

PATTERNS OF LEAF STOICHIOMETRY DURING PLANT (*DEYEUZIA ANGUSTIFOLIA* KOM.) ENCROACHMENT IN THE ALPINE TUNDRA OF THE CHANGBAI MOUNTAIN, CHINA

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Abstract. Alpine tundra ecosystems are experiencing great changes due to plant encroachment under the influence of global change, yet our understanding of the patterns of encroachment plant leaf stoichiometry is limited. This study aimed to determine the dynamic characteristics of leaf stoichiometry and the driving factors during species encroachment in the alpine tundra ecosystems. C, N, and P concentration of leaf of encroachment species as well as soil physicochemical properties and enzyme activities at different encroachment levels along an elevation gradient in the alpine tundra of the Changbai Mountain, China, were measured. The results showed that leaf stoichiometry of *D. angustifolia* and the soil properties were separated significantly by encroachment degree, and varied considerably among elevations and plant types. Differences in nutrient contents and stoichiometries in *D. angustifolia* leaves were mainly determined by soil moisture, available phosphorus, total nitrogen, and alkaline phosphatase. Results of leaf N:P ratios showed that the growth of *D. angustifolia* in the study area was mainly limited by phosphorus. Findings from this study may be useful to improve our insight on the protection of the alpine tundra ecosystems and provide a scientific basis for predicting the response of the alpine tundra ecosystem to climate change.

Keywords: global change, species encroachment, alpine tundra, ecological stoichiometry, soil factors

Introduction

Global climate change and human activities are altering the distributions of organisms worldwide (Collins et al., 2020; Du et al., 2017; Scheffers et al., 2016). High-latitude or/and high-elevation ecosystems have been a hotspot in plant range expansion research, owing to their high vulnerability to climate warming (Álvarez-Garrido et al., 2019; Ramirez et al., 2019; Shi et al., 2020). It is reported that global change is restructuring alpine tundra ecosystems via local plant encroachment (Krab et al., 2019; Løkken et al., 2020). Studies have shown that plant encroachment will cause serious consequences, including affecting aboveground productivity (usually increased), threatening biodiversity, and degradation of ecosystem functions (Alexander et al., 2016; Dukes et al., 2016). Although the effects of plant encroachment on aboveground and belowground

have been well documented (Bialic-Murphy et al., 2021; DeMarco et al., 2014; Ricciardi et al., 2017), relatively little research has been done on the patterns of leaf ecological stoichiometry of encroachment plant during the process of encroachment.

Ecological stoichiometry tries to analyze the balance of numerous elements and energy in ecosystems by combining the fundamental principles of biology, chemistry, and physics. It is a powerful resource for determining the nutrient cycling and dynamic functions of terrestrial ecosystems (Hessen et al., 2013; Moe et al., 2005; Schindler, 2003). Carbon (C), nitrogen (N), and phosphorus (P) are the three main nutritional components required for plant growth and development (Vitousek et al., 2010), thus they have always been the focus of plant stoichiometry research (Bai et al., 2012; Cao et al., 2020; Finzi et al., 2011). C:N:P stoichiometry has become increasingly popular in recent decades for studying the relationship between aboveground and belowground parts of ecosystems (Bai et al., 2019; Cao et al., 2020; Shi et al., 2021). Plant nutrient limits, nutrient cycling, and plant responses to climate change and ecological circumstances can be studied using leaf stoichiometry (Baxter and Dilkes, 2012; Tie et al., 2020; Zhu et al., 2020). At the same time, the study of leaf stoichiometric characteristics and driving factors in terrestrial ecosystems has been widely discussed at the local, regional, and global scales (Agren and Weih, 2012; Xia et al., 2014; Xiao et al., 2021). Leaf N:P ratio usually serves as a useful indicator of plant nutrient limitation (Koerselman and Meuleman, 1996). Specifically, if leaf N:P < 14, indicates that plant growth is limited by nitrogen; if leaf N:P > 16, indicates that plant growth is limited by phosphorus; and if $14 < \text{leaf N:P} < 16$, indicates that plant growth is limited by both nitrogen and phosphorus (Shi et al., 2021; Xiao et al., 2021).

Many factors can affect the stoichiometric characteristics of plant leaves, including geographical location, climate, soil characteristics, altitude, slope aspect, etc (Cao et al., 2020; Xiao et al., 2021; Zhao et al., 2018). There are extensive relationships between plant and soil stoichiometric characteristics. For example, changes in plant species abundance can alter soil nutrient stoichiometry, and soil C:N:P ratios may change with plant community dynamics (Ding et al., 2019; Pellegrini et al., 2014; Zhou et al., 2018b). Meanwhile, the status of soil resources will further affect the composition of specific plant communities and the abundance of plant species (Bell et al., 2014).

In recent years, ecological stoichiometry has been gradually applied to the study of species encroachment (Ding et al., 2019; Gao et al., 2021). Studies have shown that species encroachment can alter soil nutrient status and soil stoichiometry characteristics, but so far there is no clear conclusion (Ding et al., 2019; Feng and Bao, 2018). For example, species encroachment could reduce soil nutrient levels (Guidi et al., 2014; Jackson et al., 2002), while soil nutrient levels increased with species encroachment (Blaser et al., 2014; Ding et al., 2020; Pellegrini et al., 2014). It is also found that the trend of soil carbon, nitrogen, and phosphorus is not synchronized with species encroachment (Zhou et al., 2018b). In addition to effects on soil nutrient content and stoichiometry, plant chemical composition and stoichiometry would also change during the encroachment process (Blaser et al., 2014; Urbina et al., 2020; Wang et al., 2021), so it is often used to study plant nutrient requirements and material cycling. Encroachment plants have a high demand for phosphorus (Vitousek et al., 2002, 2010), but this nutrient limitation is often alleviated or reduced with the encroachment process (Blaser et al., 2014).

Dramatical changes has happened in the alpine tundra ecosystem (2000-2200 m) of Changbai Mountain in recent decades as a result of climate warming and atmospheric nitrogen deposition, one of which is the extensive encroachment of the local low-altitude

plant *Deyeuxia angustifolia* Kom. (*D. angustifolia*) (Zong et al., 2016). *D. angustifolia*, a herbaceous plant, was once one of the dominant plants in the Changbai Mountains' low altitude birch forest belt. Natural disasters, nutritional features, adaptability, and genetic variation are elements contributing to *D. angustifolia*'s upward expansion (Li et al., 2017; Ni et al., 2021; Zong et al., 2016). However, the dynamic changes in leaf and soil ecological stoichiometry during the encroachment of *D. angustifolia* have not yet been discussed so far. *D. angustifolia* encroachment can be divided into different stages according to its relative abundance (Catford et al., 2012), which provides a unique opportunity to study the changing patterns in leaf ecological stoichiometry during plant encroachment. Understanding leaf stoichiometry and its driving factors during species expansion are essential for understanding nutrient cycling processes and predicting ecosystem response to climate change (Cao et al., 2020; Sistla and Schimel, 2012).

Our purpose was to understand the pattern of leaf ecological stoichiometry during plant encroachment and to determine the main soil properties that affect leaf stoichiometry. Therefore, we conducted our study across different *D. angustifolia* encroachment degrees and along three elevations in the alpine tundra of Changbai Mountain. We hypothesized that: (1) leaf C, N, and P stoichiometry will differ significantly across elevations as small elevation changes will lead to huge changes in the alpine environment, (2) leaf C, N, and P stoichiometry will differ significantly across encroachment levels due to changes in community composition, and (3) changes in leaf stoichiometry may be driven by soil properties as soil conditions varied significantly across elevations and plant encroachment levels. These findings will assist in our knowledge of the nutritional condition and growth status of encroachment species, as well as provide useful information for the conservation and restoration of the alpine tundra ecosystem.

Materials and methods

Study area

Changbai Mountains National Nature Reserve (41°41' 49"–42°25' 18" N, 127°42' 55"–128°16' 48" E), located in Jilin province, northeast China, has been managed for environmental conservation for nearly six decades, making it one of the few well-preserved natural ecosystems on the planet (He et al., 2005). Vertical vegetation zones on Changbai Mountain are clearly outlined: deciduous broad-leaved forest (below 500 m), mixed deciduous broad-leaved/conifer forest (500–1,100 m), dark coniferous forest (1,100–1,700 m), betula ermine forest (1,700–1,950 m), and tundra (>1,950 m) (Li et al., 2017). Our research site is on the western slope of the alpine tundra (41°53'–42°04'N, 127°57'–128°11'E; 2,050–2,250 m), where *D. angustifolia* encroachment occurs (Fig. 1). Freezing temperatures, significant precipitation, as well as a short growing season characterize the alpine tundra climate (Zong et al., 2016). In this study, we focused on the soil properties and leaf stoichiometry of *D. angustifolia* in the two main vegetation types: shrubs and herbaceous plants.

Field sampling

In July 2018, during the plant growing season, fresh leaf samples from healthy *D. angustifolia* plants were collected within the elevation of 2,075 m (Group A: 41°59'24.78"N, 128°0'15.48"E), 2,157 m (Group B: 41°59'26.40"N, 128°0'34.80"E),

and 2,240 m (Group C: 41°59'33.66"N, 128°0'58.56"E). The soil types of these three elevations are tundra soil, which has a thin soil layer, and has more gravel and less soil and low fertility. We studied two common and contrasting plant community types where *D. angustifolia* encroachment typically occurs in the alpine tundra, one shrubs (S) and one herbaceous (H). In each of the two plant community types, we established a replicated sampling design on three encroachment levels during the process of *D. angustifolia* encroachment, which are low encroachment level (L, 10% ≤ plant cover of *D. angustifolia* ≤ 40%), medium encroachment level (M, 40% ≤ plant cover of *D. angustifolia* ≤ 70%), and high encroachment level (H, plant cover of *D. angustifolia* ≥ 70%), respectively. In each level, we collected three replicates and set a total of 54 sampling plots (1 m × 1 m, 3*2*3*3 = 54). We collected a total of 54 leaf and soil samples: 3 elevations × 2 plant community types (*D. angustifolia* encroachment occurs) × 3 encroachment levels × 3 replicates. Surface plants and litter layers were removed from each plot, and soils (top 10 cm) were collected using a soil drill (diameter 5 cm). Each sample contains 5 drillings, and the soil was mixed evenly. After collection, the samples were transported to the laboratory in an icebox, sieved (<2 mm), and removed the plant roots.

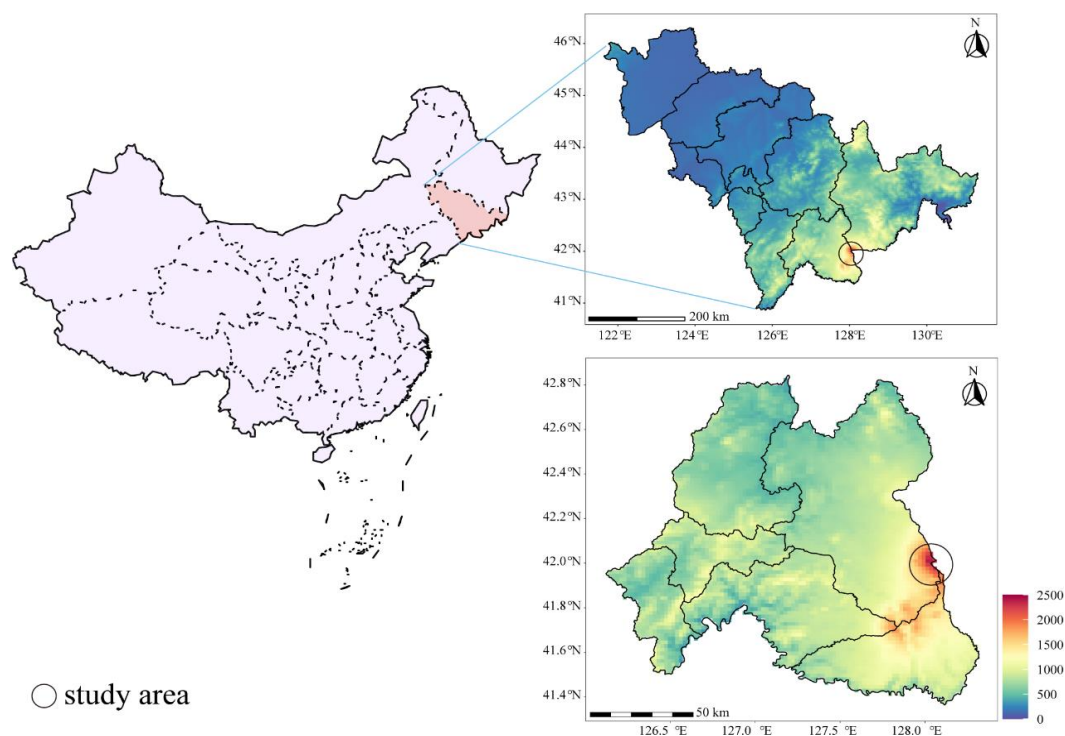


Figure 1. Location of the study area in the alpine tundra of the Changbai Mountain, China

Determination of soil properties and leaf stoichiometry

Leaf samples were oven-dried at 65 °C for 48 h, and the dried plant samples were ground to a fine powder using a mill. Leaf carbon (LC) was determined using the external heating method. Soil total organic carbon (TC) was determined by the potassium dichromate - concentrated sulfuric acid oxidation method (Li et al., 2017). Leaf nitrogen (LN) and soil total nitrogen (TN) were quantified on an automatic Kjeldahl nitrogen meter K1306 (Sonnen, Shanghai, China). Ammonium nitrogen

($\text{NH}_4^+\text{-N}$) and nitrate nitrogen ($\text{NO}_3^-\text{-N}$) were extracted with 2 M KCl, and $\text{NH}_4^+\text{-N}$ was determined by indophenol blue colorimetric method, while $\text{NO}_3^-\text{-N}$ was determined by ultraviolet spectrophotometry (SP-1900UV, Shanghai, China). Leaf phosphorus (LP) was determined colorimetrically using the molybdate method (Xu et al., 2016). Available phosphorus (AP) was determined colorimetrically based on the Olsen method. Soil pH was measured on a 1:5 (w/v) ratio in distilled water using a pH meter (MODEL828, ORION, USA). Soil moisture was determined after drying at 105 °C to a constant mass (Qin et al., 2019). The soil C:N was calculated as the TC to TN ratio. The LC:LN was calculated as the LC to LN ratio, LC:LP was calculated as the LC to LP ratio, and LN:LP was calculated as the LN to LP ratio.

The activity of catalase, alkaline phosphatase (ALP), and urease were quantified. Catalase activity was measured using the potassium permanganate titration method. ALP activity was determined spectrophotometrically at 660 nm using the p-nitrophenyl phosphate salt method. Urease activity was determined spectrophotometrically at 578 nm (Zhang et al., 2020).

Statistical analyses

Statistical analyses were performed using SPSS20.0 (IBM, Chicago, USA) and R software (version 4.0.2, <http://www.r-project.org>). Preliminary permutation multivariate analysis of variance (PERMANOVA) revealed significant differences ($P = 0.001$) between shrubs and herbaceous samples for both soil properties and leaf stoichiometry of *D. angustifolia* (Table A1); therefore, data analyses on shrubs and herbaceous samples were performed separately. Using SPSS 20.0, One-way ANOVA followed by least significant difference (LSD) was performed to test the significance of the effect of elevation and *D. angustifolia* encroachment on soil properties and leaf stoichiometry, and all statistical differences in this study were considered significant at $P < 0.05$. The R platform was used to run the following analyses and to generate plots using the “ggplot2” package. PERMANOVA was employed to assess the significance of the influential factors that differentiate plant leaf stoichiometry and soil properties (package: “vegan”, adonis function) (Taş et al., 2018; Xue et al., 2016) followed by Bray-Curtis distance. Principal component analysis (PCA) was performed to compare the differences in plant leaf stoichiometry and soil properties based on Bray-Curtis distance metric. Clustering heatmap was performed in the “pheatmap” package to reveal the differences in leaf stoichiometry and soil properties in different samples. The correlation between soil properties and plant leaf stoichiometry was estimated using Mantel tests in the “vegan” package with 9999 permutations based on Pearson’s product-moment correlation. Pearson coefficients were calculated in the “corrplot” package and used to reveal correlations between leaf stoichiometry and soil properties. Redundancy analyses (RDA) were performed by Canoco5 to determine soil properties significantly driving leaf stoichiometry of *D. angustifolia*.

Results

Effects of elevation, encroachment and their interaction on leaf stoichiometry of D. angustifolia

In shrubs communities, LN and LP at group BS were lower than that at group AS and CS, but the LC, LC:LN, LC:LP, and LN:LP were higher than those at group AS and

CS (Fig. 2). Leaf stoichiometry of *D. angustifolia* varied strongly with encroachment levels, but interestingly, the changes of most indexes were different across elevations. The LC and LC:LN increased at group BS and CS but decreased at group AS with *D. angustifolia* encroachment (Fig. 2a, d; Table A2); the LN and LP increased at group AS but decreased at group CS with *D. angustifolia* encroachment (Fig. 2b, c; Table A2); the LC:LP increased at group CS but decreased at group AS with *D. angustifolia* encroachment (Fig. 2e; Table A2); the LN:LP decreased at group CS with *D. angustifolia* encroachment (Fig. 2f; Table A2). PERMANOVA showed that elevation, encroachment, and their interaction had significant effects on the leaf stoichiometry of *D. angustifolia*, except for leaf nitrogen content ($P < 0.05$, Table 1).

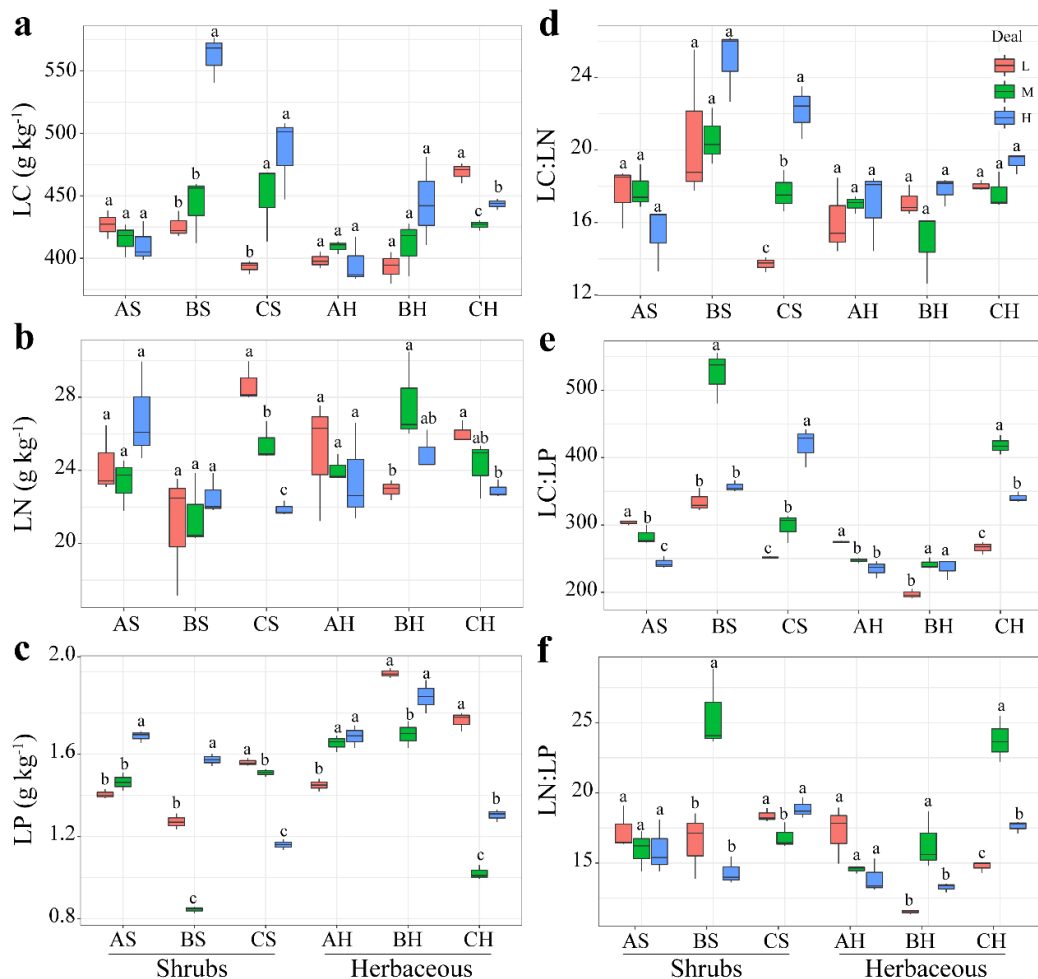


Figure 2. Leaf stoichiometry of *D. angustifolia* across encroachment levels and elevations within the study area. LC = leaf carbon concentration, LN = leaf nitrogen concentration, LP = leaf phosphorus concentration, LC:LN = LC to LN ratio, LC:LP = LC to LP ratio, LN:LP = LN to LP ratio. Different lowercase letters represent significant differences among encroachment levels within a group ($P < 0.05$)

In herbaceous communities, LC, LC:LN, LC:LP, and LN:LP at group CS were higher than that at group AS and BS; LN and LP at group BS were higher than that at group AS and CS (Fig. 2). Similar to these results in shrubs communities, leaf stoichiometry of *D. angustifolia* differed across encroachment levels and elevations.

The LC increased at group BH with *D. angustifolia* encroachment; the LN decreased at group AH and CH with *D. angustifolia* encroachment; the LP and LC:LN increased but the LC:LP and LN:LP decreased at group AH with *D. angustifolia* encroachment (Fig. 2; Table A2). In addition, LC in CHL was significantly higher than that in CHM and CHH (ANOVA: $P < 0.05$; Fig. 2a); LP in BHL and CHL was significantly lower, while LN:LP was significantly higher than the other two levels within group BH and CH, respectively (ANOVA: $P < 0.05$; Fig. 2c, f). PERMANOVA showed that elevation had significant effects on the LC, LP, LC:LN, LC:LP, and LN:LP; while encroachment significantly influenced LP, LC:LP, and LN:LP ($P < 0.05$; Table 1). The interaction between elevation and encroachment had significant effects on LC, LN, LP, LC:LP, and LN:LP ($P < 0.05$; Table 1).

Table 1. The effects of elevation and *D. angustifolia* encroachment on leaf stoichiometry of *D. angustifolia* based on PERMANOVA

| | Shrubs | | | | | | Herbaceous | | | | | |
|--------------------------|----------------|--------------|----------------|--------------|--------------------------|--------------|----------------|--------------|----------------|--------------|--------------------------|--------------|
| | Elevation | | Encroachment | | Elevation × encroachment | | Elevation | | Encroachment | | Elevation × encroachment | |
| | R ² | P | R ² | P | R ² | P | R ² | P | R ² | P | R ² | P |
| LC (g kg ⁻¹) | 0.222 | 0.001 | 0.334 | 0.001 | 0.322 | 0.001 | 0.450 | 0.002 | 0.032 | 0.293 | 0.292 | 0.005 |
| LN (g kg ⁻¹) | 0.310 | 0.001 | 0.025 | 0.506 | 0.350 | 0.004 | 0.041 | 0.508 | 0.089 | 0.222 | 0.347 | 0.041 |
| LP (g kg ⁻¹) | 0.244 | 0.001 | 0.124 | 0.001 | 0.625 | 0.001 | 0.473 | 0.001 | 0.156 | 0.001 | 0.355 | 0.001 |
| LC:LN (ratio) | 0.352 | 0.001 | 0.150 | 0.007 | 0.316 | 0.001 | 0.223 | 0.038 | 0.136 | 0.127 | 0.149 | 0.278 |
| LC:LP (ratio) | 0.400 | 0.001 | 0.100 | 0.001 | 0.466 | 0.001 | 0.626 | 0.001 | 0.117 | 0.001 | 0.234 | 0.001 |
| LN:LP (ratio) | 0.089 | 0.025 | 0.135 | 0.008 | 0.583 | 0.001 | 0.370 | 0.001 | 0.214 | 0.001 | 0.334 | 0.001 |

LC = leaf carbon concentration, LN = leaf nitrogen concentration, LP = leaf phosphorus concentration, LC:LN = LC to LN ratio, LC:LP = LC to LP ratio, LN:LP = LN to LP ratio. Values with $P < 0.05$ are in bold

Principal component analysis (PCA) showed that in shrubs and herbaceous communities, the leaf stoichiometry of *D. angustifolia* among elevations was separated along the first axis of the principal component, and the samples with different encroachment levels were distributed along the second axis of the principal component within each elevation. The first two principal components explained 94.78% (PC1 67.04% and PC2 27.74%, Fig. 3a) and 94.89% (PC1 81.29% and PC2 13.6%, Fig. 3b) of the total variation, respectively. Clustering heatmap showed that in shrubs communities, all samples can be clustered into two large branches, in which the composition of the samples of group AS and CS were similar, and both were separated from the samples of group BS. Samples with different *D. angustifolia* encroachment levels showed obvious separation within each group (Fig. A2a). In herbaceous communities, all samples could also be clustered into two large branches, and the separation in samples was not obvious among elevations, but the sample composition showed obvious separation across encroachment levels within each group (Fig. A2b). The above results showed that the leaf stoichiometry of *D. angustifolia* appeared significant heterogeneity across elevations and encroachment levels.

***D. angustifolia* encroachment shaped soil physicochemical properties and enzyme activities**

In shrubs communities, the soil TN, NH₄⁺-N, NO₃⁻-N, AP, and moisture increased but C:N, ALP, and catalase decreased with rising elevation. Additionally, TC and urease at

group BS were higher than that at group AS and CS, and pH at group AS was higher than that at group BS and CS (Table A3). Soil properties differed among *D. angustifolia* encroachment levels, and their variation trend was different among elevations. The soil TC, NH₄⁺-N, and AP increased at group CS with *D. angustifolia* encroachment; the NO₃⁻-N and catalase increased at group BS with *D. angustifolia* encroachment. The soil C:N decreased while the urease increased at group AS and BS with *D. angustifolia* encroachment. The soil moisture increased at group CS but decreased at group BS with *D. angustifolia* encroachment (Table A3). PERMANOVA showed that elevation, encroachment, and their interaction had significant effects on the soil physicochemical properties and enzyme activities, except for soil ALP ($P < 0.05$, Table 2).

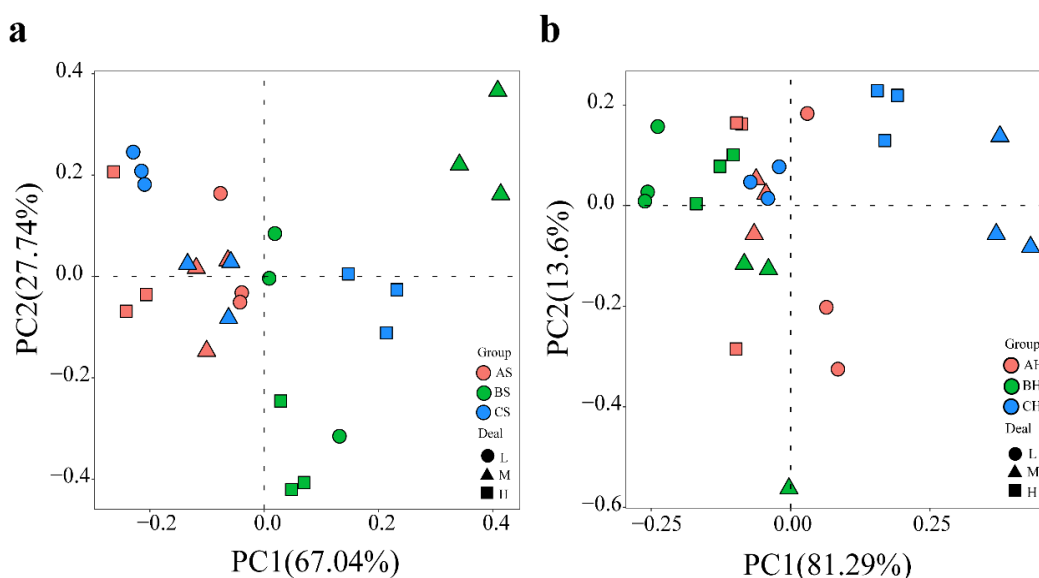


Figure 3. Principal component analysis (PCA) of leaf stoichiometry of *D. angustifolia* in shrubs (a) and herbaceous (b) communities

Table 2. The effects of elevation and *D. angustifolia* encroachment on soil properties based on PERMANOVA

| | Shrubs | | | | | | Herbaceous | | | | | |
|--|----------------|--------------|----------------|--------------|--------------------------|--------------|----------------|--------------|----------------|--------------|--------------------------|--------------|
| | Elevation | | Encroachment | | Elevation × encroachment | | Elevation | | Encroachment | | Elevation × encroachment | |
| | R ² | P | R ² | P | R ² | P | R ² | P | R ² | P | R ² | P |
| TC (g kg ⁻¹) | 0.656 | 0.001 | 0.055 | 0.001 | 0.252 | 0.001 | 0.118 | 0.001 | 0.261 | 0.001 | 0.593 | 0.001 |
| TN (g kg ⁻¹) | 0.808 | 0.001 | 0.052 | 0.001 | 0.112 | 0.001 | 0.260 | 0.001 | 0.095 | 0.001 | 0.594 | 0.001 |
| C:N (ratio) | 0.930 | 0.001 | 0.015 | 0.031 | 0.021 | 0.047 | 0.356 | 0.001 | 0.059 | 0.145 | 0.330 | 0.007 |
| NH ₄ ⁺ -N (mg kg ⁻¹) | 0.697 | 0.001 | 0.111 | 0.001 | 0.182 | 0.001 | 0.340 | 0.001 | 0.172 | 0.001 | 0.464 | 0.001 |
| NO ₃ ⁻ -N (mg kg ⁻¹) | 0.538 | 0.001 | 0.268 | 0.001 | 0.167 | 0.001 | 0.367 | 0.001 | 0.053 | 0.001 | 0.563 | 0.001 |
| AP (mg kg ⁻¹) | 0.570 | 0.001 | 0.257 | 0.001 | 0.073 | 0.034 | 0.605 | 0.001 | 0.102 | 0.016 | 0.115 | 0.043 |
| Moisture | 0.669 | 0.001 | 0.066 | 0.001 | 0.264 | 0.001 | 0.018 | 0.622 | 0.137 | 0.006 | 0.571 | 0.001 |
| pH | 0.204 | 0.003 | 0.319 | 0.001 | 0.302 | 0.001 | 0.224 | 0.001 | 0.227 | 0.001 | 0.483 | 0.001 |
| Catalase | 0.902 | 0.001 | 0.010 | 0.044 | 0.063 | 0.001 | 0.895 | 0.001 | 0.041 | 0.001 | 0.035 | 0.008 |
| ALP | 0.932 | 0.001 | 0.007 | 0.098 | 0.035 | 0.004 | 0.976 | 0.001 | 0.002 | 0.167 | 0.009 | 0.031 |
| Urease | 0.617 | 0.001 | 0.117 | 0.001 | 0.195 | 0.001 | 0.602 | 0.001 | 0.099 | 0.003 | 0.191 | 0.001 |

TC: total organic carbon, TN: total nitrogen, C:N: TC to TN ratio, NH₄⁺-N: ammonium nitrogen, NO₃⁻-N: nitrate nitrogen, AP: available phosphorus, Moisture: water content, ALP: alkaline phosphatase. *P* is the significance level. Values with $P < 0.05$ are in bold

In herbaceous communities, the soil AP increased but C:N, ALP, and urease decreased with rising elevation. Additionally, TC, TN, moisture, pH, and catalase at group BH were higher than that at group AH and CH; $\text{NH}_4^+\text{-N}$ was higher in group CH, while $\text{NO}_3^-\text{-N}$ was higher in group AH (Table A3). Similar to these results in shrubs communities, soil properties differed across *D. angustifolia* encroachment levels and elevations. The soil $\text{NO}_3^-\text{-N}$ increased at group AH but decreased at group CH with *D. angustifolia* encroachment; AP increased at group AH with *D. angustifolia* encroachment. The soil TN and pH decreased at group AH with *D. angustifolia* encroachment, while $\text{NH}_4^+\text{-N}$ decreased at group CH with *D. angustifolia* encroachment (Table A3). PERMANOVA showed that elevation, encroachment, and their interaction had significant effects on the soil physicochemical properties and enzyme activities, except for soil C:N, moisture, and ALP ($P < 0.05$, Table 2).

Principal component analysis (PCA) showed that in shrubs communities, the soil properties among elevations were separated along the first axis of the principal component, and the samples with different encroachment levels were distributed along the second axis of the principal component within each elevation. In herbaceous communities, samples among elevations and encroachment levels were distributed along the first axis of the principal component, and the first two principal components explained 85.99% (PC1 74.07% and PC2 11.92%, Fig. 4a) and 72.00% (PC1 52.36% and PC2 19.64%, Fig. 4b) of the total variation, respectively. Clustering heatmap showed that in shrubs communities, all samples can be clustered into two large branches, in which group AS was clustered into one branch, and group BS and CS were clustered into one another branch and separated from each other. Samples with different *D. angustifolia* encroachment levels showed obvious separation within each group (Fig. A3a). In herbaceous communities, all samples can also be clustered into two large branches. Unlike shrubs communities, the separation of samples at different groups was not obvious, but the composition of samples within each group showed obvious separation (Fig. A3b). The above results showed that the soil properties showed significant heterogeneity across elevations and encroachment levels.

Relationships between leaf stoichiometry and soil properties

Redundancy analyses (RDA) showed that soil properties could explain 60.04% of *D. angustifolia* leaf stoichiometric variation, among which RDA1 and RDA2 explained 52.02% and 8.02% of the total variation, respectively (Fig 5a). Soil moisture, C:N, pH, AP, and Urease had significant effects on leaf stoichiometry ($P < 0.05$, Table A4). In addition, $\text{NH}_4^+\text{-N}$ and ALP also had strong effects on leaf stoichiometry. Mantel tests showed that there was a positive correlation between soil properties and leaf stoichiometry of *D. angustifolia* (Mantle: $R = 0.073$, $P = 0.132$). Further analysis showed that soil TN, $\text{NH}_4^+\text{-N}$, AP, moisture, ALP, and urease significantly affected leaf stoichiometry (Mantle: $P < 0.05$; Fig. 5b).

Pearson correlation analysis showed that LN was positively correlated with soil $\text{NH}_4^+\text{-N}$, AP, moisture, and negatively correlated with pH and ALP. LN was negatively correlated with soil TC and moisture. LP was negatively correlated with soil TN, AP, and moisture, and positively correlated with ALP. LC:LN was positively correlated with soil AP and moisture, and negatively correlated with ALP. LC:LP and LN:LP were positively correlated with soil TN, and negatively correlated with ALP. In addition, LC:LP was positively correlated with AP and moisture ($P < 0.05$, Fig. 6). Together, these results suggested that soil properties had a great impact on the variation of leaf stoichiometry of *D. angustifolia*.

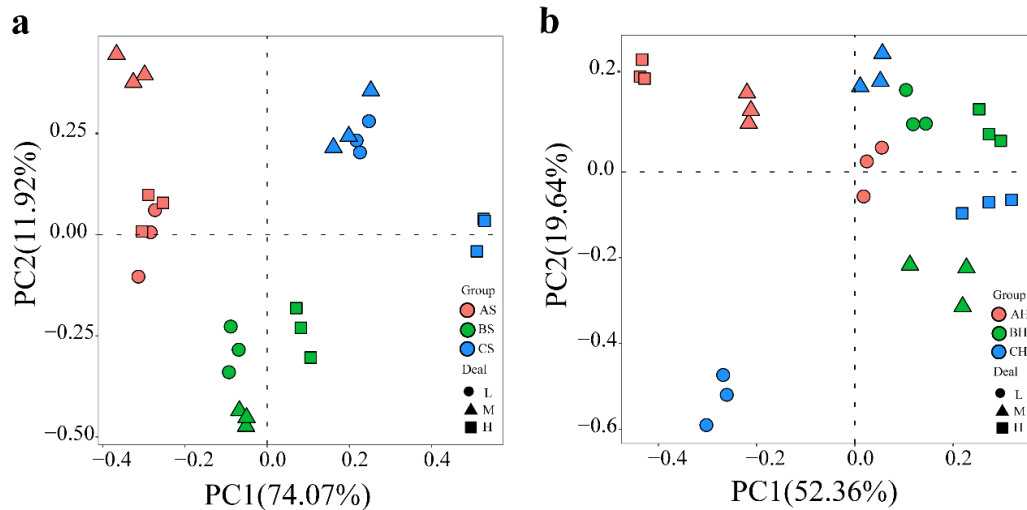


Figure 4. Principal component analysis (PCA) of soil properties in shrubs (a) and Herbaceous (b) communities

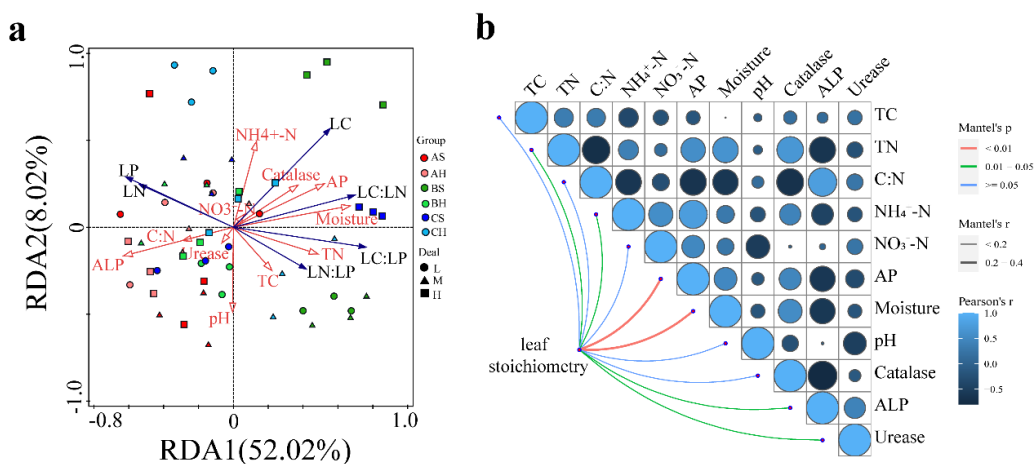


Figure 5. Redundancy analysis (RDA) (a) and Mantel tests (b) analysis of leaf stoichiometry of *D. angustifolia* and soil properties. Edge width corresponds to Mantel's r value, and the edge color denotes the statistical significance. Pairwise correlations of these variables are shown with color gradient denoting Pearson's correlations coefficients. * indicates significant differences ($P < 0.05$)

Discussion

Dynamic of leaf stoichiometry of *D. angustifolia*

Elevation had a significant effect on *D. angustifolia* leaf stoichiometry (PERMANOVA, $P = 0.001$; Table A1). Leaf C, N, P and their ratios varied with elevation, except for leaf nitrogen content in herbaceous communities ($P < 0.05$; Table 1, A2), which supported our first hypothesis. Other studies have also reported that plant leaf stoichiometry changed along elevation gradients (Cao et al., 2020; Li et al., 2018), part of which can be attributed to spatial variation of the soil environment, such as the redistribution of water and heat balance caused by topographic change, and changes in soil C, N, and P supply (Cao et al., 2020; Reich and Oleksyn, 2004).

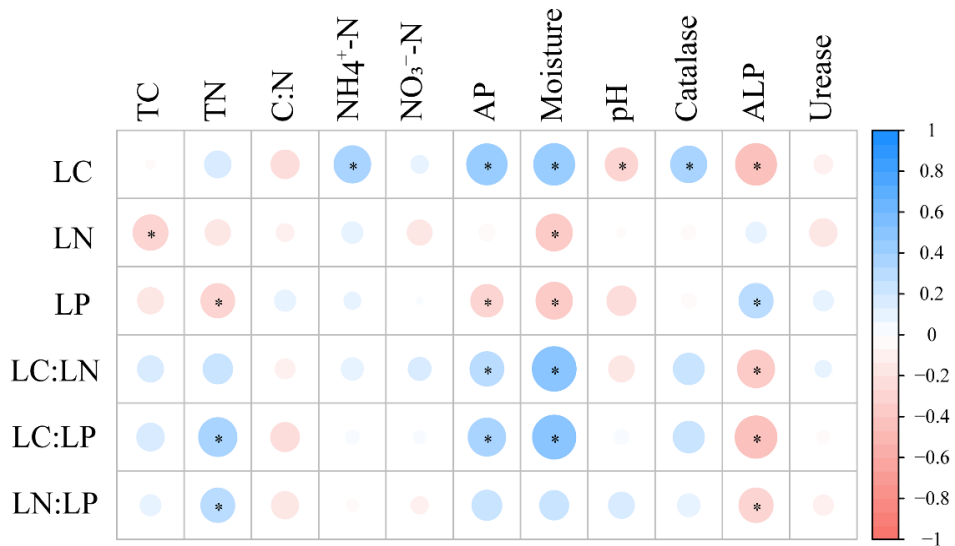


Figure 6. Pearson coefficients between leaf stoichiometry characteristics of *D. angustifolia* and soil properties. Pairwise correlations of these variables are shown with color gradient denoting Pearson's correlations coefficients. * indicates significant differences ($P < 0.05$)

Encroachment levels had a significant effect on *D. angustifolia* leaf stoichiometry (PERMANOVA, $P = 0.001$; Table A1). PCA and cluster analysis showed that there was a significant separation of leaf stoichiometric characteristics at each elevation (Figs. 3 and A2), which supported our second hypothesis. Interestingly, leaf C, N, P, and their ratios increased, decreased, or did not change significantly with the increase of *D. angustifolia* encroachment level, which depended on the elevation and vegetation community type encroached by *D. angustifolia* (Fig. 2). *D. angustifolia* leaf stoichiometry showed significant heterogeneity in elevations and encroachment levels, which indicated the adaptability of *D. angustifolia* to different environments, and has also been confirmed in the study of *Spartina alterniflora* (Zuo et al., 2021). Nutrient patterns reflect plants' adaptation to the environment. Plants must change their absorption and storage of nutrient elements to adapt to the differences in element availability, which can change within a distance of a few meters (Baxter and Dilkes, 2012). In addition, the significant heterogeneity of *D. angustifolia* leaf stoichiometry across encroachment levels may be caused by the competition with the original vegetation in the tundra zone. It is found that the interspecific competition alters the leaf stoichiometry of the encroachment plants (Zhu et al., 2020).

Responses of soil properties to *D. angustifolia* encroachment

Our results showed that *D. angustifolia* encroachment significantly affected most soil index (PERMANOVA, $P < 0.05$; Table 2), indicating that *D. angustifolia* encroachment significantly affected soil nutrient conditions, which was consistent with other studies results (Gao et al., 2021; Yang and Liu, 2019). However, this effect depended on the encroachment level, elevation, and the vegetation community type encroached by *D. angustifolia* (Figs. 4 and 5). It is reported that changes in plant species abundance had an effect on the soil nutrient characteristics, and soil nutrient contents changed with plant community dynamics (Blaser et al., 2014; Ding et al., 2019; Pellegrini et al., 2014; Zhou et al., 2018b). Given the importance of plant

functional features in soil biological processes, changes in C:N:P stoichiometry in plant tissues during encroachment are expected to have comparable effects on soil properties (Zhou et al., 2018a).

Soil nutrient concentration usually shows vertical zonal distribution characteristics, which have been widely observed on the regional scale (Cui et al., 2019; Tong et al., 2021). Our results showed that elevation significantly affected soil properties (PERMANOVA, $P = 0.001$; *Table A1*), and soil properties were significantly separated among elevations (*Fig. 4*), which was consistent with previous research results (Byars et al., 2007; Cui et al., 2019). In addition, the researchers found that the soil physicochemical characteristics of wetland ecosystems also changed significantly under small-scale elevation gradients (Li et al., 2018). These results suggest that the influence of elevation should be considered in future research to have a more comprehensive understanding of ecological stoichiometric characteristics.

Dominant factors influencing leaf stoichiometry of *D. angustifolia*

In this study, soil moisture and AP were the two most important ecological factors affecting *D. angustifolia* leaf stoichiometry in the tundra of Changbai Mountain (RDA, Mantel; *Fig. 5*). Soil moisture mainly affects the transformation and availability of soil nutrients by controlling various biogeochemical processes, thus affecting leaf stoichiometry (Anderson and Lockaby, 2011). Soil moisture was positively correlated with leaf LC, LC:LN, and LC:LP ($P < 0.05$, *Fig. 6*). The relationship between soil moisture and LC is consistent with previous reports (Cao et al., 2020), but other studies have found contrary results (Lin et al., 2019; Liu et al., 2020). For LC, it is because soil water content contributes to plant photosynthesis and growth (Li et al., 2018), and our previous results also showed that the water environment in the tundra zone of Changbai Mountain is favorable for the survival of *D. angustifolia* (Ni et al., 2021). Soil moisture was significantly negatively correlated with LN and LP ($P < 0.05$, *Fig. 6*), which is consistent with the results that soil moisture has a negative correlation on leaf nutrient content on a global scale (Ordonez et al., 2009). These results suggest that the effect of water conditions on plant stoichiometry may vary due to the selected environmental scale and species type. Soil AP was another important factor affecting leaf stoichiometry (RDA, Mantel; *Fig. 5*), as soil AP was positively correlated with LC, LC:LN, and LC:LP ($P < 0.05$, *Fig. 6*). Liu et al. also found that there was a positive correlation between soil AP and LC (Liu et al., 2020), which may be due to the fact that phosphorus is required for photosynthesis and plays a direct role in biological processes such as photophosphorylation and carbon assimilation. AP may indirectly affect the relationship between LC:LN and LC:LP through its effect on soil TC. There is a significant negative correlation between soil AP and LP ($P < 0.05$, *Fig. 6*), which is contrary to the previous results (Liu et al., 2020). Most plants obtain phosphorus through roots, and the supply of soil phosphorus can determine the concentration of plant phosphorus, thus soil AP is a factor affecting the content of phosphorus in the leaves of *D. angustifolia* (Li et al., 2018, 2014; Yan et al., 2015).

Nitrogen and phosphorus are the main limiting elements in the terrestrial ecosystem, as they play important roles in the physiology and metabolism of plant growth (Mao et al., 2016). In this study, soil TN took essential parts in the formation of *D. angustifolia* leaf stoichiometry (RDA, Mantel; *Fig. 5*), mainly by affecting LP, LC:LP, and LN:LP ($P < 0.05$, *Fig. 6*). Soil ALP significantly affected *D. angustifolia* leaf stoichiometry

(RDA, Mantel; *Fig. 5*), as ALP was negatively correlated with *D. angustifolia* LC, LC:LN, LC:LP, and LN:LP, and positively correlated with LP ($P < 0.05$, *Fig. 6*). Soil enzyme is the key to controlling the biogeochemical cycle and soil nutrient concentration (Aragon et al., 2014). The significant positive correlation between soil ALP and LP is that high phosphatase activity enables roots to obtain phosphorus in organic form in soil (Blaser et al., 2014; Venterink, 2011; Zhou et al., 2018a). The relationship between ALP and *D. angustifolia* leaf stoichiometry may be due to the influence of root phosphorus uptake on the balance of leaf nutrients. These results showed that in addition to soil moisture and AP, soil TN and ALP also play important roles in the leaf stoichiometry of *D. angustifolia*.

Elements limiting D. angustifolia growth

LN:LP plays a more important role in evaluating plant nutritional limitations than the concentration of N and P alone (Koerselman and Meuleman, 1996; Li et al., 2018; Zhu et al., 2020). In terrestrial ecosystems, when $LN:LP > 16$, plants growth is limited by P; when $14 < LN:LP < 16$, plant growth is limited by N and P, and when $LN:LP < 14$, plants is limited by N (Blaser et al., 2014; Koerselman and Meuleman, 1996). Our results showed that *D. angustifolia* LN:LP was significantly affected by the elevation, encroachment level, and their interaction (PERMANOVA: $P < 0.05$; *Table 1*). *D. angustifolia* LN:LP was between 11.52 and 25.53, indicating that the growth of *D. angustifolia* was limited by N and P during the encroachment process. However, the average $LN:LP = 16.82$ and > 16 at most sample sites (*Fig. 2f*; *Table A2*) indicated that the growth of *D. angustifolia* was mainly limited by phosphorus rather than nitrogen in the tundra of Changbai Mountain. This was consistent with other researchers' results (Cao et al., 2020; Zhou et al., 2018a) that it may be due to the high phosphorus demand of encroachment species (Binkley et al., 2003; Vitousek et al., 2002).

Conclusion

This study revealed the leaf stoichiometry and driving factors of *D. angustifolia* at different elevations and encroachment levels and is helpful for us to understand its nutritional and growth status. Elevation and encroachment degree significantly affected *D. angustifolia* leaf stoichiometry. Leaf carbon, nitrogen, and phosphorus and their ratios showed significant heterogeneity across elevations and encroachment levels, indicating the adaptability of *D. angustifolia* to different environments. Soil properties had important effects on *D. angustifolia* leaf stoichiometry, and soil moisture, AP, TN, and ALP were the main factors affecting *D. angustifolia* leaf stoichiometry in the alpine tundra of Changbai Mountain. Phosphorus was the main factor limiting the growth of *D. angustifolia* in the process of upward expansion. Results of this study improve our understanding of C:N:P stoichiometry of encroachment species, and are critical for predicting the responses of ecosystems to environmental changes.

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APPENDIX



Figure A1. Landscape photography of different vegetation types of *Deyeuxia angustifolia* Kom. (*D. angustifolia*) encroachment

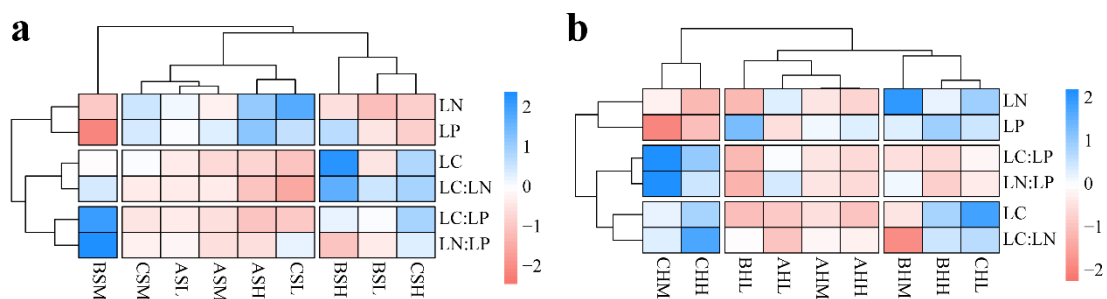


Figure A2. Clustering heatmap of leaf stoichiometry of *D. angustifolia* in different sampling sites. a, shrub ; b: herbaceous. LC: leaf carbon concentration, LN: leaf nitrogen concentration, LP: leaf phosphorus concentration, LC:LN = LC to LN ratio, LC:LP = LC to LP ratio, LN:LP = LN to LP ratio

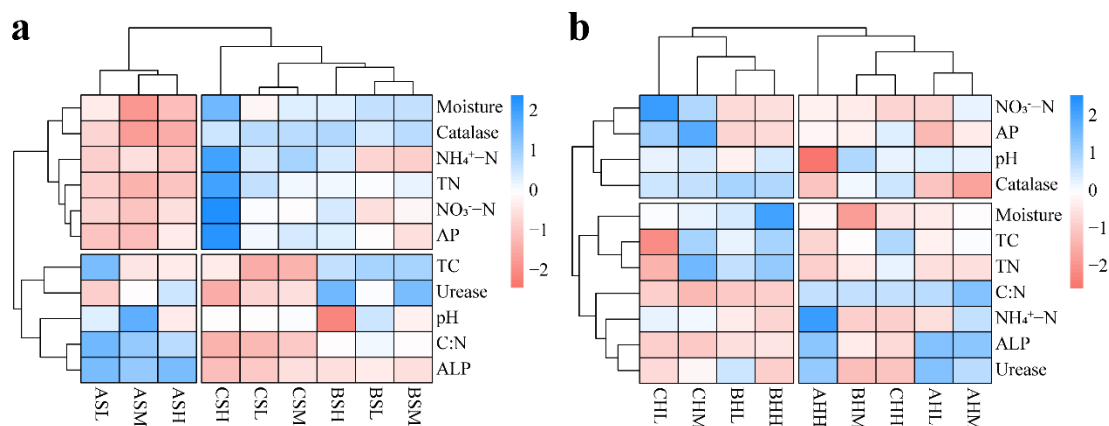


Figure A3. Clustering heatmap of soil properties in different sampling sites. *a*, shrubs ; *b*: herbaceous. TC: total organic carbon, TN: total nitrogen, C:N: TC to TN ratio, $\text{NH}_4^+\text{-N}$: ammonium nitrogen, $\text{NO}_3^-\text{-N}$: nitrate nitrogen, AP: available phosphorus, Moisture: water content, ALP: alkaline phosphatase

Table A1. Effects of plant type, elevation, and *D. angustifolia* encroachment on leaf stoichiometry of *D. angustifolia* and soil properties based on PERMANOVA

| | Leaf stoichiometry | | Soil properties | |
|---------------------------------------|--------------------|--------------|-----------------|--------------|
| | R ² | P | R ² | P |
| Elevation | 0.134 | 0.001 | 0.429 | 0.001 |
| Plant type | 0.134 | 0.001 | 0.062 | 0.001 |
| Encroachment | 0.113 | 0.001 | 0.025 | 0.001 |
| Elevation × plant type | 0.223 | 0.001 | 0.108 | 0.001 |
| Elevation × encroachment | 0.187 | 0.001 | 0.110 | 0.001 |
| Plant type × encroachment | 0.028 | 0.001 | 0.104 | 0.001 |
| Elevation × plant type × encroachment | 0.121 | 0.001 | 0.125 | 0.001 |

P is the significance level. Values with $P < 0.05$ are in bold

Table A2. Leaf stoichiometry of *D. angustifolia* across encroachment levels and elevations within the study area

| Vegetation types | | LC (g kg ⁻¹) | LN (g kg ⁻¹) | LP (g kg ⁻¹) | LC:LN (ratio) | LC:LP (ratio) | LN:LP (ratio) |
|------------------|-----|--------------------------|--------------------------|--------------------------|---------------|-----------------|---------------|
| Shrubs | ASL | 427.03 ± 11.42a | 24.33 ± 1.87a | 1.41 ± 0.02b | 17.64 ± 1.69a | 303.77 ± 3.67a | 17.32 ± 1.54a |
| | ASM | 415.23 ± 13.28a | 23.35 ± 1.42a | 1.47 ± 0.04b | 17.83 ± 1.22a | 283.61 ± 14.37b | 15.97 ± 1.44a |
| | ASH | 411.10 ± 16.27a | 26.89 ± 2.74a | 1.69 ± 0.03a | 15.40 ± 1.81a | 243.65 ± 8.76c | 15.96 ± 1.91a |
| | BSL | 425.96 ± 10.51b | 21.06 ± 3.43a | 1.27 ± 0.04b | 20.69 ± 4.23a | 335.21 ± 17.32b | 16.52 ± 2.38b |
| | BSM | 442.59 ± 26.38b | 21.53 ± 2.01a | 0.84 ± 0.02c | 20.63 ± 1.56a | 524.5 ± 39.20a | 25.53 ± 2.88a |
| | BSH | 561.65 ± 18.65a | 22.56 ± 1.11a | 1.57 ± 0.03a | 24.96 ± 1.98a | 356.95 ± 8.33b | 14.35 ± 0.96b |
| | CSL | 393.20 ± 5.31b | 28.70 ± 1.09a | 1.56 ± 0.02a | 13.71 ± 0.41c | 251.90 ± 1.52c | 18.38 ± 0.48a |
| | CSM | 449.88 ± 31.42a | 25.46 ± 1.06b | 1.51 ± 0.02b | 17.68 ± 1.15b | 297.91 ± 21.57b | 16.86 ± 0.90b |
| | CSH | 485.49 ± 33.34a | 21.89 ± 0.40c | 1.16 ± 0.02c | 22.18 ± 1.46a | 418.59 ± 29.48a | 18.88 ± 0.74a |
| Herbaceous | AHL | 398.27 ± 6.61a | 25.03 ± 3.35a | 1.45 ± 0.03b | 16.10 ± 2.11a | 274.76 ± 1.42a | 17.24 ± 2.06a |
| | AHM | 409.11 ± 5.12a | 24.05 ± 0.73a | 1.65 ± 0.04a | 17.02 ± 0.48a | 247.64 ± 3.71b | 14.55 ± 0.26a |
| | AHH | 395.95 ± 18.47a | 23.53 ± 2.73a | 1.69 ± 0.05a | 16.99 ± 2.23a | 234.91 ± 12.88b | 13.93 ± 1.21a |
| | BHL | 393.10 ± 12.54a | 22.95 ± 0.54b | 1.99 ± 0.02a | 17.14 ± 0.84a | 197.34 ± 7.29b | 11.52 ± 0.15b |
| | BHM | 410.47 ± 22.11a | 27.67 ± 2.45a | 1.70 ± 0.06b | 14.95 ± 2.00a | 241.99 ± 8.49a | 16.36 ± 2.04a |
| | BHH | 444.59 ± 35.32a | 24.95 ± 1.09ab | 1.88 ± 0.08a | 17.80 ± 0.79a | 236.50 ± 15.91a | 13.27 ± 0.32b |
| | CHL | 468.99 ± 8.05a | 26.02 ± 0.60a | 1.76 ± 0.04a | 18.02 ± 0.28a | 266.14 ± 9.38c | 14.76 ± 0.40c |
| | CHM | 426.61 ± 4.08c | 24.25 ± 1.56ab | 1.02 ± 0.03c | 17.63 ± 1.01a | 418.23 ± 14.10a | 23.77 ± 1.65a |
| | CHH | 443.42 ± 4.34b | 22.93 ± 0.48b | 1.30 ± 0.03b | 19.35 ± 0.57a | 340.50 ± 8.02b | 17.61 ± 0.44b |

Different lowercase letters represent significant differences among encroachment levels within group ($P < 0.05$)

Table A3. Soil properties and enzyme activities across encroachment levels and elevations within the study area

| | | TC (g kg ⁻¹) | TN (g kg ⁻¹) | C:N | NH ₄ ⁺ -N (mg kg ⁻¹) | NO ₃ ⁻ -N (mg kg ⁻¹) | AP (mg kg ⁻¹) | Moisture (%) | pH | Catalase | ALP | Urease |
|--------|-----|--------------------------|--------------------------|---------------|--|--|---------------------------|---------------|---------------|--------------|---------------|---------------|
| 2075 m | ASL | 113.14 ± 7.57a | 5.66 ± 0.33a | 20.10 ± 2.42a | 32.53 ± 2.96b | 13.97 ± 1.59ab | 87.14 ± 10.17a | 0.38 ± 0.00a | 4.88 ± 0.02ab | 0.76 ± 0.01a | 0.12 ± 0.00ab | 0.50 ± 0.03c |
| | ASM | 82.34 ± 1.38b | 4.48 ± 0.33c | 18.45 ± 1.21a | 40.03 ± 1.44a | 11.75 ± 1.09b | 86.42 ± 8.95a | 0.24 ± 0.00c | 5.05 ± 0.03a | 0.7 ± 0.02b | 0.12 ± 0.00b | 0.62 ± 0.02b |
| | ASH | 84.59 ± 1.85b | 5.12 ± 0.13b | 16.54 ± 0.45a | 29.92 ± 1.84b | 15.25 ± 0.99a | 103.49 ± 4.09a | 0.31 ± 0.00b | 4.81 ± 0.18b | 0.72 ± 0.01b | 0.13 ± 0.01a | 0.71 ± 0.05a |
| 2157 m | BSL | 104.87 ± 2.98a | 7.81 ± 0.38a | 13.44 ± 0.47a | 33.64 ± 1.51b | 15.76 ± 0.67c | 110.72 ± 8.07ab | 0.47 ± 0.00a | 4.91 ± 0.02a | 0.83 ± 0.00a | 0.08 ± 0.00a | 0.64 ± 0.08b |
| | BSM | 106.05 ± 2.98a | 8.40 ± 0.17a | 12.64 ± 0.59a | 33.46 ± 1.36b | 18.90 ± 0.70b | 99.41 ± 4.14b | 0.47 ± 0.00a | 4.82 ± 0.02b | 0.85 ± 0.01a | 0.08 ± 0.01a | 0.85 ± 0.06a |
| | BSH | 101.25 ± 5.89a | 7.96 ± 0.29a | 12.72 ± 0.56a | 62.40 ± 1.85a | 23.15 ± 1.51a | 121.02 ± 7.86a | 0.45 ± 0.00b | 4.59 ± 0.01c | 0.86 ± 0.01a | 0.08 ± 0.00a | 0.87 ± 0.07a |
| 2240 m | CSL | 66.72 ± 1.85b | 9.36 ± 0.84b | 7.18 ± 0.74a | 62.23 ± 1.39c | 20.45 ± 0.88b | 116.91 ± 10.20b | 0.40 ± 0.01c | 4.84 ± 0.02a | 0.85 ± 0.01a | 0.07 ± 0.00b | 0.52 ± 0.03a |
| | CSM | 66.81 ± 3.90b | 8.06 ± 0.80b | 8.37 ± 1.32a | 72.13 ± 0.62b | 19.67 ± 0.81b | 122.66 ± 11.28b | 0.45 ± 0.01b | 4.85 ± 0.04a | 0.85 ± 0.01a | 0.08 ± 0.00a | 0.54 ± 0.06a |
| | CSH | 83.80 ± 4.08a | 12.52 ± 0.29a | 6.69 ± 0.20a | 96.05 ± 2.32a | 40.08 ± 0.43a | 167.84 ± 4.45a | 0.56 ± 0.01a | 4.84 ± 0.01a | 0.84 ± 0.01a | 0.07 ± 0.00b | 0.42 ± 0.02b |
| 2075 m | AHL | 99.33 ± 0.81b | 6.75 ± 0.76a | 14.84 ± 1.67a | 51.21 ± 3.91c | 17.57 ± 0.79c | 92.21 ± 2.14b | 0.35 ± 0.01b | 4.82 ± 0.03a | 0.78 ± 0.00a | 0.13 ± 0.00a | 0.95 ± 0.03a |
| | AHM | 105.55 ± 0.41a | 6.60 ± 0.34a | 16.02 ± 0.86a | 65.16 ± 1.18a | 32.92 ± 1.64b | 106.01 ± 7.07a | 0.36 ± 0.00a | 4.81 ± 0.04a | 0.75 ± 0.01b | 0.13 ± 0.00a | 0.86 ± 0.07ab |
| | AHH | 88.00 ± 0.80c | 6.00 ± 0.06a | 14.68 ± 0.22a | 57.88 ± 1.58b | 52.52 ± 1.09a | 109.68 ± 9.53a | 0.36 ± 0.01a | 4.59 ± 0.04b | 0.78 ± 0.01a | 0.13 ± 0.00a | 0.92 ± 0.06b |
| 2157 m | BHL | 110.44 ± 4.43b | 9.54 ± 0.37b | 11.60 ± 0.88a | 52.07 ± 2.61b | 19.73 ± 0.69a | 100.60 ± 10.89a | 0.38 ± 0.00ab | 4.77 ± 0.03b | 0.88 ± 0.01a | 0.09 ± 0.00a | 0.82 ± 0.05a |
| | BHM | 103.78 ± 3.81b | 7.14 ± 0.62c | 14.65 ± 1.81a | 57.55 ± 1.61a | 14.50 ± 1.60b | 108.54 ± 6.29a | 0.30 ± 0.01b | 4.85 ± 0.02a | 0.84 ± 0.01b | 0.10 ± 0.00a | 0.58 ± 0.09b |
| | BHH | 127.11 ± 9.60a | 10.71 ± 0.16a | 11.87 ± 0.87a | 54.28 ± 1.08b | 15.74 ± 1.22b | 101.68 ± 4.53a | 0.45 ± 0.10a | 4.82 ± 0.01a | 0.87 ± 0.01a | 0.10 ± 0.00a | 0.62 ± 0.02b |
| 2240 m | CHL | 56.72 ± 3.03b | 4.83 ± 0.53c | 11.84 ± 1.24b | 92.95 ± 1.85a | 27.78 ± 0.84a | 128.88 ± 14.43a | 0.37 ± 0.01ab | 4.81 ± 0.01a | 0.86 ± 0.02a | 0.09 ± 0.00a | 0.65 ± 0.03b |
| | CHM | 126.1 ± 6.37a | 11.59 ± 1.26a | 11.00 ± 1.73b | 73.85 ± 1.71b | 27.07 ± 1.05a | 142.00 ± 6.52a | 0.37 ± 0.00a | 4.82 ± 0.01a | 0.86 ± 0.01a | 0.09 ± 0.00a | 0.73 ± 0.02a |
| | CHH | 125.02 ± 9.79a | 8.54 ± 0.31b | 14.64 ± 0.95a | 51.65 ± 0.68c | 14.22 ± 1.39b | 118.21 ± 3.18a | 0.35 ± 0.01b | 4.81 ± 0.02a | 0.86 ± 0.00a | 0.09 ± 0.00a | 0.59 ± 0.04b |

Different lowercase letters represent significant differences among encroachment levels within group ($P < 0.05$)

Table A4. The explained variance of soil properties and their significant analysis in redundancy analysis (RDA)

| Soil abiotic factor | Explains % | Contribution % | F | P** |
|--|------------|----------------|-------|--------------|
| Moisture | 23.5 | 39.2 | 16 | 0.002 |
| C:N (ratio) | 9.7 | 16.2 | 7.8 | 0.002 |
| pH | 4.2 | 7 | 4.2 | 0.026 |
| AP (mg kg ⁻¹) | 4.2 | 6.9 | 3.5 | 0.034 |
| Urease | 4.1 | 6.9 | 3.9 | 0.048 |
| NH ₄ ⁺ -N (mg kg ⁻¹) | 4.1 | 6.8 | 3.6 | 0.052 |
| ALP | 4.2 | 7 | 3 | 0.064 |
| Catalase | 2.1 | 3.5 | 2.2 | 0.112 |
| TN (g kg ⁻¹) | 2.1 | 3.4 | 2.2 | 0.126 |
| NO ₃ ⁻ -N (mg kg ⁻¹) | 1.9 | 3.1 | 1.9 | 0.152 |
| TC (g kg ⁻¹) | < 0.1 | < 0.1 | < 0.1 | 0.974 |

**Indicates the variance of each soil abiotic factor significantly contributed to the total variance. Significant values are in bold