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Response of plant-soil-microbe C–N–P stoichiometry to recovery succession in Sophora moorcroftiana shrubs in the middle reaches of the Yarlung Zangbo River

Ruizhen Dong
Shihai Yang
Xiaoli Wang
LeLe Xie
Yushou Ma (mayushou@sina.com)
Qinghai University https://orcid.org/0000-0003-2672-9222
Yanlong Wang
Litian Zhang
Min Zhang
Jinping Qin

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Abstract

Background and aims Ecological stoichiometry in plants and soils plays a crucial role in biogeochemical cycles, energy flow and functioning in ecosystems. However, the stoichiometric responses of plants and soils along successional stages in alpine desert areas remain unclear.

Methods We illustrated the C:N:P stoichiometry interactions among plants (green leaves, understorey herbs, and litter), soils, and soil microbes across three successional stages (moving dune, MD; semifixed dune, SFD; and fixed dune, FD) of the *Sophora moorcroftiana* shrub community.

Results Recovery succession increased the C:N, C:P, N:P ratios of soils, but decreased the plants. Importantly, among the stoichiometric ratios of soil resources, the C:N ratio decreased with recovery succession. Leaf C:P and N:P ratios in *S. moorcroftiana* were higher than those in the understorey herb biomass, as P concentrations gradually decreased in leaves. Leaf N:P ratio increased with succession and varied from 15.83 to 28.26, suggesting that P was the more restricted element for the growth of the *S. moorcroftiana* population. Furthermore, significant increases in plant and soil C:N and C:P ratios and decreases in soil N:P ratios were observed with shrub age, suggesting that the demand for P became increasingly critical.

Conclusion the stoichiometric ratios of plants and soils formed a complex network. Succession can balance plant and soil C:N:P ratios relationship dependent on the stoichiometry ratios of litter and microbial biomass. These findings are helpful for understanding the nutrient stoichiometry patterns and utilization strategies of C, N and P and scrubland restoration and management in alpine valley sand ecosystems.

Introduction

Carbon (C), nitrogen (N), and phosphorus (P) are three of the key elements for life on Earth (Chen and Chen 2021). Specifically, C provides the structural basis of plants and relatively steadily accounts for 50% of dry plant biomass (Schade et al. 2003; Yang et al. 2018). N is an essential component of proteins and plays an important role in plant production, photosynthesis and decomposition of litter (Daufresne 2004; Chen et al. 2016b; Yang et al. 2018). P is generally considered to be the limiting element responsible for the structure of cells and the composition of DNA and RNA, and P promotes C/N transpiration and assimilation (Tilman 1996; Xiao et al. 2009; Pii et al. 2015). Despite differences in the elemental composition of resource supply, living organisms maintain these elements around specific ratios for their growth and reproduction, commonly referred to as homeostasis (Sterner and Elser 2002; Chen and Chen 2021).

C:N:P ecological stoichiometry focuses on the interaction and balance of chemical elements in ecological processes and is often used to study feedbacks and relationships between above- and belowground components of ecosystems (Mooshammer et al. 2014; Ren et al. 2016; Zhang et al. 2019). Numerous studies have indicated that the C:N:P ratios in plants, soils and soil microorganisms affect primary

production and nutrient cycling and reveal nutrient limits in plants in terrestrial ecosystems (Sterner and Elser 2002; Manzoni et al. 2008; Huang et al. 2013; Chen and Chen 2021). For instance, the relative supplies of nutrients (i.e. soil nitrogen and phosphorus) are a key driver of C:N:P ratios in plants (Plum et al. 2015), and plant C:N:P ratios may be transformed by litter into soil inputs and microbes (Zechmeister-Boltenstern et al. 2015). Researchers have also demonstrated increases in the C:nutrient ratios of plants and associated litter, which in turn may increase the soil microbial biomass C:N and C:P ratios because microorganisms may adjust their biomass C:substrate N:P ratios to suit the litter (Mooshammer et al. 2014; Zechmeister-Boltenstern et al. 2015; Chen and Chen 2021). Thus, the determination of stoichiometric imbalances in the plant–soil–microbe system remains to be clarified (Mooshammer et al. 2014; Zhang et al. 2019). Examples include how plant diversity, successional stage, and environmental factors affect the redistribution of nutrients (C, N, and P) between plants and soils and how relationships between above- and belowground elemental stoichiometry influence terrestrial ecosystem processes (Li et al. 2016; Zhang et al. 2019; Liu et al. 2020; Chen and Chen 2021). Therefore, evaluating the C:N:P stoichiometry of plants, soils, and soil microbial biomass will provide insights into element cycling and promote ecosystem sustainability (Chen and Chen 2021).

Plant succession tends to exhibit relatively rapid and predictable trajectories in terms of species diversity and composition (Foster and Tilman 2000). Previous studies have demonstrated that succession-induced increases in soil nutrients and silt content as well as microbial biomass and activity are associated with increases in plant productivity and diversity (Lozano et al. 2014). However, there are currently insufficient studies to accurately predict the feedback effects of shrub/understorey species interactions (Bai et al. 2018). Understorey herbaceous plants have an overall negative, neutral, and positive effect on shrub growth and reproductive output, showing antagonistic, commensalistic, and facultative mutualistic relationships (Bai et al. 2018), and they play a crucial role in increasing soil nutrient content and influencing nutrient cycling between plants and soil systems (Zhang et al. 2019). In addition, Fan et al. (2015) found that plant C:N:P stoichiometry varies with plant size. For instance, the findings that the C:N, C:P, and N:P ratios in leaves and roots decreased with stand age (Zeng et al. 2016). Moreover, in karst regions, Zhang et al. (2015) demonstrated that the foliar N:P ratios of forest communities remained stable at different successional stages. These studies revealed that the relationship between shrub and understorey species significantly influences shrub growth and that the C:N:P ratios in plants may differ based on successional stage. Therefore, it is very important to understand the biochemical cycle of ecosystems by using stoichiometry theory to clarify the internal relationship between understorey species and shrubs and the relationship between them and soil elements along a successional stage.

The Tibetan Plateau is characterized by excessive land desertification and is one of the most ecologically vulnerable regions (Shen et al. 2012; Liao et al. 2021). As a negative consequence, the degradation of alpine grasslands leads to a reduction in ecosystem services, such as the loss of biodiversity, water erosion and reduced productivity (Du and Gao 2021). In dune habitats, shrub species are the key elements in rehabilitating a degraded ecosystem (Liao et al. 2021), which contributes to shrubs having specific and obvious “morphological, physiological or behaviour” effects in these extreme and harsh

environmental conditions; these adaptations not only strongly affect the substrate stability and sand movement but also the basal soil organic matter (SOM), nutrient contents, salinity and other conditions and affect the activity, quantity and diversity of soil microorganisms (Yu and Steinberger 2012; Wang et al. 2019). In recent years, natural restoration has mainly involved the secondary succession of shrubs and grasslands after the prohibition of overgrazing, extensive reclamation, and excessive firewood collection on the Tibetan Plateau (Liao et al. 2021). However, the ecological effects of the interrelationships among desert shrubs, understorey species, litter, soil and microbes in the succession process from the perspective of C:N:P stoichiometry have rarely been evaluated.

In this study, we determined the dynamics of C, N, and P contents and stoichiometry in plants (including green leaves, understorey species, and litter), soils and microbial biomass from three different successional stages (mobile, semifixed, and fixed dunes) in an *S. moorcroftiana* population. We hypothesized that the C:N:P stoichiometry in the shrub, understorey species, litter, soils and microbes might be affected by the successional stage, and the C, N, and P concentrations and their ratios in the soils, plants and microbes might be highly coupled because they coevolved. In addition, we also predicted that the C:N:P stoichiometry in plants, soils and microbes might respond to shrub age characteristics. Therefore, our objective was to (1) examine the effect of successional stage on C:N:P stoichiometry in plants, soils, and microbes; (2) explore the relationships of C:N:P stoichiometry between plants, soils, and microbes; and (3) determine the response of C:N:P stoichiometry in plants, soils, and microbes to shrub age characteristics.

Materials And Methods

Study area

This study was conducted in the middle reaches of the Yarlung Zangbo River (28°59'–29°26' N, 88°04'–93°18' E) (Fig. 1, Table 1), southern Tibetan Plateau. The terrain of the catchment is higher in the northwest and lower in the southeast, with an altitude of 2900–4550 m, which provides a significant volume of negative topographic conditions for the accumulation of eolian sediments, and eolian deposits (e.g. eolian sand and sandy loess) are widely distributed in the basin. In addition, there are strong winds in the basin during winter and spring. The wind velocity is extremely high at 32.5 m/s, the monthly maximum wind velocity is 4.7 m/s, and the number of windy days is as high as 172 days per year (Shen et al. 2012). The climate in this region is a temperate semihumid and semiarid plateau monsoon climate. The average annual temperature is approximately 6–8 °C, with summer temperatures of >15 °C and winter temperatures below -2 °C, with low total accumulated temperature. The average annual precipitation is 300–600 mm, and the precipitation in the basin is unevenly distributed throughout the year, mainly concentrated from June to September, accounting for 86.6% of the annual precipitation. The soil in this area is dominated by mountainous shrub steppe soil and alpine meadow soil, as well as alpine cold desert soil, subalpine meadow soil, and subalpine steppe soil. The vegetation on the ground is low and sparse, with grassland as the main type. It mainly includes the species *Sophora*

moorcroftiana, *Ceratostigma minus*, *Aristida triseta*, *Orinus thoroldii*, *Pennisetum centrasiaticum*, *Stipa purpurea*, and *Artemisia*.

Experimental design

Field plant and soil sampling was conducted in August 2020. According to the vegetation coverage and degree of soil surface fixation in the Yarlung Zangbo River basin (Shen et al. 2012). We selected three vegetation successional stages, with stages of fixed dune (FD), semifixed dune (SFD), and moving dune (MD), in which *S. moorcroftiana* was the dominant species. The moving dunes are in an early successional stage, which has < 10% vegetation cover. At the second successional stage, the importance value of *S. moorcroftiana* gradually increased, and herbaceous species gradually recovered in the presence of the nurse shrub into the semifixed dune stage, with vegetation cover of 10%–30%. The fixed dunes are in the last successional stage, which had > 30% vegetation cover, no mobile sand. Three independent replicate plots of 10 m × 10 m were randomly set up at each site, with five subplots (1 m × 1 m) in the centre and opposite corners of each plot for the subsequent plant investigation and soil sampling. All the selected sites had similar topographies (flat sandy land) to ensure comparability of the plant and soil samples. Basic information about each successional stage of *S. moorcroftiana* shrubs is presented in Table 1.

Soil and plant sampling

For soil sampling, after removing the litter layer, stones, and biological crusts, nine soil cores (5 cm diameter) were taken at each plot at a depth of 20 cm along an “S” shape under the canopy of the shrub (Fig. 1) and then thoroughly mixed and pooled as one complete soil sample. Each soil sample was sieved through a 2 mm mesh, and visible gravel, roots and plant litter were removed and then subsequently divided into two parts: a portion of the fresh soil from the field was at 4 °C for microbial biomass (carbon, nitrogen, and phosphorus), inorganic N (NH_4^+-N and NO_3 -N) and available phosphorus (AP) analyses, and the other part was air-dried and stored at room temperature for soil physicochemical property analysis.

For in situ plant investigations, five subplots (1 m × 1 m) were used to survey the name, height, coverage, and number of each species to collect litter. After the basic vegetation survey, we chose 8–10 vigorous *S. moorcroftiana* shrubs in each plot, collected samples of fully expanded leaves, and measured the diameter at breast height (DBH), height (SH), and shrub crown size (SCS) to characterize the shrub age class (Pugnaire et al. 1996; Hortal et al. 2013). Additionally, all the plant samples were dried at 105 °C for approximately 15 min to minimize respiration and decomposition. Subsequently, the plant samples were oven-dried to a constant mass at 65 °C. The plant samples were measured as previously described (Higgins et al. 2012). In addition, changes in the plant characteristics and soil properties among different successional stages are presented in Table 2.

Analysis of plant and soil physicochemical properties

Soil bulk density (BD) (0–20 cm) was measured by collecting soil in a steel ring (Volume 100 cm^3), oven drying at 105 °C (± 2 °C) for 24 h, and weighing the dry mass. Soil pH was determined in a 1:5 (soil:water ratio) after 30 min shaking at room temperature. The soil moisture (SM) content was measured by oven drying at 105 °C to constant mass. The organic carbon concentrations of soils and plant samples were assayed by the $K_2Cr_2O_7$ oxidation and $FeSO_4$ titration methods (Huang et al. 2014). Total nitrogen (TN) was determined by Cleverchem 200+ (Chilehaus A, Fischertw iete2-20095 Hamburg, Germany) after digestion with H_2SO_4-H_2O_2. The total phosphorus (TP) concentration was determined with a molybdenum antimony colorimetric method following digestion with H_2SO_4-$HClO_4$. Soil dissolved organic carbon (DOC) was extracted with 0.5 M K_2SO_4 and analysed with vario TOC (Elementar-Straβe1·63505, Langenselbold, Germany). The soil inorganic N concentration was extracted with 1 M KCl for 30 min on a shaker at room temperature, and the filtrates were analysed by Cleverchem 200+ (Chilehaus A, Fischertw iete2-20095 Hamburg, Germany). The AP concentration was measured using the molybdenum blue method (Yang and Liu 2019).

Soil microbial biomassdetermination

Soil microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) were determined by the chloroform fumigation extraction method (Brookes et al. 1985; Vance et al. 1987; Wu et al. 2000). Before analyses, the fresh soil samples were adjusted to 40% water holding capacity (WHC) and then incubated for 7–15 days at 25 °C in the dark. For each soil sample, three 30 g soil subsamples were fumigated with chloroform for 24 hours under dark conditions at 25 °C. At the same time, three replicates of the same amount of soil were collected without fumigation, and then MBC and MBN were extracted with 0.5 M K_2SO_4 (soil solution ratio of 1:2) for 30 min on a shaker. The extracts were measured for total organic C and total N with a TOC analyser (vario TOC, Elementar, Germany). Soil microbial biomass P (MBP) was extracted with 0.5 M $NaHCO_3$ (pH = 8.5, soil solution ratio of 1:20) for 30 min on a shaker. The extractions were determined colorimetrically in a spectrophotometer (Analytikjena, SPECORD 210, Germany) at 880 mm after the $NaHCO_3$ filtrates (25 ml) were digested with 1 ml 33% H_2SO_4 and 4 ml 5% potassium persulfate at 120 °C for 30 min (high temperature, high pressure, humid-heat treatment). Soil microbial biomass C, N and P were estimated as the difference between fumigated and unfumigated soil measurement results and based on extraction measurement efficiency (conversion coefficient k) (k_C = 0.45 for MBC, k_{EN} = 0.45 for MBN, k_P = 0.40 for MBP) (Brookes et al. 1985; Vance et al. 1987; Joergensen and Mueller 1996)

Statistical analysis

Before analysis, all data were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Levene's test), and expressed as the mean ± standard error (SE). Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) and least significant difference (LSD) tests were used to examine differences in plant characteristics (leaf, understorey herb biomass and litter C:N:P stoichiometry, DBH, SCS, H, SH, understorey grass coverage,

and *S. moorcroftiana* coverage) and soil properties (BD, SM, DOC, AP, and inorganic N) among the three successional stages. Pearson's correlation was used to investigate the stoichiometric relationships among plants, soils, and microbial biomass. In addition, Structural equation modelling (SEM) was used to determine the effects of successional stage on the plant-soil-microbe C–N–P stoichiometry. The final variables used in SEM were comprehensively determined based on Pearson's correlation analysis and PCA ordinations. We tested the fit of the models using the χ^2 goodness of fit test and the RMSEA test. The SEM analyses were performed using AMOS version 24.0 software (IBM SPSS Inc.) and redundancy analysis (RDA) was conducted using CANOCO 5.0 to evaluate the associations of the C, N, and P concentrations between soil and leaf, understorey herb biomass, litter, and soil microbes. Origin 2021 software (Origin Lab Inc., Northampton, USA) plotting. $P < 0.05$ was considered statistically significant.

Results

Vegetation composition of the *Sophora moorcroftiana* shrub community

The climax of the succession of sandy vegetation in the middle reaches of the Yarlung Zangbo River is the shrub community dominated by *S. moorcroftiana* (Table 1). *P. centrasiaticum*, an important companion species, was distributed in all three successional stages, but *Agriophyllum squarrosum* was distributed only in the SFD and MD stages. In the SFD and MD stages, *P. centrasiaticum* and *A. wellbyi* were the main companion species. In the FD stages, a number of new species, for instance, *Thermopsis fabacea*, *Setaria viridis*, *Erodium stephanianum*, and *Achnatherum splendens*, emerged, with *P. centrasiaticum*, *Arisaema flavum*, *A. wellbyi*, *O. thoroldii*, and *Artemisia stechmanniana* as the main companion species.

C:N:P stoichiometry in leaves, understorey herb biomass and litter

As shown in Fig. 2, the C concentrations in leaves, understorey herb biomass and litter were not significantly different among the three successional stages, varying from 473.62 to 491.28 g kg^{-1}, from 402.57 to 424.34 and from 306.49 to 364.79 g kg^{-1}, respectively ($P > 0.05$). The N concentrations of leaves varied from 22.55 to 22.99 g kg^{-1}, and the N and P concentrations had the same variation tendency in the understorey herb biomass and litter, which were ranked in the following order: the FD stages were significantly higher than those in the SFD and MD stages ($P < 0.05$). Additionally, the P concentrations in leaves in the SFD stage were significantly higher than those in the FD and MD stages ($P < 0.05$), whereas the P concentrations in the understorey herb biomass and litter were highest in FD among the three successional stages, with the order SFD > MD > FD.

The C:N ratio in leaves and the N:P ratio in understorey herb biomass did not differ significantly among the three successional stages ($P > 0.05$) (Fig. 2d and f), while the C:N ratio in understorey herb biomass and litter in the FD stage was significantly lower than that in the other stages ($P < 0.05$) (Fig. 2d). The C:P ratio in understorey herb biomass and litter were highest in MD, the C:P ratio varied from 351.31.50 to 507.82, from 656.53 to 1551.78, respectively, and the C:P ratio showed the order MD > SFD > FD ($P <$

0.05) (Fig. 2e). In addition, the C:P and N:P ratios in leaves were significantly lower in SGL stage than that in the other successional stages ($P < 0.05$) (Fig. 2e and f). However, the N:P ratio in litter in MD stage were significantly higher than that in FD stage ($P < 0.05$) (Fig. 2f).

C:N:P stoichiometry in soil and microbial biomass

The successional stage had a significant effect on C, N and P concentrations and ratios in soil and microbial biomass; however, it did not affect soil C:P ratio. Fig. 3 shows that the C, N and P concentrations in the soil and microbial biomass tended to increase with recovery succession stage ($P < 0.05$), and were significantly higher in the FD stage than in MD stage (Fig. 3a-f).

The soil C:N ratios in SFD and MD stages were significantly higher than those in FD ($P < 0.05$). The soil N:P ratio showed opposite trends as the soil C:N ratio ($P < 0.05$). The MBN:MBP ratio ranged, with the highest in SGL stage. The microbial biomass C:N, C:P and N:P ratios ranged from 8.19 to 14.45, from 19.49 to 45.28 and from 2.18 to 3.18 and were significantly higher in FD than in SFD and MD stages ($P < 0.05$).

Correlations among soil, microbial biomass, and plant C:N:P ecological stoichiometry

The C, N, and P concentrations of plant components and microbial biomass and their stoichiometries responded differently to soil C, N, and P concentrations and ratios (Fig. 4 and S1, Table 3). The Soil C concentrations were positively correlated with the C, N, and P concentrations of understorey herb biomass, litter, and microbial biomass and C concentration of litter ($P < 0.05$). Soil TN concentrations were positively correlated with N and P concentrations in understorey herb biomass, litter, and microbial biomass and C concentrations in understorey herb biomass and microbial biomass and negatively correlated with C concentration in leaves ($P < 0.05$). Soil P concentrations were positively correlated with C concentrations in leaves and microbial biomass and N and P concentrations in understorey herb biomass ($P < 0.05$). The C, N, and P concentrations of microbial biomass were positively correlated with The C, N, and P concentrations of understorey herb biomass and litter. There was no significant correlation between the C, N, and P concentrations in leaves and the C, N, and P concentrations in soil (Fig. 4 and S1).

Leaf C:N ratio was significantly correlated with soil C:N and N:P ratios. Understorey herb biomass C:P and N:P ratios were significantly correlated with soil C:P and N:P ratios. Litter C:N, C:P and N:P ratios were significantly correlated with soil C:N and N:P ratios. Microbial biomass C:N and C:P ratios were significantly correlated with soil C:N and N:P ratios (Table 3). Additionally, the simple linear relationships among the C:N:P ecological stoichiometry in leaves, understorey herb biomass, litter, microbial biomass, and soil are shown in Fig. 5. The relationships between the C:N and N:P ratios in soil were significant and negative linear relationships, and C:N and N:P ratios in litter and the C:P and N:P ratios in leaves, understorey herb biomass, litter, microbial biomass and soil were significant positive linear relationships.

The SEMs showed that leaf N:P ratio may have indirectly affected soil N:P ratio by affecting microbial biomass C:N ratio or by affecting litter and microbial biomass C:N ratio (Fig. 5). Soil N:P ratio had a direct negative effect on microbial biomass C:P ratio. Understorey herb biomass N:P ratio had a direct negative effect on soil C:N and N:P ratios and might also have indirectly affected soil N:P ratio by affecting litter and microbial biomass C:N ratio. Understorey herb biomass N:P ratio had a direct positive effect on leaf N:P ratio, whereas soil C:N ratio had a direct negative effect on soil N:P ratio. The SEMs also indicated that succession might have indirectly affected soil N:P and microbial biomass C:P ratios by affecting the C:N ratio in soil, litter and microbial biomass.

Effect of shrub age on plant and soil C:N:P ecological stoichiometry

Shrub age characteristics (diameter at breast height, height and shrub crown size) showed separate responses to soil and leaf C:N:P stoichiometry (Fig. 6). *S. moorcroftiana* shrub height was significantly negatively correlated with the C:N and C:P ratios of understorey herb biomass (Fig. 6a and c), whereas it was significantly positively correlated with the C:N ratio of leaves (Fig. 6a). In addition, both the diameter at breast height and shrub crown size were positively related to the C:N and C:P ratios of litter and the N:P ratio of litter (Fig. 6d and l, g-i). Conversely, both the diameter at breast height and shrub crown size were significantly negatively correlated with soil N:P (Fig. 6° and r), whereas both of them were significantly positively correlated with soil C:N ratio (Fig. 6m and n). Shrub crown size was significantly negatively correlated with and microbial biomass C:N and C:P ratios (Fig. 6m and n), and there was no correlation between the soil C:N:P ratio and shrub height (Fig. 6j-l). In short, compared to plants, the C:N:P ratio in soil was significantly correlated with the shrub crown size and diameter at breast height.

Discussion

C:N:P stoichiometry in plants among the different successional stages

Nitrogen and phosphorus are the most common limiting nutrient elements of plant growth, and play important roles in controlling plant growth, reproduction and metabolism in most terrestrial ecosystems (Chapin 1980; Reich et al. 1997; Han et al. 2005). Subsequent studies have revealed that the leaf N:P ratio in plant biomass gives an indicator of the relative N or P limitation at the community level, whereas the shifts in limitation lead to changes in plant functional traits, vegetation composition and botanical diversity (Koerselman and Meuleman 1996; Roem and Berendse 2000; Güsewell 2004). For N and P, the “law of the minimum” states that there is a “critical N:P ratio” below which growing plants are limited only by N and conversely above which growing plants are limited only by P (Güsewell 2004). According to a wide report, N:P ratios > 16 often indicate P limitation, whereas N:P ratios < 14 signify N limitation, and with intermediate values, plant growth is colimited by N and P (Aerts and Chapin 2000; Reich 2005; Fan et al. 2015). Güsewell (2004) found that when the N:P ratio in leaves was < 10, the plant was relatively restricted by nitrogen, whereas when the N:P ratio was > 20, the plant was relatively limited by phosphorus, and when the N:P ratio was between 10 and 20, the plant growth was N- or P-limited according to plant-specific conditions.

In our study, the leaf N:P ratio ranged from 18.53 to 28.26 (Fig. 2), and the mean foliar N:P ratio was 25.70, higher than the value reported by Han et al. (2005) (average N:P ratio of China's flora = 16.3) and the global averages (12.7 and 13.8) (Reich and Oleksyn 2004; Elser et al. 2000). The leaf N concentrations varied from 22.55 to 22.99 g kg^{-1}, and the P concentrations > 1.0 mg g^{-1} varied from 0.95 to 1.33 g kg^{-1} (Fig. 2), suggesting that P was the more restricted element for the *S. moorcroftiana* population in the study area. This finding supports the view that Chinese vegetation probably reflects a greater degree of P limitation by measuring 753 species (Han et al. 2005). A potential cause for such high leaf N:P ratios (low P content) may be the low P content of soils in China (Han et al. 2005). Plant and soil phosphorus are generally coupled at the ecosystem scale (Aerts and Chapin 2000; Han et al. 2005). Another possibility for this P limitation is that leguminous plants can fix N from the air and cause a higher N:P ratio (Yang et al. 2018), whereas P is a 'rock-derived' element and cannot be obtained largely from the soil (Walker and Syers 1976). Simultaneously, recent studies have also shown that understorey species are more able to obtain P from the soil than dominant tree species (Huang et al. 2013), resulting in P limitation in overstory trees (Zhang et al. 2019). Our results also indicated that understorey plants were limited by P and had a higher P content than leaves (Fig. 2c). A possible explanation for this observation is that understorey plants can preferentially obtain phosphorus returned by litter (Zhang et al. 2019).

Litter is a nutrient pool (Xiao et al. 2015; Liu and Wang 2021), and its C:N:P stoichiometry ratio affects the nutrient soil availability and quality in terrestrial ecosystems (Villalobos-Vega et al. 2011; Zhang et al. 2017). Previous studies showed that when the C:N ratio in litter was < 40, the litter began to undergo mineralized decomposition and the net release of nitrogen, and the lower the ratio of C:N and C:P was, the faster the decomposition rate (Parton et al. 2007). In our study, the C:N ratio in litter was lower than 40, indicating that decomposition mineralization and the release of nitrogen from litter were relatively fast. In addition, the N:P ratio is a vital indicator restricting the decomposition of litter, and the lower the ratio is, the more beneficial to the decomposition of litter (Sun et al. 2019). Our results showed that the N:P ratio in litter ranged from 29.94 to 46.07 and was much larger than 25 (Güsewell and Verhoeven 2006), also indirectly demonstrating the nutrient limitations of *S. moorcroftiana* shrub community growth in the study area.

C:N:P stoichiometry in soils and soil microbes among the different successional stages

Soil C:N:P ratios generally reflect the stoichiometric ratio of their sources (i.e. plant and microbial residues) (Chen et al. 2021). Our study indicated that the C:N:P stoichiometry in soil varied significantly and that the C:N ratio and N:P ratio varied in an opposite pattern among the three successional stages (Fig. 3). The large differences in soil C:N and N:P ratios can be ascribed to the varied plant characteristics and soil properties (Table S1). In addition, the average soil C:N ratio (34.12) was higher than both the value reported by Han et al. (2005) (average C:N ratio of China's soil varied from 10.1 to 12.1) and the global average ratio (12.2) (McGroddy et al. 2004; Tian et al. 2010; Chen and Chen 2021). The soil C:N ratio is an important indicator of microbial activity, which determines the mineralization rate of SOM and the release of available N and can directly regulate the competition between microorganisms and plants

for N and indirectly affect plant photosynthesis (Brust 2019; Qi et al. 2022). In our study, the soil C:N ratio of the SFD and MD stages was significantly higher than that of the FD stage (Fig. 3g), suggesting that microbial decomposition and mineralization were faster in the FD stage (Qi et al. 2022). The average soil C:P ratio was 77.34, which is higher than the average ratio in China (61.0), and the N:P ratio (3.78) is lower than the global average ratio (5.9) (Han et al. 2005; Yang et al. 2018), indicating that the soil N (C:N = 36.8) and P (C:P = 62.5) (Han et al. 2005) contents are relatively poor sites. This is possibly due to fine particles containing nutrient components (such as carbon, nitrogen and phosphorus) being eroded and transported by suspension during aeolian processes, resulting in surface soils with decreased nutrients (Alfaro et al. 1998; Larney et al. 1998; Wang et al. 2016).

Soil microbes are key players in biogeochemical cycles (Mooshammer et al. 2014), and microbial biomass acts as a key indicator of soil health (Liu et al. 2020). In general, soil microbes can maintain stoichiometric homeostasis through compensatory regulation (Chen and Chen 2021). The soil microbial biomass C:N:P stoichiometry was 15:1.2:1 in this study, which was narrower than shrub-land (43:6:1), desert (31:4:1) and the global average (42:6:1) (Xu et al. 2013). This large discrepancy is largely because of the lower SOC content in deserts (Wang et al. 2022); consequently, the stoichiometric variability decreases with soil OM processing (Mooshammer et al. 2014). A meta-analysis from Xu et al. (2013) reported that microbial biomass C:N ratios averaged 7.6 and ranged from 4.5 to 12.5, and microbial N:P ratios averaged 5.6, ranging from 3.5 to 10.6. The soil samples at our study site had a significantly higher microbial biomass C:N (13.4) and a lower N:P (2.9) ratio (Fig. 3g and i), largely due to relatively lower N availability to soil microbes. A greater microbial biomass C:N ratio suggests a lower level of N in the soil and its mineralization rate due to a lack of sufficient detrital material and the desired type of microbial population (Arunachalam et al. 2002). Furthermore, a higher microbial biomass C:N ratio also indicates that fungi dominate the microbial biomass (Anderson et al. 1980). The microbial biomass C:P ratios averaged at the 24 sites were 35.9 (Fig. 3h); however, they were well within the range (24.3–275.0) reported by Xu et al. (2013).

The foliar N:P ratio may provide a reliable index of nutrient limitation in terrestrial ecosystems; however, it varies widely between individual plant species. (Cleveland and Liptzin 2007). Therefore, a meta-analysis from Cleveland and Liptzin (2007) suggested that a more complete understanding of nutrient limitation within ecosystems requires measuring not only N:P ratios in plants but also assessing N:P ratios in soil and the soil microbial biomass. The lower N:P ratio of soil microbes may reflect higher P requirements of soil bacteria relative to marine algae (Cleveland and Liptzin 2007). The relatively higher microbial biomass N:P ratios suggest that P limits soil microbial biomass and activity (Cleveland et al. 2002; Cleveland and Townsend 2006). Nevertheless, Xu et al. (2013) argued that the soil microbial biomass N:P ratio might not be a more appropriate indicator for ecosystem nutrient limitation. According to the study of Paul (2007), in contrast to plants, soil microbes can thrive in phosphorus-deficient conditions compared to plants. Moreover, when phosphorus availability in ecosystems decreases, relatively more phosphorus is stored in soil microbial biomass, which enhances phosphorus limitation by plants, resulting in relatively lower microbial biomass N:P ratios and severe P limitation in plants (Xu et al. 2013).

Microbial biomass is the most active nutrient pool in soils (Yan et al. 2021). A recent global-scale analysis showed that soil organic carbon is the main factor affecting microbial biomass C, N, and P in the top 0–30 cm soil depth (Wang et al. 2022). In agreement with this, we observed significantly positive correlations between the microbial biomass C, N, and P and SOC (Table S1). Soil microbial biomass C is usually positively correlated with microbial biomass N, as higher microbial biomass leads to higher microbial N requirements (Deng et al. 2016; Fig. 4). Schleuss et al. (2021) indicated that the soil microbial biomass C:N ratio was not affected by nutrient (N and P) additions, demonstrating that microbial communities adjust the acquisition, absorption, turnover, distribution and release of elements according to their stoichiometric needs, which allows soil microorganisms to maintain their biomass stoichiometry even when the ratio of bioavailable elements in their environment changes (Zechmeister-Boltenstern et al. 2015; Spohn, 2016; Schleuss et al. 2021).

Correlations with the C:N:P stoichiometry

In this study, we found that plant C and N concentrations were closely related to soil C and N concentrations but weakly correlated with soil P concentrations (Fig. 4, Table S1), suggesting that the C and N contents in understorey herb plants and litter have a strong coupling relationship with soil nutrients. This interaction may be because the soil C content can be determined by the coefficient of humus and organic matter entering the soil, while the N in decomposed litter is mainly returned to the soil, and P is mainly retained in the litter (Robbins et al. 2019). In addition, the soil C:N ratio was closely related to the C:N ratios in leaves, litter and microbial biomass (Table 3). On the one hand, the litter in the surface layer can act as the main input to the soil, leading to the accumulation of C and N (Yang and Liu 2019). On the other hand, nitrogen-fixing plants are good for absorbing N and SOC (Li et al. 2014). The effects of diversity on plant C:N:P ratios may converted into soil nutrients inputs and microorganisms through litter (Zechmeister-Boltenstern et al. 2015; Chen and Chen 2021). In the present research, plants N:P ratio may have indirectly affected soil N:P and microbial biomass C:N:P ratios by affecting litter C:N ratio, and succession may have indirectly affected soil N:P and microbial biomass C:N:P ratios by affecting the litter C:N ratio (Fig. 7). Soil C:N:P ratios usually reflect stoichiometric ratios of their sources: plant and microbial residues (Chen and Chen 2021) (Fig. 7). By contrast, higher litter inputs could increase the soil nutrient retention, resulting in soil microbial C: nutrient reduction (Chen et al. 2021). Moreover, the N:P and C:P ratios in the plants and soil presented a significantly positive linear relationship ($P < 0.001$) (Fig. 5), which agrees with previous studies (Yang et al. 2018). A previous study also reported that in P-poor systems, slow-growing shrubs transfer P from deeper soil layers to shallow soils, which helps meet the high leaf P needs of fast-growing understorey herbs (He et al. 2014; Luo et al. 2021); as such, the leaf P content was closely correlated with the understorey herb biomass P content and the C:P and N:P ratios (Table S1), indicating that there is a coupling relationship between the P levels of the plants and soil.

Forest stand age had distinct influences on the C:N:P stoichiometry in plants and soil (Fan et al. 2015; Liu et al. 2020). In this study, there was an obvious pattern of strong negative relationships between the understorey herb biomass C:P ratio and SH and between the soil N:P ratio and SCS and DBH (Fig. 6c, o,

and r). A possible explanation was that the roots of understorey herbs are mainly distributed in the upper soil layer, and therefore, understorey plants can preferentially absorb P returned by litter from the soil (Ren et al. 2017; Zhang et al. 2019). As the afforestation age increases, the roots of *Robinia pseudoacacia* (RP) are distributed deeper into the soil (Jiao et al. 2016). As a result, Chen et al. (2016a) reported that deep-rooted systems of RP cannot preferentially access P and other resources returned by plants, resulting in a higher N:P ratio in leaves. Our results are also consistent with this finding. In addition, in arid regions, insufficient rainfall events and high evaporation limit the infiltration of N and P into the soil (Zhang et al. 2018), thereby causing the most serious restriction on the uptake and cycling of plant nutrients (Luo et al. 2021), which in turn leads to a negative correlation between shrub age and the N:P ratio.

Conclusion

We observed that soil C, N and P increase along a recovery succession stage, which leads to changes in soil stoichiometry. The leaf C:P and N:P ratios in *S. moorcroftiana* were higher than those in the understorey plants because of the low P concentrations in *S. moorcroftiana* leaves. We demonstrate that

variations in the effects of succession on plant, soil and microbial biomass C:N:P ratios can be explained by the respective stoichiometric ratios of litter C, N, and P. Furthermore, significant increases in plant and soil C:N and C:P ratios and decreases in soil N:P ratios were observed with shrub age, suggesting that the demand for P became increasingly critical. The N:P threshold theory indicates that P is the limiting element for the *S. moorcroftiana* population in the study region. In total, these findings provide important references for understanding nutrient stoichiometry patterns and management strategies of plants and soils in alpine desert ecosystems on the Tibetan Plateau.

Declarations

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Declaration of competing interest

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

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Tables

Table 1 Main geographic characteristics and floristic compositions of the *Sophora moorcroftiana* shrubs sampling sites among the middle reaches of the Yarlung Zangbo River basin.

Sites	Longitude (E)	Latitude (N)	Elevation (m)	Main companion species	Minor species
FD1	88°31'54.74"	29°11'00.39"	3957	*P. centrasiaticum*	*Poaannua*, *A. membranaceus*
FD2	88°38'04.54"	29°09'11.07"	3964	-	-
FD3	89°20'30.35"	29°05'17.94"	3886	*P. centrasiaticum*	*A. membranaceus*
FD4	87°27'38.31"	29°06'09.21"	3998	*P. centrasiaticum*	*T. fabacea*
FD5	88°09'27.16"	29°21'14.97"	3886	*P. centrasiaticum*	*A. membranaceus*, *A. scoparia*
FD6	89°23'52.30"	29°22'09.29"	3806	*A. flavum*	*P. centrasiaticum*, *D. schraderiana*, *A. scoparia*, *O. thoroldii*
FD7	89°35'44.24"	28°55'46.39"	4019	*P. centrasiaticum*	*A. stechmanniana*, *A. wellbyi*, *A. membranaceus*
FD8	89°45'24.52"	29°18'15.28"	3745	-	*P. centrasiaticum*, *A. flavum*, *A. scoparia*, *S. viridis*
FD9	90°10'39.67"	29°26'16.11"	3785	*P. centrasiaticum*	*A. wellbyi*
FD10	90°50'54.31"	29°16'51.66"	3543	*P. centrasiaticum*	*A. membranaceus*, *T. terrestris E. stephanianum*,
FD11	90°54'15.75"	29°20'00.87"	3539	*P. centrasiaticum*	*A. wellbyi*, *H. gouldii*, *O. sericopetala*
FD12	91°21'19.16"	29°18'24.35"	3534	*A. wellbyi*	*P. centrasiaticum*, *C. minus*, *A. splendens*
FD13	91°53'45.30"	29°16'57.26"	3535	*P. centrasiaticum*, *O. thoroldii*	*A. stechmanniana*, *A. wellbyi*, *A. demissa*, *A. splendens*,
FD14	92°43'02.36"	29°06'11.87"	3126	*A. stechmanniana*	*A. wellbyi*, *A. flavum*
FD15	93°18'21.68"	28°59'37.45"	3032	*A. wellbyi*, *A. stechmanniana*	*P. centrasiaticum*, *Poaannua*, *A. membranaceus*
SFD1	91°05'06.60"	29°20'15.46"	3543	-	*O. sericopetala*, *A. wellbyi*
SFD2	91°09'09.82"	29°19'35.75"	3543	*O. thoroldii*	*P. centrasiaticum*, *A.*

					membranaceus, *O. sericopetala*, *A. squarrosum*, *T. hookeri*
SFD3	91°30'03.92"	29°19'58.66"	3530	*P. centrasiaticum*, *A. wellbyi*	*O. sericopetala*, *A. membranaceus*
SFD4	91°31'42.81"	29°15'19.90"	3523	*P. centrasiaticum*	*D. schraderiana*, *A. wellbyi*, *Poaannua*
MD1	88°04'20.50"	29°21'53.67"	3884	-	*O. sericopetala*, *A. membranaceus*, *A. scoparia*, *P. centrasiaticum*
MD2	89°30'27.56"	29°21'40.85"	3750	*P. centrasiaticum*	*O. sericopetala*
MD3	91°21'05.25"	29°18'45.76"	3553	*P. centrasiaticum*	*A. squarrosum*, *A. wellbyi O. sericopetala*
MD4	91°31'42.81"	29°15'19.90"	3523	*A. wellbyi*	*O. sericopetala*, *T. terrestris*, *P. centrasiaticum*, *D. schraderiana*
MD5	91°35'20.60"	29°15'14.44"	3548	*A. squarrosum*	*P. centrasiaticum*, *S. arenaria*, *D. schraderiana*

FD: fixed dune, SFD: semifixed dune, MD: moving dune. *P. centrasiaticum*: *Pennisetum centrasiaticum*, *A. membranaceus*: *Astragalus membranaceus*, *T. fabacea*: *Thermopsis fabacea*, *A. flavum*: *Arisaema flavum*, *A. scoparia*: *Artemisia scoparia*, *O. thoroldii*: *Orinus thoroldii*, *D. schraderiana*: *Dysphania schraderiana*, *A. stechmanniana*: *Artemisia stechmanniana*, *A. wellbyi*: *Artemisia wellbyi*, *S. viridis*: *Setaria viridis*, *T. terrestris*: *Tribulus terrestris*, *E. stephanianum*: *Erodium stephanianum*, *H. gouldii*: *Heteropappus gouldii*, *O. sericopetala*: *Oxytropis sericopetala*, *C. minus*: *Ceratostigma minus*, *A. splendens*: *Achnatherum splendens*, *A. demissa*: *Artemisia demissa*, *O. sericopetala*: *Oxytropis sericopetala*, *A. squarrosum*: *Agriophyllum squarrosum*, *T. hookeri*: *Trikeraia hookeri*, *S. arenaria*: *Saussurea arenaria*

Table 2 Characteristics of the plant and soil under three successional stages.

Types	FD	SFD	MD	F	*P*
Shrub coverage (%)	52.11 ± 2.62a	17.21 ± 1.43b	5.72 ± 0.36c	72.697	**<0.001**
Shrub crown size (m^2)	1.19 ± 0.75b	1.78 ± 0.42a	1.96 ± 0.28a	5.714	**0.005**
Diameter at breast height (cm)	9.88 ± 0.25b	9.93 ± 0.80b	12.55 ± 0.92a	7.433	**0.001**
Understorey herb coverage (%)	21.76 ± 2.14a	6.67 ± 1.18b	4.07 ± 0.49b	17.250	**<0.001**
Understorey herb biomass (g m^{-2})	69.60 ± 11.86a	16.44 ± 3.35b	9.19 ± 1.17b	5.917	**0.004**
Shrub height (cm)	56.38 ± 2.50a	43.52 ± 5.32b	51.76 ± 3.13ab	3.056	0.054
Soil moisture (%)	4.99 ± 0.54a	4.08 ± 0.29a	3.47 ± 0.29a	1.663	0.197
pH	9.93 ± 0.08a	9.60 ± 0.12a	9.58 ± 0.14a	3.586	0.066
BD (g cm^{-3})	1.52 ± 0.01a	1.55 ± 0.02a	1.56 ± 0.01a	1.692	0.192
DOC (mg kg^{-1})	93.34 ± 7.53a	37.79 ± 4.83b	30.84 ± 4.69b	19.484	**<0.001**
NH_4^+-N (mg kg^{-1})	2.10 ± 0.17a	1.49 ± 0.09ab	1.14 ± 0.08b	6.445	**0.003**
NO_3^--N (mg kg^{-1})	3.80 ± 0.28a	3.19 ± 0.76a	3.49 ± 0.54a	0.470	0.627
Available P (mg kg^{-1})	2.23 ± 0.16a	2.08 ± 0.15ab	1.57 ± 0.11b	3.320	0.055

FD: fixed dune, SFD: semifixed dune, MD: moving dune, BD: soil bulk density, DOC: dissolved organic carbon. Different lowercase letters indicate significant difference among the successional stages at $p < 0.05$.

Table 3 Correlation coefficients between soil ecological stoichiometry and plant, soil microbial biomass ecological stoichiometry.

	Soil C:N	Soil C:P	Soil N:P
Leaf C:N	0.335**	0.190	-0.381**
Leaf C:P	0.178	-0.209	-0.268*
Leaf N:P	0.004	-0.170	-0.116
Herb C:N	0.273*	0.118	-0.082
Herb C:P	0.062	-0.258*	-0.315**
Herb N:P	-0.118	-0.393**	-0.294*
Litter C:N	0.456**	0.000	-0.413**
Litter C:P	0.464**	-0.009	-0.418*
Litter N:P	0.294*	-0.005	-0.354**
SMB C:N	-0.438**	0.137	0.534**
SMB C:P	-0.285*	0.149	0.318**
SMB N:P	-0.012	0.168	0.101

SMB: soil microbial biomass.

Figures

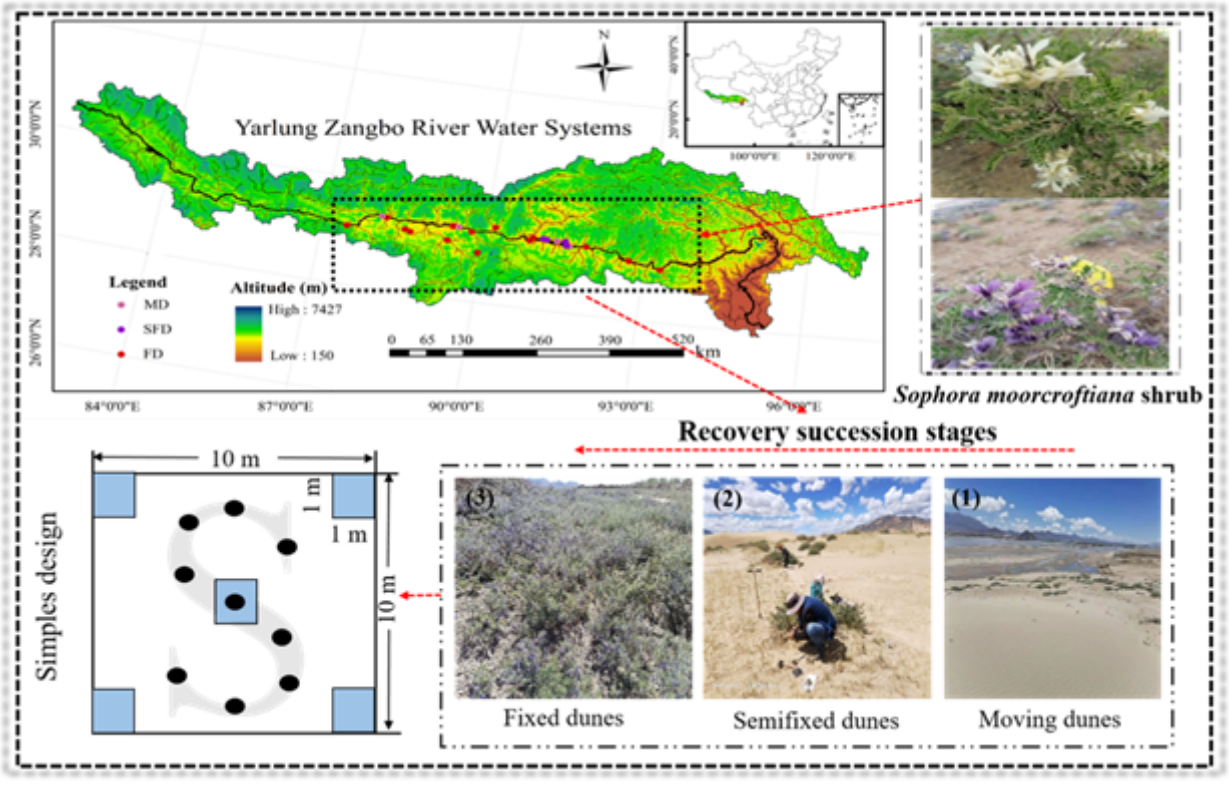

Figure 1

Location of the middle reaches of the Yarlung Zangbo River on the southern Tibetan Plateau of China and layout of the *Sophora moorcroftiana* shrub (plotted by Arcgis9.3).

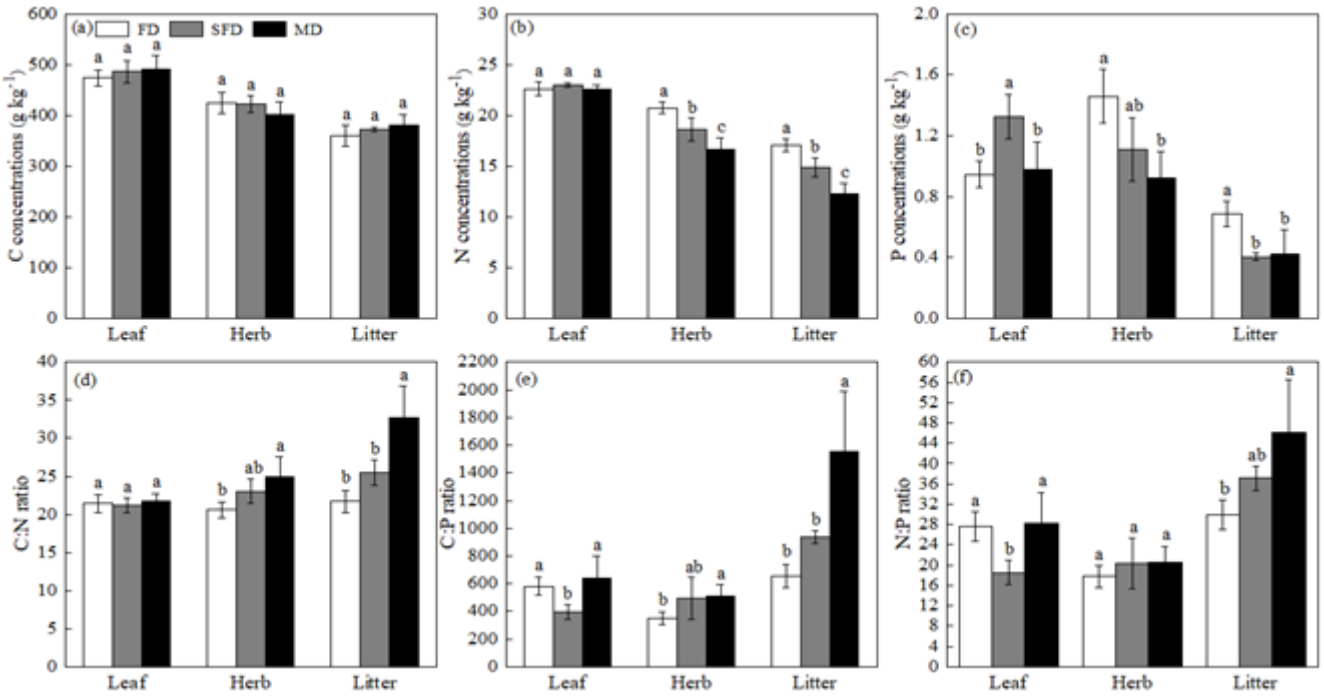

Figure 2

C, N, and P concentrations and stoichiometry in different components of *Sophora moorcroftiana* shrub under different successional stages. Different lowercase letters indicate significant difference under different successional stages based on analysis of variance and least significant difference tests ($P < 0.05$). FD: fixed dune, SFD: semifixed dune, MD: moving dune, Herb: understorey herb biomass.

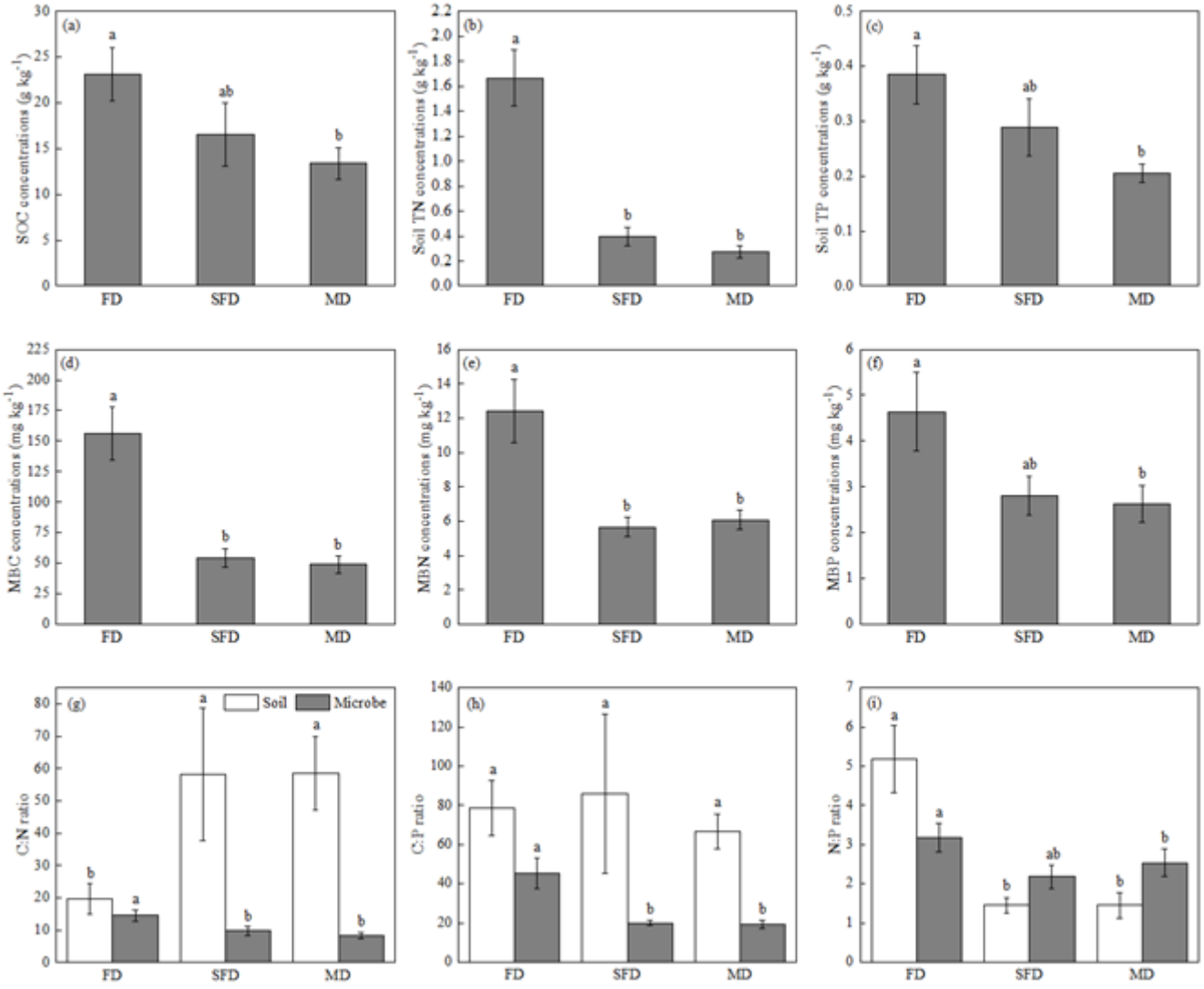

Figure 3

C, N, and P concentrations and stoichiometry in soils and microbes of *Sophora moorcroftiana* population in 0–20 cm soil layer under different successional stages. Different lowercase letters indicate a significant difference under different successional stages ($P < 0.05$). FD: fixed dune, SGL: sandy gravel land, SFD: semifixed dune, MD: moving dune.

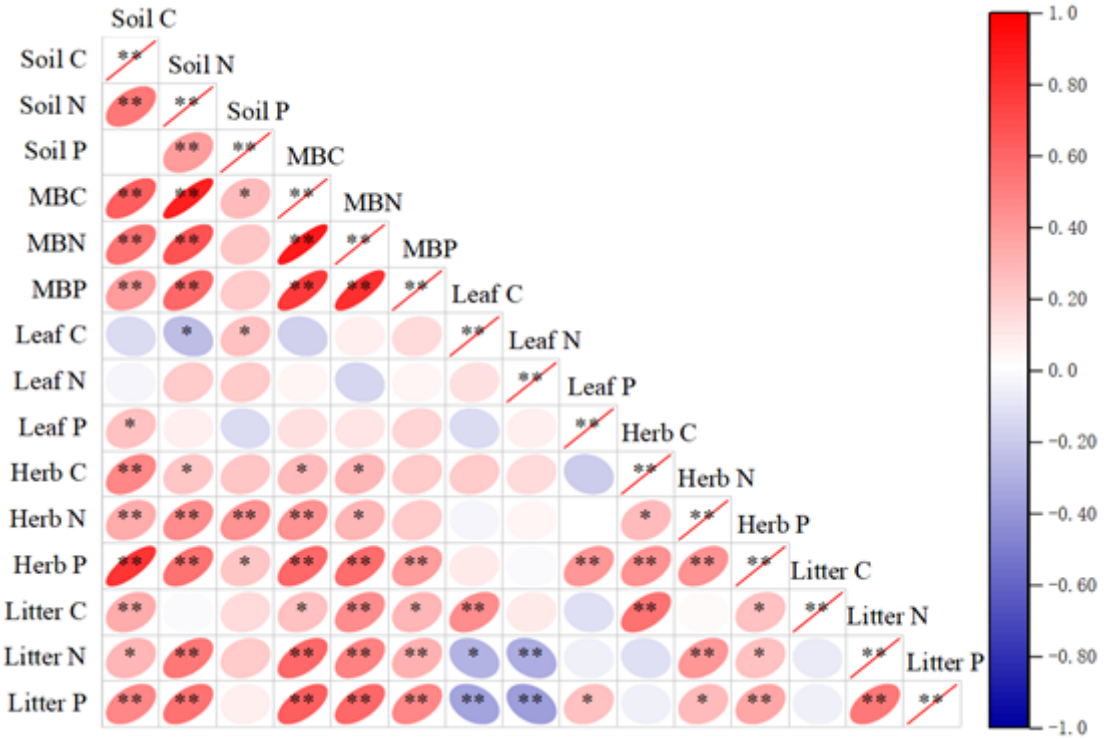

Figure 4

Correlations between soil, soil microbial biomass, *Sophora moorcroftiana* leaf, herb, and litter C, N, and P concentrations. Herb: understorey herb biomass. * $P < 0.05$, ** $P < 0.01$.

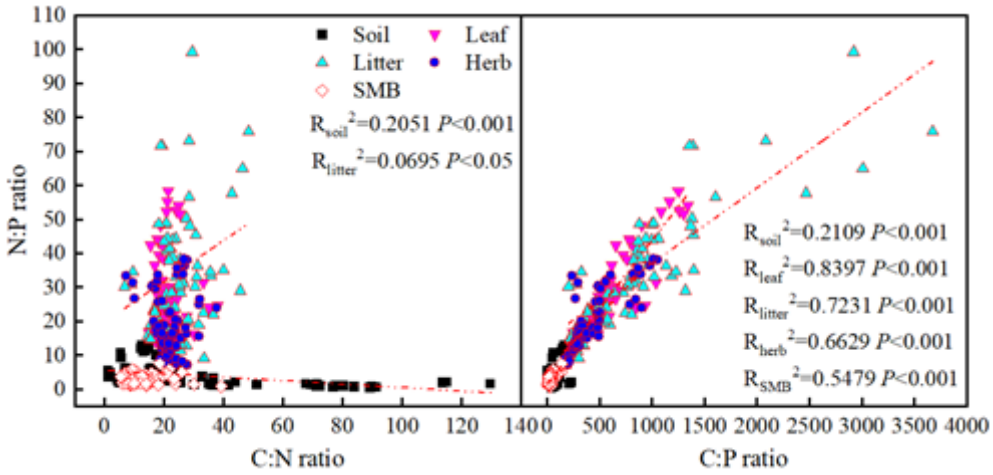

Figure 5

Correlations between the leaf, herb, litter, soil microbial biomass and soil C, N, P ecological stoichiometry. Herb: understorey herb biomass, SMB: soil microbial biomass.

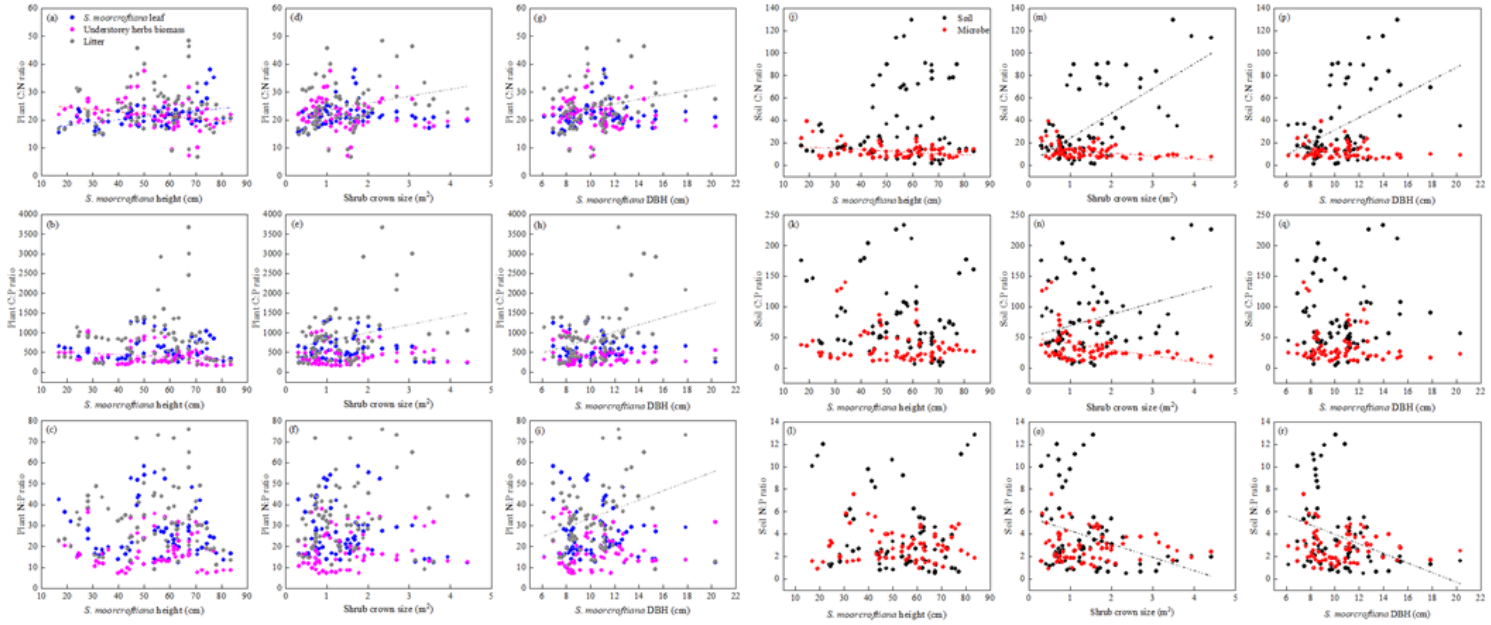

Figure 6

Relationships between plant and soil C:N:P stoichiometry and *Sophora moorcroftiana* height, shrub crown size, Diameter at breast height (DBH), respectively.

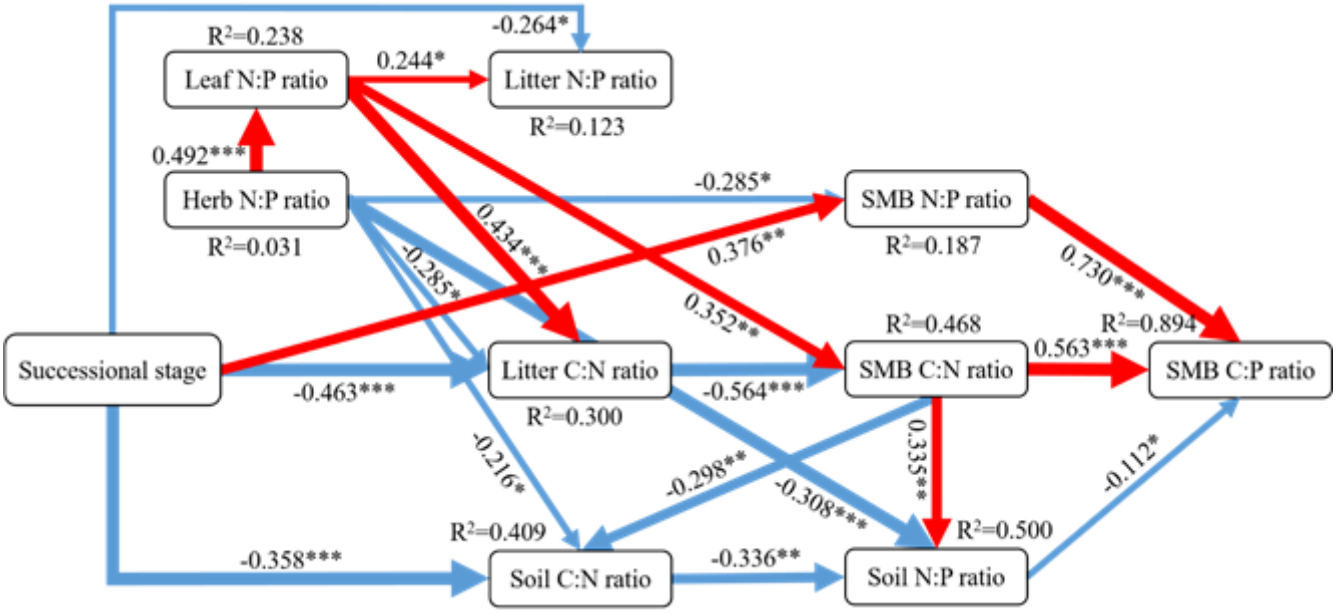

Figure 7

Structural equation modelling (SEM) analysis of the effects of successional stage on the plant-soil-microbe C–N–P stoichiometry. Results of model fitting: χ^2 = 8.700, df = 8, P = 0.368, GFI = 0.976, CFI = 0.998, RMSEA = 0.035. Numbers on arrows are standardized path coefficients. Red and blue arrows indicate positive and negative effects, respectively, with the thickness representing the extent of influence. R^2 values represent the proportion of the variance explained for each endogenous variable. Significance levels are as follows: *P < 0.05, **P < 0.01, and ***P < 0.001.

Supplementary Files

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- Supplementarydata.docx