

The mitigation of microbial carbon and nitrogen limitations by shrub encroachment: extracellular enzyme stoichiometry of the alpine grassland on the Qinghai-Tibetan Plateau

Ting Zhang

Southwest Minzu University

Yu Tian

Southwest Minzu University

wenming ma (✉ mawmtf@swun.edu.cn)

Southwest Minzu University

Song Bai

Southwest Minzu University

Zuoma Dengzheng

Southwest Minzu University

Dong Zhang

Southwest Minzu University

Xiangli Ma

Southwest Minzu University

Xianrun Mu

Southwest Minzu University

Research Article

Keywords: Extracellular enzyme stoichiometry, Rhizosphere, Microbial nutrient limitation, Shrub encroachment, Qinghai-Tibetan Plateau

Posted Date: March 15th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2604519/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at Biogeochemistry on August 8th, 2023. See the published version at <https://doi.org/10.1007/s10533-023-01075-2>.

Abstract

Shrub encroachment changes the patterns of nutrition allocation in the belowground and aboveground grassland ecosystem. However, influence of shrub encroachment on microbial carbon (C) and nitrogen (N) limitations remains unclear. Using the extracellular enzyme stoichiometry model, microbial nutrition limitations in bulk and rhizosphere soils at various soil layers were investigated at non-shrub alpine grasslands (GL) and shrub-encroached alpine grasslands including *Spiraea alpina* lands (SA), *Caragana microphylla* lands (CM), *Potentilla fruticosa* lands (PF) on the Qinghai-Tibetan Plateau. We determined C-acquisition (β -1,4-glucosidase (BG); β -D-fibrinase (CBH)), N-acquisition (β -1,4-N-acetylglucosaminidase (NAG); leucine aminopeptidase (LAP)) and phosphorus (P)-acquisition (acid phosphatase (AP)) enzyme activities. Soil organic carbon (SOC) content in both topsoil and subsoil significantly increased under shrub encroachment. Interestingly, (LAP+NAG) activities increased in subsoil after shrub encroachment. $E_{C:N}$ in subsoil decreased after shrub encroachment. Microbial C and N limitations were found in both encroached and non-encroached alpine grasslands. The limitations were gradually mitigated following shrub encroachment, reducing the decomposition rate of SOC by microorganisms, indicating that shrub encroachment might potentially contribute to SOC storage. Furthermore, microbial C and N limitations of bulk in topsoil decreased after shrub encroachment. microbial N limitations in subsoil decreased after shrub encroachment. This result indicates that shrub encroachment mitigated microbial C and N limitations. In addition, the structural equation modeling (SEM) shows that the increasing content of SOC and NH_4^+ -N were key factors in the mitigation of microbial C and N limitations after shrub encroachment. This study provides available information on the environmental variables affecting the stoichiometry of extracellular enzymes under shrub encroachment, and the theoretical basis for the study of C and N cycling in alpine grasslands.

Introduction

Many shrub encroachment phenomena have occurred in arid and semi-arid regions due to global climate change (Eldridge et al. 2011). The soil nutrient distribution pattern can be changed after shrub encroachment, however, changing this pattern through various ways, is still being explored. Alpine grassland on the Qinghai-Tibetan Plateau is a significant carbon sink area and plays a crucial role in carbon emissions in China, and even in the world (Yan et al. 2015). shrub encroachment is notably spreading in alpine grasslands on the eastern of the Qinghai-Tibetan Plateau, with a 39% raise in size from 1990 to 2009 (Brandt et al. 2013). The structure of the vegetation community, especially, is changed due to this severe shrub encroachment, affecting the distribution pattern of soil nutrients (Fayiah et al. 2019). The influences of shrub encroachment on SOC storage remains unclear at the ecosystem level, with few studies showing an increase of SOC storage in some ecosystems (Liao et al. 2006b), while others demonstrated a decrease instead (Mureva et al. 2018). Meanwhile, the increase of SOC at deep layers following shrub encroachment was only found in a few investigations. SOC in roots following shrub encroachment is the major nutrient source of C input at deep layers (Rumpel et al., 2011). It means that, the changes in SOC storage at different layers following shrub encroachment have not been measured and evaluated thoroughly, and the study of SOC storage in top- and subsoil is crucial for understanding the SOC dynamics in alpine grasslands.

Soil microorganisms mainly secrete extracellular enzymes to decompose SOC for acquisition C and other nutrients (Soong et al. 2020). Specifically, β -1,4-glucosidase (BG) and β -D-fibrinase (CBH) hydrolyze cellulose and hemicellulose and acquired C sources for microbe (Sinsabaugh & Shah 2012). β -1,4-N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) catalyze chitin, protein, and urea, which are significant indicators of the requirements of microbial N (Jian et al. 2016; Moorhead & Sinsabaugh 2006). Acid phosphatase (AP) hydrolyzes phosphate monomer under acidic conditions, which can reflect the enrichment reaction of microorganisms to phosphorus (P) (Tischer et al. 2015). Soil extracellular enzyme stoichiometry can determine the demand of microorganisms for soil nutrients (Yang et al. 2020), which has increasingly become a research highlight in the past ten years (Zhang et al. 2019). A global meta-analysis suggests that the stoichiometry ratio of soil C-, N-, and P-acquisition enzymes is 1:1:1 at the global average level (Sinsabaugh et al. 2009). However, due to the great variety of soil extracellular enzyme stoichiometry among different ecosystems, the ratio was not followed in all regions (Sinsabaugh & Shah 2012). In addition, Moorhead et al. (2016) employed the ratio of the soil C- and N-acquisition enzyme, and of the soil C and P- acquisition enzyme to calculate vector length (relative to C limitations) and angle (relative to P vs N limitations) to characterize microbial nutrient limitations (Moorhead et al., 2016). In contrast, a recent study pointed out that when cellulose is the predominant carbon source for soils rather than chitin, peptidoglycan, and protein, BG:NAG (or BG:(NAG + LAP)) can represent microbial C limitations vs N limitations (Mori 2020). On the shrub-encroached alpine grasslands at the eastern of the Qinghai-Tibetan Plateau, soil litter enriches cellulose, which is a primary C source for microbial growth (He et al. 2015). Therefore, the application of soil extracellular enzyme stoichiometry is critical to reveal the characteristics of microbial metabolism in alpine grasslands.

Rhizosphere is a hotspot in the researches of the interactions between soil extracellular enzymes and microorganisms (Zheng et al. 2019). Rhizosphere soils are usually relatively nutrient-rich and have a higher microbial metabolic rate than bulk soils (Ai et al. 2012). Root exudates and rhizosphere metabolism change the composition of soil microbial communities (Bi et al. 2021). Therefore, the variations in microbial community structure are very likely to change the metabolic process and further result in the variations in the amount of microbial acquisition

nutrients (Paterson 2003). Consequently, determining metabolic characteristics of microbial communities in rhizosphere environments is of vital importance for understanding the function of root systems in C cycling with the development of shrub encroachment in alpine grassland ecosystems.

Shrub encroachment is the primary factor affecting the carbon pool balance in alpine grasslands (Chen et al. 2022). The changes in soil extracellular enzyme stoichiometry following shrub encroachment are not clear. It is necessary to further study soil extracellular enzyme stoichiometry at bulk and rhizosphere in shrub-encroached and non-encroached alpine grasslands. By studying the characteristics of extracellular enzyme stoichiometry in bulk and rhizosphere soils, microbial nutrient limitations can be determined, which will greatly promote the understanding of the nutrient conversion process following shrub encroachment. Therefore, we proposed the following hypotheses: (1) The contents of SOC massively increase following shrub encroachment; (2) Microbial C and N limitations are found in shrub-encroached and non-shrub grasslands. (3) Microbial C and N limitations are mitigated following shrub encroachment. (4) Microbial C and N limitations are notably higher in rhizosphere soils than that in bulk soils following shrub encroachment, which is probably due to C and N competition between plants and microorganisms in rhizosphere environments. Thus, in this study, soil extracellular enzyme stoichiometry is used to study the characteristics of microbial nutrient limitations at bulk and rhizosphere in different layers in shrub-encroached and non-shrub alpine grasslands on the Qinghai-Tibetan Plateau. Understanding the characteristics of soil extracellular enzyme stoichiometry following shrub encroachment is beneficial to further determining nutrient cycling (eg. C, N, P) on the Qinghai-Tibetan Plateau.

Materials And Methods

Study sites

This study is conducted in the Qinghai-Tibetan Plateau Research Base of Southwest Minzu University in western Sichuan Province, China (32°49'38"N, 102°34'21"E, 3485 m elevation), belonging to the cold temperate monsoon climate of the continental plateau. The temperature reaches its peak in July, with 25.6°C as the highest record and 10.9°C as the average. The annual relative humidity is 60-70%, and the moisture coefficient is 1.26. The annual average rainfall is 650-800 mm, which is mainly concentrated from May to August every year, during when the average rainfall is about 80% of the annual total. Alpine grassland soil is the main soil type, and the soil parent material is mostly residual slope deposits and slope deposits. Its vegetation type belongs to alpine grassland, mainly consisting of herbaceous and shrubs. In the study area, shrub encroachment can be deemed serious. The encroached grasslands are mainly composed of *Spiraea alpina*, *Potentilla fruticosa*, and *Caragana microphylla*. The herbaceous plants are mainly composed of *Kobresia filifolia*, and *Elymus nutans* is the most common one.

Sampling design

Field sampling for this experiment was carried out in July 2021. A 10 m×10m plot of shrub-encroached alpine grassland including *Spiraea alpina* lands (SA), *Caragana microphylla* lands (CM), *Potentilla fruticosa* lands (PF) was randomly selected from the sampling area, and three 1 m×1 m small plots heavily encroached by more than 75% were set in the large plot. All aboveground biomass in the plot was collected with scissors. The root depth of the grassland is about 0-40 cm. The average root depth of *Spiraea alpina*, *Caragana microphylla* and *Caragana microphylla* is 0-80 cm. Considering the root depth of grassland and three shrubs, bulk and rhizosphere soils in topsoil (0-10 cm) and subsoil (30-40cm) were collected respectively. Rhizosphere soils were sampled using the shaking-off method (Fujii et al. 2005). Bulk soils were sampled at least 5mm away from roots. Soil samples (physicochemical properties and enzyme samples) were put into zip-lock bags. Soil enzyme samples were kept in zip-lock bags, and then immediately transferred into the ice box with ice packs. A 10 m×10 m plot in non-shrub grassland (GL) was selected near where shrubs grew. Soil samples in non-encroached grassland plots were sampled in the same way as those from encroached grassland plots. Aboveground biomass and soil samples from each plot were brought back to the laboratory. Aboveground biomass was classified and measured. Soil physicochemical properties samples were weighed and baked at 105°C for 24 hours. Natural drying treatment is carried out after the calculation of soil water content. Soil enzyme samples were stored at -4°C to determine the extracellular enzyme activities.

Soil properties measurements

Soil water contents (SWC) are determined by the natural drying method. A pH meter was used to measure soil pH, and the ratio of the soil and water was 2.5:1.0. The contents of SOC, total nitrogen (TN) and dissolved organic C (DOC) were determined by the Elementar Variomax CNS analyzer (Germany) (Jones & Willett 2006). The TP and available P (Olsen-P) contents were determined by NaOH alkali fusion-Molybdenum-antimony resistance colorimetry. The NO₃⁻-N and NH₄⁺-N contents were determined by a Flow Analyzer.

Assays of soil extracellular enzyme activity

Using the Saiya-cork fluorimetric test method, C-acquisition enzymes (BG and CBH), N-acquisition enzymes (NAG and LAP), and P-acquisition enzymes (AP) were identified (Saiya-Cork et al., 2002). To measure the enzyme activity, the 125 ml of deionized water and 1 g of fresh soil were

homogenized for 2 hours at a speed of 180 rotations per minute on a rotary shaker. Then, 200 μL of each sample was put into a 96-well microplate. We added 50 μL of the standard substrate (40 $\mu\text{mol L}^{-1}$ 4-MUB and 10 $\mu\text{mol L}^{-1}$ 7-amino-4-methylcoumarin) and 200 μL of test suspension to the extinguished standard microplates. We added 50 μL standard substrate and 200 μL deionized water to the negative control microplates. The 96-well microplates were brooded in obscurity at 25 °C for 4 hours (German et al. 2011). Soil extracellular enzyme stoichiometry was computed using formulas on $\ln(\text{BG} + \text{CBH})$: $\ln(\text{NAG} + \text{LAP})$, $\ln(\text{BG} + \text{CBH})$: $\ln(\text{AP})$ and $\ln(\text{NAG} + \text{LAP})$: $\ln(\text{AP})$, respectively.

Quantification of soil microbial metabolic limitation

Based on the relative proportion of untransformed soil extracellular enzyme activities, such as $(\text{BG} + \text{CBH})$: $(\text{BG} + \text{CBH} + \text{AP})$, vector lengths and angles were used to quantify microbial nutrition limitations (Moorhead et al. 2013; Moorhead et al. 2016).

Equations 1 and 2 were used to compute vector lengths and angles, with x and y representing for, respectively, $(\text{BG} + \text{CBH})$: $(\text{BG} + \text{CBH} + \text{AP})$ and $(\text{BG} + \text{CBH})$: $(\text{BG} + \text{CBH} + \text{NAG} + \text{LAP})$. The microbial C limitation is represented by vector lengths. Microbial C limitations would be enhanced with vector lengths. The microbial N or P limitation is represented by vector angles. Vector angles lower than 45° indicate microbial N limitations. Microbial N limitations increased with the decrease of vector angles. Vector angles greater than 45° imply microbial P limitations. Microbial P limitations would be enhanced with vector angles.

$$\text{Length} = \sqrt{x^2 + y^2} \quad (1)$$

$$\text{Angle}^\circ = \text{DEGREES}(\text{ATAN2}((x, y))) \quad (2)$$

Statistical analysis

The experimental data were collected by Microsoft Excel 2019 and analyzed by IBM SPSS Statistics 26.0. Data conforming to normal distribution were analyzed by one-way variance analysis and an independent-sample t-test. The effects of different vegetation types on soil physicochemical properties, extracellular enzyme activities, extracellular enzyme stoichiometry, and microbial nutrient limitations were examined using one-way variance analysis (Tukey's multiple comparison test) ($p < 0.05$). The effects of soil depths and locations (bulk and rhizosphere) on soil physicochemical properties, extracellular enzyme activity, extracellular enzyme stoichiometry, and microbial metabolic limitations were examined using an independent-sample t-test ($p < 0.05$). Canoco 5.0 was used to conduct the Redundancy analysis (RDA) to evaluate the relationship between soil physicochemical properties vs extracellular enzyme activity and soil physicochemical properties vs extracellular enzyme stoichiometry, respectively. A generalized linear regression analysis was used to determine the relationship between environmental factors and microbial nutrient limitations. Origin 2021 was used to conduct a generalized linear regression analysis. non-encroached grasslands, *Spiraea alpina* lands, *Caragana microphylla* lands (CM) and *Potentilla fruticosa* lands were ranked by continuous variable (1, 2, 3 and 4), respectively. Structural equation modeling (SEM) is used to identify the potential trends of shrub encroachment's effects on microbial nutrient limitations. Amos v.23 was used to conduct the model.

Results

Soil physical property and available nutrients

Vegetation types affect physical property and available nutrients. Generally, in topsoil, the bulk and rhizosphere SWC, NH_4^+ -N, NO_3^- -N and DOC in CM were significantly higher than that in GL (Table 1). The bulk pH in SA, CM and PF was significantly higher than that in GL. The bulk Olsen-P in SA, CM and PF was significantly lower than in GL ($p < 0.05$). The bulk and rhizosphere SWC, NH_4^+ -N, NO_3^- -N and DOC in CM were significantly higher than in GL. In subsoil, the bulk pH in CM was significantly higher than that in GL ($P < 0.05$). The rhizosphere pH was significantly higher in PF than in GL. The rhizosphere Olsen-P in SA was significantly higher than that in GL, CM and PF ($P < 0.05$). Therefore, in top- and subsoil, *Caragana microphylla* has a significant effect on the contents of SWC, NH_4^+ -N, NO_3^- -N and DOC in non-shrub grassland.

Soil depths affected physical property and available nutrients. In bulk and rhizosphere soil, NO_3^- -N and DOC in topsoil were significantly higher than that in subsoil in GL (Table 1, $P < 0.05$). The contents of pH, NH_4^+ -N, NO_3^- -N and DOC in topsoil were significantly higher than that in subsoil in SA and CM ($P < 0.05$). SWC content in topsoil was significantly higher than that in subsoil in PF. The content of DOC in topsoil was significantly lower than that in subsoil in PF ($P < 0.05$).

Rhizosphere affect physical property and available nutrients. In top- and subsoil, pH in rhizosphere was significantly higher than that in bulk in GL and CM ($P < 0.05$). NH_4^+ -N in rhizosphere was significantly higher than that in bulk in CM ($P < 0.05$). NO_3^- -N in rhizosphere was significantly higher than in bulk of GL and SA ($P < 0.05$). DOC in rhizosphere was significantly higher than that in bulk in GL and CM. Olsen-P in rhizosphere was significantly higher than in bulk in GL, SA, CM and PF.

Soil total nutrients and nutrient ratios

Vegetation types affect soil total nutrients and nutrient ratios. In top- and subsoil, the bulk and rhizosphere SOC, C:P and N:P in CM were significantly higher than that in GL ($p < 0.05$, Table 2). In topsoil, bulk TN in CM was significantly higher than in GL ($p < 0.05$). In topsoil, the TP of bulk and rhizosphere in SA, CM and PF was significantly lower than that in GL ($p < 0.05$). In subsoil, rhizosphere TP in SA and CM was significantly lower than in GL ($p < 0.05$). In deep soil, the bulk and rhizosphere SOC:TN in PF was significantly higher than that in GL ($p < 0.05$).

Soil depths affected soil total nutrients and nutrient ratios. In bulk and rhizosphere soil, the contents of SOC, TP, SOC:TN and SOC:TP in topsoil were significantly higher than that in subsoil in GL ($p < 0.05$). In SA, the contents of SOC, TN and TN:TP in topsoil were significantly higher than that in subsoil in SA ($P < 0.05$); SOC:TN content in topsoil was significantly lower than that in subsoil ($p < 0.05$). SOC, TN, TP and TN:TP contents in topsoil were significantly higher than that in subsoil in CM ($p < 0.05$). In PF, the SOC and SOC:TP contents in topsoil were significantly higher than that in subsoil ($p < 0.05$); The contents of TN and TN:TP in topsoil were significantly lower than those in subsoil ($p < 0.05$).

Rhizosphere affected soil total nutrients and nutrient ratios. In top- and subsoil, pH in rhizosphere was significantly higher than that in bulk in GL and CM. $\text{NH}_4^+\text{-N}$ in rhizosphere was significantly higher than that in bulk in CM. $\text{NO}_3^-\text{-N}$ in rhizosphere was significantly higher in bulk in GL. DOC in rhizosphere was significantly higher than that in bulk in GL and CM ($p < 0.05$). The rhizosphere Olsen-P was significantly higher than that of the bulk in GL, SA, CM and GL.

Soil extracellular enzyme activities (EEAs)

Vegetation types affect EEAs (Table 3). In top- and subsoil, rhizosphere (BG+CBH) activities in CM were significantly lower than that in GL ($p < 0.05$). In top- and subsoil, bulk (LAP+NAG) activities in CM were significantly higher than that in GL ($p < 0.05$). In topsoil, rhizosphere (LAP+NAG) activities in PF were significantly lower than that in GL ($p < 0.05$). In subsoil, rhizosphere (LAP+NAG) activities in CM were significantly higher than that in GL ($p < 0.05$). In topsoil, bulk AP activities in CM were significantly higher than that in GL ($p < 0.05$). In subsoil, rhizosphere AP activities in SA, CM and PF were significantly higher than that in GL ($p < 0.05$).

Soil depths affected EEAs (Table 3). In SA, rhizosphere (BG+CBH) activities in topsoil were significantly higher than that in subsoil ($p < 0.05$). In GL, rhizosphere (LAP+NAG) activities in topsoil were significantly higher than that in subsoil ($p < 0.05$). In SA, rhizosphere (LAP+NAG) and AP activities in topsoil were significantly lower than that in subsoil ($p < 0.05$). In GL and PF, bulk AP activities in topsoil were significantly lower than that in subsoil ($p < 0.05$). In GL, rhizosphere AP activities in topsoil were significantly higher than that in subsoil ($p < 0.05$).

The rhizosphere affected EEAs (Table 3). In top- and subsoil, (BG+CBH) activities in rhizosphere were significantly higher than that in bulk in GL and SA ($p < 0.05$). In topsoil, (LAP+NAG) activities in rhizosphere were significantly higher than that in bulk in GL ($p < 0.05$). In subsoil, (LAP+NAG) activities in rhizosphere were significantly higher than that in bulk in SA ($p < 0.05$). In topsoil, AP activities in rhizosphere were significantly higher than that in bulk in GL and PF; AP activities in rhizosphere were significantly lower than that in bulk in SA ($p < 0.05$). In subsoil, AP activities in rhizosphere were significantly lower than that in bulk in GL ($p < 0.05$).

In topsoil, in the redundancy analysis of soil physicochemical properties and extracellular enzyme activity, the interpretation rates of RDA1 and 2 axes were 68.06% and 22.02%, respectively, which amounted to 90.08% (Fig. 1). TP, DOC and $\text{NH}_4^+\text{-N}$ were the three factors with the highest explanatory degree, explaining 27.7%, 25.6% and 13.9% of the variables respectively. (BG+CBH) activities were correlated with TP positively, and with SWC, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, DOC and C:P negatively ($p < 0.05$, Fig. 1). (LAP+NAG) activities were correlated with $\text{NH}_4^+\text{-N}$, DOC and TP, positively, and with SWC, $\text{NO}_3^-\text{-N}$ and C:P negatively ($p < 0.05$, Fig. 1). AP activities were correlated with DOC and TP, positively, and with SWC, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and C:P negatively ($p < 0.05$, Fig. 1). In subsoil, in the redundancy analysis of soil physicochemical properties and extracellular enzyme activities, the interpretation rates of RDA1 and 2 axes were 49.39% and 36.68% respectively, totaling 86.07% (Fig. 2). $\text{NH}_4^+\text{-N}$, Olsen-P and SOC are the three factors with the highest explanatory power, accounting for 38.6%, 23.6% and 8.9% of the variables respectively. (BG+CBH) activities were correlated with Olsen-P contents positively, and with $\text{NH}_4^+\text{-N}$ and SOC negatively ($p < 0.05$, Fig. 2). (LAP+NAG) activities were correlated with SOC and $\text{NH}_4^+\text{-N}$ positively, and with Olsen-P negatively ($p < 0.05$, Fig. 2). AP activities were correlated with SOC and $\text{NH}_4^+\text{-N}$ positively, and with Olsen-P negatively ($p < 0.05$, Fig. 2).

Soil extracellular enzymatic stoichiometry (EES)

Vegetation types had effects on EES (Table 4). In topsoil, bulk $E_{C:N}$ in SA were significantly lower than that in GL ($p < 0.05$) in subsoil, rhizosphere $E_{C:N}$ in SA and PF were significantly lower than that in GL ($p < 0.05$). In topsoil, bulk $E_{C:P}$ in SA were significantly lower than that in GL ($p < 0.05$) in subsoil, rhizosphere $E_{C:P}$ in PF were significantly lower than that in GL ($p < 0.05$). In topsoil, rhizosphere $E_{N:P}$ in PF were significantly lower than that in GL ($p < 0.05$).

Soil depths affected EES (Table 3). In GL, bulk $E_{C:P}$ and $E_{N:P}$ in topsoil were significantly higher than that in subsoil; rhizosphere $E_{C:N}$ and $E_{C:P}$ in topsoil were significantly lower than that in subsoil ($p < 0.05$). In SA, bulk $E_{C:N}$ in topsoil were significantly lower than that in subsoil; bulk $E_{N:P}$ in topsoil were significantly higher than that in subsoil ($p < 0.05$). In CM, rhizosphere $E_{C:N}$ and $E_{C:P}$ in topsoil were significantly higher than that in subsoil ($p < 0.05$). In PF, bulk $E_{C:P}$ in topsoil were significantly higher than that in subsoil ($p < 0.05$). Moreover, most soil $E_{C:N}$ ratios were less than 1, $E_{C:P}$ and $E_{N:P}$ were greater than 1 in top- and subsoil of shrub-encroached and non-shrub grasslands. The stoichiometric ratios of C-, N- and P-acquisition enzymes in top- and subsoil were 1.28:1.45:1-1.44:1.36:1 and 1.27:1.48:1-1.54:1.44:1, respectively, and the mean values of top- and subsoil were 1.37:1.45:1 and 1.34:1.43:1, respectively. It deviated from the global average level of 1:1:1, indicating the existence of microbiological C and N limitation, and the microbiological limitation on N was strong, and the microbiological C limitation in topsoil was higher than that in subsoil.

The rhizosphere affected EEAs (Table 4). In subsoil, $E_{C:N}$ in rhizosphere were significantly higher than that in bulk in GL; $E_{C:N}$ in rhizosphere were significantly lower than that in bulk in CM ($p < 0.05$). In topsoil $E_{C:P}$ in rhizosphere were significantly higher than that in bulk in SA and CM ($p < 0.05$). In subsoil $E_{C:P}$ and $E_{N:P}$ in rhizosphere were significantly higher than that in bulk in GL and SA ($p < 0.05$). In topsoil, $E_{N:P}$ in rhizosphere were significantly lower than that in bulk in PF ($p < 0.05$).

In topsoil, in the redundancy analysis of soil extracellular enzyme stoichiometry and environmental factors, RDA1 and 2 axes were 55.82% and 30.52% respectively, the explanation of a total of 86.34% (Fig. 3). TP, SWC and NH_4^+-N are to interpret the highest degree of the three factors, explains the variables were 36.2%, 10.2% and 8.0% respectively. soil $E_{C:N}$ and $E_{C:P}$ were correlated with TP and C:N positively, and with the contents of SWC, NH_4^+-N and SOC negatively ($p < 0.05$, Fig. 3). Soil $E_{N:P}$ were correlated with TP positively, and with SWC, NH_4^+-N , SOC, and C:P negatively ($p < 0.05$, Fig. 3). In subsoil, in the redundancy analysis of soil extracellular enzyme stoichiometry and environmental factors, the interpretation rates of RDA1 and 2 axes were 65.45% and 18.94%, respectively, which amounted to 84.39% (Fig. 4). Among them, C:N and N:P are the two factors with the highest explanatory degree, explaining 26.0%, 20.1% and 11.8% of the variables respectively. Soil $E_{C:N}$ and $E_{C:P}$ were correlated with C:N and N:P negatively ($p < 0.05$, Fig. 4). Soil $E_{N:P}$ were correlated with C:N and N:P positively ($p < 0.05$, Fig. 4).

The characteristics of microbial C and N limitations

There were microbial C and N limitations in both shrub-encroached and non-shrub grasslands, as shown by the fact that all data points fell below the 1:1 line ($p < 0.01$; Fig. 5 a and b). Vegetation types affected microbial C and N limitations ($p < 0.05$; Fig. 6 and 7). In topsoil, bulk vector length in CM were significantly lower than that in GL; bulk vector angle in SA and CM were significantly lower than that in GL. In subsoil, rhizosphere vector length in CM and PF were significantly lower than that in GL; bulk and rhizosphere vector angle in CM and PF were significantly lower than that in GL. Therefore, shrub encroachment mitigated microbial C and N limitations.

Soil depths affected microbial C and N limitations. In GL, bulk vector length in topsoil were significantly higher than that in subsoil; rhizosphere vector length and vector angle in topsoil were significantly lower than that in subsoil ($p < 0.05$). In SA, bulk vector angle in topsoil were significantly lower than that in subsoil ($p < 0.05$). In CM, rhizosphere vector length and vector angle in topsoil were significantly higher than that in subsoil ($p < 0.05$). In PF, rhizosphere vector length in topsoil were significantly higher than that in subsoil ($p < 0.05$).

Rhizosphere affected microbial C and N limitations. In top- and subsoil, vector length in rhizosphere were significantly higher than that in bulk of SA ($p < 0.05$). In topsoil, vector length in rhizosphere were significantly higher than that in bulk of CM; vector angle in rhizosphere were significantly lower than that in bulk of GL; vector angle in rhizosphere were significantly higher than that in bulk of PF ($p < 0.05$). In subsoil, vector length in rhizosphere were significantly higher than that in bulk of GL; vector angle in rhizosphere were significantly lower than that in bulk of CM ($p < 0.05$).

In addition, in topsoil and subsoil, there was a greatly positive correlation between microbial C and N limitations. ($p < 0.001$; Fig. 5). By linear regression analysis, in topsoil, microbial C limitations were correlated with SWC, pH, NH_4^+-N , NO_3^-N , DOC, SOC, TN, C:P and N:P negatively, and with TP and Olsen-P positively ($p < 0.05$; Fig. 8). In subsoil, microbial C limitations were significantly correlated with the contents of pH, NH_4^+-N , NO_3^-N , DOC and SOC, C:N and C:P negatively, and with TP and Olsen-P positively ($p < 0.05$; Fig. 9). So, the effects of physicochemical properties on microbial C limitations were higher in topsoil than that in subsoil. Meanwhile, linear regression analysis further showed that microbial N limitations were highly correlated with pH, DOC and TN, and N:P negatively, and with TP and Olsen-P in topsoil positively ($p < 0.05$; Fig. 10). In subsoil, microbial N limitations were significantly correlated with SWC, pH, NH_4^+-N , NO_3^-N , DOC, SOC, TN, C:N, C:P and N:P negatively, and with TP and Olsen-P positively ($p < 0.05$; Fig. 11). Therefore, the effects of physicochemical properties on microbial N limitations were higher in subsoil than that in topsoil.

The SEM was conducted to analyze the relationships between microbial C and N limitations and physiochemical properties, and nutrient stoichiometry following shrub encroachment, in topsoil and in subsoil, respectively (Fig. 12). In topsoil, SWC and DOC had positive and

negative correlations with Olsen-P respectively; SWC and C:N had positive and negative correlations with $\text{NH}_4^+\text{-N}$ respectively; Olsen-P and $\text{NH}_4^+\text{-N}$ had positive and negative impacts on microbial N limitations respectively (Fig. 12a). In addition, SWC contents had negative correlations with C:N; SWC had positive correlations with SOC; C:N and SOC had positive and negative impacts on microbial C limitations (Fig. 12b). In subsoil, SWC had positive correlations with $\text{NH}_4^+\text{-N}$; pH had positive correlations with Olsen-P; Olsen-P and $\text{NH}_4^+\text{-N}$ had positive and negative impacts on microbial N limitations, respectively (Fig. 12c). Additionally, the pH had positive correlations with C:N and SOC; SOC had positive and negative impacts on microbial C limitations (Fig. 12d). $\text{NH}_4^+\text{-N}$ and SOC had strong effect on microbial N and C limitations in topsoil and subsoil following shrub encroachment, respectively.

Discussion

The effect of shrub encroachment on soil nutrition

Our study showed that shrub encroachment greatly affected physicochemical properties. In bulk physicochemical properties, except DOC and Olsen-P, TN, TP and C:N, other physicochemical properties in topsoil were significantly higher in the shrub-encroached than in the non-shrub grassland. These results indicate that shrub encroachment has a great influence on physicochemical properties of bulk in topsoil. This may be because grass roots are generally distributed in the topsoil (Mordelet et al. 1997), causing the nutrient content of non-shrub grassland in topsoil was higher than that of shrub-encroached grassland. In terms of rhizosphere physicochemical properties, only $\text{NO}_3^-\text{-N}$, SOC and C:P in topsoil were significantly higher in shrub-encroached grassland than in non-shrub grassland ($p < 0.05$). SWC, $\text{NH}_4^+\text{-N}$, DOC, SOC and C:P in subsoil were significantly higher in shrub-encroached grassland than in non-shrub grassland ($p < 0.05$). These results indicate that shrub encroachment has great influence on physicochemical of rhizosphere in subsoil. This may be because the root system of the shrub is deep and the microorganisms in the rhizosphere are more active, which is easy to push nutrients from shallow layer to deeper soil and maintain nutrient metabolism in deep roots (Caldwell et al. 1996). We found that in the subsoil, the pH, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ of the bulk and rhizosphere soil in shrub-encroached grassland were significantly higher than in non-shrub grassland. The pH increase after shrub encroachment may be due to the fixed root system of shrubland, after rainfall, little leaching loss occurs in shrubland, which weakens the nitrification effect (Pellegrini et al. 2021). In alkaline environments, plant residues can reduce soil pH through nitrification (Binkley & Richter 1987). However, shrub encroachment weakens nitrification and does not reduce soil pH, could increase the pH of grassland. Our results showed that the rhizosphere soil DOC content of *Caragana microphylla* was the highest compared with that of non-shrub grassland. The loss of DOC depends on the concentration of dissolved organic carbon in soil pore water and hydrological conditions. Under low moisture conditions, DOC concentrations may decrease (Dieleman et al. 2016). However, our study showed that soil moisture in *Caragana microphylla* was generally higher than that in non-shrub grassland (Table 1). Therefore, high soil moisture conditions may lead to an increase in DOC concentration. At the same time, we found that soil SOC content increased significantly after shrub encroachment, which supports our first hypothesis. This is consistent with the conclusions by Gómez-Rey et al. and Liao et al., that shrub encroachment led to the increase of soil C storage (Gómez-Rey et al. 2013; Liao et al. 2006c). The reason may be that shrub litter is not easy to be decomposed by soil microorganisms, leading to the accumulation of complex organic matter on the alpine grassland eroded by shrubs (Liao et al. 2006a). In addition, the root growth of perennial shrubs often needs to consume more carbon to resist long-term environmental stress, so there will be more root sediments and nutrient enrichment on the shrub alpine grassland (Bardgett et al. 2014). Moreover, TN content in *Caragana microphylla* was significantly higher than that in non-shrub grassland. This is probably because mycorrhizal plants' ability to fix nitrogen increased the accumulation of soil N (Cao et al. 2011). In addition, our study found that the content of TP in shrub-encroached and non-shrub grassland was much lower than that of SOC and TN, and was in a state of soil P limitation.

The effect of shrub encroachment on soil extracellular enzymes

Our results showed that bulk (BG+CBH) activities increased after shrub encroachment, while rhizosphere bulk (BG+CBH) activities decreased. This may be due to the fact that our sampling time was August. In fact, there was no litter mass loss in the deep roots of the shrub between 6 and 12 months., and the (BG+CBH) catalyzed the decomposition rate of soluble organic compounds and sugars decreased, and its activity decreased compared to previous months. This is consistent with previous findings by Hewins et al. (Hewins et al. 2017). (LAP+NAG) activities in topsoil showed an increasing trend after shrub encroachment. This is consistent with the results of Akinyemi et al. (Akinyemi et al. 2020). The formation of "fertile islands" under the shrub canopy contributed to the increase in organic matter, which may be responsible for the increased activity of (LAP+NAG) (Akinyemi et al. 2020). These "fertile islands" in shrub patches may be associated with higher quality litter because shrubs usually have relatively high leaf area and they provide more decomposable and nitrogen-rich litter, which makes shrub patches likely to contain more substrates that stimulate (LAP+NAG) activities (Sardans et al. 2017). At the same time, our study also found that the rhizosphere (LAP+NAG) activities of *Caragana microphylla* shrub was significantly higher than that of non-shrub grassland. This may be due to the existence of nitrogen-fixation related mycorrhiza in shrub, and the mycorrhizal nodules of mycorrhizal fungi accelerate the secretion of (LAP+NAG) enzyme, thus increasing the activity of (LAP+NAG) around the shrub roots (Wallenstein et al. 2010). Our study also showed that the AP activities of *Caragana microphylla* in subsoil was significantly higher than that in non-shrub grassland. This may be due to the fact that the

deep roots of shrubs may be colonized by more arbuscular mycorrhizal fungi than the roots of gramineous plants (Yu-Ying & Wei 2004). Arbuscular mycorrhizal mycelium increases soil volume, from which plants can obtain phosphorus and produce AP in the rhizosphere (Javot et al. 2007). RDA analysis showed that there was a significant positive correlation between the activity of AP and TP content in topsoil. This is consistent with the results of Jing et al., in an ecosystem with limited phosphorus, phosphatase activity may increase as demand of soil microorganisms phosphorus (Spohn & Kuzyakov 2013). In addition, RDA analysis found that (LAP+NAG) activities in top- and subsoil were significantly positively correlated with $\text{NH}_4^+\text{-N}$. This may be because plants allocate more resources for the growth of their aboveground and subsurface biomass, and input from available nitrogen sources (Root exudates) increases, which leads to accelerated metabolism of soil microorganisms and the release of more (LAP+NAG) into the soil (Zuo et al. 2018).

The effect of shrub encroachment on soil extracellular enzyme stoichiometry and microbial nutrient limitations

Soil extracellular enzyme stoichiometry can highlight factors limiting C and N cycling and predict the stability of alpine grassland ecosystem (Hu et al. 2021). In our study, the stoichiometry ratios of C-, N-, and P-acquisition enzymes ranged from 1.27:1.48:1 to 1.54:1.44:1, and the average was 1.36:1.44:1, which deviates from the 1:1:1 ratio at the global average level (Cui et al., 2021; Sinsabaugh et al., 2008). Moreover, $E_{\text{C:N}}$ were much lower than the average values of major terrestrial enzymes in the world, with $E_{\text{C:N}}$ being 1.41. $E_{\text{C:P}}$ and $E_{\text{N:P}}$ were much higher than the average values of major terrestrial enzymes in the world, that is, $E_{\text{C:P}}$ was 0.62, and $E_{\text{N:P}}$ was 0.44 (Sinsabaugh et al., 2009). Our finding is consistent with Luster et al and Tapia-Torres et al, that soil extracellular enzyme stoichiometry can greatly change in different ecosystems, especially alpine ecosystems (Luster et al., 2009; Turpault et al., 2005). Our results showed that $E_{\text{C:N}}$ in subsoil decreased after shrub encroachment. This means that the shrub-encroached grassland has a lower microbial N limitation than the non-shrub grassland. At the same time, the soil rhizosphere $E_{\text{C:N}}$ and $E_{\text{C:P}}$ in topsoil showed a downward trend after shrub encroachment, indicating that the shrub-encroached grassland was subject to lower microbial C limitation than the non-shrub grassland (Koranda et al. 2011).

Microbial nutrient limitation plays a crucial role in soil carbon cycle, especially in the decomposition of SOC (Sinsabaugh et al. 2009). In our study, the enzyme vector angle was less than 45° . At the same time, the microbial nutrient limitation characteristics of figure showed that the data points were below the 1:1 line in both topsoil and subsoil (Fig. 5), which indicated that there were microbial C and N limitations in the shrub-encroached and non-shrub grasslands. This supports our second hypothesis. These microbial limitations indicated that C- and N-acquisition enzyme activities were higher than P-acquisition enzyme activities in both shrub-encroached and non-encroached grasslands. This may be because more C- and N-acquisition enzymes are produced during microbial metabolism to obtain C and N resources to maintain their respiration and growth under high water conditions. In particular, high SWC content in non-shrub grasslands can accelerate plant growth and increase plant demand for soil elements C and N, and plants will compete with microorganisms for C and N, resulting in microbial C and N limitations (Wang et al. 2020). Our results showed a significant positive correlation between microbial C limitation and microbial N limitation in topsoil and subsoil (fig. 5). This further proves that microbes cannot get C without nitrogen, and there is a strong coupling between the two elements (Soussana & Lemaire 2014). Previous studies have shown that when microbial C and N limitations increase, microbial community metabolism shifts from growth to maintenance of respiration. It can accelerate the decomposition of SOC by extracellular enzymes, thus promoting the release of soil carbon (Manzoni et al. 2012). Therefore, microbial C and N limitations may be detrimental to microbial assimilation of SOC, and thus affect soil carbon retention. In our study, the limitation of soil microbial C and N in the shrub alpine grassland were lower than those in the non-shrub grassland, while the soil nutrient did not show a similar trend. This supports our third hypothesis. The reason may be the imbalance of soil nutrients, which affects the utilization of nutrients by microorganisms, resulting in microbial C and N limitation (Cui et al. 2018). Ecological enzyme stoichiometry is usually affected by soil nutrient status (Sinsabaugh et al. 2009). EEA vector analysis showed that shrub development mitigated microbial C limitation in alpine grassland. This means that vegetation type indirectly affects microbial nutrient limitations by affecting microbial community structure and soil nutrient supply. Litter from herbaceous plants is more easily decomposed by microorganisms than litter from shrubs because herbaceous plants have higher carbohydrates and cellulose and lower lignin (Domenach et al. 1994). However, the carbon content of shrubs is sufficient to sustain the growth of microorganisms due to the nitrogen fixation of shrub root nodule fungi (Carrasco et al. 2011). Therefore, the decomposition rate of cellulose by microorganisms to non-encroached grassland was much higher than that to shrub-encroached grassland, resulting in the increase of C-acquisition enzyme. The rhizosphere is a key region for the interaction between plant roots and soil microorganisms, which plays a crucial role in regulating soil extracellular enzyme stoichiometry and affecting soil carbon and nitrogen content (Luster et al. 2009). Nutrient competition among roots will reduce the availability of soil nutrients and hinder microbial access to nutrients, resulting in increased microbial C and N limitations in rhizosphere soil (Kuzyakov & Xu 2013). Our results showed that microbial C and N were limited by differences between rhizosphere and bulk soils after shrub encroachment, which supports our third hypothesis. This may be due to the interaction between plants and microorganisms in the rhizosphere environment (Zhu et al. 2020). At the same time, our results show that microbial C and N limitations in *Caragana microphylla* differ significantly between bulk and rhizosphere soils. This suggests that compared with the bulk region, the rhizosphere region of *Caragana microphylla* requires more C input from root secretions (Zhang et al. 2011) due to higher microbial activity and faster nutrient turnover rate, which may accelerate the secretion of C-acquisition enzymes and N-acquisition enzymes. Linear regression analysis showed that the restriction of shallow microbial C was significantly negatively correlated with SWC content (Fig. 8). This may be because the anoxic environment caused by high soil moisture

inhibits microbial metabolic activities (Grandy et al. 2007), thus affecting microbial demand for soil C. At the same time, the restriction of C and N of most microorganisms in non-shrub grassland was higher than that in the scrub grassland. This is probably due to the higher soil water content in non-shrub alpine grasslands, and the more susceptible to microbial carbon and nitrogen constraints in wet grasslands (Hooper & Johnson 1999). Linear regression analysis further showed that microbial C restriction was significantly negatively correlated with $\text{NH}_4^+\text{-N}$, NO_3^- -N, DOC and SOC contents in shallow and deep soils (Fig 10 and 11). Therefore, the results suggest that changes in microbial C limitation may be driven by changes in soil nutrient levels. Our results are consistent with previous studies on the effects of soil nutrients on soil extracellular enzyme stoichiometry (Li et al. 2019; Yang et al. 2020). Through generalized linear correlation analysis, we found that soil C:N and N:P were significantly negatively correlated with microbial C and N limitation (Figure 8), indicating the influence of nutrient stoichiometry on microbial nutrient acquisition. That is, soil nutrient stoichiometric imbalance, especially when soil P is much smaller than soil C and N, microorganisms will increase their investment in P and affect their acquisition rate of C and N (Cui et al. 2022; Gao et al. 2021). In addition, the structural equation model also showed that changes in soil nutrients could explain changes in microbial C and N limitation (Figure 12). In particular, the increase of SOC and $\text{NH}_4^+\text{-N}$ content alleviated microbial C and N limitation after shrub encroachment, respectively. According to the theory of microbial resource allocation. This may be due to the fact that soil microorganisms do not need to secrete more C- and N- acquisition enzymes to obtain C and N elements when the increased content of soil organic carbon and available nitrogen is sufficient to maintain the growth of shrubs after shrub encroachment, thus reducing microbial C and N limitations. In summary, changes in microbial C and N limitations may depend on soil nutrients and nutrient stoichiometry.

Conclusions

The results demonstrated the ratio of log-transformed C-, N-, and P-acquisition enzyme activities ranged from 1.27:1.48:1 to 1.54:1.44:1, and the average was 1.36:1.44:1, which deviates from the 1:1:1 at the global average level. Our study revealed that soil microbial C and N limitations were found in shrub-encroached and non-encroached alpine grasslands on the Qinghai-Tibetan Plateau. Most microbial C and N limitations were higher in non-shrub alpine grasslands than that in shrub-encroached grasslands. It indicates that shrub encroachment mitigated microbial C and N limitations in non-shrub grasslands. At the same time, linear regression further revealed that microbial C and N limitations were directly mediated by soil nutrients and nutrient stoichiometry. Meanwhile, our results showed that microbial C and N limitations were different between bulk and rhizosphere soils in shrub-encroached and non-shrub grassland. It suggests the influences of vegetation roots on microbial C and N metabolisms. Meanwhile, the results of SEM show that the increasing contents of SOC and $\text{NH}_4^+\text{-N}$ directly mitigated microbial C and N limitations following shrub encroachment, respectively. Our results emphasized the change of microbial C and N limitations and the response to environmental factors following shrub encroachment.

Declarations

Acknowledgments

We thank Professor Brian McGarvey for providing language help and proofreading for this article.

Funding

This research was financially supported by the National Science Foundation of China [Grant No. 31600378] and a Special fund for Basic scientific research of central universities [2022NYXS043]. We are grateful to the staff in our research group for their help with field sampling.

Conflict of Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in the manuscript entitled, "The mitigation of microbial carbon and nitrogen limitations by shrub encroachment: extracellular enzyme stoichiometry of the alpine grassland on the Qinghai-Tibetan Plateau"

References

1. Ai C, Liang G, Sun J, Wang X, Zhou W (2012) Responses of extracellular enzyme activities and microbial community in both the rhizosphere and bulk soil to long-term fertilization practices in a fluvo-aquic soil. *Geoderma* 173: 330-338
2. Akinyemi DS, Zhu Y, Zhao M, Zhang P, Shen H, Fang JJGE, Conservation (2020) Response of soil extracellular enzyme activity to experimental precipitation in a shrub-encroached grassland in Inner Mongolia. 23: e01175
3. Bardgett RD, Mommer L, De Vries FT (2014) Going underground: root traits as drivers of ecosystem processes. *Trends Ecol Evol* 29(12): 692-699

4. Bi B, Wang K, Zhang H, Wang Y, Fei H, Pan R, Han F (2021) Plants use rhizosphere metabolites to regulate soil microbial diversity. *Land Degradation Development* 32(18): 5267-5280
5. Binkley D, Richter D (1987) Nutrient cycles and H⁺ budgets of forest ecosystems. In: *Advances in ecological research*. Elsevier. p 1-51
6. Brandt JS, Haynes MA, Kuemmerle T, Waller DM, Radeloff VC (2013) Regime shift on the roof of the world: Alpine meadows converting to shrublands in the southern Himalayas. *Biological Conservation* 158: 116-127
7. Caldwell MM, Manwaring JH, Durham SL (1996) Species interactions at the level of fine roots in the field: influence of soil nutrient heterogeneity and plant size. *Oecologia* 106: 440-447
8. Cao C, Jiang S, Ying Z, Zhang F, Han X (2011) Spatial variability of soil nutrients and microbiological properties after the establishment of leguminous shrub *Caragana microphylla* Lam. plantation on sand dune in the Horqin Sandy Land of Northeast China. *Ecological Engineering* 37(10): 1467-1475
9. Carrasco L, Azcón R, Kohler J, Roldán A, Caravaca F (2011) Comparative effects of native filamentous and arbuscular mycorrhizal fungi in the establishment of an autochthonous, leguminous shrub growing in a metal-contaminated soil. *Science of the Total Environment* 409(6): 1205-1209
10. Chen H, Ju P, Zhu Q, Xu X, Wu N, Gao Y, Feng X, Tian J, Niu S, Zhang Y, Peng C, Wang Y (2022) Carbon and nitrogen cycling on the Qinghai–Tibetan Plateau. *Nature Reviews Earth & Environment* 3(10): 701-716
11. Cui Y, Bing H, Moorhead DL, Delgado-Baquerizo M, Ye L, Yu J, Zhang S, Wang X, Peng S, Guo X (2022) Ecoenzymatic stoichiometry reveals widespread soil phosphorus limitation to microbial metabolism across Chinese forests. *Communications Earth Environment* 3(1): 184
12. Cui Y, Fang L, Guo X, Wang X, Zhang Y, Li P, Zhang X (2018) Ecoenzymatic stoichiometry and microbial nutrient limitation in rhizosphere soil in the arid area of the northern Loess Plateau, China. *Soil Biology Biochemistry* 116: 11-21
13. Dieleman CM, Lindo Z, McLaughlin JW, Craig AE, Branfireun BA (2016) Climate change effects on peatland decomposition and porewater dissolved organic carbon biogeochemistry. *Biogeochemistry* 128: 385-396
14. Domenach A-M, Moiroud A, Jocteur-Monrozier L (1994) Leaf carbon and nitrogen constituents of some actinorhizal tree species. *Soil Biology and Biochemistry* 26(5): 649-653
15. Eldridge DJ, Bowker MA, Maestre FT, Roger E, Reynolds JF, Whitford WG (2011) Impacts of shrub encroachment on ecosystem structure and functioning: towards a global synthesis. *Ecology letters* 14(7): 709-722
16. Fayiah M, Dong S, Li Y, Xu Y, Gao X, Li S, Shen H, Xiao J, Yang Y, Wessell K (2019) The relationships between plant diversity, plant cover, plant biomass and soil fertility vary with grassland type on Qinghai-Tibetan Plateau. *Agriculture, Ecosystems & Environment* 286:
17. Fujii Y, Furubayashi A, Hiradate S Rhizosphere soil method: a new bioassay to evaluate allelopathy in the field. In: *Proceedings of the 4th World Congress on Allelopathy: Establishing the Scientific Base*. 2005. p 21-26
18. Gao X-I, Li X, Zhao L, Kuzyakov Y (2021) Shrubs magnify soil phosphorus depletion in Tibetan meadows: Conclusions from C: N: P stoichiometry and deep soil profiles. *Science of The Total Environment* 785: 147320
19. German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD (2011) Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biology and Biochemistry* 43(7): 1387-1397
20. Gómez-Rey MX, Madeira M, Gonzalez-Prieto SJ, Coutinho J (2013) Soil C and N dynamics in a Mediterranean oak woodland with shrub encroachment. *Plant and Soil* 371(1-2): 339-354
21. Grandy AS, Neff JC, Weintraub MN (2007) Carbon structure and enzyme activities in alpine and forest ecosystems. *Soil Biology and Biochemistry* 39(11): 2701-2711
22. He W, Wu F, Zhang D, Yang W, Tan B, Zhao Y, Wu Q (2015) The effects of forest gaps on cellulose degradation in the foliar litter of two shrub species in an alpine fir forest. *Plant and Soil* 393(1): 109-122
23. Hewins DB, Sinsabaugh RL, Archer SR, Throop HL (2017) Soil–litter mixing and microbial activity mediate decomposition and soil aggregate formation in a sandy shrub-invaded Chihuahuan Desert grassland. *Plant ecology* 218: 459-474
24. Hooper DU, Johnson L (1999) Nitrogen limitation in dryland ecosystems: responses to geographical and temporal variation in precipitation. *Biogeochemistry* 46(1): 247-293
25. Hu Z, Li J, Shi K, Ren G, Dai Z, Sun J, Zheng X, Zhou Y, Zhang J, Li G (2021) Effects of Canada goldenrod invasion on soil extracellular enzyme activities and ecoenzymatic stoichiometry. *Sustainability* 13(7): 3768
26. Javot H, Pumplin N, Harrison MJ (2007) Phosphate in the arbuscular mycorrhizal symbiosis: transport properties and regulatory roles. *Plant, Cell & Environment* 30(3): 310-322
27. Jian S, Li J, Chen J, Wang G, Mayes MA, Dzantor KE, Hui D, Luo Y (2016) Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. *Soil Biology and Biochemistry* 101: 32-43

28. Jones DL, Willett VB (2006) Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology and Biochemistry* 38(5): 991-999
29. Koranda M, Schneckner J, Kaiser C, Fuchslueger L, Kitzler B, Stange CF, Sessitsch A, Zechmeister-Boltenstern S, Richter A (2011) Microbial processes and community composition in the rhizosphere of European beech—the influence of plant C exudates. *Soil Biology and Biochemistry* 43(3): 551-558
30. Kuzyakov Y, Xu X (2013) Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. *New Phytologist* 198(3): 656-669
31. Li J, Liu Y, Hai X, Shangguan Z, Deng L (2019) Dynamics of soil microbial C: N: P stoichiometry and its driving mechanisms following natural vegetation restoration after farmland abandonment. *Science of the Total Environment* 693: 133613
32. Liao J, Boutton T, Jastrow J (2006a) Organic matter turnover in soil physical fractions following woody plant invasion of grassland: Evidence from natural ¹³C and ¹⁵N. *Soil biology and biochemistry* 38(11): 3197-3210
33. Liao J, Boutton T, Jastrow J (2006b) Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. *Soil biology and biochemistry* 38(11): 3184-3196
34. Liao JD, Boutton TW, Jastrow JD (2006c) Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. *Soil Biology and Biochemistry* 38(11): 3184-3196
35. Luster J, Göttlein A, Nowack B, Sarret G (2009) Sampling, defining, characterising and modeling the rhizosphere—the soil science tool box. *Plant and Soil* 321(1): 457-482
36. Manzoni S, Taylor P, Richter A, Porporato A, Ågren GI (2012) Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist* 196(1): 79-91
37. Moorhead DL, Sinsabaugh RL (2006) A theoretical model of litter decay and microbial interaction. *Ecological Monographs* 76(2): 151-174
38. Mordelet P, Menaut JC, Mariotti A (1997) Tree and grass rooting patterns in an African humid savanna. *Journal of Vegetation Science* 8(1): 65-70
39. Mori T (2020) Does ecoenzymatic stoichiometry really determine microbial nutrient limitations? *Soil Biology and Biochemistry* 146: 107816
40. Mureva A, Ward D, Pillay T, Chivenge P, Cramer M (2018) Soil organic carbon increases in semi-arid regions while it decreases in humid regions due to woody-plant encroachment of grasslands in South Africa. *Scientific Reports* 8(1): 15506
41. Paterson E (2003) Importance of rhizodeposition in the coupling of plant and microbial productivity. *European journal of soil science* 54(4): 741-750
42. Pellegrini E, Boscutti F, Alberti G, Casolo V, Contin M, De Nobili M (2021) Stand age, degree of encroachment and soil characteristics modulate changes of C and N cycles in dry grassland soils invaded by the N₂-fixing shrub *Amorpha fruticosa*. *Science of the Total Environment* 792: 148295
43. Sardans J, Bartrons M, Margalef O, Gargallo-Garriga A, Janssens IA, Ciais P, Obersteiner M, Sigurdsson BD, Chen HY, Peñuelas J (2017) Plant invasion is associated with higher plant–soil nutrient concentrations in nutrient-poor environments. *Global Change Biology* 23(3): 1282-1291
44. Sinsabaugh RL, Hill BH, Follstad Shah JJ (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature* 462(7274): 795-798
45. Sinsabaugh RL, Shah JJF (2012) Ecoenzymatic stoichiometry and ecological theory. *Annual Review of Ecology, Evolution, and Systematics* 43(313): 2012
46. Soong JL, Fuchslueger L, Marañón-Jimenez S, Torn MS, Janssens IA, Penuelas J, Richter A (2020) Microbial carbon limitation: the need for integrating microorganisms into our understanding of ecosystem carbon cycling. *Global change biology* 26(4): 1953-1961
47. Soussana J-F, Lemaire G (2014) Coupling carbon and nitrogen cycles for environmentally sustainable intensification of grasslands and crop-livestock systems. *Agriculture, Ecosystems & Environment* 190: 9-17
48. Spohn M, Kuzyakov Y (2013) Distribution of microbial-and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation—Coupling soil zymography with ¹⁴C imaging. *Soil Biology and Biochemistry* 67: 106-113
49. Tischer A, Blagodatskaya E, Hamer U (2015) Microbial community structure and resource availability drive the catalytic efficiency of soil enzymes under land-use change conditions. *Soil Biology and Biochemistry* 89: 226-237
50. Wallenstein M, Allison SD, Ernakovich J, Steinweg JM, Sinsabaugh R (2010) Controls on the temperature sensitivity of soil enzymes: a key driver of in situ enzyme activity rates. In: *Soil enzymology*. Springer. p 245-258
51. Wang X, Cui Y, Zhang X, Ju W, Duan C, Wang Y, Fang LJ, SotTE (2020) A novel extracellular enzyme stoichiometry method to evaluate soil heavy metal contamination: evidence derived from microbial metabolic limitation. *Science of the Total Environment* 738: 139709

52. Yan L, Zhou G, Wang Y, Hu T, Sui X (2015) The spatial and temporal dynamics of carbon budget in the alpine grasslands on the Qinghai-Tibetan Plateau using the Terrestrial Ecosystem Model. *Journal of Cleaner Production* 107: 195-201
53. Yang Y, Liang C, Wang Y, Cheng H, An S, Chang SX (2020) Soil extracellular enzyme stoichiometry reflects the shift from P-to N-limitation of microorganisms with grassland restoration. *Soil Biology and Biochemistry* 149: 107928
54. Yu-Ying B, Wei Y (2004) Arbuscular mycorrhizae and their structural types on common plants in grasslands of mid-western Inner Mongolia. *Biodiversity Science* 12(5): 501
55. Zhang C, Liu G, Xue S, Song Z (2011) Rhizosphere soil microbial activity under different vegetation types on the Loess Plateau, China. *Geoderma* 161(3-4): 115-125
56. Zhang W, Xu Y, Gao D, Wang X, Liu W, Deng J, Han X, Yang G, Feng Y, Ren G (2019) Ecoenzymatic stoichiometry and nutrient dynamics along a revegetation chronosequence in the soils of abandoned land and Robinia pseudoacacia plantation on the Loess Plateau, China. *Soil Biology and Biochemistry* 134: 1-14
57. Zheng T, Liang C, Xie H, Zhao J, Yan E, Zhou X, Bao X (2019) Rhizosphere effects on soil microbial community structure and enzyme activity in a successional subtropical forest. *FEMS microbiology ecology* 95(5): fiz043
58. Zhu X, Liu M, Kou Y, Liu D, Liu Q, Zhang Z, Jiang Z, Yin H (2020) Differential effects of N addition on the stoichiometry of microbes and extracellular enzymes in the rhizosphere and bulk soils of an alpine shrubland. *Plant and Soil* 449(1): 285-301
59. Zuo Y, Li J, Zeng H, Wang W (2018) Vertical pattern and its driving factors in soil extracellular enzyme activity and stoichiometry along mountain grassland belts. *Biogeochemistry* 141: 23-39

Tables

Table 1. The effects of vegetation types, locations (bulk and rhizosphere), and soil depths on physical property and available nutrients on the Qinghai-Tibetan Plateau.

Layer (cm)	vegetation types	SWC	pH	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	DOC (mg kg ⁻¹)	Olsen-P (mg kg ⁻¹)
0-10	GL bulk	47.13±1.66Ad	5.15±0.03Ac	13.62±1.66Ad	3.47±0.1Ad	61±0.89Ab	1.87±0.02Aa
	GL rhizosphere	51.93±0.79Ab	5.25±0.01Aab*	17.65±0.4Ab	6.87±0.45Ad**	89.18±0.29Ac*	2.56±0.15Aa*
	SA bulk	57.36±2.25Ac	5.44±0.01Ab	31±0.62Ab	38.32±0.6Ab	119.2±2.23Aab	0.16±0.02Bc
	SA rhizosphere	58.62±5.06Aab	5.59±0.07Aab	32.55±0.96Aab	44.48±1.21Ab*	121.07±1.09Ab	1.81±0.25Ba*
	CM bulk	76.59±1.18Aa	5.61±0.01Aa	37.5±1.15Aa	53.4±1.21Aa	133.42±0.65Aa	0.38±0.04Bb
	CM rhizosphere	78.89±1.42Aa	5.71±0.03Aa*	45.16±0.62Aa**	55.75±0.19Aa	137.72±1.15Aa*	2.3±0.21Aa*
	PF bulk	65±0.74Ab	5.37±0.02Ab	19.57±0.71Ac	29.29±0.87Ac	81.8±0.42Bab	0.25±0.02Ac
	PF rhizosphere	70.55±1.92Aab	5.19±0.02Bb**	20.47±2.3Aab	34.1±0.91Ac*	85.47±1.58Bc	1.89±0.22Aa*
30-40	GL bulk	42.41±0.54Ac	5.08±0.01Ab	10.94±0.09Ab	1.94±0.13Bb	54.77±1.24Bd	1.49±0.23Aab
	GL rhizosphere	45.4±1.59Bc	5.14±0.01Bb*	12.74±0.67Bc	5.06±0.2Bb*	69.26±1.17Bd**	3.44±0.29Ab**
	SA bulk	55.22±1.29Ab	5.33±0.01Bab	18.91±0.35Bab	20.43±1.46Bab	80.67±1.16Bc	3.25±0.33Aa
	SA rhizosphere	57.4±0.88Ab	5.41±0Bab*	25.27±1.3Bb**	29.35±2.72Bab*	90.26±1.34Bc**	5.08±0.11Aa**
	CM bulk	66.24±1.27Ba	5.55±0.01Ba	21.83±0.06Ba	36.63±0.84Ba	112.73±0.73Ba	0.9±0.08Aab
	CM rhizosphere	75.65±2.65Aa*	5.61±0.01Bab*	40.88±1.32Ba***	42.63±0.29Ba**	118.86±0.97Ba**	1.85±0.21Ac*
	PF bulk	42.25±0.64Bc	5.26±0.05Aab	18.91±0.97Aab	26.69±0.38Aab	92±1.24Ab	0.51±0.1Ab
	PF rhizosphere	48.2±0.86Bc**	5.62±0.02Aa**	27.06±0.64Ab**	25.87±4.15Aab	100.05±0.42Ab**	2.38±0.2Ac**

Note: Values are given as mean $\pm$ standard error (n=3). The different letters in columns represent significant differences among four vegetation types and soil depths ($p < 0.05$). GL, non-shrub alpine grasslands; SA, *Spiraea alpina* lands; CM, *Caragana microphylla* lands; PF, *Potentilla fruticosa* lands. Different lowercase letters represent significant differences ($p < 0.05$) among four vegetation lands in topsoil or subsoil. Different uppercase letters represent significant differences ($p < 0.05$) between topsoil and subsoil among four vegetation lands. * indicates the differences between bulk and rhizosphere soils within four vegetation lands and soil depths (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Table 2. The effects of vegetation types, locations (bulk and rhizosphere), and soil depths on total nutrients and nutrient ratios on the Qinghai-Tibetan Plateau.

Layer (cm)	vegetation type	SOC (g kg ⁻¹)	TN (g kg ⁻¹)	TP (g kg ⁻¹)	C:N	C:P	N:P
0-10	GL bulk	43.54 $\pm$ 0.7Ad	2.22 $\pm$ 0.16Ab	1.86 $\pm$ 0.02Aa	19.83 $\pm$ 1.2Aab	23.38 $\pm$ 0.33Ad	1.19 $\pm$ 0.09Bd
	GL rhizosphere	52.63 $\pm$ 2.47Ad*	4.22 $\pm$ 0.03Aab**	2.07 $\pm$ 0Aa***	12.48 $\pm$ 0.65Aab**	25.48 $\pm$ 1.22Ad	2.04 $\pm$ 0.01Ab***
	SA bulk	69.48 $\pm$ 0.3Ac	9.44 $\pm$ 0.1Aab	1.33 $\pm$ 0.03Ac	7.36 $\pm$ 0.08Bb	52.38 $\pm$ 1Ac	7.12 $\pm$ 0.21Ab
	SA rhizosphere	74.09 $\pm$ 2.68Ac	9.64 $\pm$ 0.33Aab	1.63 $\pm$ 0.04Abc**	7.68 $\pm$ 0.05Bb*	45.43 $\pm$ 2.47Ac	5.91 $\pm$ 0.32Aa*
	CM bulk	102.22 $\pm$ 1.24Aa	9.96 $\pm$ 0.03Aa	1.26 $\pm$ 0.02Ac	10.27 $\pm$ 0.15Bab	81.04 $\pm$ 1.61Aa	7.9 $\pm$ 0.16Aa
	CM rhizosphere	124.32 $\pm$ 1.35Aa***	10.5 $\pm$ 0.29Aa	1.51 $\pm$ 0.04Ac**	11.86 $\pm$ 0.24Aab**	82.47 $\pm$ 2.75Aa	6.96 $\pm$ 0.28Aa*
	PF bulk	97.02 $\pm$ 0.71Ab	3.58 $\pm$ 0.27Bab	1.46 $\pm$ 0.01Ab	27.45 $\pm$ 2.41Aa	66.24 $\pm$ 0.43Ab	2.45 $\pm$ 0.2Bc
	PF rhizosphere	104.96 $\pm$ 0.99Ab**	3.77 $\pm$ 0.27Bb	1.7 $\pm$ 0.04Ab**	28.14 $\pm$ 2.22Aa	61.83 $\pm$ 1.21Ab*	2.23 $\pm$ 0.2Bb
30-40	GL bulk	24.89 $\pm$ 0.42Bb	2.78 $\pm$ 0.21Ab	1.33 $\pm$ 0.04Bab	9.05 $\pm$ 0.73Bc	18.67 $\pm$ 0.44Bd	2.08 $\pm$ 0.11Ab
	GL rhizosphere	29.21 $\pm$ 1.8Bb	3.77 $\pm$ 0.13Ad*	1.9 $\pm$ 0.03Ba***	7.78 $\pm$ 0.66Bc	15.33 $\pm$ 0.78Bc*	1.98 $\pm$ 0.07Ad
	SA bulk	60.64 $\pm$ 2.15Bab	5.53 $\pm$ 0.03Bab	1.4 $\pm$ 0.08Aa	10.95 $\pm$ 0.33Ac	43.45 $\pm$ 1.24Bc	3.98 $\pm$ 0.21Bab
	SA rhizosphere	60.48 $\pm$ 0.34Bab	7 $\pm$ 0.02Bb***	1.48 $\pm$ 0.02Bb	8.65 $\pm$ 0.07Ac**	40.98 $\pm$ 0.75Ab	4.74 $\pm$ 0.07Bb*
	CM bulk	88.49 $\pm$ 0.46Ba	6.71 $\pm$ 0.31Ba	1.14 $\pm$ 0.02Bb	13.23 $\pm$ 0.51Ab	77.85 $\pm$ 1.35Aa	5.9 $\pm$ 0.22Ba
	CM rhizosphere	92.26 $\pm$ 3.08Ba	7.79 $\pm$ 0.25Ba*	1.31 $\pm$ 0.03Bb**	11.87 $\pm$ 0.57Ab	70.37 $\pm$ 3.45Aa	5.93 $\pm$ 0.09Ba
	PF bulk	73.37 $\pm$ 0.55Bab	4.08 $\pm$ 0Aab	1.25 $\pm$ 0.05Bab	17.98 $\pm$ 0.15Aa	58.98 $\pm$ 2.57Bb	3.28 $\pm$ 0.12Aab
	PF rhizosphere	87.14 $\pm$ 0.55Bab***	5.33 $\pm$ 0.09Ac***	1.77 $\pm$ 0.06Aa**	16.37 $\pm$ 0.37Ba*	49.34 $\pm$ 1.53Bb*	3.02 $\pm$ 0.12Ac

Note: Values are given as mean $\pm$ standard error (n=3). The different letters in columns represent significant differences among four vegetation types and soil depths ($p < 0.05$). GL, non-shrub alpine grasslands; SA, *Spiraea alpina* lands; CM, *Caragana microphylla* lands; PF, *Potentilla fruticosa* lands. Different lowercase letters represent significant differences ($p < 0.05$) among four vegetation lands in topsoil or subsoil. Different uppercase letters represent significant differences ($p < 0.05$) between topsoil and subsoil among four vegetation lands. * represents the differences between bulk and rhizosphere soils within four vegetation lands and soil depths (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Table 3. The effects of vegetation types, locations (bulk and rhizosphere), and soil depths on extracellular enzyme activities on the Qinghai-Tibetan Plateau.

Layer (cm)	vegetation type	C-acquisition enzymes (nmol g ⁻¹ h ⁻¹)	N-acquisition enzymes (nmol g ⁻¹ h ⁻¹)	P-acquisition enzymes (nmol g ⁻¹ h ⁻¹)
0-10	GL bulk	266.49±33.82Aa	300.23±17.95Ab	48.92±1.95Bc
	GL rhizosphere	440.13±3.16Aa*	573.54±12.49Aa*	65.92±1.47Aa**
	SA bulk	218.46±11.07Aa	384.48±36.04Aab	58.24±1.49Ab
	SA rhizosphere	367.29±30.17Aab*	411.5±40.77Aab	67.38±5.32Aa
	CM bulk	234.75±0.97Aa	428.3±22.74Aa	73.75±2.38Aa
	CM rhizosphere	264.69±3.84Ab**	418.13±17.34Bab	62.12±1.67Ba*
	PF bulk	225.14±0.73Aa	352.99±19.81Aab	50.99±1.28Bbc
	PF rhizosphere	316.02±56.56Aab	326.32±9.04Ab	69.65±2.37Aa**
30-40	GL bulk	217.72±8.7Aa	285.6±25.79Ab	69.54±0.31Aa
	GL rhizosphere	448.24±8.59Aa***	336.47±31.94Bb	49.96±2.36Bb**
	SA bulk	219.57±0.66Aa	312.12±14.84Ab	64.53±3.03Aa
	SA rhizosphere	420.46±8.89Aa**	433.24±72.16Aab	70.42±3.39Aa
	CM bulk	237.89±4.87Aa	436.52±20.82Aa	57.85±6.92Aa
	CM rhizosphere	244.58±2.86Bb	573.46±27.44Aa*	72.79±2.36Aa
	PF bulk	228.47±4.04Aa	430.69±33.3Aa	65.95±4.99Aa
	PF rhizosphere	242.98±5.66Ab*	451.28±58.63Aab	75.09±1.16Aa

Note: Values are given as mean ± standard error (n=3). The different letters in columns represent significant differences among four vegetation types and soil depths ($p < 0.05$). GL, non-shrub alpine grasslands; SA, *Spiraea alpina* lands; CM, *Caragana microphylla* lands; PF, *Potentilla fruticosa* lands. C-acquisition enzymes, BG+CBH; N-acquisition enzymes, NAG+LAP; P-acquisition enzyme, AP. Different lowercase letters represent significant differences ($p < 0.05$) among four vegetation lands in topsoil or subsoil. Different uppercase letters represent significant differences ($p < 0.05$) between topsoil and subsoil among four vegetation lands. * represent the differences between bulk and rhizosphere soils within four vegetation lands and soil depths (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Table 4. The effects of vegetation types, locations (bulk and rhizosphere), and soil depths on extracellular enzyme stoichiometry on the Qinghai-Tibetan Plateau.

Layer (cm)	vegetation types	ln(BG+CBH):ln(NAG + LAP)	ln(BG+CBH):ln(AP)	ln(NAG + LAP):ln(AP)
0-10	GL bulk	0.98±0.01Aa	1.43±0.04Aa	1.47±0.03Aa
	GL rhizosphere	0.96±0Ba	1.45±0.01Ba	1.52±0.01Aa
	SA bulk	0.91±0.01Bab	1.33±0.02Aab	1.46±0.03Aa
	SA rhizosphere	0.98±0.03Aa*	1.4±0.02Aa*	1.43±0.04Aab
	CM bulk	0.9±0.01Ab	1.27±0.01Ab	1.41±0.02Aa
	CM rhizosphere	0.92±0.01Aa	1.35±0.01Aa**	1.46±0.01Aab
	PF bulk	0.92±0.01Aab	1.38±0.01Aab	1.49±0.01Aa
	PF rhizosphere	0.99±0.03Aa	1.35±0.04Aa	1.36±0.01Ab**
30-40	GL bulk	0.95±0.01Aa	1.27±0.01Ba	1.33±0.02Ba
	GL rhizosphere	1.05±0.01Aa**	1.56±0.02Aa***	1.49±0.04Aa*
	SA bulk	0.94±0.01Aa	1.29±0.02Aa	1.38±0.01Ba
	SA rhizosphere	1±0.03Aa	1.42±0.02Aab*	1.42±0.03Aa*
	CM bulk	0.9±0Aa	1.36±0.04Aa	1.51±0.05Aa
	CM rhizosphere	0.87±0.01Bb*	1.28±0.01Bab	1.48±0Aa
	PF bulk	0.9±0.01Aa	1.3±0.02Ba	1.45±0.04Aa
	PF rhizosphere	0.9±0.02Ab	1.27±0.01Ab	1.41±0.02Aa

Note: Values are given as mean ± standard error (n=3). The different letters in columns represent significant differences among four vegetation types and soil depths ($p < 0.05$). GL, non-shrub alpine grasslands; SA, *Spiraea alpina* lands; CM, *Caragana microphylla* lands; PF, *Potentilla fruticosa* lands. Different lowercase letters represent significant differences ($p < 0.05$) among four vegetation lands in topsoil or subsoil. Different uppercase letters represent significant differences ($p < 0.05$) between topsoil and subsoil among four vegetation lands. * represents the differences between bulk and rhizosphere soils within four vegetation lands and soil depths (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Figures

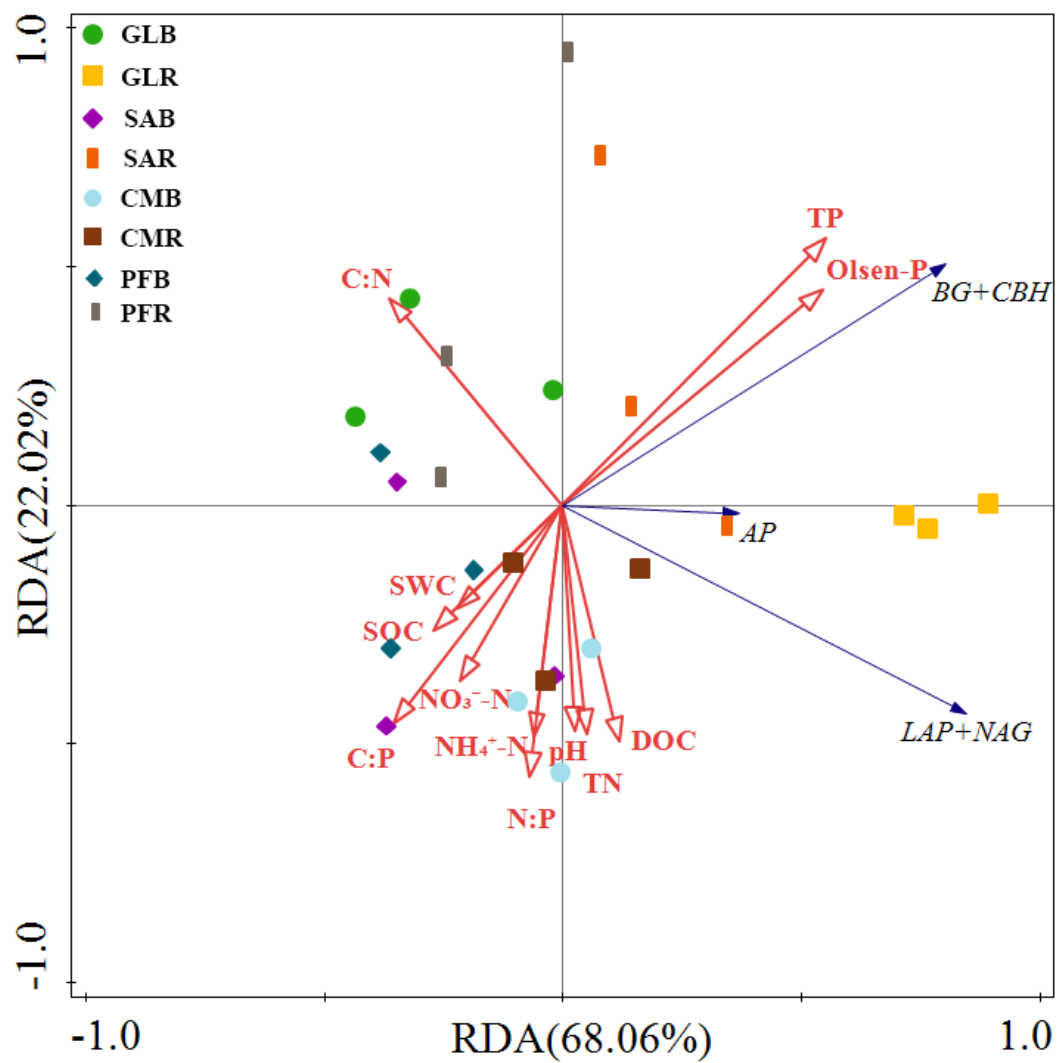


Figure 1

Legend not included with this version

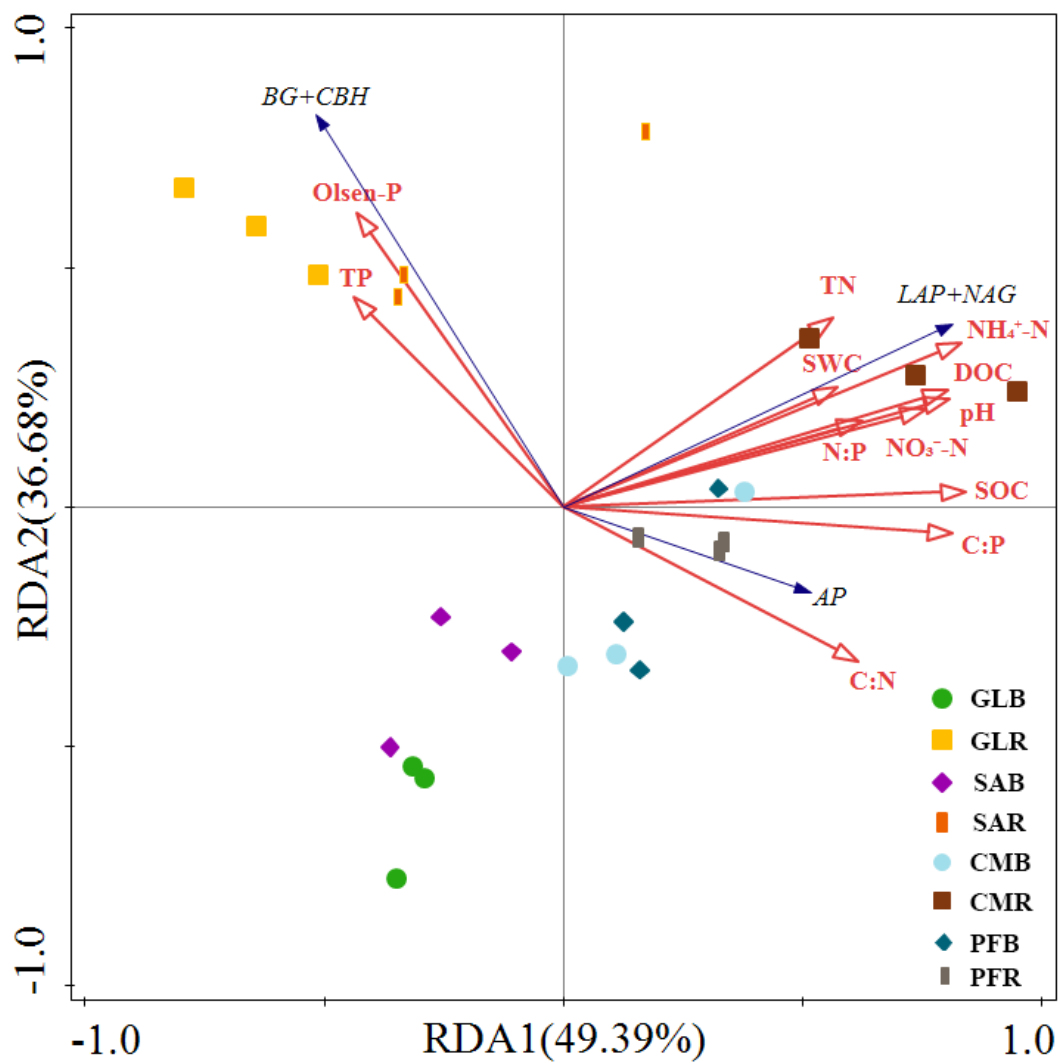


Figure 2

Legend not included with this version

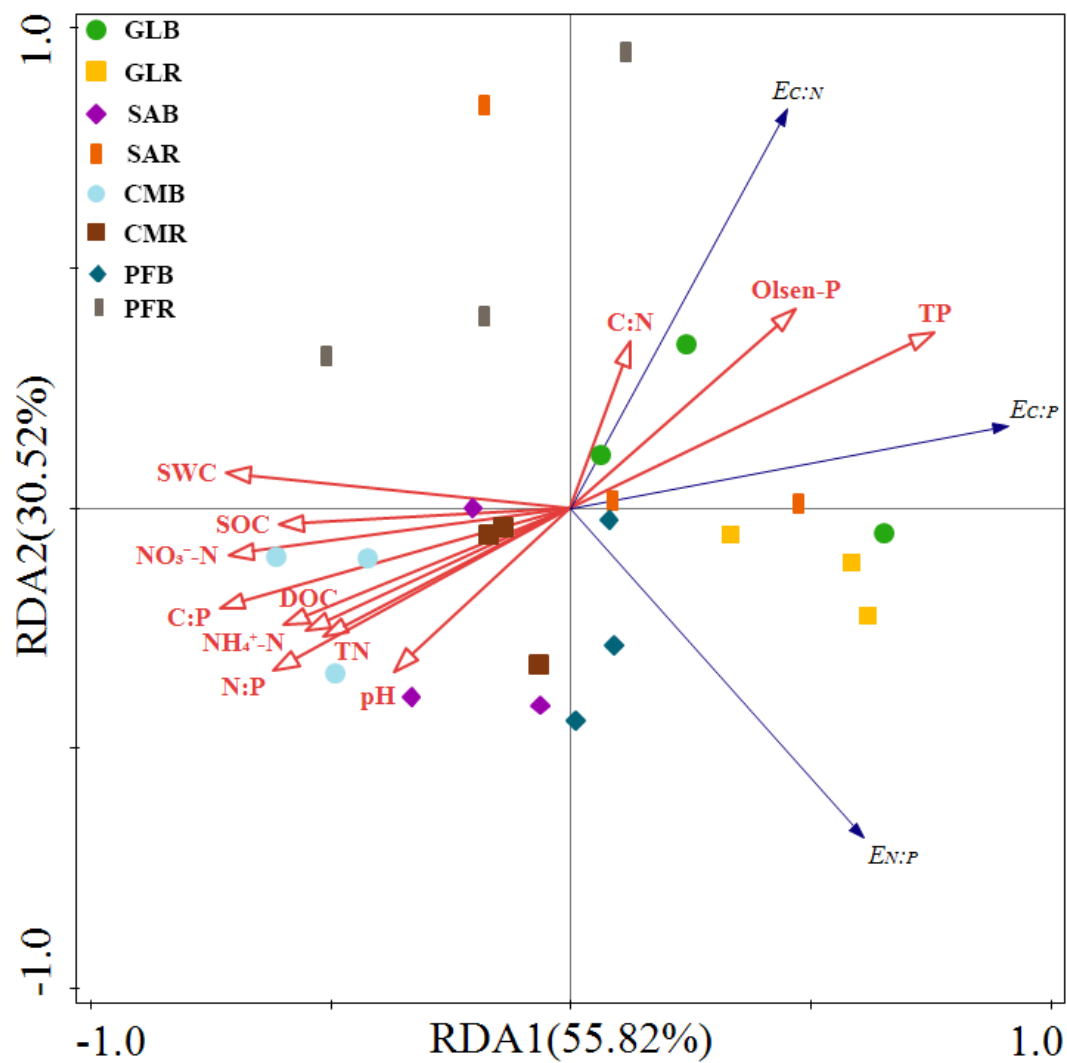


Figure 3

Legend not included with this version

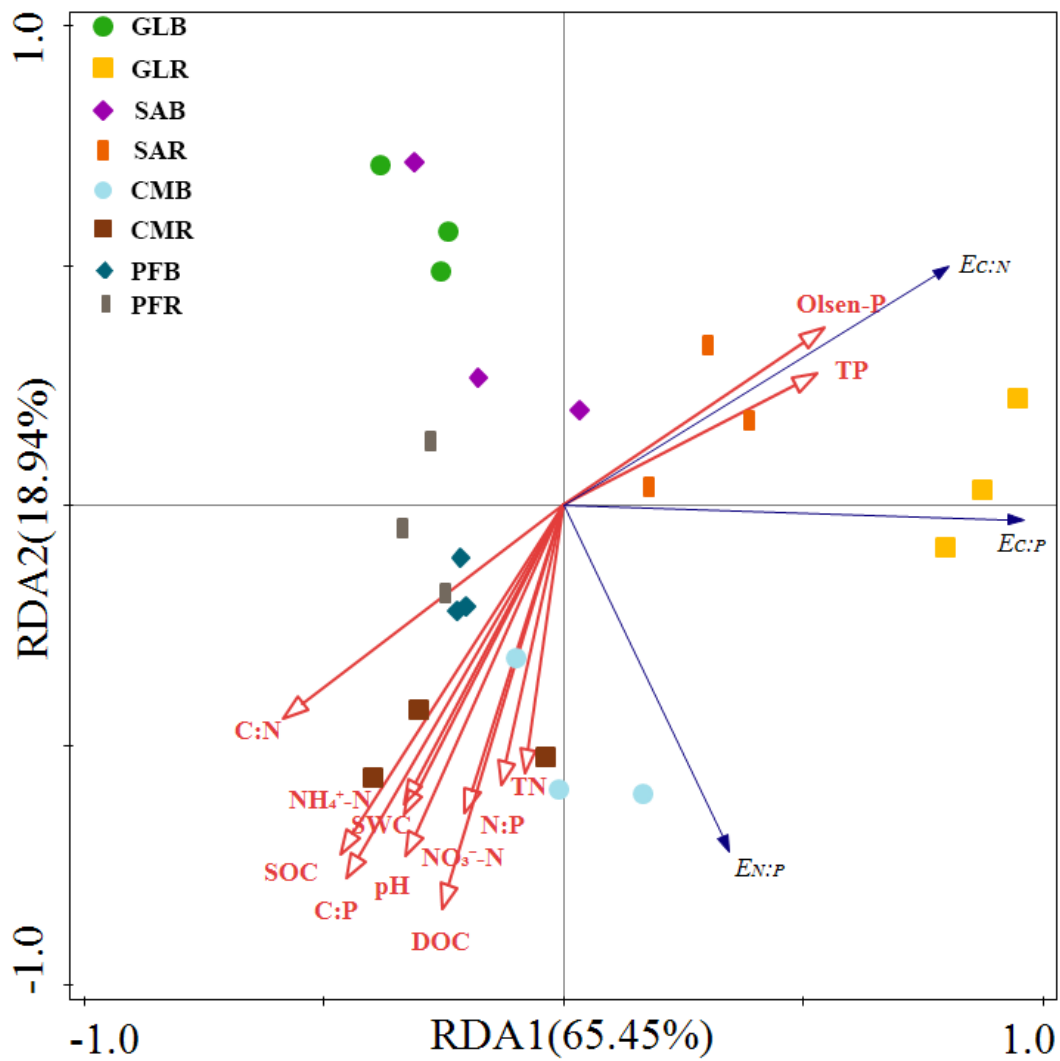


Figure 4

Legend not included with this version

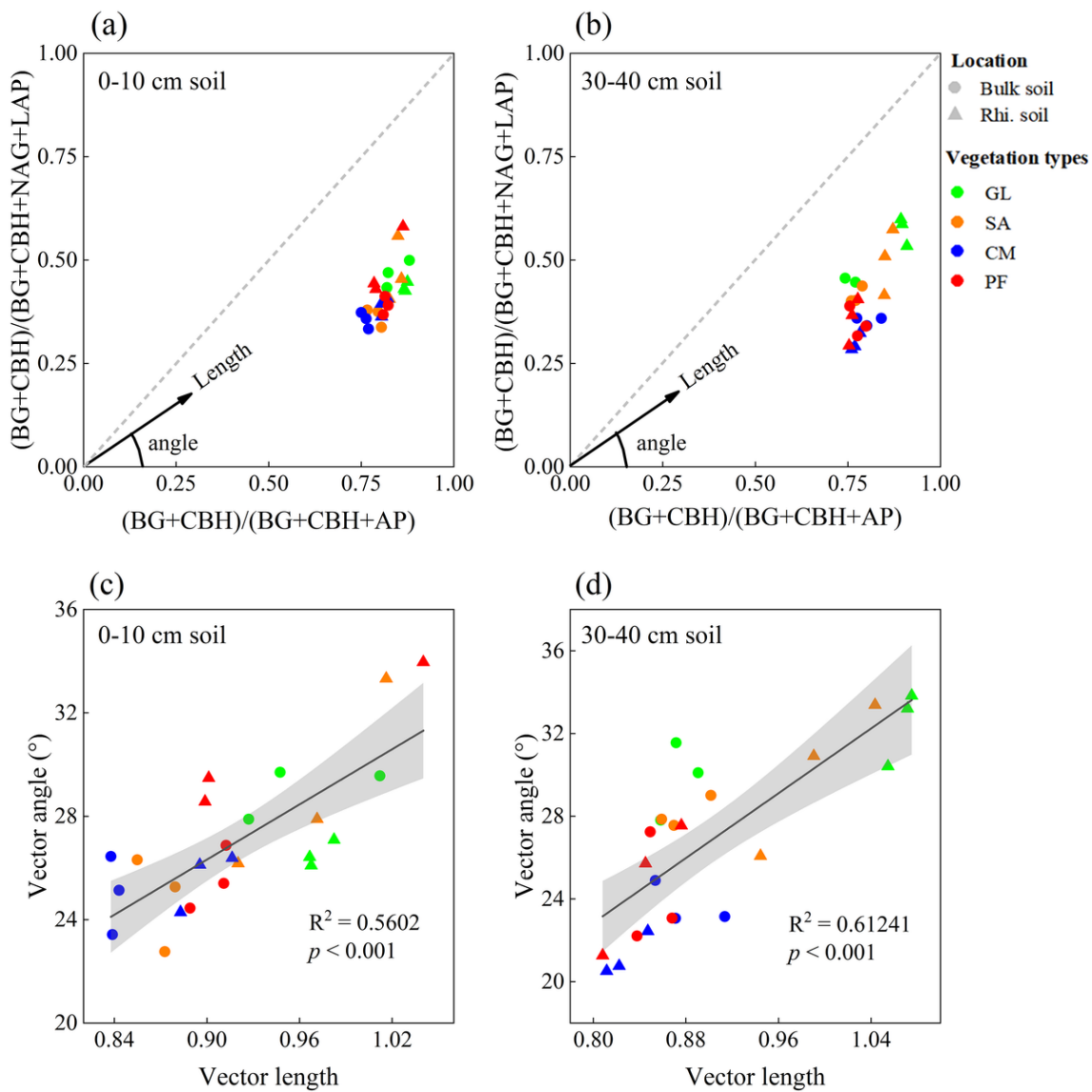


Figure 5

Legend not included with this version

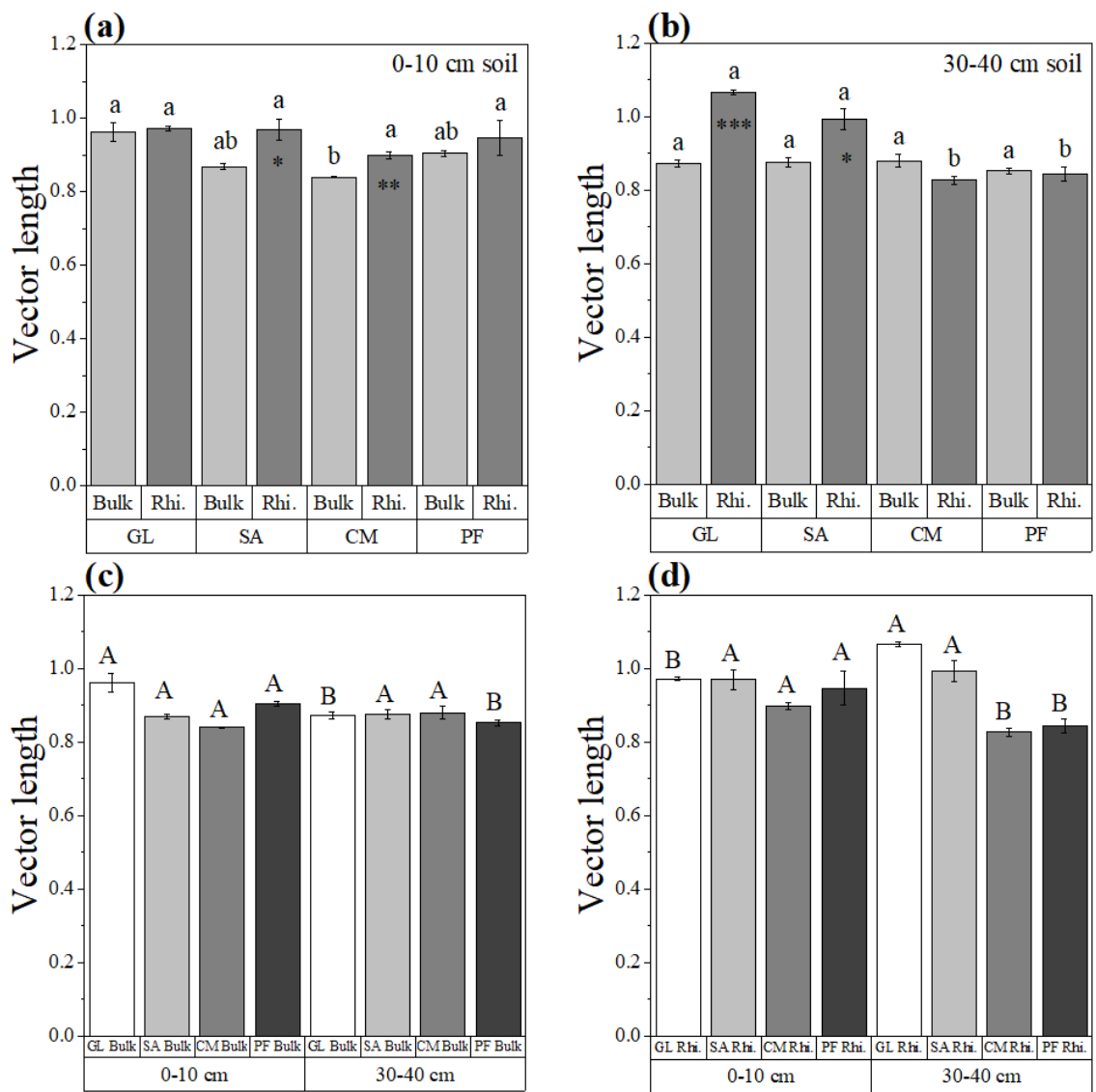


Figure 6

Legend not included with this version

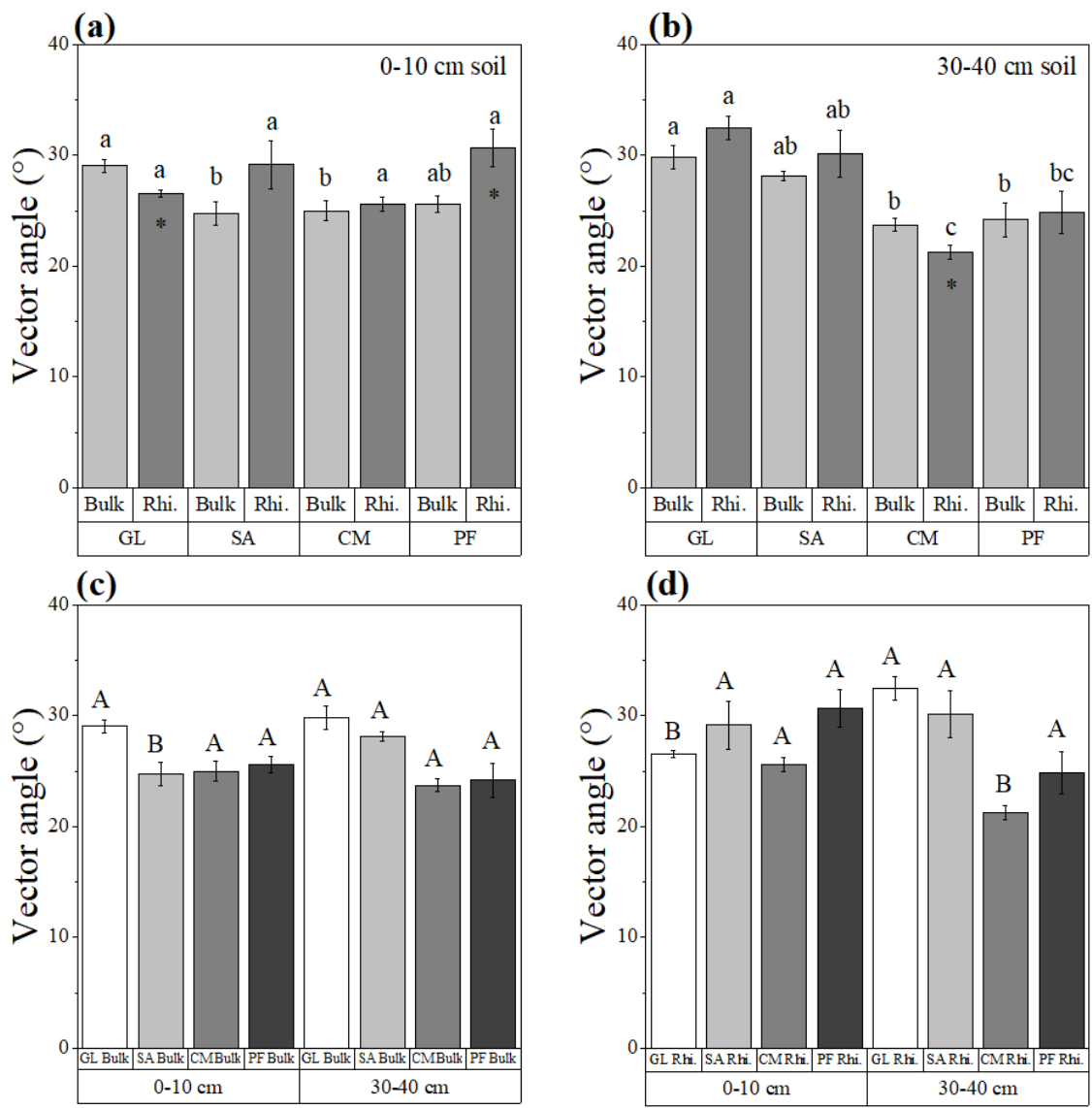


Figure 7

Legend not included with this version

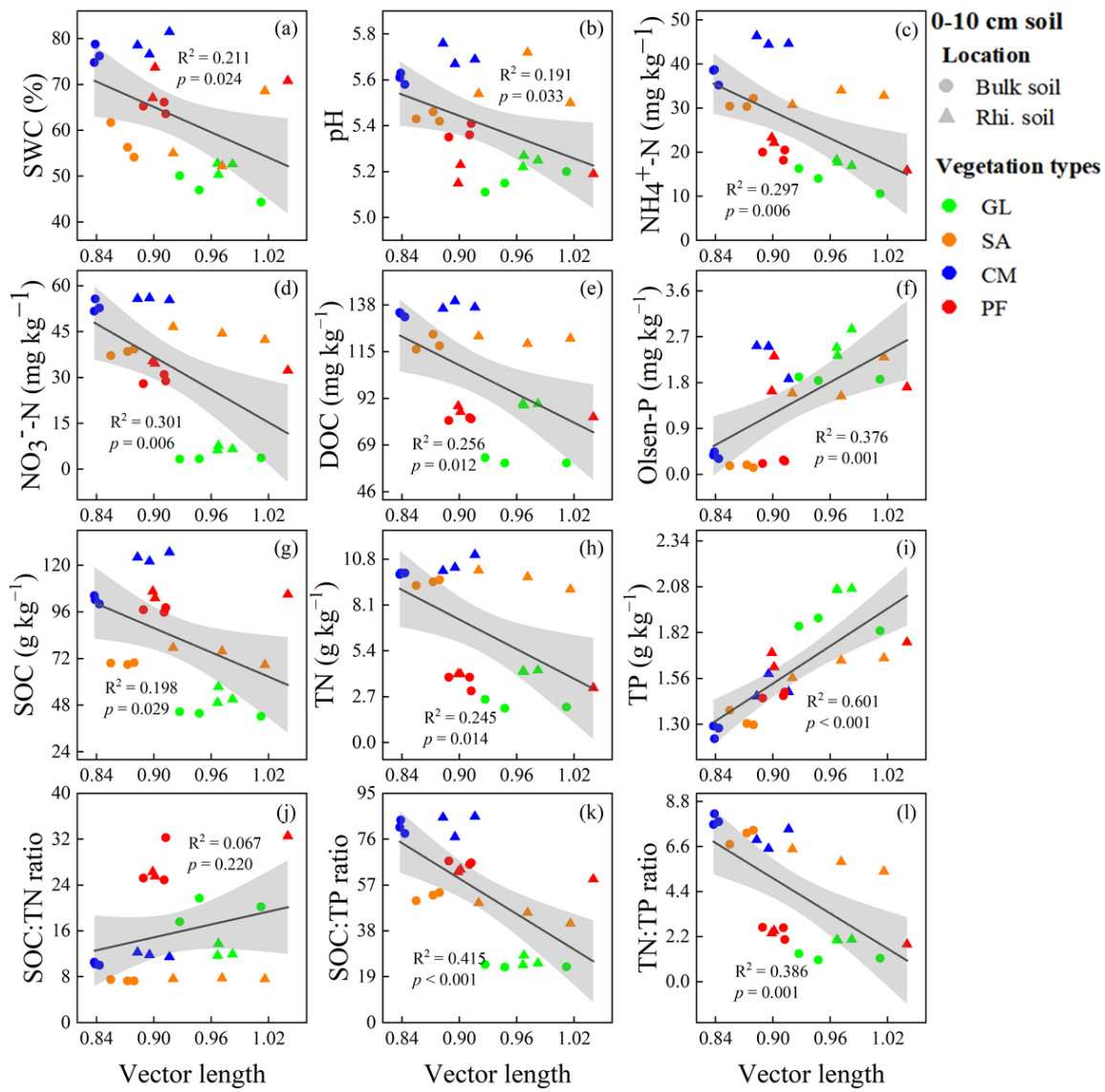


Figure 8

Legend not included with this version

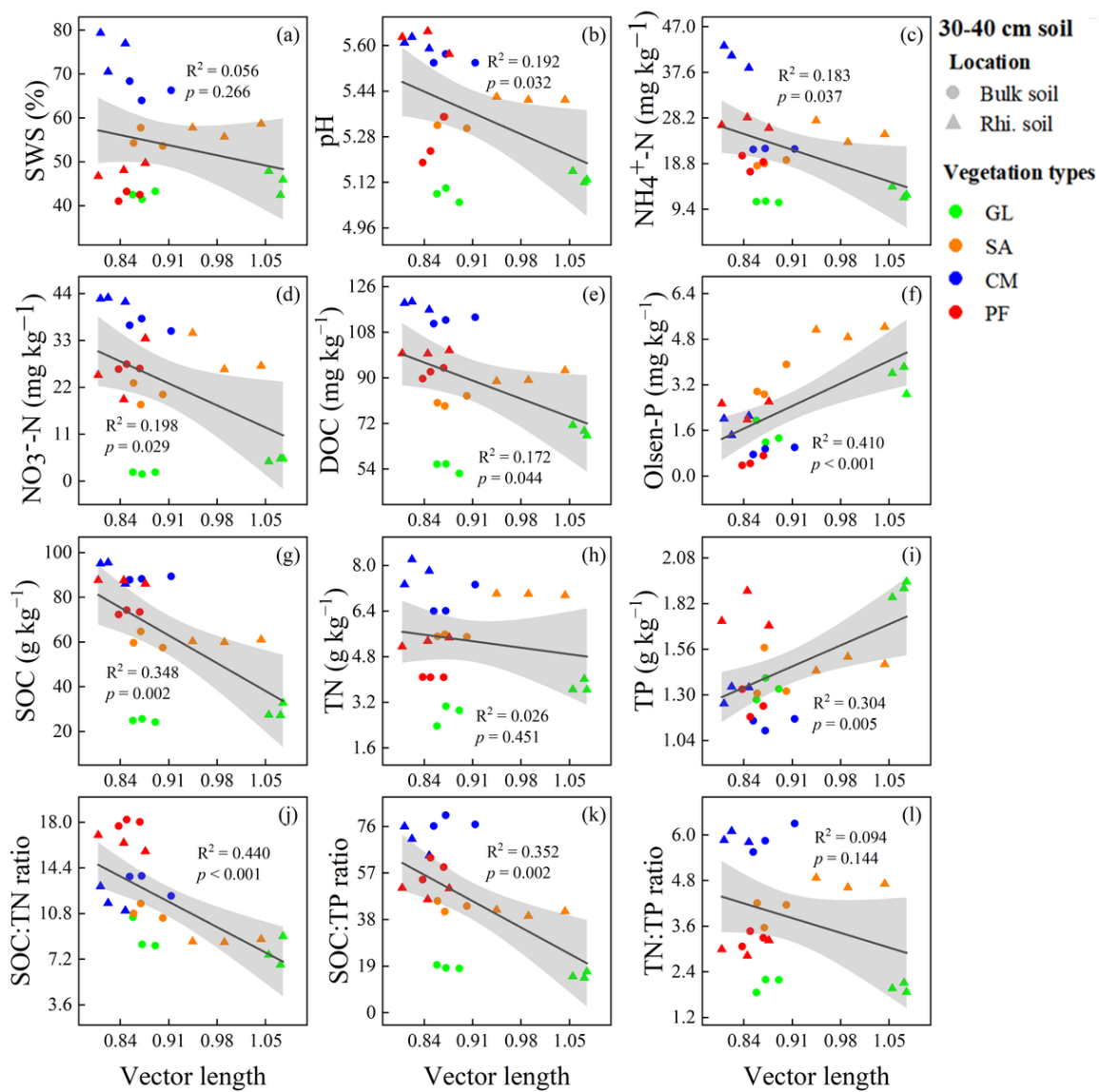


Figure 9

Legend not included with this version

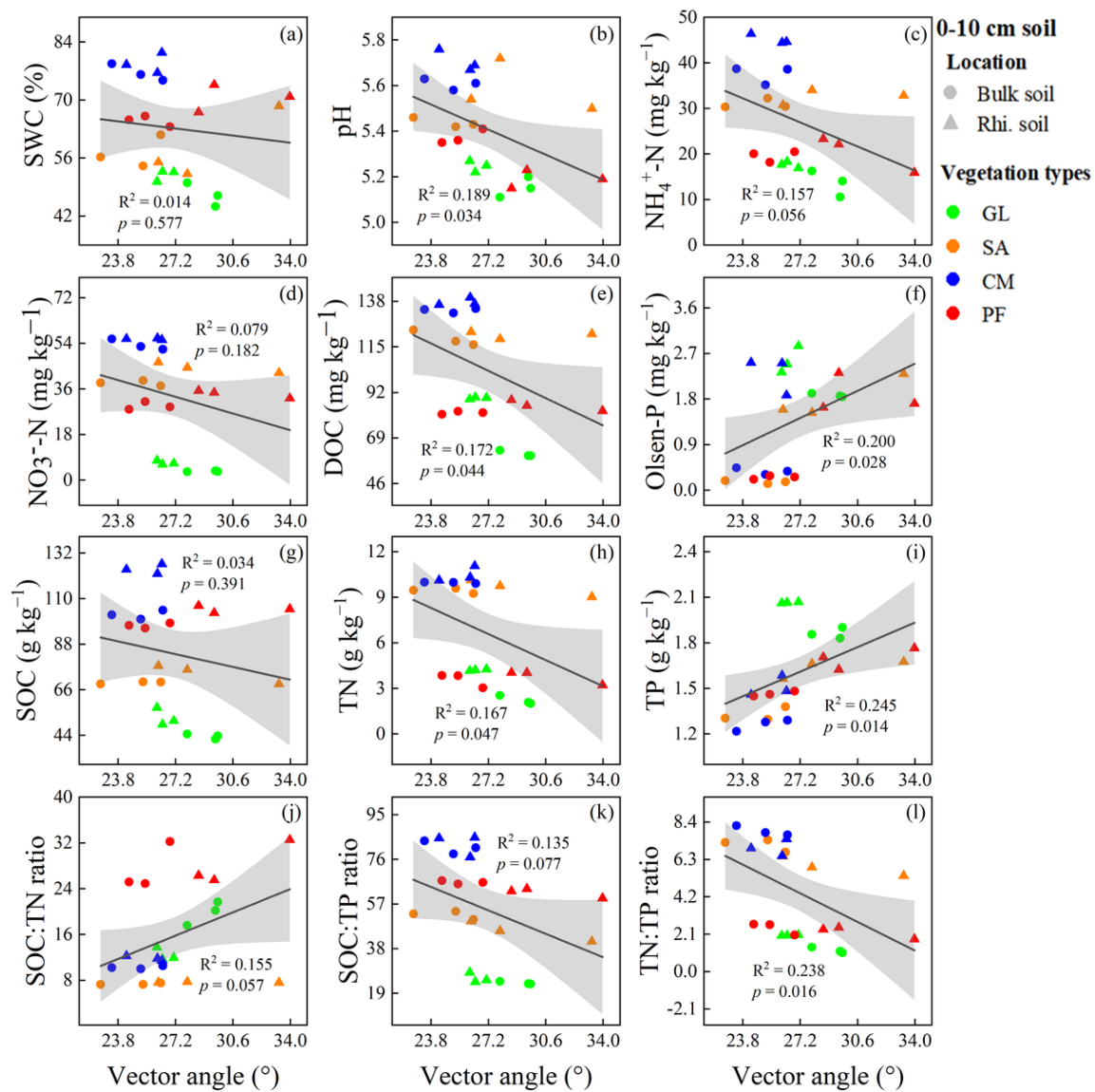


Figure 10

Legend not included with this version

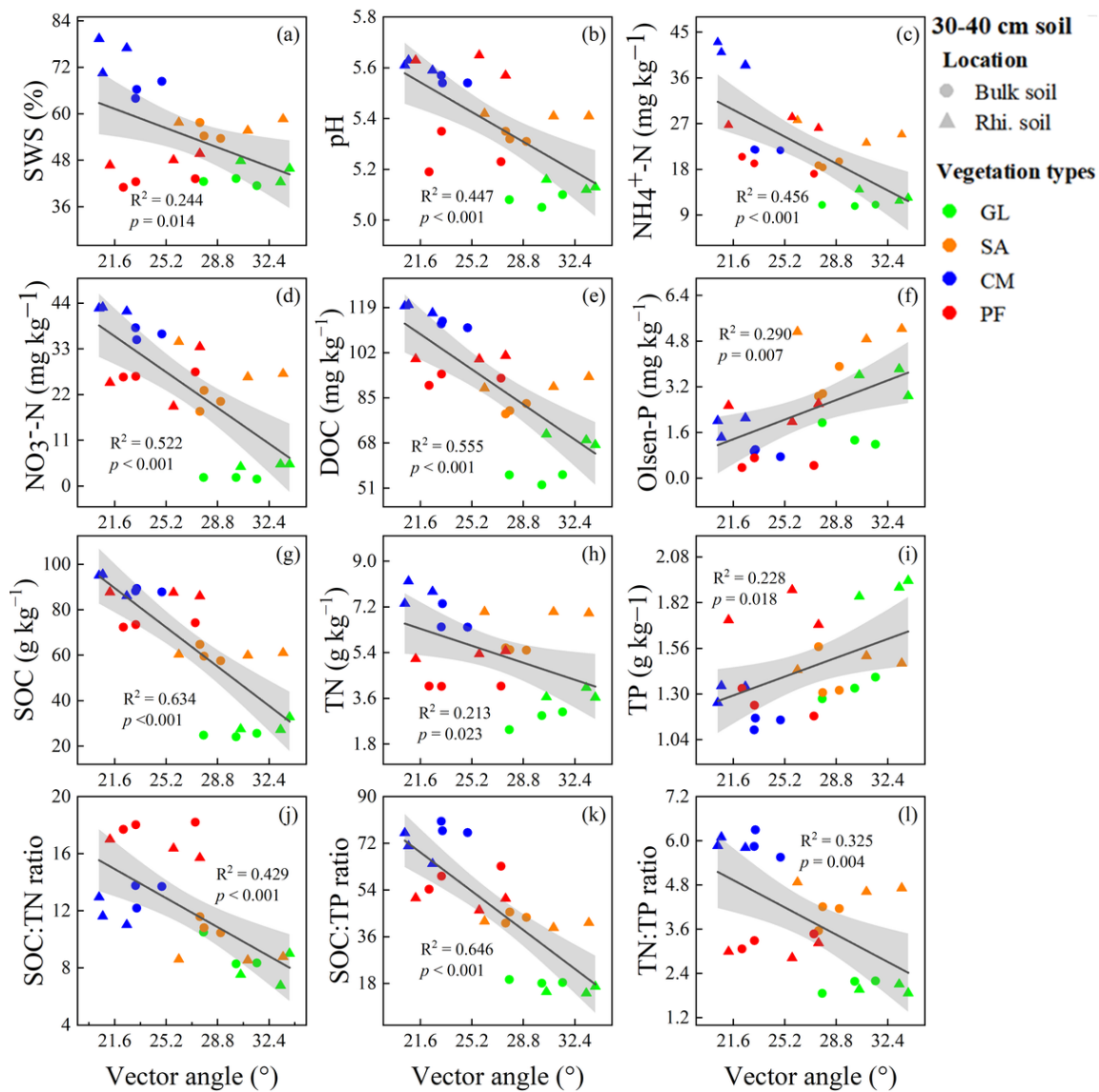


Figure 11

Legend not included with this version

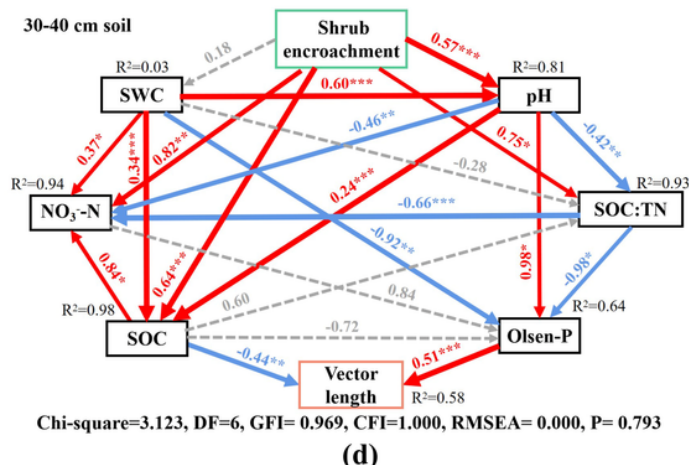
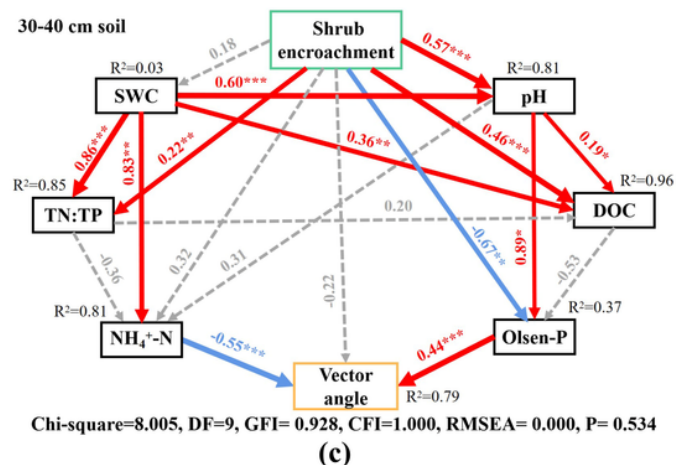
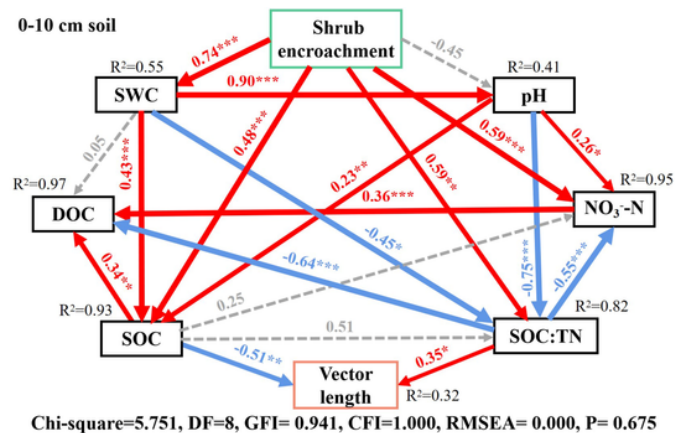
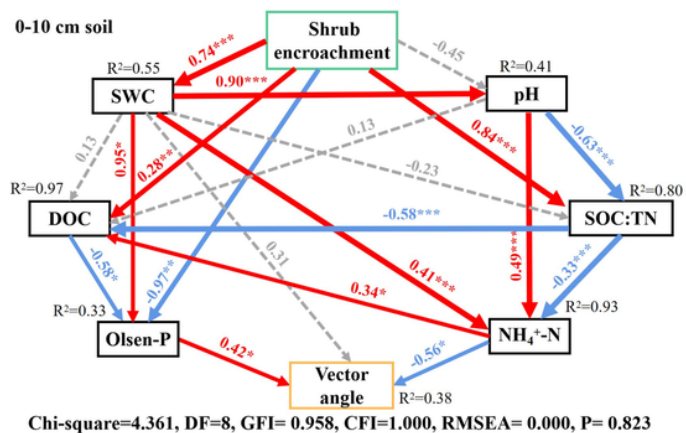


Figure 12

Legend not included with this version