

Microbial responses to stoichiometric imbalances regulate soil organic carbon loss in degrading alpine ecosystems

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ABSTRACT

Alpine grassland degradation is a major threat to global carbon cycles, yet the microbial mechanisms driving soil organic carbon (SOC) loss remain poorly understood. Ecological stoichiometry theory provides a framework for understanding how resource imbalances constrain microbial activity and metabolism. Here, we investigated how grassland degradation altered the stoichiometric imbalances between soil microbes and their resources and how microbes coped with such imbalances, as well as the implications of their responses for SOC stock. We established a degradation gradient (non-, light, moderate, and heavy) in both an alpine meadow and an alpine steppe on the Qinghai-Tibet Plateau, China, with analyzing vegetation nutrient storage, soil physicochemical properties, microbial biomass, dissolved organic nutrients, extracellular enzyme activities, and nutrient mineralization rates. Our results showed that C:N stoichiometric imbalance exhibited a hump-shaped response to grassland degradation with a maximum around moderate degradation, while C:P and N:P stoichiometric imbalances significantly decreased with increasing grassland degradation levels in both ecosystems. However, microbial responses were ecosystem-specific: meadow microbes showed strong C:N:P homeostasis, while steppe microbes showed weaker C:N and C:P homeostasis, indicating higher stoichiometric plasticity. Mechanistically, microbes coped with these shifting imbalances by adjusting extracellular enzyme stoichiometry, net N mineralization, and soil microbial respiration. For instance, C:P and N:P stoichiometric imbalances were strongly linked to the relative production of P-acquiring enzymes across both ecosystems, with slightly stronger correlations in meadows. These response mechanisms were significantly correlated with SOC stock, suggesting that microbial metabolic adjustments are a key pathway regulating the 14.8–71.5% decline in SOC stock decline observed during degradation. Our findings provide a mechanistic link between grassland degradation, microbial stoichiometric response, and carbon cycling, highlighting that ecosystem-specific microbial strategies are critical determinants of SOC vulnerability in these sensitive high-altitude ecosystems.

1. Introduction

Globally, the soil carbon (C) stock within the 0–3 m depth interval in terrestrial ecosystems is roughly three times higher than that in the atmosphere (Schmidt et al., 2011; He et al., 2022), and thus, even slight

alterations of this C pool could have significant impacts on climate change and atmospheric carbon dioxide (CO₂) (Balesdent et al., 2018). Soil microbes, as the primary decomposers of soil organic matter (SOM) in terrestrial ecosystems, play a crucial role in regulating nutrient cycles and energy flow through a series of nutrient utilization processes

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(Sterner and Elser, 2002; Sinsabaugh et al., 2009; Zechmeister-Boltenstern et al., 2015). Based on ecological stoichiometry theory (EST), the process by which soil microbes acquire nutrients from the environment exhibits a supply–demand balance, with a relatively stable ratio of their demand for various nutrients (Sterner and Elser, 2002). This balance not only affects their growth and metabolic activity but also drives the cycling and sequestration of soil C and nutrients (Sterner et al., 2007; Manzoni et al., 2012; Kaiser et al., 2014; Mooshammer et al., 2014a). Typically, soil microbes will grow at the maximum possible rate when the stoichiometric ratio between the environmental resource supply and their demand is matched (i.e., stoichiometric balance) (Mooshammer et al., 2014b; Wutzler et al., 2017; Prater et al., 2024). However, the stoichiometric ratios of SOM, the primary substrate source for soil microbes, often do not meet the optimal stoichiometric requirements for microbial growth (i.e., stoichiometric imbalance), leading to growth limitations imposed by C and/or nutrients such as nitrogen (N) and phosphorus (P) (Mooshammer et al., 2014b; Zhang et al., 2023). In this context, soil microbes will cope correspondingly through unique physiological and metabolic adjustments in the uptake, assimilation, and turnover of these scarce resources (Mooshammer et al., 2014b; Yuan et al., 2019; Yang et al., 2024). Thus, a better understanding of the mechanisms underlying how soil microbes cope with stoichiometric imbalances is essential for projecting the changes in the key ecosystem processes and functions.

Soil microbes cope with stoichiometric imbalances through several mechanisms, most notably by regulating the production of extracellular enzymes (Mooshammer et al., 2014b; Zechmeister-Boltenstern et al., 2015). As predicted by resource allocation theory, microbes invest in extracellular enzymes that specifically target their most limiting nutrient within complex organic matter (Allison and Vitousek, 2005). For instance, when high N availability induces C-limitation, microbes upregulate C-acquiring enzymes while downregulating N-acquiring ones to rebalance the C:N ratio of available resources (Yuan et al., 2019). This strategic enzymatic response is therefore a fundamental *in vivo* strategy for acquiring scarce nutrients. By contrast, releasing excess elements and efficiently utilizing limiting elements to maintain a relatively constant element composition within the cell are important *ex vivo* strategies by which microbes achieve nutrient turnover (Mooshammer et al., 2014b; Manzoni et al., 2021). Releasing excess elements is primarily achieved through soil microbial respiration and nutrient mineralization (Yuan et al., 2019), while efficiently utilizing limiting elements can be accomplished by regulating microbial C and nutrient use efficiency (Mooshammer et al., 2014a; Sinsabaugh et al., 2016; Guo et al., 2020). Emerging insights have shown that as C:N and C:P imbalances increase, soil microbes reduce C storage by increasing their respiration or decreasing organic N/P mineralization (Yuan et al., 2019; Cui et al., 2020; Manzoni et al., 2021; Zhao et al., 2021). Conversely, soil microbes can increase the release of N into the environment through net N mineralization when the availability of N is high (Schleuss et al., 2021; Wang et al., 2023). More importantly, a few recent studies have found that soil microbes can simultaneously utilize two or three of the above-mentioned adaptive strategies to simultaneously deal with stoichiometric imbalances, ultimately driving soil C cycling (Yuan et al., 2019; Zhong et al., 2020; Manzoni et al., 2021). Moreover, strategies by which microbes cope with stoichiometric imbalances may be influenced by the type of ecosystem (Mooshammer et al., 2014b). For example, soil microbes can respond to C:N imbalances by adjusting the fungal:bacterial ratio in subtropical forest ecosystems (Guo et al., 2020), while they mainly modulate the allocation ratio of C- and N-acquiring extracellular enzymes in temperate semi-arid grassland ecosystems (Yuan et al., 2019; Zhong et al., 2020). However, our understanding of how soil microbes in C-rich alpine ecosystems respond to stoichiometric imbalances is insufficient.

Empirical evidence has shown that grassland degradation results in reduced plant biomass and nutrient reserves (Guo et al., 2019; Wang et al., 2022), decreased input of plant-derived organic matter such as

litter and root exudates into the soil (Bai et al., 2020; Yang et al., 2023), and decreased ratios of the soil organic carbon (SOC) to total phosphorus (TP) and the total N (TN) to TP (Liu et al., 2018; Zhang et al., 2019; Li et al., 2024). By contrast, the ratios of soil microbial nutrient stoichiometry remain relatively stable with increasing grassland degradation, indicating that the nutrient composition within microbes is characterized by stoichiometric homeostasis (Wang et al., 2020). Moreover, grassland degradation alters soil extracellular enzyme activity (Dong et al., 2019), nutrient mineralization (Abdalla et al., 2018), and microbial respiration (Li and Zhou, 2018). The results of the above-described studies suggest that grassland degradation may alter the stoichiometric balance between soil microbes and their resources; and under such conditions, soil microbes could regulate their extracellular enzyme production and element use efficiency to adapt, ultimately affecting C stock. However, very few studies have investigated the impacts of grassland degradation on soil microbial nutrient utilization strategies and their regulation of SOC stock from the perspective of stoichiometric imbalances.

The Qinghai-Tibet Plateau is a critical component of the global C cycle, containing an estimated 55 % of China's grassland C stocks (Ni, 2002). Despite its importance, this region faces significant environmental challenges. Driven by climate fluctuations and overgrazing, nearly half of the grasslands have undergone degradation, with a substantial portion (16–54 %) classified as severe (Shang and Long, 2007; Harris, 2010). These degraded areas offer an ideal platform to study the responses of soil microbial communities to stoichiometric imbalances in carbon-rich alpine ecosystems. The investigation is further enriched by the plateau's two predominant grassland ecosystems: alpine meadows and steppes. These ecosystems present contrasting conditions in terms of water availability, soil properties, and vegetation composition, which may in turn support distinct microbial communities employing divergent strategies to cope with degradation (He et al., 2022; Mao et al., 2024). Therefore, to address this, we determined the soil microbial biomass and dissolved organic nutrients in both an alpine meadow and an alpine steppe with four degrees of degradation (non-, light, moderate, and heavy degradation) on Qinghai-Tibet Plateau and compared the effects of degradation on stoichiometric imbalances between soil microbes and their resources. Other variables, including plant nutrients, soil physicochemical properties, and microbial physiological and metabolic activities (extracellular enzyme activities, N and P mineralization), were also determined to explore the mechanisms underlying the response of the microbial community to stoichiometric imbalances under grassland degradation and its modulating effect on SOC stock. The objectives of this study were to address two questions. First, how does grassland degradation affect stoichiometric imbalances between soil microbes and their resources across alpine ecosystems? Second, what microbial response mechanisms mediate the relationship between stoichiometric imbalances and SOC stocks, and how do these mechanisms differ between alpine meadow and steppe? We hypothesized that: (1) grassland degradation would decrease C:P and N:P imbalances due to preferential P retention but increase C:N imbalances due to differential C and N losses; (2) microbes would respond through coordinated adjustments in extracellular enzyme stoichiometry and nutrient mineralization, with meadow communities maintaining stronger homeostasis than steppe communities, leading to differences in SOC stock.

2. Materials and methods

2.1. Site description and experimental design

This study was conducted in Tianjun County, Qinghai Province, on the northeastern Qinghai-Tibet Plateau (36°53'–48°39'N, 96°49'–99°41'E; 3,500 m a.s.l.). The region is characterized by a typical plateau continental climate. Mean annual precipitation is 360 mm, most of which occurs from May to September (Ren et al., 2019). Mean annual evaporation ranges from 819.5 to 1,203.5 mm. Over 1961–2015, mean

annual air temperature was $-1.5\text{ }^{\circ}\text{C}$; during the growing season (May–August), monthly mean temperatures ranged from $7\text{ to }11\text{ }^{\circ}\text{C}$. The area lacks a frost-free period and displays no pronounced four-seasonality; pastoral production is traditionally partitioned into cold and warm seasons, and the growing period spans approximately 127–185 days. Dominant livestock includes yak, sheep, and horses. Local grasslands have an average theoretical stocking capacity of one sheep unit per hectare. Since the 1980 s, economic pressures have driven severe overstocking of these natural rangelands. At the county scale, the current stocking rate exceeds 1.8 million sheep units, a number that substantially surpasses the theoretical carrying capacity. This overstocking has caused the degradation of 7.67×10^5 ha of grassland, representing 59.5 % of the total usable area (Li, 2018). Grasslands are primarily classified as alpine meadow and alpine steppe. The alpine meadow was dominated by sedges, including *Kobresia humilis*, *Kobresia graminifolia*, and *Carex spp.* and the alpine steppe was dominated by *Stipa purpurea* Griseb (Wang et al., 2020). According to the World Reference Database of Soil Classification, the soils in the alpine meadow and steppe grasslands were classified as Mattic Grylic Cambisols and Grylic Calcic Aridisols, respectively (IUSS-WRB, 2014).

Since 2001, rangelands have been allocated to individual families by the government (Yao et al., 2016). The allocated rangelands have subsequently been fenced and managed by graziers. We used grazer-managed rangelands as sampling sites and collected plant and soil samples from alpine meadows and alpine steppes representing four degradation levels. Accordingly, these sites share a comparable

temporal framework of management since allocation and enclosure. Because grassland degradation reduces plant community cover, soil fertility, and soil texture quality, we assessed degradation status using a combination of plant and soil variables (Wang et al., 2007; Zhang et al., 2024). Briefly, plant variables included the relative cover of three groups: climax species, degradation indicator species, and palatable species (Liu et al., 2008). Soil variables included SOC content, TN content, and sand content (Bardgett et al., 2021; Zhang et al., 2024).

The grassland degradation index (GDI) was used to quantify the degree of grassland degradation:

$$\text{GDI} = (C_1 + C_2 \times 1/3 + C_3 \times 2/3) \times 1/3 + (\text{SOC} \times 1/2 + \text{TN} \times 1/2) \times 1/3 + (1 - \text{Sand}) \times 1/3 \quad (1)$$

where C_1 , C_2 , and C_3 represent the relative covers of the climax species, palatable species, and degradation indicator species, respectively; SOC and TN represent the contents of soil organic carbon and total nitrogen, respectively, standardized to 0–1 using $(X - X_{\min})/(X_{\max} - X_{\min})$. Here, X is the target variable, and X_{\min} and X_{\max} are its minimum and maximum values across all samples, respectively. Sand represents the soil sand content. Based on GDI, we classified the alpine meadow and steppe grassland areas as non-degraded (ND), lightly degraded (LD), moderately degraded (MD), and heavily degraded (HD) grasslands. Detailed information about the geographic and plant community characteristics of these two types of grassland is presented in Table 1.

Table 1

Basic characteristics of plant community and soil physicochemical properties at four degradation levels in the alpine meadow and alpine steppe.

Grassland types	Parameters	Non-degraded grassland	Lightly degraded grassland	Moderately degraded grassland	Heavily degraded grassland
Alpine meadow	Latitude and longitude	37°33'23" N, 99°08'55" E	37°07'18" N, 99°12'35" E	37°42'08" N, 98°33'54" E	37°32'07" N, 98°40'06" E
	Altitude (m)	3646	3427	3662	3570
	Dominant species composition	<i>Kobresia humilis</i>	<i>Kobresia humilis</i> , <i>Carex moorcroftii</i>	<i>Kobresia humilis</i> , <i>Carex moorcroftii</i> , <i>Potentilla bifurca</i> L.	<i>Carex moorcroftii</i> ; <i>Potentilla bifurca</i> L.
	Plant community cover (%)	92.3 ± 1.5	86.3 ± 0.6	57.0 ± 2.0	36.3 ± 5.0
	Relative cover of climax species (%)	74.03 ± 2.01	67.57 ± 2.39	59.51 ± 5.63	47.06 ± 2.44
	Relative cover of degradation indicators (%)	1.92 ± 0.06	8.11 ± 1.17	22.82 ± 0.46	46.45 ± 2.26
	Relative cover of available species (%)	24.04 ± 2.07	24.31 ± 1.71	20.00 ± 3.64	6.47 ± 0.93
	Sand content (%)	20.85 ± 1.01	27.69 ± 3.95	29.07 ± 0.99	38.92 ± 3.92
	SOC (g kg ⁻¹)	84.90 ± 2.15	58.57 ± 0.91	38.12 ± 0.09	20.70 ± 1.27
	TN (g kg ⁻¹)	5.24 ± 0.12	5.01 ± 0.26	3.03 ± 0.11	1.96 ± 0.09
	Grassland degradation index (GDI)	0.85 ± 0.01	0.75 ± 0.01	0.61 ± 0.02	0.48 ± 0.01
	BD (g cm ⁻³)	0.78 ± 0.01	0.94 ± 0.01	1.14 ± 0.01	1.31 ± 0.01
	TP (g kg ⁻¹)	0.76 ± 0.03	0.98 ± 0.01	0.72 ± 0.01	0.75 ± 0.02
Alpine steppe	Latitude and longitude	37°25'11" N, 99°10'01" E	37°27'48" N, 98°33'46" E	37°28'48" N, 98°31'21" E	37°43'11" N, 98°33'49" E
	Altitude (m)	3490	3603	3607	3571
	Dominant species composition	<i>Stipa purpurea</i> Griseb., <i>Poa alpigena</i> (Bulytt) L.	<i>Stipa purpurea</i> Griseb., <i>Carex moorcroftii</i>	<i>Stipa purpurea</i> Griseb., <i>Potentilla bifurca</i> L.	<i>Dasiphora fruticosa</i> (L.) Rydb., <i>Stipa purpurea</i> Griseb
	Plant community cover (%)	90.0 ± 3.6	83.7 ± 0.6	49.7 ± 4.9	26.0 ± 5.3
	Relative cover of climax species (%)	55.59 ± 0.95	45.79 ± 2.44	27.15 ± 4.15	24.28 ± 3.52
	Relative cover of degradation indicators (%)	6.28 ± 0.24	6.76 ± 1.03	38.86 ± 4.73	61.90 ± 1.56
	Relative cover of available species (%)	38.11 ± 0.83	47.43 ± 3.39	33.98 ± 2.57	13.80 ± 3.81
	Sand content (%)	39.19 ± 1.35	30.53 ± 3.73	44.78 ± 5.52	89.67 ± 2.79
	SOC (g kg ⁻¹)	45.17 ± 0.53	27.43 ± 0.95	19.13 ± 0.89	9.70 ± 0.60
	TN (g kg ⁻¹)	3.68 ± 0.13	2.26 ± 0.09	1.56 ± 0.02	0.56 ± 0.01
	Grassland degradation index (GDI)	0.82 ± 0.01	0.69 ± 0.01	0.60 ± 0.01	0.34 ± 0.01
	BD (g cm ⁻³)	1.08 ± 0.01	1.24 ± 0.01	1.33 ± 0.01	1.44 ± 0.01
	TP (g kg ⁻¹)	0.79 ± 0.02	0.83 ± 0.01	0.75 ± 0.01	0.60 ± 0.01

Note: All values are means ± SE (n = 3). SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; BD, soil bulk density.

2.2. Field sampling of vegetation and soils

Within each grassland degradation level, we established three 1 m × 1 m quadrats along transects in flat areas, with quadrats serving as replicates, in July 2017 (peak growing season). To ensure representativeness of the local climate and original vegetation, quadrats were spaced at least 500 m apart. Plant coverage was quantified within the same 1 × 1 m quadrats using a metal frame subdivided into 100 equally distributed grids positioned above the canopy. At each pin-point, the number of contacts between live organs and the pin was recorded for each species (Niu et al., 2018). Species richness was quantified as the number of plant species recorded within each measured quadrat. Aboveground plant biomass was then harvested by clipping at ground level. Following the harvesting of aboveground plant biomass and removal of the litter layer, two soil cores (0–10 cm soil depth) were collected with a root auger (12.5 cm inner diameter) to determine belowground plant biomass. This depth encompasses the primary zone of fine root and microbial activity on the Qinghai-Tibet Plateau (Dai et al., 2019; He et al., 2022). In addition, four soil cores (0–10 cm) were sampled using a soil auger (inner diameter of 7.5 cm) within each quadrat. After this, all of the soil samples were placed in individual plastic bags and stored in a cooler with ice for transportation to the laboratory, and fresh soil samples were stored at 4°C and analyzed within two weeks of collection. In the laboratory, each soil sample was completely homogenized and sieved through a 2 mm mesh sieve to remove the plant tissues and stones and obtain one composite sample. Each combined soil sample was divided into two parts. One part was stored at 4°C for measurements of the soil extracellular enzymes, microbial biomass, dissolved organic nutrients, SOC mineralization, and net nutrient mineralization. The other part was air-dried for measuring the soil physicochemical properties. We collected soil bulk density samples at depths of 0–10 cm using a standard container with a fixed volume size of 100 cm³.

2.3. Plant nutrient measurements

Belowground plant samples were washed through a 0.5 mm mesh. Subsequently, both aboveground and belowground samples were oven-dried at 70 °C for 48 h and weighed to quantify above- and belowground biomass. The total C and N contents of the plant samples were determined using a CHN elemental analyzer (Flash EA1112, Thermo Scientific, West Palm Beach, FL, USA). After plant samples were digested in H₂SO₄ and H₂O₂ in a heating-block system (Fanin et al., 2013), the plant total phosphorous (P) concentration was analyzed via colorimetry using an automatic discrete analyzer (Smartchem450, AMS, Italy). The C and nutrient storage (g m⁻²) of the plant samples were calculated by multiplying the biomass by the nutrient concentrations.

2.4. Soil analysis

2.4.1. Soil property analyses

Soil pH was determined in a 1:2.5 mix of dry soil and deionized water using a pH meter (PHS-3C, Shanghai, China). Soil water content (% SWC) was measured via oven-drying at 105°C for 48 h. Soil water-holding capacity (% WHC) was determined gravimetrically using the Whatman funnel method (Govindasamy, et al., 2023). After removal of organic matter and carbonates, soil texture was analyzed with a particle size analyzer Mastersizer 2000 (Malvern Instruments, UK). Bulk density was calculated as oven-dry mass (after drying container at 105 °C to constant weight) divided by the container volume. SOC and TN contents were quantified with a CHN elemental analyzer (Flash EA1112, Thermo Scientific, West Palm Beach, FL, USA). TP content was measured using the molybdenum blue method after digestion using sulfuric acid and perchloric acid. Nitrate-N (NO₃⁻-N) and ammonium-N (NH₄⁺-N) were extracted from 10 g of fresh soil with 50 ml of 2 M KCl and quantified using the modified indophenol blue enzyme assay and the Griess reagent

enzyme assay, respectively (Zhang et al., 2025). Available P was extracted from 5 g of air-dried soil with 50 ml of 0.5 M NaHCO₃ (pH 8.5) and measured colorimetrically by the molybdate–ascorbic acid method. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were extracted from 10 g of homogenized fresh soil with 50 ml of 2 M KCl (120 rpm, 1 h), filtered, and analyzed using a TOC/TN analyzer (Elementar vario TOC select, Hanau, Germany (Wang et al., 2023)). Total dissolved phosphorus (TDP) was extracted from 5 g of fresh soil with 50 ml of 0.5 M NaHCO₃ (pH 8.5), digested with acid persulfate, and determined by ammonium molybdate spectrophotometry (Lajtha et al., 1999). The dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) were calculated as the differences between the concentrations of the total dissolved and inorganic dissolved forms (Tapia-Torres et al., 2015).

2.4.2. Soil microbial biomass analysis and extracellular enzyme assays

The soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphorus (MBP) were determined using the chloroform fumigation extraction method (Brookes et al., 1985). The potential activities of four soil extracellular enzymes, namely a C-acquiring enzyme (β-1,4-glucosidase, BG), N-acquiring enzymes (β-1,4-N-acetylglucosaminidase, NAG; leucine aminopeptidase, LAP), and a P-acquiring enzyme (alkaline phosphatase, AP), were measured according to previously described protocols (Verchot and Borelli, 2005; Yuan et al., 2019). Briefly, 2.5 g of fresh soil was homogenized in 50 ml of 50 mM acetate buffer (Ph = 8.0) in a 100 ml plastic bottle and then shaken in a thermostat shaker (25 °C) at 180 rpm for about 40 min. Then, 2 ml of slurry and 2 ml of substrate solution were added to each centrifuge tube. In addition, we prepared three soil controls and three substrate controls for each assay. The soil controls consisted of 2 ml of buffer and 2 ml of soil slurry, and the substrate controls consisted of 2 ml of buffer and 2 ml of substrate solution. The tubes were vortexed in a magnetic stirring apparatus for 10 s and then placed in a rotary shaker (25 °C) to incubate. The incubation time for the BG, NAG, and LAP activities was 4 h, and that for the AP activity was 2 h. The AP activity assay needed to incubate in the dark. Following incubation, the test tubes were centrifuged for 5 min at 2000 rpm, and 1 ml of supernatant was transferred from each tube into a 10 ml volumetric flask containing 0.2 ml of 1 M NaOH to stop the reaction and was then diluted to 10 ml using deionized water. Then, the absorbance (410 nm) was measured using a spectrophotometer (UV-1601, Shimadzu Inc.). Finally, all the extracellular enzyme activities were expressed in μmol h⁻¹ g⁻¹ of dry soil.

2.4.3. Soil microbial respiration and net N and P mineralization rates

To determine the soil microbial respiration (SMR), fresh soil samples (20 g dry weight) were placed in incubation bottles and adjusted to 60 % of their water-holding capacity. All of the sample bottles were sealed with preservative films, into which several small holes were made for ventilation. The samples were then pre-incubated at 20 °C for seven days to activate the soil microbes and avoid any pulse effects on the microbial activity. After pre-incubation, the SMR was measured at 25°C using an automatic sampling and analysis system (LI-8800; PRE-ECO, Beijing, China), which was newly developed through modification of the method described by He et al. (2013). The SMR was measured on days 1, 3, 5, and 7, and deionized water was regularly added to maintain a constant soil moisture content. The details of this method have been described by He et al. (2013).

The SMR was calculated from the slope of the CO₂ concentration and the corresponding transformation factors using the following equation:

$$SMR = \frac{C \times V}{22.4} \times \frac{12}{44} \times \frac{86,400}{m} \quad (2)$$

where SMR is the rate of soil microbial respiration (μg CO₂-C g⁻¹ soil d⁻¹), C is the slope of CO₂ concentration (ppm s⁻¹), V is the volume of

the incubation bottle and gas tube (L), m is the dry weight of the soil (g), 12/44 is the relative atomic mass of C, 86,400 is the conversion factor from days to seconds, and 22.4 is the standard gas volume at 25 °C and 1 atm (L mol⁻¹) (He et al., 2013; Liu et al., 2021).

To measure the net N and P mineralization, we used fresh soil samples (20 g dry weight) in the SMR measurements. The differences of inorganic N and PO₄³⁻ concentrations between the initial pre-incubation and final incubation were used to calculate the net N mineralization rate (N_{\min}) and P mineralization rate (P_{\min}) (Fanin et al., 2016).

2.5. Calculations

The stoichiometric imbalance (SI) between the soil microbial communities and their available resources was calculated as the C:N_{resource} (or C:P, N:P) ratio for the soil dissolved organic matter divided by C:N_{microbes} (or C:P, N:P) of the soil microbial biomass using the following equations (Mooshammer et al., 2014a; Yuan et al., 2019):

$$SI_{C:N} = \frac{R_{C:N}}{MB_{C:N}} \quad (3)$$

$$SI_{C:P} = \frac{R_{C:P}}{MB_{C:P}} \quad (4)$$

$$SI_{N:P} = \frac{R_{N:P}}{MB_{N:P}} \quad (5)$$

where $SI_{C:N}$, $SI_{C:P}$, and $SI_{N:P}$ are the C:N imbalance, C:P imbalance, and N:P imbalance, respectively. $R_{C:N}$, $R_{C:P}$, and $R_{N:P}$ are the C:N:P ratios of the soil dissolved organic matter. $MB_{C:N}$, $MB_{C:P}$, and $MB_{N:P}$ are the C:N:P ratios of the soil microbial biomass.

The SOC stock at the depth of 0–10 cm was calculated as follows:

$$\text{SOC stock} = \text{SOC concentration} \times \text{BD} \times D \times \frac{(1 - C)}{100} \quad (6)$$

where SOC stock is the soil organic carbon stock at depth of 0–10 cm (kg C m⁻²), SOC concentration is the soil organic carbon concentration (g kg⁻¹), BD is the soil bulk density (g cm⁻³), D is the soil thickness (cm), and C is >2 mm rock content (%).

2.6. Statistical analyses

The effects of grassland degradation on vegetation biomass and nutrient storage, soil physicochemical properties, SOC stock, soil dissolved organic nutrients and their stoichiometry, microbial biomass and its stoichiometry, stoichiometric imbalances, soil extracellular enzyme activity and their stoichiometry, nutrient mineralization rate, and SMR were analyzed using linear or nonlinear models in the alpine meadow and alpine steppe. Pearson correlation analysis was conducted to analyze the relationships of soil dissolved organic nutrients and their stoichiometry, microbial biomass and its stoichiometry, and stoichiometric imbalances with the vegetation nutrient storage and soil properties across all of the grassland degradation levels in the alpine meadow and alpine steppe areas. In addition, linear or nonlinear models were constructed to fit the changes in soil extracellular enzyme stoichiometry, nutrient mineralization rate, and SMR with increasing stoichiometric imbalances across all of the grassland degradation levels in the alpine meadow and alpine steppe areas. Moreover, we used linear regression to evaluate the relationships between SOC and the stoichiometric imbalances, soil extracellular enzyme stoichiometry, N_{\min} , and SMR across all of the grassland degradation levels. All of the statistical analyses were performed using R (version 4.4.1).

3. Results

3.1. Vegetation nutrient storage, SOC stock, soil dissolved organic nutrients, and microbial biomass

In both the alpine meadow and alpine steppe, the vegetation biomass and nutrient storage, SOC stock, and available nutrient stoichiometry exhibited the same variation trends with increasing grassland degradation (Figs. 1 and 2). Specifically, aboveground and belowground plant biomass, as well as their associated C, N, and P storages significantly decreased in response to increasing grassland degradation (all $P < 0.05$; Fig. 1). With increasing grassland degradation, SWC, SOC stock, DOC, DON, MBC, MBN, and MBP decreased significantly, whereas pH and DOP exhibited the opposite trend (all $P < 0.05$; Fig. 2a-i). With grassland degradation intensified, $R_{C:N}$ exhibited a hump-shaped response, whereas $R_{C:P}$ and $R_{N:P}$ decreased significantly in both grassland types (all $P < 0.05$; Fig. 2j-l). In addition, the response of the soil microbial biomass stoichiometry to grassland degradation depended on the type of grassland (Fig. 2m-o). The microbial biomass stoichiometry was not significantly affected by grassland degradation in the alpine meadow, except that the $MB_{C:P}$ exhibited a decrease-then-increase pattern ($P < 0.05$). By contrast, the $MB_{C:N}$ and $MB_{C:P}$ exhibited a unimodal pattern, reaching a minimum at intermediate degradation levels (both $P < 0.05$), while no change was observed for the $MB_{N:P}$ in the alpine steppe.

3.2. Stoichiometric imbalances and stoichiometric homeostasis

The $SI_{C:N}$ exhibited a hump-shaped response to grassland degradation, with a maximum at MD, while $SI_{C:P}$ and $SI_{N:P}$ significantly decreased with increasing grassland degradation levels in both alpine meadow and alpine steppe (all $P < 0.05$; Fig. 3). To examine the strength of stoichiometric homeostasis, we further analyzed the relationships between the microbial biomass stoichiometry and those between the soil resources. The correlations between the log $R_{C:N}$ and log $MB_{C:N}$, log $R_{C:P}$ and log $MB_{C:P}$, and log $R_{N:P}$ and log $MB_{N:P}$ were not significant in the alpine meadow when all of the data were analyzed together (Fig. 4), suggesting that the microbial community was characterized by relatively strong C:N, C:P, and N:P homeostasis. In addition, the correlations between the log $R_{C:N}$ and log $MB_{C:N}$ and log $R_{C:P}$ and log $MB_{C:P}$ were significant, while there was no significant correlation between log $R_{N:P}$ and log $MB_{N:P}$ in the alpine steppe (Fig. 4).

3.3. Dominant controls of stoichiometric imbalances

To explore the factors controlling the stoichiometric imbalances, we analyzed the relationships between the potential factors (i.e., vegetation C and nutrient storage and soil properties) and the stoichiometric imbalances and their components (Fig. 5). Our results found that soil dissolved organic nutrients and their stoichiometry and stoichiometric imbalances were significantly associated with the vegetation nutrient storage and soil pH in the alpine meadow, whereas the soil microbial biomass and its stoichiometry was weakly correlated with these examined factors (Fig. 5a).

For the alpine steppe, $SI_{C:P}$ and $SI_{N:P}$ were mainly correlated with the root nutrient storage, and $SI_{C:N}$ was not significantly correlated with the examined factors (Fig. 5b). Regarding the dissolved organic nutrients and their stoichiometry, DOC, DON, DOC:DOP, and DON:DOP exhibited significant correlations with the vegetation nutrient storage and soil properties (Fig. 5b). Significant correlations were observed between soil microbial biomass and vegetation nutrient storage and soil properties, but the corresponding stoichiometric metrics were only weakly associated (Fig. 5b). The results of the variation partitioning analysis (VPA) showed that the vegetation nutrient storage and soil properties explained 91.4 %, 85.9 %, and 93.5 % of the variances of the $SI_{C:N}$, $SI_{C:P}$, and $SI_{N:P}$ in the alpine meadow, respectively, and 88.4 %, 77.5 %, and 95.8 % of the total variances of $SI_{C:N}$, $SI_{C:P}$, and $SI_{N:P}$ in the alpine steppe,

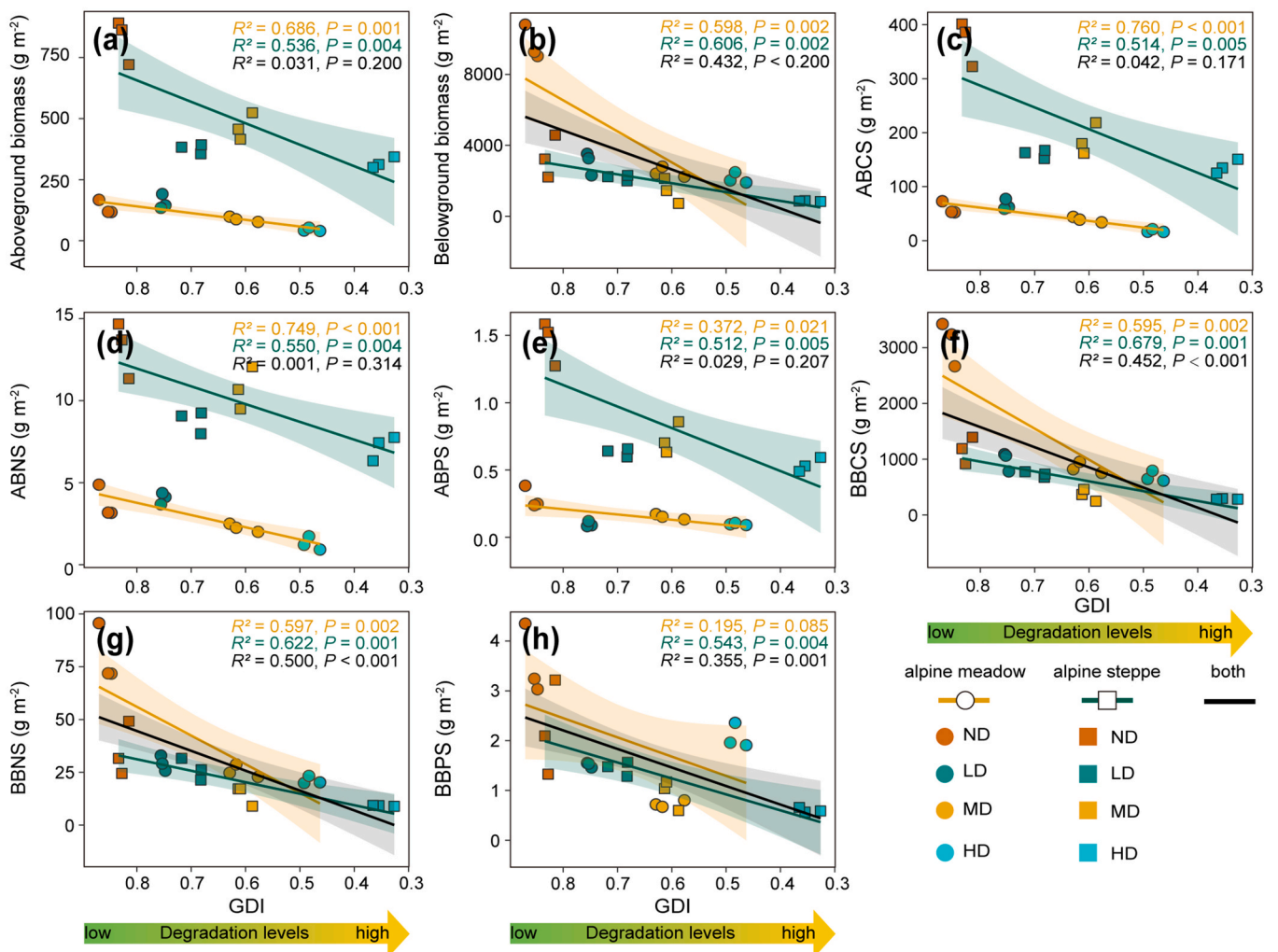


Fig. 1. Effects of grassland degradation on vegetation biomass and nutrient storage in alpine meadow and alpine steppe, including (a) Aboveground biomass, (b) Belowground biomass, (c) ABCS, (d) ABNS, (e) ABPS, (f) BBCS, (g) BBNS, and (h) BBPS. Curves in different colors were fitted using linear or nonlinear regression models, and shaded areas denote the corresponding 95% confidence intervals. ABCS, carbon storage in the aboveground biomass; ABNS, nitrogen storage in the aboveground biomass; ABPS, phosphorus storage in the aboveground biomass; BBCS, carbon storage in the belowground biomass; BBNS, nitrogen storage in the belowground biomass; BBPS, phosphorus storage in the belowground biomass. ND, non-degraded; LD, lightly degraded; MD, moderately degraded; HD, heavily degraded.

respectively (Fig. S5).

3.4. Soil extracellular enzymes, net nutrient mineralization, and microbial respiration

Grassland degradation significantly altered the C-, N-, and P-acquiring enzyme activities and their stoichiometry (all $P < 0.05$; Fig. 6). In both the alpine meadow and alpine steppe, the C-acquiring enzyme (i.e., BG) activity initially decreased and then increased with increasing grassland degradation, and the lowest value occurred under MD ($P < 0.05$; Fig. 6a). The activities of the N-acquiring enzymes (i.e., NAG + LAP) and P-acquiring enzyme (i.e. AP) significantly decreased with increasing grassland degradation (both $P < 0.05$; Figs. 6b, c). In addition, the ratio of BG:(NAG + LAP) ($EEA_{C:N}$) and BG:AP ($EEA_{C:P}$) initially decreased and then increased with degradation, and the lowest values occurred under LD in both the alpine meadow and alpine steppe ($P < 0.05$; Fig. 6d). The ratio of (NAG + LAP):AP ($EEA_{N:P}$) presented a hump-shaped response to grassland degradation in the alpine meadow, whereas it increased significantly in the alpine steppe (both $P < 0.05$; Figs. 6e, f).

In addition to these changes in the soil enzyme activities and their

stoichiometry, nutrient mineralization was also strongly affected by the grassland degradation (Fig. 3). Specifically, N_{min} significantly decreased with increasing grassland degradation in the alpine meadow and alpine steppe (both $P < 0.05$; Fig. 6g). P_{min} showed no significant response to grassland degradation (Fig. 6h). In addition, in both the alpine meadow and alpine steppe, the SMR exhibited a hump-shaped response to grassland degradation, and the maximum value occurred under LD ($P < 0.05$; Fig. 6i).

3.5. Associations of stoichiometric imbalances with extracellular enzymes stoichiometry, net nutrient mineralization, and microbial respiration

In both the alpine meadow and alpine steppe, the $SI_{C:N}$ imbalance was negatively correlated with N_{min} ($P < 0.05$; Fig. 7d) but did not exhibit any significant relationship with $EEA_{C:N}$ and SMR (Figs. 7a, j). The $SI_{C:P}$ was negatively correlated with the $EEA_{C:P}$ ($P < 0.05$; Fig. 7b) in both the alpine meadow and alpine steppe, and positively correlated with the SMR in the alpine steppe ($P < 0.05$; Fig. 7h). In both types of grassland, the $SI_{N:P}$ was negatively correlated with the $EEA_{N:P}$ ($P < 0.05$; Fig. 7c), positively correlated with the N_{min} ($P < 0.05$; Fig. 4i), and not significantly correlated with P_{min} (Fig. 7f).

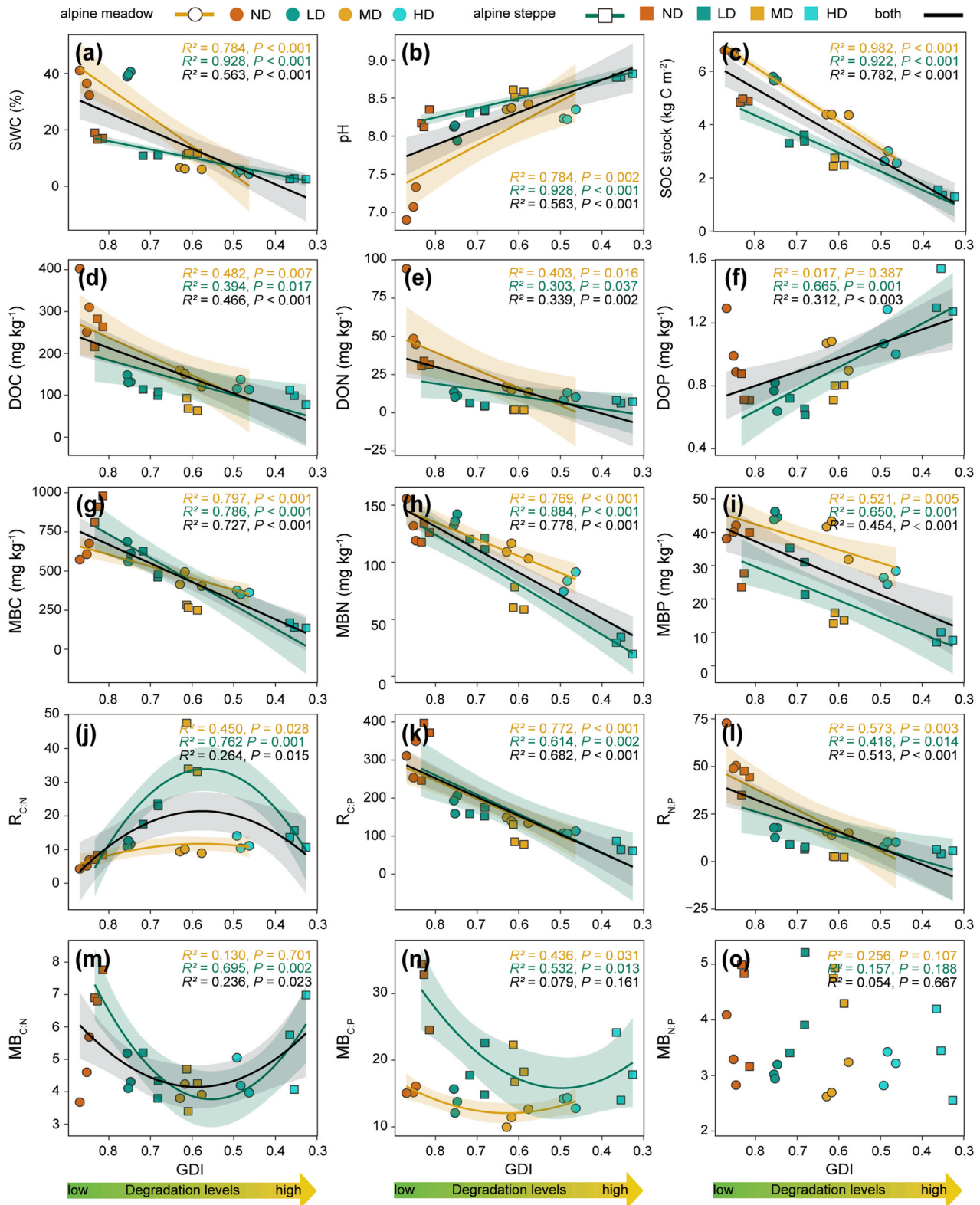


Fig. 2. Effects of grassland degradation on soil physicochemical properties, SOC stocks, soil available nutrients, and microbial biomass in alpine meadow and alpine steppe, including (a) SWC, (b) pH, (c) SOC stock, (d) DOC, (e) DON, (f) DOP, (g) MBC, (h) MBN, (i) MBP, (j) $R_{C:N}$, (k) $R_{C:P}$, (l) $R_{N:P}$, (m) $MB_{C:N}$, (n) $MB_{C:P}$, and (o) $MB_{N:P}$. Curves in different colors were fitted using linear or nonlinear regression models, and shaded areas denote the corresponding 95% confidence intervals. See Fig. 1 for treatment abbreviations. SWC, soil water content; SOC stock, soil organic carbon stock; DOC, soil dissolved organic carbon; DON, soil dissolved organic nitrogen; DOP, soil dissolved organic phosphorus; MBC, soil microbial biomass carbon; MBN, soil microbial biomass nitrogen; MBP, soil microbial biomass phosphorus; $R_{C:N}$, soil dissolved organic C:N ratio; $R_{C:P}$, soil dissolved organic C:P ratio; $R_{N:P}$, soil dissolved organic N:P ratio; $MB_{C:N}$, soil microbial biomass C:N ratio; $MB_{C:P}$, soil microbial biomass C:P ratio; $MB_{N:P}$, soil microbial biomass N:P ratio.

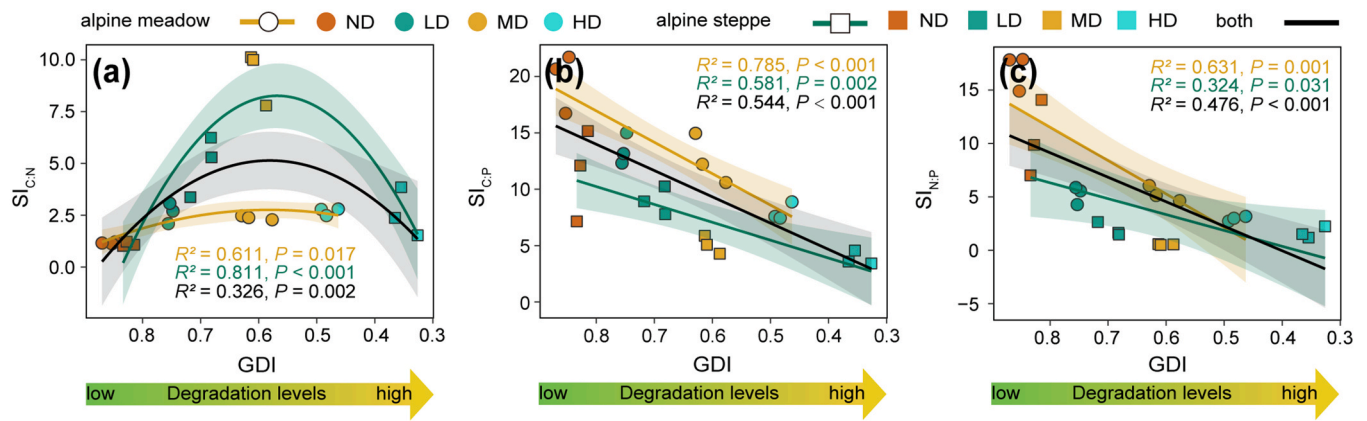


Fig. 3. Effects of grassland degradation on stoichiometric imbalances between soil microbial communities and their resources in alpine meadow and alpine steppe, including (a) $SI_{C:N}$, (b) $SI_{C:P}$, and (c) $SI_{N:P}$. Curves in different colors were fitted using linear or nonlinear regression models, and shaded areas denote the corresponding 95% confidence intervals. See Fig. 1 for treatment abbreviations. $SI_{C:N}$, C:N imbalance between microbes and resources; $SI_{C:P}$, C:P imbalance between microbes and resources; and $SI_{N:P}$, N:P imbalance between microbes and resources.

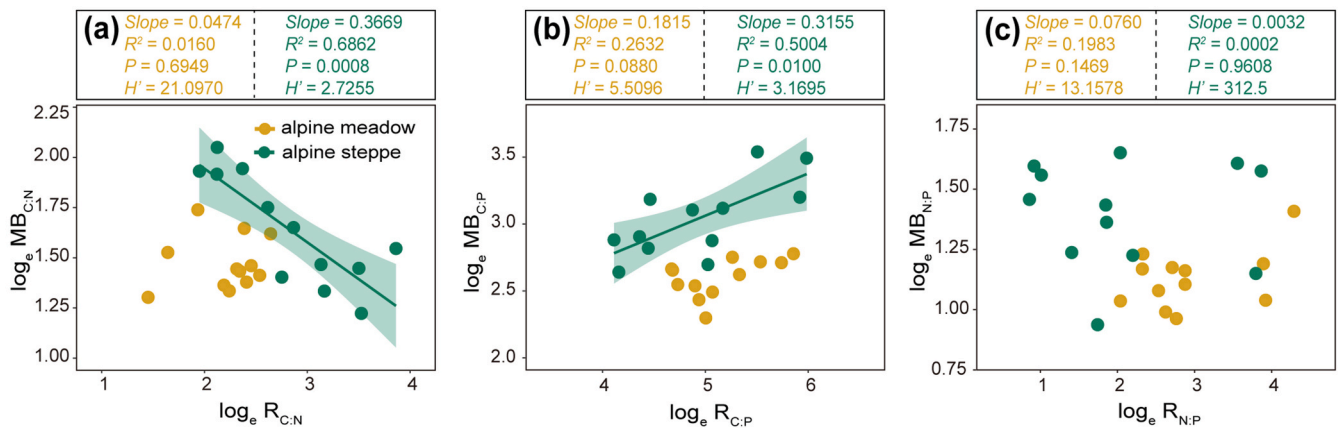


Fig. 4. Relationships between the log-transformed soil microbial biomass and their resources for C:N homeostasis (a), C:P homeostasis (b), and N:P homeostasis (c) across all of the grassland degradation levels in alpine meadow and alpine steppe sites. Curves in different colors were fitted using linear regression models, and shaded areas denote the corresponding 95% confidence intervals.

3.6. Effects of microbial response mechanisms to stoichiometric imbalances on SOC stock

The results of the linear regressions indicated that the SOC stock was negatively correlated with the $SI_{C:N}$ in the alpine meadow but not in the alpine steppe ($P < 0.01$; Fig. 8a), while it was positively correlated with the $SI_{C:P}$ and $SI_{N:P}$ in both types of grassland (both $P < 0.01$; Figs. 8b, c). In addition, the SOC stock was negatively correlated with the $E_{EA_{C:P}}$ and $E_{EA_{N:P}}$, positively correlated with the N_{min} , and not significantly correlated with the SMR in the alpine meadow (Fig. 8d-g). For the alpine steppe, the SOC content was negatively associated with the $E_{EA_{C:P}}$, positively correlated with the N_{min} and SMR, and not correlated with the $E_{EA_{N:P}}$ (Fig. 8d-g).

4. Discussion

4.1. Effects of grassland degradation on stoichiometric imbalances

A widespread stoichiometric imbalance has been observed between soil microbes and their resources in most empirical studies conducted in terrestrial ecosystems (Frost et al., 2005; Elser et al., 2007; Mooshammer et al., 2014b), and the magnitudes of such imbalances are influenced by changes in the soil environmental resources (Yuan et al., 2019). Consistent with these findings, the results of this study suggest that

grassland degradation significantly decreased the C:P imbalance ($SI_{C:P}$) and N:P imbalance ($SI_{N:P}$) in both types of grassland studied (Figs. 3b, c), which is consistent with our initial hypothesis. A lower consumer biomass C:P ratio compared with their resource suggests that the amount of P in the food resources is insufficient to meet the consumers' P demand, leading to high $SI_{C:P}$ values, and vice versa (Cross et al., 2003; Zhao et al., 2021). Moreover, ecological stoichiometry theory (EST) suggests that soil microbes with relatively high P contents compared with C and N have a high P demand, making them more susceptible to P limitation (Sterner and Elser, 2002). In this study, it was found that the soil microbial biomass C:P ratios (meadow: 11.3–15.4; steppe: 18.7–30.6) and N:P ratios (meadow: 2.8–3.4; steppe: 3.4–4.7) were generally below the global average microbial biomass C:P (48) and N:P (6.7) values (Gao et al., 2022). The microbial biomass C:P ratios were also lower than the soil DOC:DOP (Fig. 2), indicating a high P demand by the soil microbes in the alpine grasslands studied. Some studies have suggested that microbial P limitation occurs when the resource C:P ratio exceeds 50–100 (Scott et al., 2012). In this study, the soil resource C:P ratios ranged from 109.4 to 304.6 in the alpine meadows and from 70.6 to 338.4 in the alpine steppes (Fig. 2), confirming the microbial growth limitation by P in the study area. As the grassland degradation intensified, the $SI_{C:P}$ and $SI_{N:P}$ values decreased, suggesting an increase in the P content relative to the C and N contents in the soils (Fig. 2), potentially alleviating microbial growth limitation by P. In contrast, with increasing

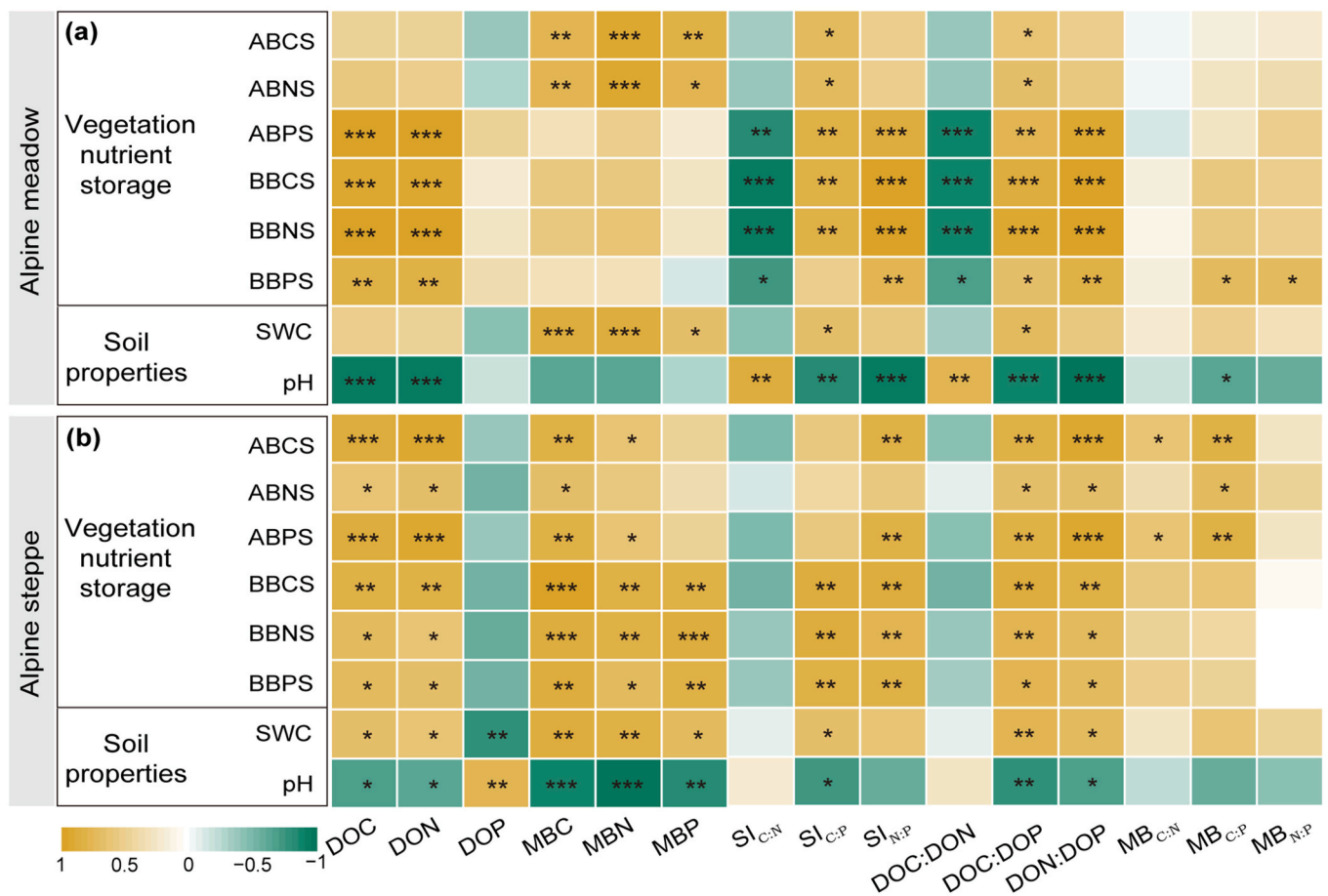


Fig. 5. Relationships of soil dissolved organic nutrients and their stoichiometry, microbial biomass and its stoichiometry, and stoichiometric imbalances with the vegetation nutrient storage and soil properties across all of the grassland degradation levels in (a) alpine meadow and (b) alpine steppe. *, **, and *** denote significance at the $P < 0.05$, $P < 0.01$, and $P < 0.001$ levels, respectively.

grassland degradation, the C:N imbalance ($SI_{C:N}$) exhibited a hump-shaped response, peaking at moderate degradation in both types of grassland (Fig. 3a). This pattern may arise because, under light grassland degradation, plant-derived C inputs partially offset soil C deficits, leading to a smaller decline in DOC than in DON; however, as degradation intensifies, plant-derived C inputs can no longer meet microbial demand, resulting in rapid depletion of soil DOC. This interpretation is further supported by the hump-shaped response of the DOC:DON ratio to increasing grassland degradation (Fig. 2j).

In natural ecosystems, the C:N:P ratios of the resources (ranging from 287:17:1 to 14103:40:1) are typically higher than those of soil microbes (42:6:1), resulting in significant C:N:P imbalances between them (Mooshammer et al., 2014b). Such stoichiometric imbalances are primarily influenced by microbial element demands and available resources. In this study, we found that the soil resource stoichiometry contributed more to the variations in the stoichiometric imbalances than the microbial biomass stoichiometry (Fig. S4), which is consistent with the results of previous research (Yuan et al., 2019; Wang et al., 2023). In addition, C:N:P imbalances can be influenced by vegetation nutrients and soil environmental factors (Zhao et al., 2021; Wang et al., 2023). Our results demonstrate that vegetation nutrient stocks, particularly root nutrient stocks, significantly affected the $SI_{C:N}$, $SI_{C:P}$, and $SI_{N:P}$ in both the alpine meadows and steppes (Fig. 5). This may be due to the fact that the vegetation nutrient stock reflects the quantity and quality of the plant-derived organic matter input into the soil, thus affecting soil C and nutrient availability, as well as microbial nutrients (Day et al., 2008; Castellano et al., 2015). Furthermore, the soil pH was positively correlated with the $SI_{C:N}$ and negatively correlated with the $SI_{C:P}$ and $SI_{N:P}$

(Fig. 5), suggesting that an increase in the soil pH due to grassland degradation would increase the $SI_{C:N}$ and decrease the $SI_{C:P}$ and $SI_{N:P}$. The increase in the soil pH under grassland degradation may facilitate the neutralization of acidic components in dissolved organic matter containing calcium and magnesium compounds, ultimately reducing their contents (Liu et al., 2018). In addition to the vegetation nutrient stock and soil pH, the soil moisture content is another crucial factor that affects the soil nutrients and microbial nutrient content within an ecosystem. It can directly affect the adsorption and leaching of soluble organic matter and the decomposition of organic matter by microbes (Tapia-Torres et al., 2015; Huang et al., 2023). The significant positive correlation between the soil moisture content and the dissolved organic nutrients and microbial biomass observed in this study supports this deduction (Fig. 5). In summary, grassland degradation modifies the stoichiometric imbalance between soil microbes and their resources in alpine grasslands, a process collectively regulated by soil resources, microbial nutrient demands, vegetation nutrients, and soil physicochemical properties. Moreover, given that seasonal and interannual variations in vegetation nutrients, soil physicochemical properties, and microbiological properties can be substantial in alpine ecosystems (Maslov and Maslova, 2021; Yuan et al., 2023), the findings presented here, based on single time-point sampling at peak plant growth, represent only a snapshot of these dynamic relationships. Future research incorporating multiple sampling times across seasons and years would be valuable to elucidate the temporal stability of these stoichiometric patterns and microbial response mechanisms.

Based on the ecological stoichiometric theory (EST), we analyzed the C:N, C:P, and N:P relationships between soil microbes and their

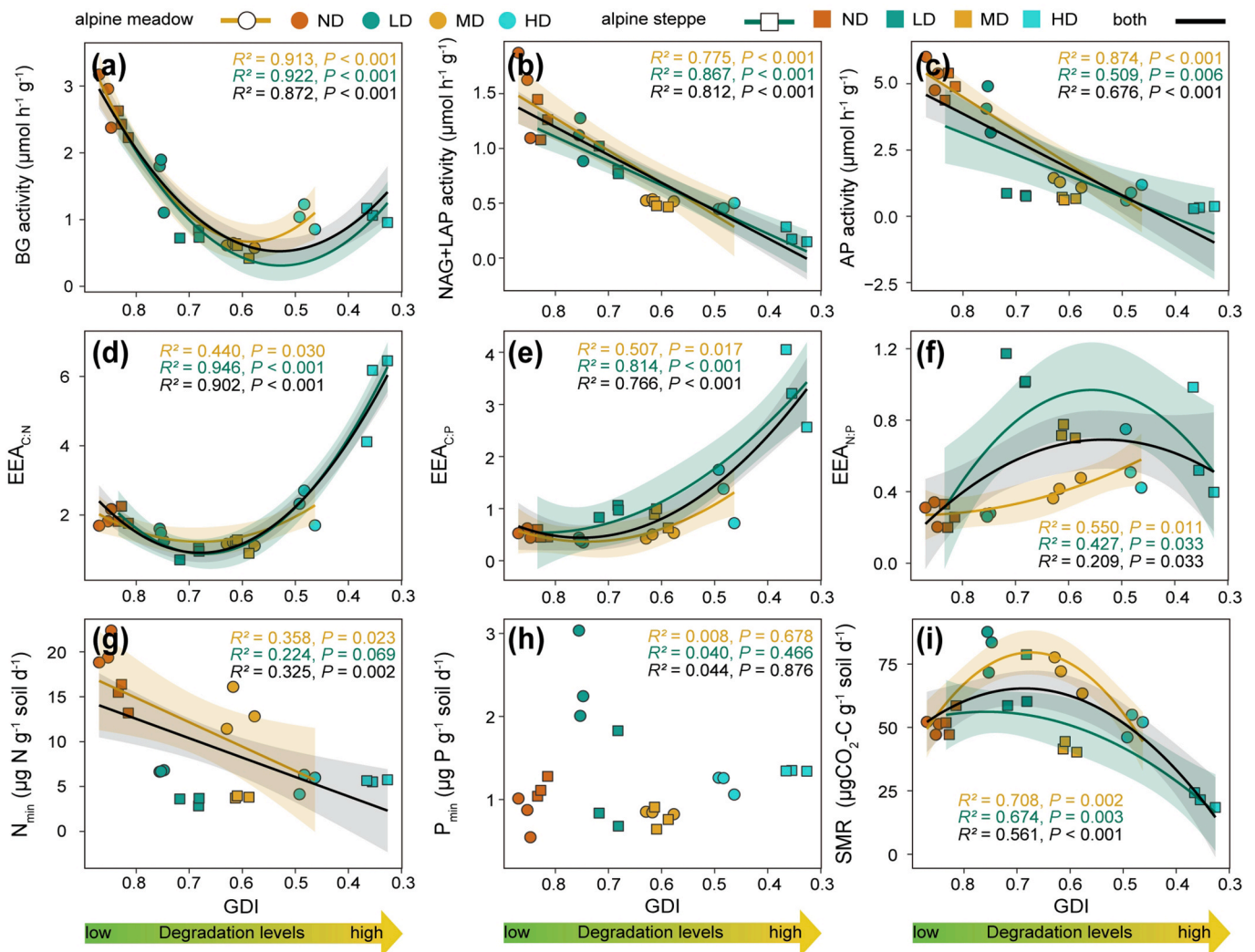


Fig. 6. Effects of grassland degradation on soil extracellular enzyme activity, soil extracellular enzyme stoichiometry, soil net nutrient mineralization, and microbial respiration in alpine meadow and alpine steppe, including (a) BG activity, (b) NAG + LAP activity, (c) AP activity, (d) $EEA_{C:N}$, (e) $EEA_{C:P}$, (f) $EEA_{N:P}$, (g) N_{min} , (h) P_{min} , and (i) SMR. Curves in different colors were fitted using linear or nonlinear regression models, and shaded areas denote the corresponding 95 % confidence intervals. See Fig. 1 for treatment abbreviations. BG, β -glucosidase; NAG, β -1,4-N-acetylglucosaminidase; LAP, leucine aminopeptidase; AP, alkaline phosphatase; $EEA_{C:N}$, ratio of BG to (NAG + LAP); $EEA_{C:P}$, ratio of BG to AP; $EEA_{N:P}$, ratio of (NAG + LAP) to AP; N_{min} , soil net nitrogen mineralization rate; P_{min} , soil net phosphorus mineralization rate; SMR, soil microbial respiration.

available nutrients to evaluate the homeostasis index (H') (Sternier and Elser, 2002). The results showed that the H' values for C:N, C:P, and N:P in the alpine meadow were all greater than the homeostasis threshold of 4 (Persson et al., 2010) (Fig. 4), indicating that despite significant changes in the C:N:P stoichiometry of soil resources induced by grassland degradation, the C:N:P stoichiometry of soil microbes remained invariant. This result supports the perspective that soil microbial stoichiometric homeostasis is common not only in alpine grasslands (Fan et al., 2021) but also across most terrestrial ecosystems (Cleveland and Liptzin, 2007; Griffiths et al., 2012; Tapia-Torres et al., 2015). Microbes can actively regulate the uptake, assimilation, and release of elements through a series of physiological and biochemical processes to maintain the relative constancy of their elemental composition (Frost et al., 2005; Schleuss et al., 2021). However, some studies have shown that soil microbes are not always strictly homeostatic (Xue et al., 2019), and that homeostasis can be either strong or weak, or even non-homeostatic (Persson et al., 2010; Forstner et al., 2019). In this study, unlike the alpine meadow, the alpine steppe exhibited lower H' values for C:N and C:P and higher H' values for N:P (Fig. 4), suggesting that soil microbes in the alpine steppe display weaker homeostasis for C:N and C:P, and thus

greater stoichiometric plasticity. This finding is consistent with previous research demonstrating that C:N homeostasis in the alpine steppe is lower than in the alpine meadow (Fan et al., 2021), likely because differences in hydrothermal conditions and soil resources between meadow and steppe lead to divergent microbial strategies in balancing soil C and N.

4.2. Responses of soil microbial communities to stoichiometric imbalances under grassland degradation

According to resource allocation theory, microbes can enhance extracellular enzyme secretion by targeting scarce elements, which have been identified as one of the crucial strategies employed by microbes to cope with stoichiometric imbalances (Allison and Vitousek, 2005; Moorhead et al., 2016). In this study, $EEA_{C:P}$ and $EEA_{N:P}$ were significantly correlated with $SI_{C:P}$ and $SI_{N:P}$, respectively, in alpine steppe. However, in alpine meadow, $EEA_{C:P}$ was significantly correlated with $SI_{C:P}$, whereas the relationship between $EEA_{N:P}$ and $SI_{N:P}$ was not significant (Figs. 7b, c).

These results suggest that soil microbial extracellular enzyme

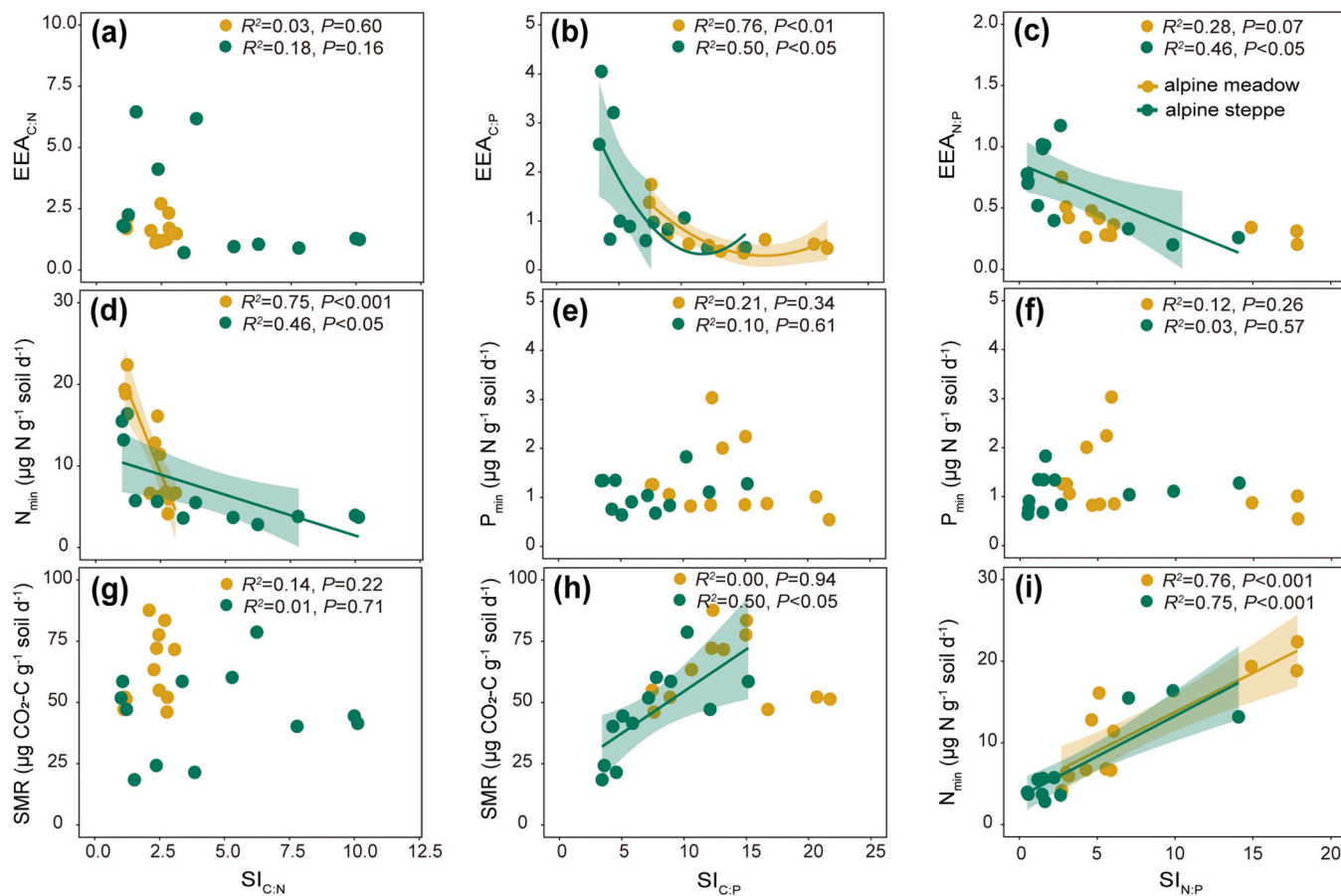


Fig. 7. Relationships of stoichiometric imbalances with extracellular enzyme C:N:P ratios, net nutrient mineralization, and microbial respiration across all of the grassland degradation levels in alpine meadow and alpine steppe. Curves in different colors were fitted using linear or nonlinear regression models, and shaded areas denote the corresponding 95% confidence intervals.

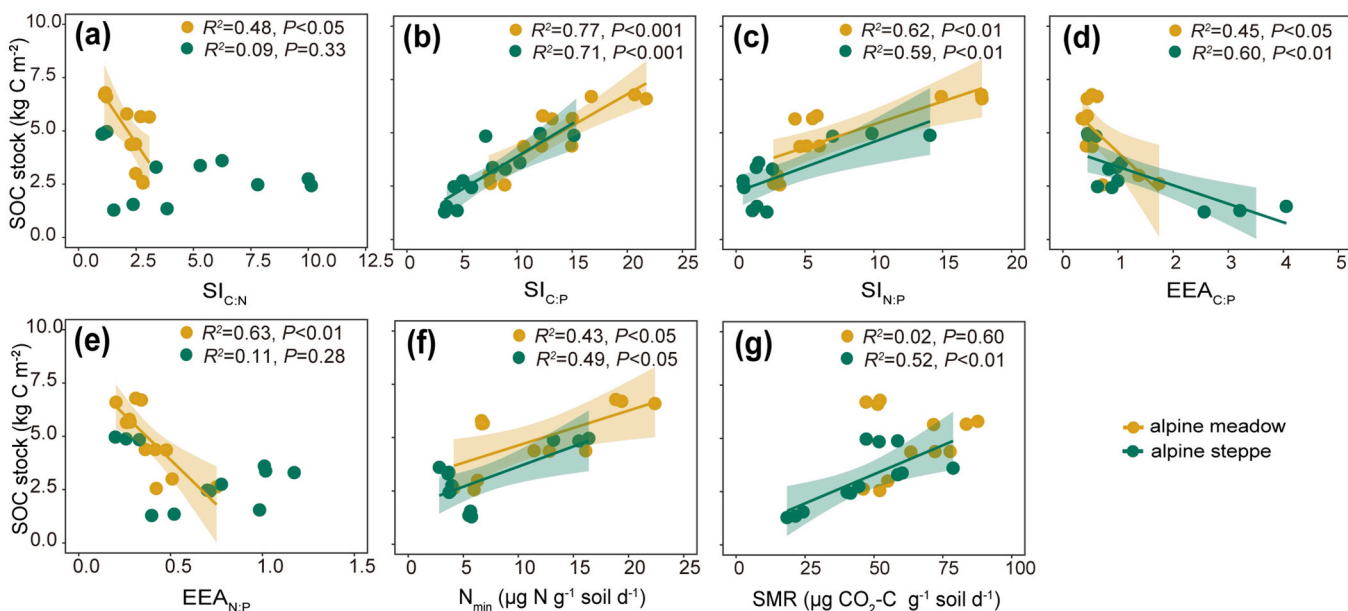


Fig. 8. Relationships of soil organic carbon (SOC) stock with the stoichiometric imbalances, soil extracellular enzyme C:N:P ratios, soil net nitrogen mineralization (N_{min}), and soil microbial respiration (SMR) across all of the grassland degradation levels in alpine meadow and alpine steppe. Curves in different colors were fitted using linear or nonlinear regression models, and shaded areas denote the corresponding 95% confidence intervals.

strategies in alpine meadow and alpine steppe are consistent in response to $SI_{C:P}$, but differ in response to $SI_{N:P}$. As grassland degradation intensifies, the consequent reductions in $SI_{C:P}$ and $SI_{N:P}$ result in a decrease in the P-acquiring extracellular enzyme activity. The substantial decreases in the availability of DOC and DON relative to DOP as a result of grassland degradation could induce soil microbes to decrease the production of P-acquiring extracellular enzymes compared with C- and N-acquiring extracellular enzymes to counterbalance the imbalances between the DOC and DON and DOP (Figs. 6e, f). However, the insignificant negative correlation between $EEA_{C:N}$ and $SI_{C:N}$ suggests that the regulation of the C:N imbalance is not influenced by the relative productions of soil C- and N-acquiring extracellular enzymes in both grassland types (Fig. 7a). This observation is consistent with the results of several previous studies (Forstner et al., 2019; Zhao et al., 2021), indicating that soil microbes regulate extracellular enzyme production based on the return on investment principle. In this principle, soil microbes evaluate whether the energy gained from secreting extracellular enzymes to acquire resources exceeds the energy expended during enzyme production (Schimel and Weintraub, 2003; Mooshammer et al., 2014b). This principle may drive soil microbes to cope with changes in the $SI_{C:N}$ through alternative strategies in addition to extracellular enzymes (Camenzind et al., 2021; Zhao et al., 2021). Therefore, the regulation of soil microbial extracellular enzyme production during the degradation of alpine grasslands is an essential mechanism for coping with $SI_{C:P}$ and $SI_{N:P}$ in alpine grassland ecosystem.

Soil microbes can mitigate stoichiometric imbalances in their resources by releasing excess elements and enhancing their utilization efficiency of the limiting elements, thus maintaining their stoichiometric homeostasis (Mooshammer et al., 2014a; Manzoni et al., 2021). In this study, the soil microbes in the alpine meadow exhibited strong C:N, C:P, and N:P homeostasis, while those in the alpine steppe exhibited weaker C:N and C:P non-homeostasis and strong N:P homeostasis (Fig. 4). Changes in the stoichiometry of soil resources can influence the homeostatic/non-homeostatic characteristics of soil microbes, which are not only linked to shifts in the microbial community composition but are primarily associated with the regulation of element retention and release by microbes (Scott et al., 2012; Fanin et al., 2013; Mooshammer et al., 2014a). Our findings reveal that the relationship between the $SI_{C:N}$ imbalance and SMR was not significant in alpine meadow and alpine steppe (Fig. 7g), suggesting that the soil microbes did not adjust their respiration to deal with the increase in the $SI_{C:N}$. This observation might be due to the fact that the soil microbes were limited by C rather than N in alpine grassland, leading to the absence of C release through respiration. In contrast to the $SI_{C:N}$, the $SI_{C:P}$ in the alpine steppe exhibited a significant positive correlation with the SMR, but this relationship was not significant in the alpine meadow (Fig. 7h). This indicates that adjusting respiration was a strategy by which microbes in the alpine steppe coped with the $SI_{C:P}$, but this was not true in the alpine meadow.

Furthermore, we found that the net N mineralization rates (N_{min}) decreased with increasing $SI_{C:N}$ but increased with increasing $SI_{N:P}$ in alpine meadow and alpine steppe (Figs. 7d, i). These results indicate that as the relative N availability decreased compared with C and P, the soil microbes reduced N_{min} to minimize N loss, and vice versa (Manzoni et al., 2021). In addition, we observed that the soil net P mineralization rate (P_{min}) did not significantly change as $SI_{C:P}$ and $SI_{N:P}$ increased (Figs. 7e, f), indicating that the regulation of P mineralization was not adopted by the soil microbes to address the imbalance between P and C and N. The reason for this may be that despite the increased availability of P relative to C and N due to grassland degradation, indicated by the decreases in DOC:DOP and DON:DOP (Figs. 2k, l), the soil microbial growth was still limited by P. Overall, as the grassland degradation intensified, the soil microbes adjusted N_{min} to cope with the increase in the $SI_{C:N}$ imbalance, regulated SMR in response to the decrease in the $SI_{C:P}$, and adjusted N_{min} to counteract the decrease in the $SI_{N:P}$ in both alpine grassland types.

4.3. Relative contributions of the response mechanisms to SOC stock

Consistent with previous findings that grassland degradation accelerates SOC loss (Abdalla et al., 2018; Guo et al., 2019; Breidenbach et al., 2022), we observed that compared with the undegraded grassland, the SOC stocks of the alpine meadows and alpine steppe decreased by 14.8–59.2 % and 30.0–71.5 % with increasing grassland degradation, respectively (Fig. 2c). The SOC stocks were controlled by the balance between the inputs from the plant-derived organic matter and the anabolic (biomass synthesis and necromass formation) and catabolic (C release as CO_2) processes of soil heterotrophic organisms (Manzoni et al., 2012; Liang et al., 2017; Bai and Cotrufo, 2022). In this study, the positive relationships between the vegetation nutrient storage and the DOC and MBC suggest that as the grassland degradation intensified, the significant reduction of the vegetation nutrient stocks significantly decreased the availability and storage of soil C (Fig. 5). Soil pH is a key determinant of SOC sequestration (Wu et al., 2025). Our results show that degradation in both alpine meadows and alpine steppes increased soil pH (Fig. 2b). This pattern is likely attributable to reduced vegetation cover and biomass during degradation, which diminishes plant-derived acidity (Wu and Tiessen, 2002), together with enhanced evaporative demand and lower vegetation cover that promote the upward movement of bicarbonates/carbonates and consequent soil alkalization (Maestre et al., 2015). We observed opposite trends in the responses of pH and SOC stock to grassland degradation (Figs. 2b, c), consistent with previous findings that higher soil pH generally corresponds to lower SOC (Zhang et al., 2020). Mechanistically, elevated pH can shift microbial communities toward bacteria, increasing the decomposition of labile C and thereby accelerating SOC turnover (Rousk et al., 2010), while also weakening mineral-associated protection of SOC (Kleber et al., 2015). Unlike the soil pH, the trend of soil moisture content was consistent with the SOC stock changes (Fig. 2a), which is supported by the results of several previous studies (Craine and Gelderman, 2011; Kpemoua et al., 2023). This may be related to the increase in the conversion of plant-derived organic matter into SOC under high moisture content conditions.

Apart from the plant-derived C input and soil physicochemical properties, microbial metabolism activity also plays an important role in determining the soil C storage (Lange et al., 2015; Liang et al., 2017; Chen et al., 2018). In this study, we found that there were significant relationships between the SOC stock and C:N:P stoichiometric imbalances (Fig. 8a–c), which may be due to the regulation of microbial anabolism and catabolism by the stoichiometric imbalances. For example, recent studies have shown that stoichiometric imbalances can directly or indirectly affect the SMR through soil extracellular enzymes (Yuan et al., 2019; Guo et al., 2020; Zhong et al., 2020). Our findings confirm that the soil microbes responded to the changes in the C:N:P stoichiometric imbalance by adjusting their production of extracellular enzymes, N mineralization, and SMR (Fig. 7). Notably, these response mechanisms significantly affected the SOC stocks (Fig. 8). Among them, the SOC stocks exhibited negative correlations with the $EEA_{C:P}$ and $EEA_{N:P}$ (Figs. 8d, e), indicating that the relative increases in the C- and N-acquiring enzymes reduced the soil C storage. The reason for this may be that the increases in the C- and N-acquiring enzymes accelerated the SOC decomposition, thereby increasing CO_2 emission (Schimel and Weintraub, 2003; Chen et al., 2018). In addition, producing and secreting these enzymes require microbes to consume C and nutrients, which further promotes SOC decomposition (Schimel and Weintraub, 2003; Manzoni et al., 2012).

In addition to the extracellular enzymatic decomposition pathway, another key pathway for soil microbial-mediated SOC transformation and formation is internal synthesis and decomposition, including the formation of biomass and C and nutrient mineralization (Liang et al., 2017; Bai and Cotrufo, 2022). The results of this study showed that there was a significant positive correlation between the SOC stocks and N_{min} (Fig. 8f), indicating that the decrease in N_{min} caused by the grassland

degradation decreased the soil C stock. Typically, microbial N mineralization and C mineralization are closely related and exhibit coupling (Weintraub and Schimel, 2003). For example, previous studies have shown that an increase in the C mineralization rate can promote the release of bound N, thereby leading to an increase in N_{min} (Tian et al., 2017). However, the relationship between soil C and N mineralization can become decoupled when subjected to external disturbances, manifesting as a negative correlation or lack of correlation (Yuan et al., 2023). In this study, the relationship between the two was decoupled, with an overall decrease in N_{min} and a hump-shaped variation in SMR with increasing grassland degradation (Figs. 6g, i). Therefore, the reduction in soil N turnover during grassland degradation accelerated C decomposition, leading to a decrease in SOC stocks. In general, increased soil C mineralization enhances the release of C in the form of CO_2 , leading to a decrease in C storage (Manzoni et al., 2012; Zechmeister-Boltenstern et al., 2015). In this study, there was a significant positive correlation between the SMR and SOC stock in the alpine

steppe but not in the alpine meadow (Fig. 8g), indicating that the changes in the SMR in the alpine steppe with increasing degradation level affected the soil C stocks. Moreover, given that microbial carbon use efficiency is considered a key physiological parameter regulating soil C storage (Manzoni et al., 2012; Tao et al., 2023), as well as a crucial microbial strategy for coping with stoichiometric imbalances (Mooshammer et al., 2014b; Yuan et al., 2019), future studies should integrate isotope tracing approaches (Schimel et al., 2022) to elucidate how microbes regulate carbon use efficiency to narrow imbalances with soil resources and ultimately mediate soil C stock. Our overall results support the second hypothesis, showing that microbial responses to stoichiometric imbalances regulate SOC stock. These regulatory processes may differ between alpine meadow and alpine steppe, with meadows exhibiting stronger homeostasis and thus more stable SOC mediation under moderate degradation, while steppes show higher plasticity leading to amplified SOC loss in heavy degradation (Fig. 9). This highlights the need for ecosystem-specific management in high-

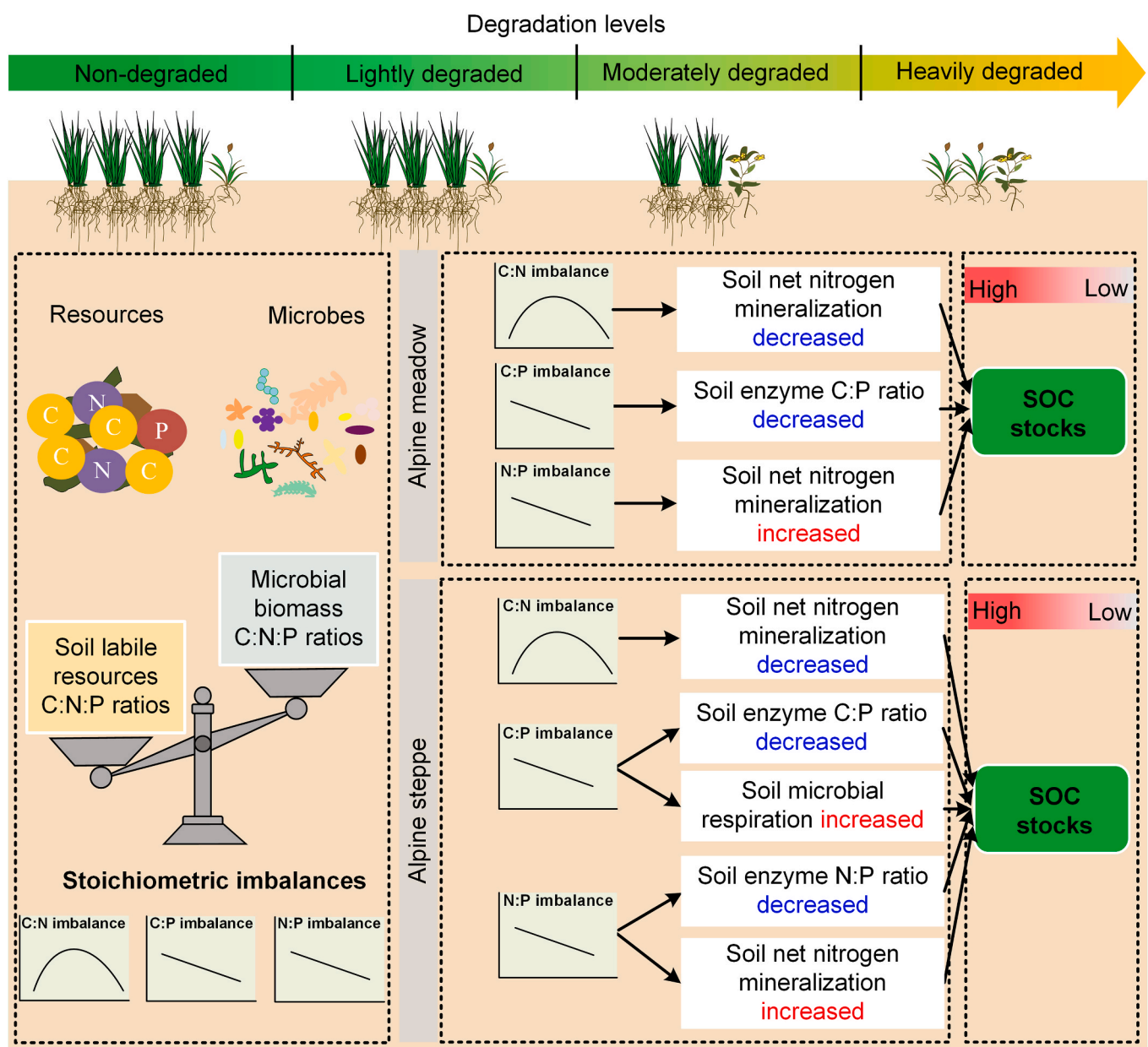


Fig. 9. Conceptual diagram of the response mechanisms of the soil microbial communities to stoichiometric imbalances, and the regulation of surface SOC stock in degraded alpine grasslands.

altitude grasslands.

5. Conclusions

We found that grassland degradation significantly changed the stoichiometric imbalances between soil microbes and their resources in alpine meadow and alpine grassland ecosystems. With increasing grassland degradation, the C:N imbalance presented a hump-shaped response, whereas the C:P and N:P imbalances decreased in both types of grassland. These changes in the stoichiometric imbalances were closely associated with the vegetation nutrient stocks and soil physico-chemical characteristics. Soil microbes in the alpine meadow exhibited C:N, C:P, and N:P homeostasis, while those in the alpine steppe displayed weaker homeostasis for C:N and C:P. Our results suggest that soil microbes coped with the stoichiometric imbalances by regulating the soil extracellular enzyme stoichiometry, net nitrogen mineralization rates, and microbial respiration, ultimately modulating the surface SOC stock. Overall, the results of this study improve our understanding of the connections between plants, soil resources, soil microbial physiology and metabolism, and C stock in alpine ecosystems and provide new insights into microbial regulation of soil C cycling under grassland degradation.

CRedit authorship contribution statement

Xiaobo Yuan: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ying Wang:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Yuan Li:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Dong-Gill Kim:** Writing – review & editing, Formal analysis. **Yunqiao Ma:** Visualization, Methodology, Investigation, Formal analysis. **Ze Ren:** Methodology, Investigation, Formal analysis. **Decao Niu:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Hua Fu:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2025.117560>.

Data availability

Data will be made available on request.

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