may be attributed to the favorable conditions for microbial biomass growth, including high-quality forest litter, optimal soil moisture, slightly acidic pH, combined with enhanced microbial nutrient use efficiency (Xu et al. 2022; Jiang et al. 2023).

Cumulative effect of disturbances, soil depth, and season on EEAs and associated stoichiometry in soils

Soil DHA, C-, N-, and P- acquiring enzyme activities responded significantly to disturbance regimes, driven by changes in soil C and nutrient levels, which were positively correlated with variations in EEAs (Wan et al. 2019; Wang et al. 2023; Huang et al. 2024). Varying substrate inputs, such as litter biomass resulting from disturbance, directly or indirectly influence microbial community structure and the synthesis of soil ecoenzymes, leading to stand-specific heterogeneity in EEAs (Zhou et al. 2020; Zheng et al. 2022). These enzymes are crucial for energy transformation by breaking down organic materials, thus promoting microbial growth and regulating microbial nutrient allocation strategies in soil systems (Xu et al. 2022; Zeng et al. 2023). MD stands consistently showed higher enzyme activities across all soil C-, N-, and P- acquiring enzyme types, likely due to greater resource availability (e.g., FRB) and improved microenvironmental conditions (e.g., moisture, SOC, and nutrients) compared to other disturbed stands. This evidence aligns with recent studies that work on different intensities of disturbed forest ecosystems by Kim et al. (2019) and Qui et al. (2021) indicates moderate disturbances (30-40%), enhance moisture, nutrients contents, and reduced bulk density of soil by significant growth of FRB, understory vegetation diversity, and biomass, which create favorable condition for microbial growth and EEAs production. Additionally, increased SOC content promotes microbial biomass growth, potentially stimulating the secretion of C-acquiring enzymes like BG, CBH, which are primarily produced by microbes (Wan et al. 2019; Qui et al. 2021; Xu et al. 2021). The findings consistently supported by our data as soil BG and CBH activity had a positive correlation with the SOC and MBC (Fig. 6). Recent studies by Wang et al. (2023) and Jiang et al. (2023) suggest that N- and P-acquiring enzyme activities are closely linked to nutrient availability but can shift in response to disturbance-induced nutrient limitations, thereby influencing microbial preferences for C substrates. Consequently, soil

499 microorganisms in MD stands increased N- and P-acquiring enzyme secretion to meet higher nutrient demands by
500 vegetation and microbes for their growth and reproduction, aligning with the greater availability of soil N and P compared
501 to other disturbance regimes (Waring et al. 2014; Kim et al. 2019; Qui et al. 2021). Previous studies have emphasized that
502 soil P limitation can significantly constrain plant and microbial growth, particularly in ecosystems such as the Loess
503 Plateau of China (Xu et al. 2022), sal forests (Shankar and Garkoti 2025), and other tropical forests (Singh et al. 2017;
504 Jiang et al. 2023; Zeng et al. 2023).

505 Seasonality significantly influences soil C-, N-, and P-acquiring EEAs in tropical forests (Singh et al. 2017; Lull et al.
506 2020; Manral et al. 2023). However, the observed variations in EEA types across disturbance regimes were strongly
507 dependent on season, indicating that seasonal patterns of soil EEAs are regulated by complex microenvironmental factors
508 such as temperature, moisture, and vegetation dynamics (Wan et al. 2019; Hernández-Cáceres et al. 2022;
509 Xue et al. 2023). These differential seasonal effects likely stem from disturbance-induced variations in the understory
510 vegetation layer, which significantly influence soil enzyme activities through: (1) changes in soil nutrients and
511 physicochemical properties during vegetation growth, (2) shifts in root biomass dynamics, particularly FRB, and (3)
512 changes in microbial biomass and community composition (Wang et al. 2019; Jiang et al. 2023; Zeng et al. 2023). In
513 particular, soil C-, N-, and P-acquiring EEAs were significantly higher during the monsoon season compared to summer
514 and winter, supporting our second hypothesis. This seasonal pattern may be driven by "source-sink" dynamics, where
515 microorganisms act as nutrient sinks during dry-cold seasons and sources during wet seasons (Singh et al. 2017; Manral et
516 al. 2023). Moreover, favorable conditions of high moisture and temperature during the monsoon benefit vegetation,
517 resulting in the production of high-quality plant litter with low C: N ratios. This litter decomposes more readily, providing
518 abundant substrates for microorganisms to produce substrate-specific enzymes. This shift fosters a transition in the
519 microbial community from oligotrophic to eutrophic dominance (Cao et al. 2021; Wang et al. 2023; Huang et al. 2024).
520 Our findings align with studies in Himalayan forest ecosystems (Kumar et al. 2021; Manral et al. 2023) and other
521 mountainous regions (Cao et al. 2021), but contrast with tropical forests (Singh et al. 2017) and other ecosystems (Qui et

al. 2021), where peak enzyme activity occurred in seasons other than monsoon/rainy seasons. Our findings support this pattern, demonstrating that high moisture and low bulk density enhance pore connectivity, thereby facilitating faster litter decomposition, greater nutrient accumulation, and increased microbial biomass. This is consistent with recent findings showing that elevated moisture levels can stimulate EEAs (Qui et al. 2021; Gao et al. 2024; Zhao et al. 2024).

Soil ecoenzymatic stoichiometry (EES) is widely regarded as a vital indicator for evaluating soil structure, function, nutrient allocation, and microbial resource acquisition strategies within plant-soil ecosystems (Waring et al. 2014; Wei et al. 2024; Zhao et al. 2024). The seasonal variations in log-transformed C-, N-, and P-acquiring EEA ratios showed distinct differences across disturbance-affected stands and soil depths, attributed to changes in environmental factors like soil moisture, pH, nutrient status, and microbial biomass. These factors collectively influence soil enzyme stoichiometry through complex interactions between disturbance and seasonal dynamics (Cao et al. 2021; Qui et al. 2021; Zeng et al. 2023). Consequently, soil enzyme stoichiometry serves as a reliable indicator for microbial homeostasis in forest ecosystems (He et al. 2020; Xu et al. 2022; Wang et al. 2023). Supporting our third hypothesis, we observed two seasonal peaks (summer and monsoon) in enzyme C: N and C: P ratios, which were significantly higher in MD-affected stands, indicating increased microbial nutrient demands for enzyme production. This aligns with greater differences in biomass litter content (substrate supply), affecting soil SOC and nutrient availability, and resulting in microbes investing more in C compounds to acquire N and P (Waring et al. 2014; Jiang et al. 2021; Wang et al. 2023). In disturbance-affected stands with higher SOC, microbes produced more N- and P-acquiring enzymes to address N and P limitations in microbial metabolism. The significant positive correlations between enzyme C: N and C: P ratios with SOC, N, and P (Fig. 6) support this, indicating that increased SOC and nutrients positively impact N- and P-acquiring enzymes. Increased tree density and understory species in MD stands enhanced below-ground biomass (e.g., FRB), facilitating effective soil exploitation for nutrient and water uptake, particularly in surface layers (Zeng et al. 2023; Shankar et al. 2025). This is consistent with previous findings that soils under leguminous species have higher soluble/bioavailable N and P content due to symbiotic microorganisms like rhizobia, which mobilize P by secreting P-acquiring enzymes (Singh et al. 2017;

545 Huang et al. 2024). Thus, C-, N-, and P-acquiring enzyme activity is strongly influenced by understory vegetation
546 attributes following seasonal changes across disturbances, supporting our hypotheses and highlighting the critical role of
547 vegetation and soil nutrient conditions in shaping soil enzyme dynamics.

548 **Environmental drivers of microbial resource limitations in soils of different disturbance regimes and depths**

549 We quantified the metabolic limitations of soil microorganisms using vector analysis, where vector length represents C-
550 limitation, and vector angle indicates N or P limitation (Zheng et al. 2022; Gao et al. 2024). Across all disturbance-affected
551 stands in sal forest ecosystems, vector length exceeded 1, indicating a consistent C-limitation. Similarly, vector angles
552 surpassed 45° (except in surface soils of ND and MD stands), signifying a widespread P limitation. This finding is
553 consistent with studies in arid grasslands (Huang et al. 2024), subtropical forests (Waring et al. 2014; Singh et al. 2017;
554 Shankar and Garkoti 2023), and other forest ecosystems globally, where soil microbes are predominantly limited by P
555 availability (Xu et al. 2022; Wang et al. 2023; Wang et al. 2024). In our study area, microbial activity was primarily
556 limited by P rather than N. This may be due to microorganisms allocating more C resources to acquire P, particularly in
557 stands with high SOC content. Increased vegetation diversity and fine root biomass (FRB) enhance SOC, stimulating
558 microbial production of P-acquiring enzymes to alleviate P limitations on metabolism (Singh et al. 2017; Lucas-Borja and
559 Delgado-Baquerizo 2019; Zheng et al. 2022). Notably, in sal forests, P-acquiring enzyme activity showed a significant
560 positive correlation with SOC, indicating that P limitation in microbial metabolism is strongly influenced by SOC
561 availability (Fig. 6). These findings underscore the critical role of P limitation in the maintenance of elemental
562 homeostasis by microbial C-N-P nutrient cycling process (Waring et al. 2014; Singh et al. 2017).

563 Microbial resource limitations were directly influenced by understory vegetation attributes, soil nutrients, and microbial
564 biomass, with varying effects of disturbance levels and soil depths (Fig. 8). Disturbances act as a key external driver,
565 shaping the complex interactions that regulate microbial nutrient acquisition strategies in these ecosystems. The overall
566 impact of disturbances can alter the resistance and resilience of forest ecosystems by modifying coexisting vegetation

structures and functions, which are critical for maintaining soil nutrient dynamics and belowground communities (Kim et al. 2019; Lull et al. 2020; Zeng et al. 2023). Unexpectedly, microbial metabolic C and P limitations were more pronounced in HD stands than in ND and MD stands (Fig. 5), suggesting that understory vegetation in HD stands did alleviate microbial nutrient limitations as anticipated. This may be due to resource competition between vegetation and soil microorganisms in HD stands for nutrient allocation for growth and reproduction. Soils in these stands exhibited lower SOC and microbial biomass, which increased the microbial reliance on C to sustain catabolic processes (e.g., respiration) and overall activity. This, in turn, intensified microbial C limitation compared to ND and MD stands (Waring et al. 2014; Kim et al. 2019; Qui et al. 2021). Several mechanisms explain the stronger microbial P limitations observed in the sal forest soils. First, the close relationship between microbial C and P limitations highlights the synchronized requirement for these nutrients to maintain a stable microbial C:P ratio (15.9–60.6) (Table 1). Low SOC levels restrict microbial P acquisition by limiting the energy available for extracellular enzyme production (Lucas-Borja and Delgado-Baquerizo 2019; Xu et al. 2022). Second, the diverse and abundant understory vegetation in HD stands competes with belowground microorganisms for available nutrients without contributing to increased nutrient availability (N or P) (Table S1 and Fig. 8). Although soil microorganisms have a greater potential than plants for nutrient uptake, their turnover is rapid, and mineral nutrients are re-released after microbial death (Zhou et al. 2020). Meanwhile, vegetation and microorganisms both continuously and unidirectionally require nutrients (N or P) for physiological processes and structural growth (Koné and Yao 2021; Hernández-Cáceres et al. 2022). Thus, competition between vegetation and soil microorganisms drives nutrient flows within the ecosystem. Combined, these factors contribute to stronger microbial C and P limitations in soils under vegetation cover, particularly in high-intensity disturbed stands. These findings highlight that disturbances intensify competitive dynamics in soil ecosystems by reinforcing microbial C-N-P homeostasis, thereby inducing tight co-regulation of enzymes responsible for balancing nutrient supply and vegetation-microbial demand.

Soil physicochemical properties indirectly influence microbial nutrient limitations by regulating vegetation attributes, soil nutrient availability, and microbial biomass (Fig. 8). Changes in disturbance intensity can modify forest canopy structure,

leading to the consistent redistribution of soil temperature and moisture (Lull et al. 2020; Zeng et al. 2023; Xue et al. 2023). Soil moisture is a critical driver of nutrient cycling, vegetation abundance, and microbial activity across diverse ecosystems globally (Lucas-Borja and Delgado-Baquerizo 2019; Li et al. 2023; Shankar and Garkoti 2025). It not only supports microbial growth and enzyme production but also enhances nutrient availability and microbial access to organic matter on the forest floor (Kumar et al. 2021; Cao et al. 2021; Shankar and Garkoti 2024). Similarly, soil structure, particularly bulk density (BD) and pH, significantly impacts belowground vegetation growth (e.g., fine root abundance), root morphology, organic matter mineralization rates, and microbial community composition and diversity (He et al. 2020; Xu et al. 2022; Huang et al. 2024). These interconnected processes underscore the indirect role of soil physicochemical properties in shaping microbial nutrient acquisition and limitations. Our findings align with the fourth hypothesis, demonstrating that vegetation attributes, soil nutrient status, and the indirect effects of soil physicochemical properties collectively influence microbial resource limitations across different disturbance regimes in sal forests.

Conclusion

The present findings support our hypothesis that moderate disturbance (MD), by enhancing nutrient availability and soil moisture, promotes microbial biomass and the activity of C-, N-, and P-acquiring enzymes in sal forest ecosystems. Microbial biomass and enzymatic activities were markedly higher during the monsoon and summer seasons compared to winter, with the most pronounced shifts observed in highly disturbed (HD) stands. These seasonal and disturbance-driven changes reflect alterations in vegetation structural diversity and soil properties, particularly nutrient status and microbial biomass. Based on the EES and vector analyses of EEAs set the ecological indicators that indicate microbial metabolism in *Shorea robusta* forests are co-limited by C and P, with P limitations being more pronounced under HD conditions and at greater soil depths. The observed stoichiometric imbalances suggest that disturbance-induced shifts in vegetation from tree-dominated to understory-dominated communities intensify nutrient competition and reduce soil moisture, thereby constraining microbial activity and nutrient acquisition strategies. Overall, soil moisture, nutrient content, and microbial biomass emerge as key ecological factors co-regulating microbial metabolism and nutrient cycling. These findings suggest

613 that microbial and enzymatic stoichiometry in response to disturbance is governed more by resource availability than by
614 strict homeostatic control.

615 Our study demonstrates that microbial resource limitations are primarily shaped by vegetation attributes, nutrient
616 availability, and microbial biomass, further modulated by underlying soil physicochemical conditions. Disturbance
617 intensity, seasonal fluctuations, and soil depth collectively influence the intricate interactions among soil and microbial
618 nutrient dynamics. These findings deepen our understanding of nutrient regulation in forest ecosystems and offer practical
619 guidance for forest management by elucidating microbial elemental demands in relation to resource availability, ultimately
620 supporting soil health and optimizing ecosystem functioning.

621 **Acknowledgment**

622 The authors express gratitude to the DFOs and the Forest Department of Sirmour Himachal Pradesh staff for their valuable
623 assistance and field support throughout the study. Additionally, the authors appreciate the School of Environmental
624 Sciences at Jawaharlal Nehru University for providing the essential facilities that enabled the successful completion of the
625 research. The authors gratefully acknowledge financial support from the University Grants Commission (UGC), India.

626 **Author contributions**

627 **Satish Chandra Garkoti:** Supervision, Conceptualization, Writing- review & editing, Supervision. **Anand Shankar:**
628 Conceptualization, Methodology, Fieldwork, Validation, Formal analysis, Data curation, Data visualization, Writing-
629 original draft, review & editing.

630 **Data availability**

631 The datasets generated during and/or analyzed during the current study are available from the corresponding author on
632 reasonable request.

633 **Declaration of Competing Interest**

634 The authors declare that they have no known competing financial interests or personal relationships that could have

635 appeared to influence the work reported in this paper.

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Figures

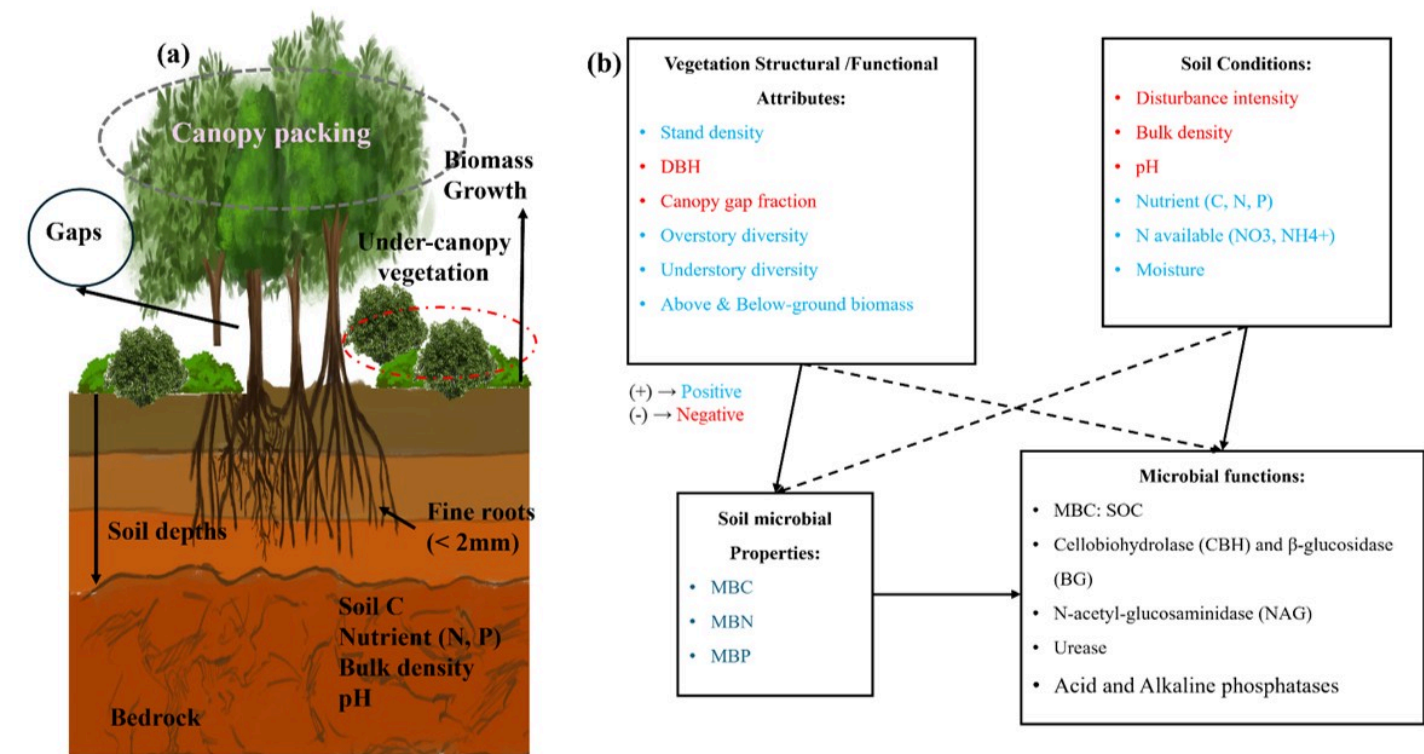


Figure 1

Conceptual diagram presents the influence of vegetation and soil environmental factors induced by forest thinning on belowground microbial properties and functions, in the context of hypothesis testing. (a) outlines the evaluated environmental parameters and (b) illustrates potential correlations between these factors and microbial traits.

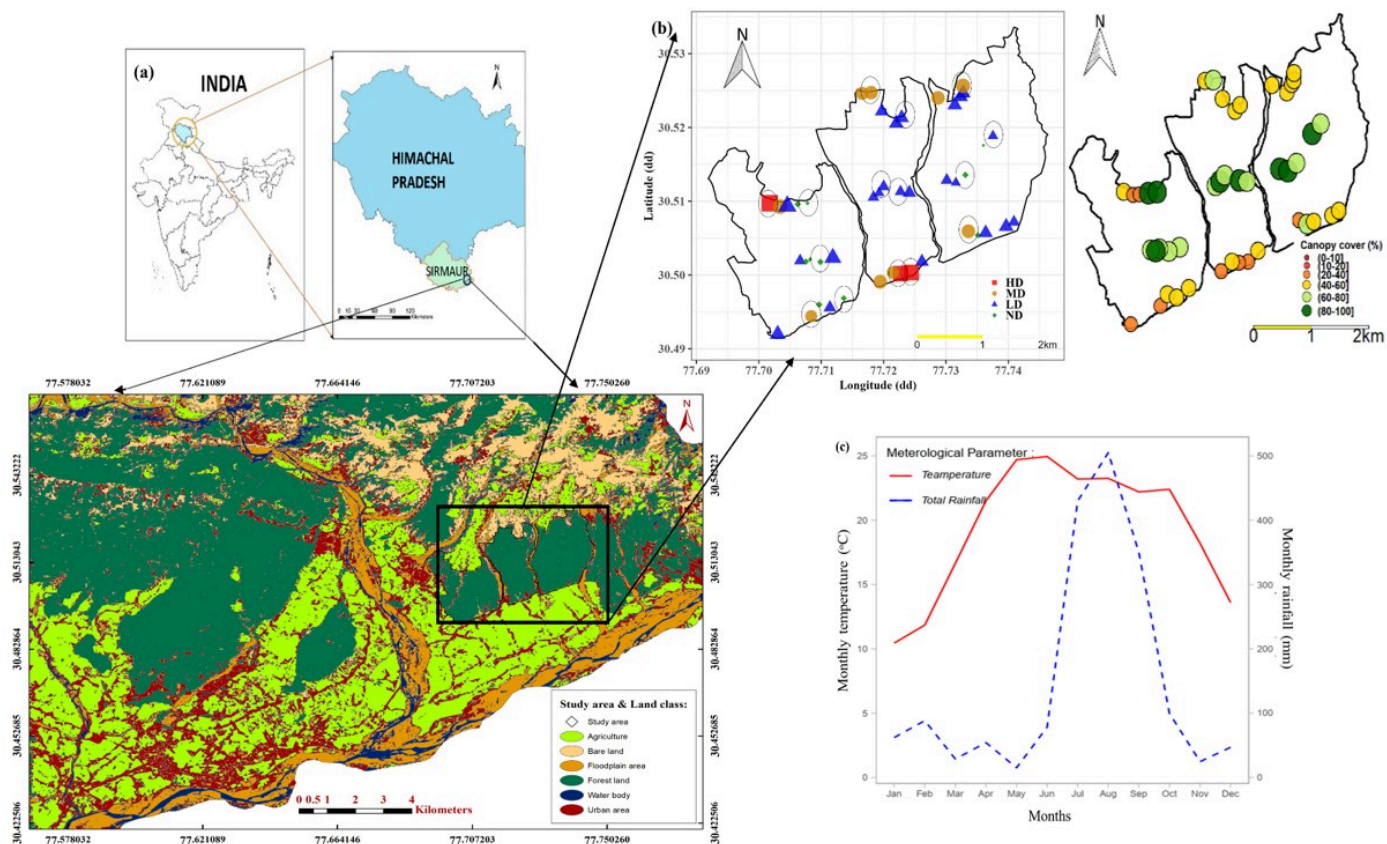


Figure 2

(a) Map location of study area; **(b)** disturbance intensity gradient and canopy cover (%) of sampling sites, and **(c)** local climatic pattern (rainfall and temperature). Distinct colors represent the disturbance gradients and canopy cover percentage. Solid red and dashed blue lines represent the temperature and precipitation. Stand characteristics: ND = no disturbance; LD = low disturbance; MD = moderate disturbance; and HD = high disturbance. Black circles indicate the selected sampling stands.

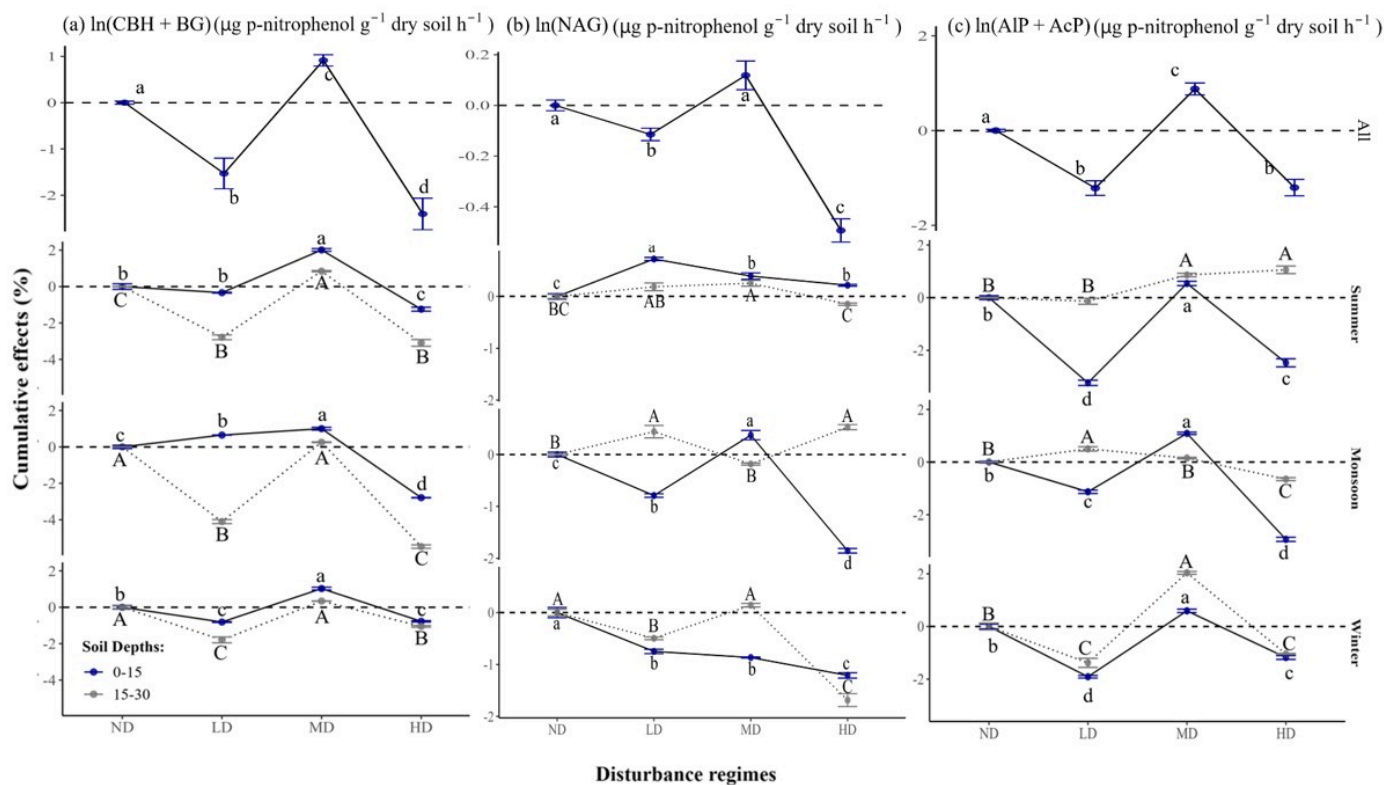


Figure 3

Cumulative effects of disturbances (LD, MD, and HD), soil depths (0 - 30 cm), and seasons (summer, monsoon, and winter) on **(a-c)** extracellular enzyme activities (EEA) compared to control soil (ND) (Two-way ANOVA, $P < 0.05$). Bars were represented as mean $\pm$ standard error. Small letters indicate significant differences between the disturbances under the same seasons of depth (0-15 cm), and capital letters indicate significant differences between the disturbances under the same seasons of depth (15-30 cm). The dotted line marks the horizontal axis, distinguishing between positive (above) and negative (below) patterns in element-acquiring EEAs. BG = β -1,4-glucosidase; CBH = β -1,4-cellobiosidase; NAG = β -1,4-N-acetyl-glucosaminidase; AIP = alkaline phosphatase; AcP = acid phosphatase.

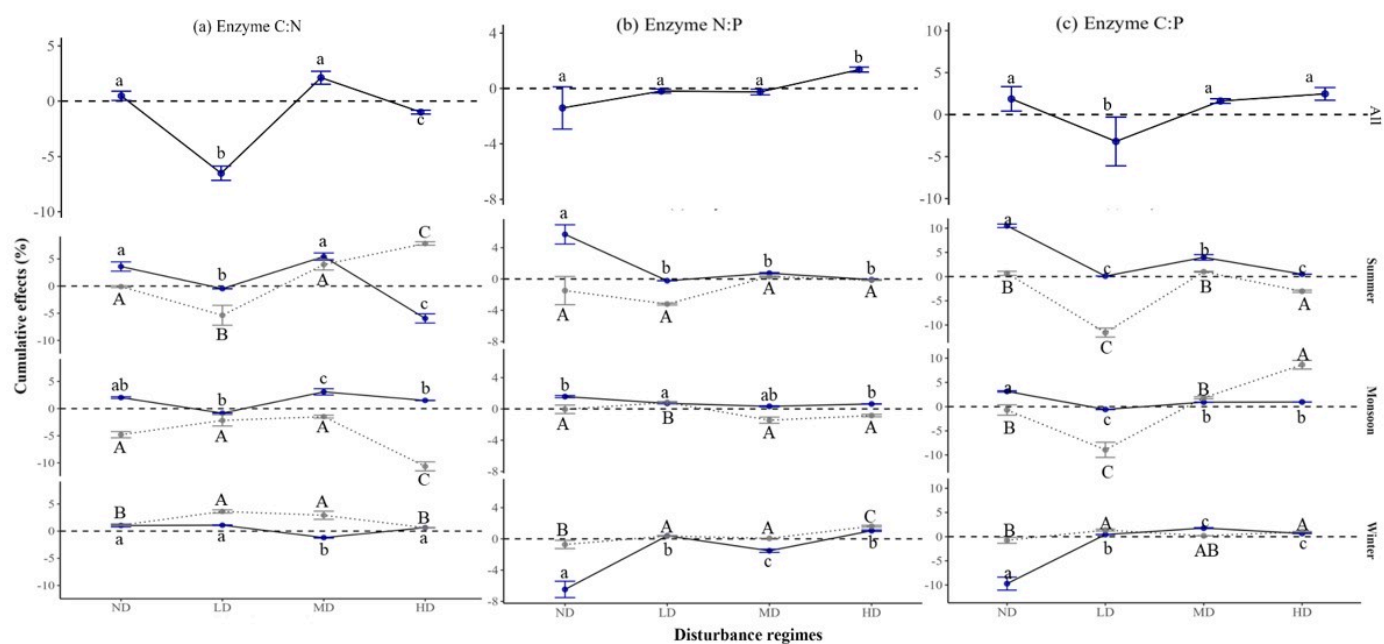


Figure 4

Cumulative effects of disturbances (LD, MD, and HD), soil depths (0 - 30 cm), and seasons (summer, monsoon, and winter) on **(a-c)** element-acquiring EEA stoichiometry compared to control soil (ND) (Two-way ANOVA, $P < 0.05$). Values were represented as mean $\pm$ standard error. Small letters indicate significant differences between the disturbances under the same seasons of depth (0-15 cm), and capital letters indicate significant differences between the disturbances under the same seasons of depth (15-30 cm). The dotted line marks the horizontal axis, distinguishing between positive (above) and negative (below) patterns in element-acquiring EEAs stoichiometry. C: N = $\ln(\text{CBH} + \text{BG}) : \ln(\text{NAG})$; N: P = $\ln(\text{NAG}) : \ln(\text{AIP} + \text{AcP})$; and C: P = $\ln(\text{CBH} + \text{BG}) : \ln(\text{AIP} + \text{AcP})$.

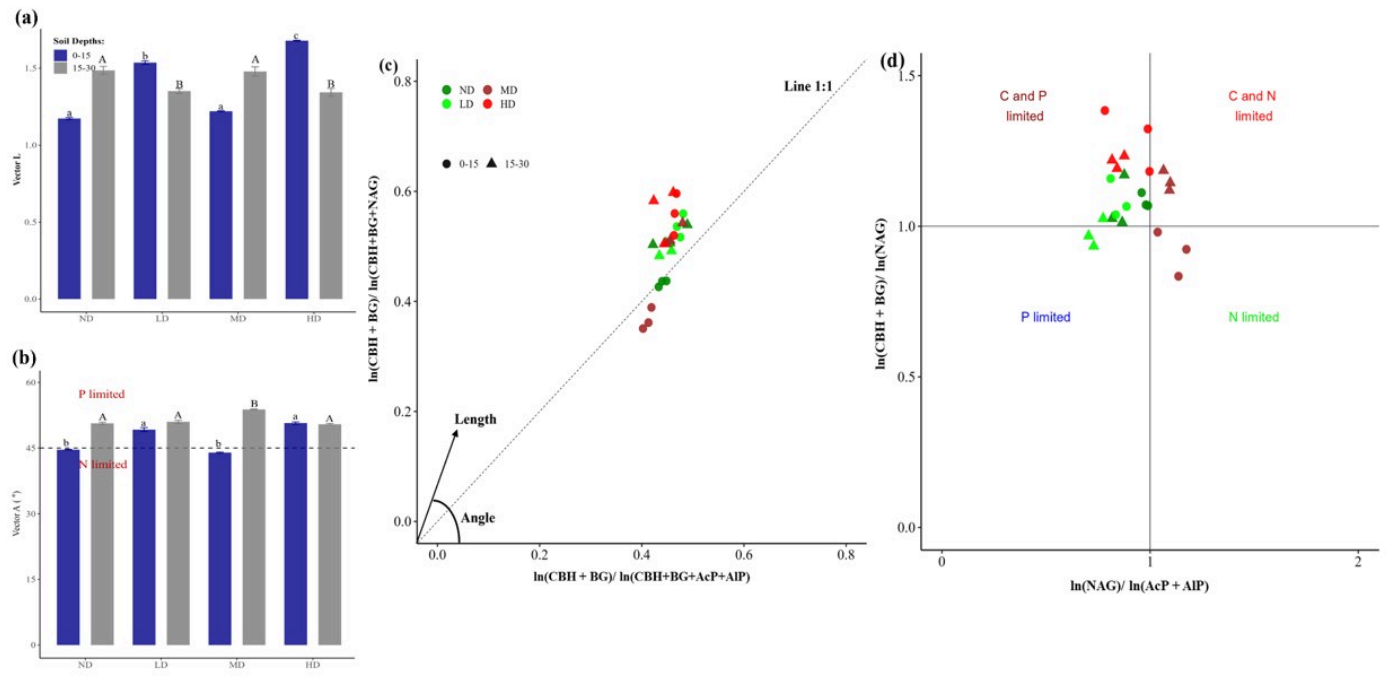


Figure 5

Effect of disturbance regimes and soil depths on soil microbial resources limitation based on: **(a-b)** vector length and angle in three distinct seasons (Two-way ANOVA, $P < 0.05$). Each bar was presented as mean $\pm$ standard error. Small letters indicate significant differences between the disturbances in the same upper soil depth (0-15 cm) and capital letters indicate significant differences between the disturbances in the same lower soil depth (15-30 cm). **(c-d)** microbial resource acquisition strategies by relative proportion of element-acquiring EEAs on both X and Y axes. A 1:1 dotted line indicates equal microbial acquisition efforts by the element-acquiring EEAs.

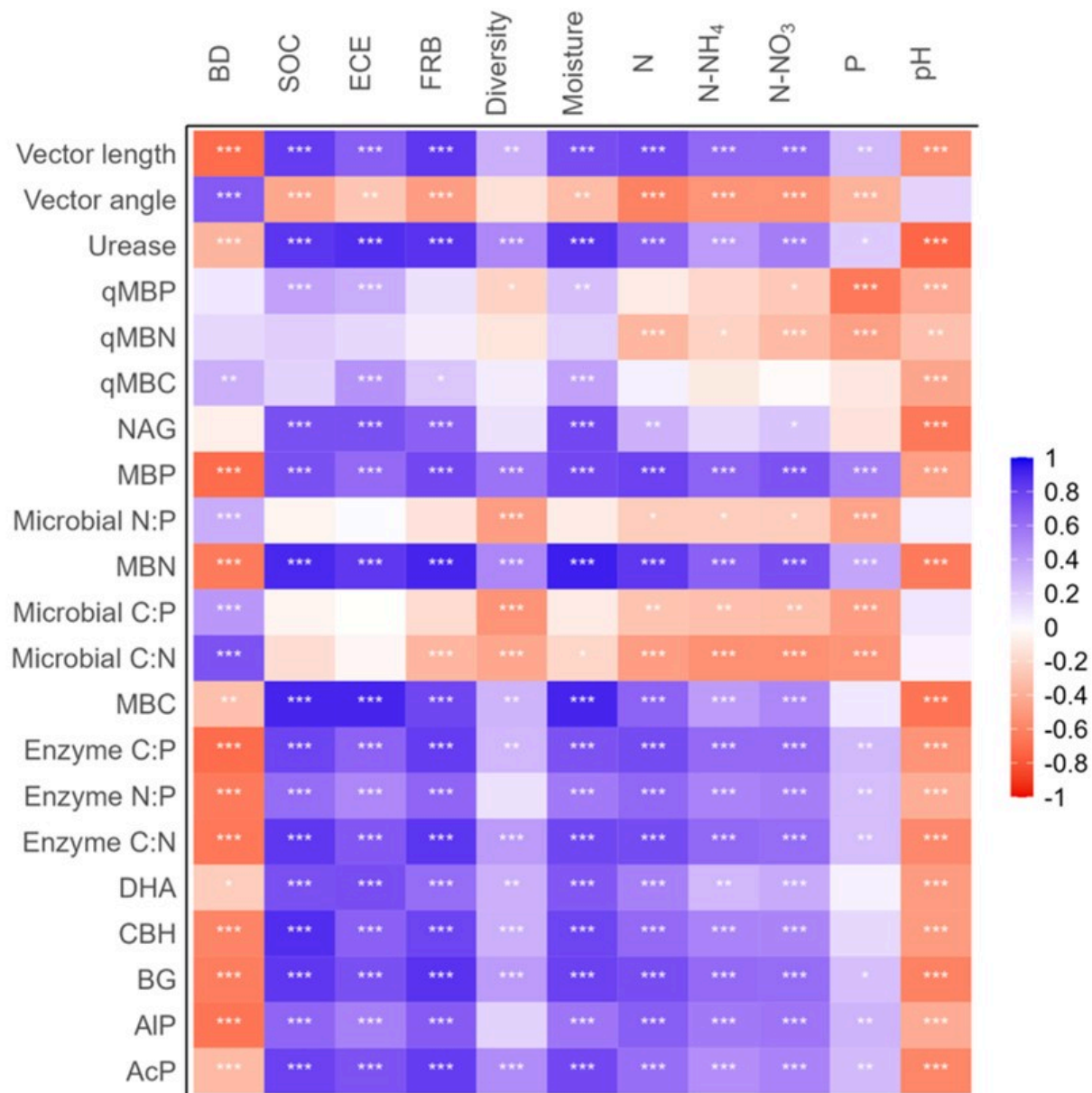


Figure 6

Pearson correlation heatmap illustrates correlation coefficients between selected environmental factors for element-acquiring EEAs, enzyme C: N: P stoichiometry, and microbial biomass C: N: P stoichiometry. BD: bulk density, ECE: electric conductivity, SOC: soil organic carbon, N: total nitrogen, FRB: fine root biomass. *P < 0.05, **P < 0.01, ***P < 0.001, and ^{ns}P ≥ 0.05.

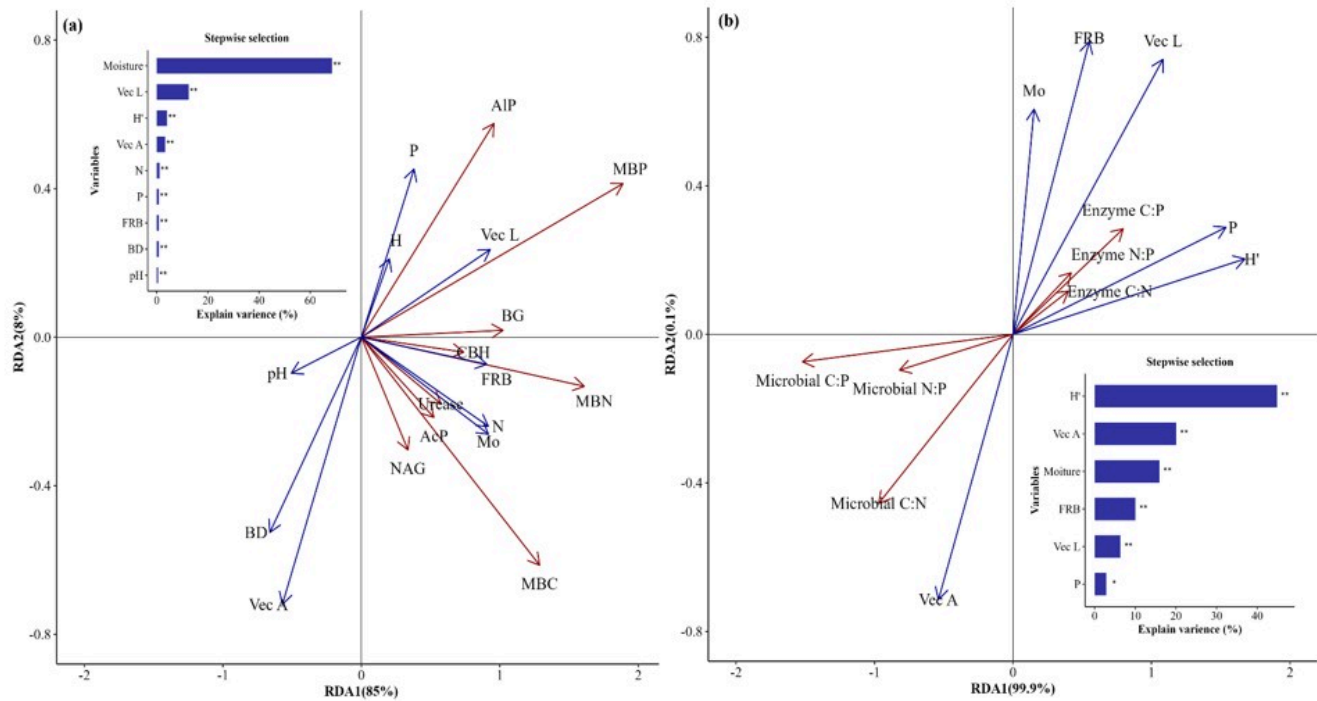


Figure 7

Redundancy analysis (RDA) regarding: **(a)** element-acquiring EEAs and microbial biomass (C, N, and P), and **(b)** element-acquiring EEAs and microbial biomass stoichiometry constrained by the soil physicochemical and vegetation properties. The blue arrow indicates the activity of element-acquiring EEAs, soil microbial biomass, and their stoichiometry, and the red arrow indicates significant explanatory variables, respectively. The bar indicates the significant variance of forward-selected explanatory variables. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Vec A: vector angle, Vec L: vector length, P: available phosphorus, SR: species richness, H': understory diversity, Mo: moisture, BD: bulk density, FRB: fine root biomass.

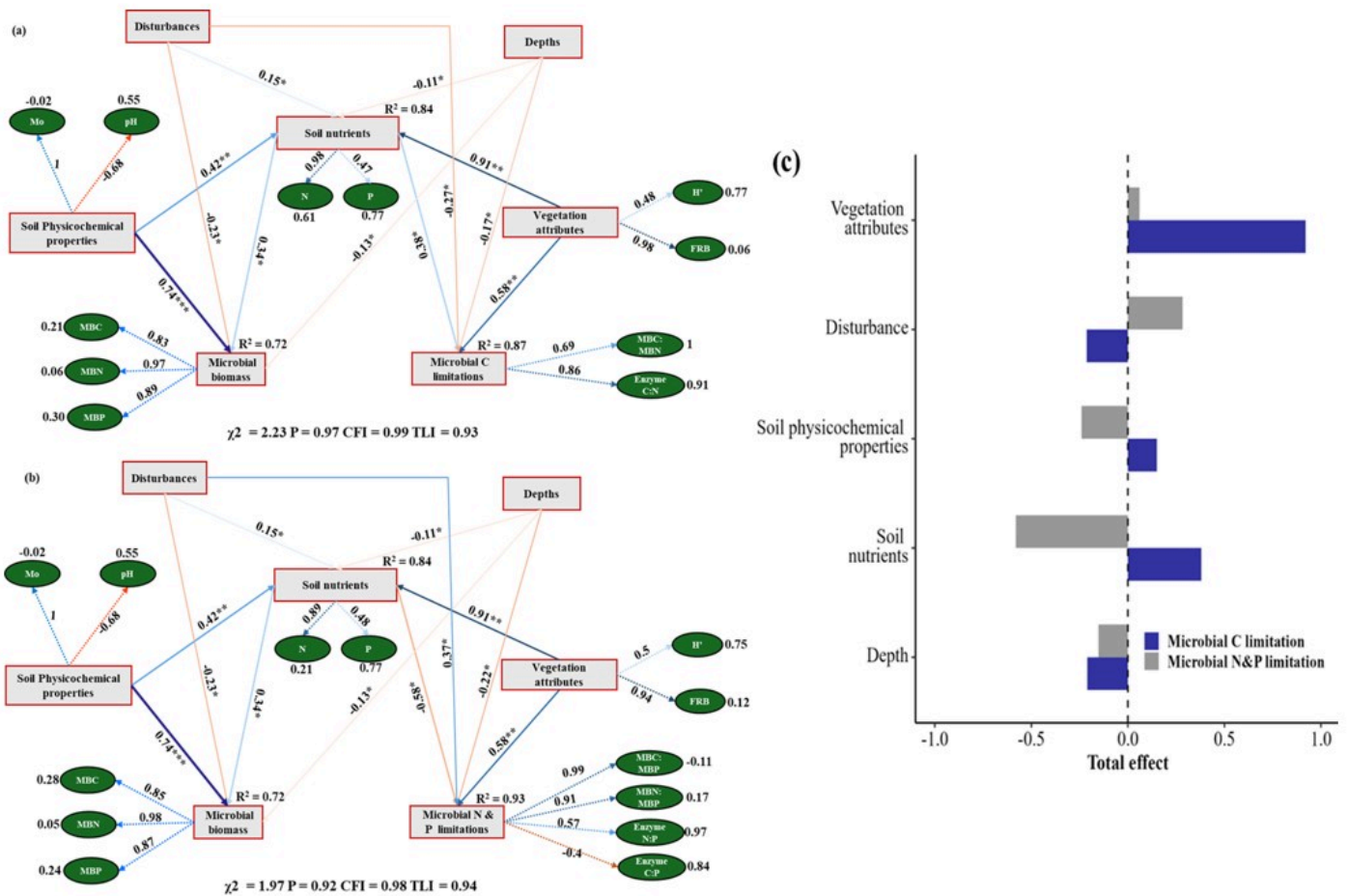


Figure 8

Structural equation models (SEMs) evaluate the direct and indirect effects of disturbance regimes and depths on **(a)** soil microbial C limitation, **(b)** microbial N and P limitation, and **(c)** their total effects. Blue and red arrows indicate the positive and negative effects, where numbers adjacent to the arrows are standardized path coefficients. The proportion of variance explained (R^2) appears next to the respective response variables. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. P: available phosphorus, SR: understory species richness, H': understory diversity, N: total nitrogen, Mo: available moisture, FRB: fine root biomass.

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