



## OPEN The ecological stoichiometry shifts induced by *Ligularia virgaurea* invasion in alpine meadows of the Qinghai-Tibetan plateau

Meiling Song<sup>✉</sup>, Yuqin Wang<sup>✉</sup>, Rui Zhou, Hongsheng Wang, Chunping Zhang & Bing Li

The invasion of alpine meadows on the Qinghai-Tibetan Plateau by *Ligularia virgaurea* raises serious concerns about its impact on ecosystem functions. This study investigated how its spread influences ecological stoichiometry and nutrient transitions in native plant communities. We investigated four invasion density levels in a typical alpine meadow: no invasion (LN), low (LL), moderate (LM), and high (LH) density. A replicated sampling design was used to assess the C: N:P stoichiometry of different plant functional groups and its correlation with soil nutrients. Our results demonstrate that increasing *L. virgaurea* density led to a significant decline in plant community nitrogen content and a rise in C: N ratios, mirroring the response of grasses. Phosphorus content in community plants and key groups (legumes, forbs, and *L. virgaurea* itself) initially increased then slightly decreased, peaking at moderate invasion density. Under high-density invasion, plant organic carbon content decreased significantly. Concurrently, soil inorganic nitrogen declined, while soil available phosphorus, organic carbon, and microbial biomass (C, N, P) increased markedly. These findings suggest that *L. virgaurea* successfully invades by outcompeting natives for nitrogen and enhancing phosphorus availability via stimulation of the soil microbial pool. Furthermore, different functional groups exhibited distinct stoichiometric strategies; sedges maintained competitiveness through efficient nutrient retention, while grass faced intensified nitrogen limitation. Overall, *L. virgaurea* invasion induces a fundamental shift in the nutrient cycling of alpine meadows, driven by plant-soil-microbe feedback that reinforces its dominance.

**Keywords** *Ligularia virgaurea*, Ecological stoichiometry, Alpine meadows, Qinghai-Tibetan plateau, Toxic weed, Invasion

Carbon (C), nitrogen (N), and phosphorus (P) are the essential elements utilized by plants<sup>1</sup>. Ecological stoichiometry, which explores the balance of energy and chemical elements in biological systems, provides a powerful framework for understanding nutrient cycling and the functioning of terrestrial ecosystems<sup>2,3</sup>. The study of leaf ecological stoichiometry in particular has become an effective method for determining plant growth rates, nutrient utilization efficiency, and the availability of soil nutrients for plant growth<sup>4,5</sup>. Among these essential elements, C is the most important for dry matter accumulation and serves as the substrate and energy source for various critical physiological processes<sup>6</sup>. The C: N and C: P ratios represent the ability of plants to assimilate C while simultaneously absorbing both N and P; in contrast, the N: P ratio reflects the dynamic balance between soil nutrients and plant nutrition demands<sup>7,8</sup>. Ecosystem C: N:P stoichiometry varies significantly among ecosystem types, land-use categories, and environmental gradients<sup>9,10</sup>. Recently, most studies on C: N:P stoichiometry have focused on the plant organ level, with a scarcity of studies conducted at the plant functional group or community levels in grasslands<sup>11</sup>.

A plant community is formed through the assemblage of species adapted to a specific environment and through mutual competition. Thus, plants with different strategies compete for limited resources, such as water, light, and nutrients<sup>12,13</sup>. Changes in community traits are often weighted by the relative abundances of species and are mostly driven by dominant species rather than other species<sup>14</sup>. Therefore, any changes in community

Key Laboratory of the Alpine Grassland Ecology in the Three River Region, Ministry of Education, Academy of Animal and Veterinary Sciences, Qinghai University, Xining 810016, China. ✉email: meilings@163.com; gsdnwangyuqin@126.com

composition can have significant implications for community-level nutrient resources<sup>15,16</sup>. Previous studies in high-altitude grasslands have shown that net plant–plant interactions shift from competitive to facilitative in response to environmental changes<sup>17,18</sup>. However, little is known about the changes in soil nutrient availability and plant C: N:P stoichiometry associated with the progression of community succession, which limits our understanding of nutrient geochemical cycles in ecosystems<sup>19</sup>.

The Qinghai–Tibetan Plateau, popularly known as the “Roof of the World,” contains unique and fragile ecosystems that harbor diverse plant species adapted to extreme environmental conditions<sup>20</sup>. Alpine meadows, a dominant vegetation type of the plateau, support a variety of plant functional groups, including grasses, sedges, legumes, and forbs, which each play crucial roles in maintaining ecosystem services, such as nutrient cycling, carbon sequestration, and forage production<sup>21</sup>. However, in recent decades, the plateau has experienced profound changes caused by climate warming and overgrazing, leading to widespread degradation of alpine meadows<sup>22</sup>. These ecological pressures often promote the invasion of exotic plant species, which can in turn create positive feedback loops that maintain and exacerbate the degraded state by which they are caused<sup>23</sup>. On one hand, toxic plants in grassland are harmful to grazing animals which induces substantial losses of animal husbandry<sup>24</sup>. Moreover, these toxic weeds can form intraspecific aggregations that enhance their competitive ability against other species<sup>16,25</sup>. Therefore, the wide expansion of toxic plants under climate change and human activities has been concerned globally<sup>23,26</sup>. Recent research on the spread of toxic plants has focused mainly on key contemporary factors such as grazing livestock and climate change<sup>27</sup>. However, how toxic plants adapt to the poor soil conditions of degraded grasslands remains unknown.

One of the most notorious invasive species in the Qinghai–Tibetan Plateau is *Ligularia virgaurea*, a perennial herbaceous plant native to Central Asia that has gradually spread across alpine grasslands<sup>28</sup>. *Ligularia virgaurea* can become dominant in grazed alpine communities owing to its toxicity to grazers. Its invasion is associated with significant shifts in species composition, community structure, and ecosystem processes<sup>6</sup>. The allelochemicals released by *L. virgaurea* alter soil microbial communities, thus affecting soil nutrients and creating positive feedback effects<sup>25,29</sup>. There is also evidence that allelopathic effects of volatile or aqueous leaf or root extracts of *L. virgaurea* inhibit the seed germination and growth of forb species native to the Qinghai–Tibet Plateau<sup>28</sup>. Consequently, it alters the growth of associated plant species and the overall functioning of invaded alpine meadows.

Previous studies have documented the effects of *L. virgaurea* on nutrient cycling, plant community composition, and soil properties<sup>26,29</sup>. However, a comprehensive understanding of how the spread of *L. virgaurea* impacts the ecological stoichiometry of associated plants remains elusive. However, addressing this knowledge gap is crucial for developing effective management strategies to mitigate the impacts of invasive species and restore the resilience of alpine meadows on the Qinghai–Tibetan Plateau. This study was conducted to investigate the effect of *L. virgaurea* spread on the ecological stoichiometry of associated plants in alpine meadows of the Qinghai–Tibetan Plateau. Specifically, we aimed to (1) quantify the changes in C, N, and P contents and stoichiometric ratios (C: N, C: P, and N: P) of different plant functional groups in response to increasing *L. virgaurea* density; (2) analyze the impacts of *L. virgaurea* spread on soil nutrient contents and their relationships with plant stoichiometric traits; and (3) identify the underlying mechanisms responsible for the observed changes in plant ecological stoichiometry under *L. virgaurea* invasion.

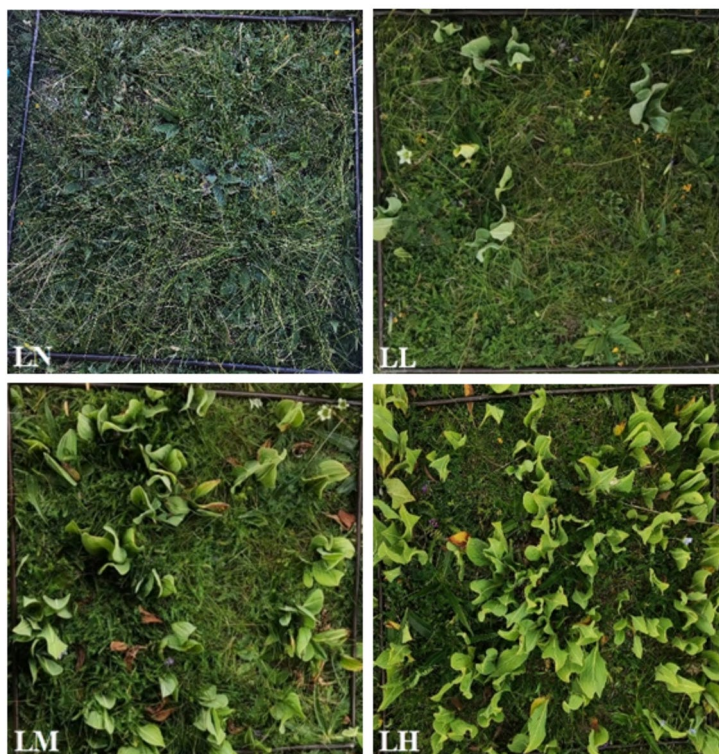
## Materials & methods

### Study site

The study was conducted in an alpine meadow located at an elevation of 3,532 m in Henan County (34°42′ N, 101°36′ E), Huangnan Tibetan Autonomous Prefecture, Qinghai Province, China. The region has a typical plateau continental climate, with a mean annual solar radiation of 2,580 h, an average annual temperature of 0.4–3.4 °C, and an annual precipitation accumulation of 579–616 mm (most of which falls between May and September). The vegetation is typical of nearby alpine meadows, dominated by *Carex alataensis*, *C. capillifolia*, and *Stipa aliena*. Other commonly occurring species include *C. atrofusca*, *Poa pratensis*, *Melissitus ruthenica*, and *Lancea tibetica*. The study site is utilized as winter rangeland by local herders (grazing from September to May) with a heavy grazing intensity of about 7.94 sheep units per hectare. Over the past few decades, *Ligularia virgaurea* has invaded the grassland and gradually become the dominant species (reaching an average density of up to 133.6 plants per m<sup>2</sup>)<sup>30</sup>, posing serious degradation challenges to the grassland. The permission (granted by Grassland Working Station of Henan county, Huangnan, China and local herdsmen) had been obtained for field study and plant materials’ collection.

### Sampling and chemical analysis

To focus on the effect of *L. virgaurea* density and minimize the influence of confounding environmental gradients, the study was deliberately conducted within a contiguous area characterized by uniform topography, consistent soil type, and a homogenous macro-climate. To isolate the effect of *L. virgaurea* invasion from the confounding effects of contemporary grazing, the entire study area was fenced in May 2022, one year prior to sampling, to exclude livestock. Three plots (each 80 × 80 m) with varying naturally occurring densities of *L. virgaurea* were established, ensuring a sufficient distance of approximately 30–40 m between them. In August 2023, within each plot, four levels of *L. virgaurea* density were selected based on plant counts per square meter: LN (no *L. virgaurea*), LL (low density, 30–40 plants/m<sup>2</sup>), LM (moderate density, 100–120 plants/m<sup>2</sup>) and LH (high density, 200–240 plants/m<sup>2</sup>) (Fig. 1). These density categories were defined according to the Local Standards of Qinghai Province (Grassland Toxic Weed Management Guidelines, DB63/T 241–2021), which align with the invasion intensities commonly observed in local grasslands. Three quadrats (0.5 m × 0.5 m) were randomly established for each density level. Aboveground tissues of all species within each quadrat were sampled and sorted into five mutually exclusive functional groups: grasses, sedges, legumes, forbs, and *L. virgaurea*. Species identification was



**Fig. 1.** The sets for different density levels of *Ligularia virgaurea*. LN: None of *L. virgaurea*, LL: Low density of *L. virgaurea*, LM: Moderate density of *L. virgaurea*, LH: High density of *L. virgaurea*.

undertaken by collectors according to Hou<sup>31</sup> et al. To minimize sampling bias and ensure the representativeness of the chemical analysis, the aboveground tissues for each plant functional group collected from the three quadrats of the same *L. virgaurea* density level within a plot were pooled to form one composite sample per group per density per plot. Each composite sample was then homogenized and split into three analytical subsamples for subsequent nutrient determination. At the community level, another three quadrats were randomly surveyed within each *L. virgaurea* density level, and aboveground tissues of all observed species were collected and pooled as one sample per quadrat. Root samples were collected using a root auger (7-cm diameter) from each quadrat from depths of 0–10 cm and 10–20 cm and then washed to remove soil and other impurities. After sampling, all tissues and roots were oven-dried at 85 °C to a constant mass, weighed, and ground for further nutrient analysis at the laboratory facilities of Qinghai University, Xining, Qinghai, China. Soil samples were also collected from 0 to 20 cm deep using a soil auger (3.5-cm diameter) within each quadrat, and all the soil samples of three quadrats in a plot were pooled into one sample. After air-drying, soil samples were passed through a 1-mm sieve for nutrient analysis.

The total soil and plant organic carbon content was determined using the oil bath- $K_2CrO_7$  titration method (oxidization with dichromate in the presence of  $H_2SO_4$ , heated to 180 °C for 5 min followed by titration with  $FeSO_4$ )<sup>32</sup>. The total nitrogen content of soil and plant samples was analyzed using the Kjeldahl digestion method with a Nitrogen Analyzer System (Kjeltec 2300 Auto System II, Foss Tecator AB, Höganäs, Sweden). Total phosphorus content was determined using the molybdate blue colorimetric method with a spectrophotometer (SP-723; Analytikjena, Germany) after digestion with  $H_2SO_4$  and  $H_2O_2$ . Soil  $NH_4^+-N$  and  $NO_3^--N$  levels were measured using a FIAstar 5000 Analyzer (Foss Tecator AB). Available phosphorus in soil was analyzed using the Molybdenum-antimony anti-colorimetric method<sup>16,32</sup>.

### Statistical analysis

Data analyses were conducted using SPSS (version 17.0; SPSS Inc., Chicago, IL, USA). As necessary, data were transformed to meet the homogeneity of variance and normality assumptions of ANOVA. Independent sample *t*-tests were used to determine significant differences between various *L. virgaurea* density levels for all parameters. For all two-way ANOVAs that revealed significant main or interaction effects ( $P < 0.05$ ), Tukey's Honest Significant Difference (HSD) post-hoc test was subsequently applied to identify significant pairwise differences among the levels of functional groups and *L. virgaurea* density. Statistical significance was determined at the 95% level ( $P < 0.05$ ). To examine the overall relationships between the multivariate profile of plant stoichiometric traits and soil nutrient parameters, Mantel tests were performed using the linkET package in R. Separate Euclidean distance matrices were constructed for the dataset of plant stoichiometric traits (including nitrogen, phosphorus, and carbon contents, and C: N, C: P, N: P ratios in both aboveground and root tissues) and the dataset of soil variables (including TN,  $NH_4^+-N$ ,  $NO_3^--N$ , TP, AP, TOC, MBC, MBN, MBP). The correlation

between these two distance matrices was assessed using the Spearman rank correlation coefficient. The statistical significance of the Mantel correlation statistic ( $r$ ) was tested with 999 permutations. A significance threshold of  $P < 0.05$  was applied, with correlations at  $P < 0.01$  considered highly significant.

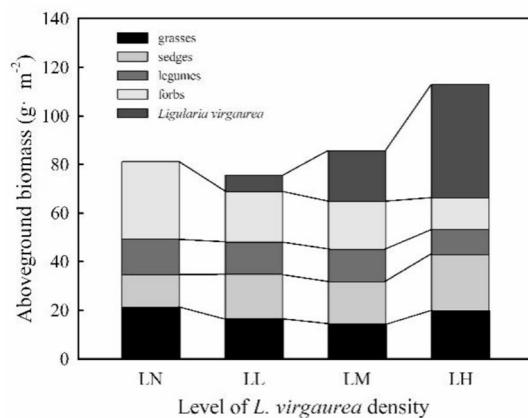
## Results

### The biomass and stoichiometry of different functional group plants under different *L. virgaurea* density levels

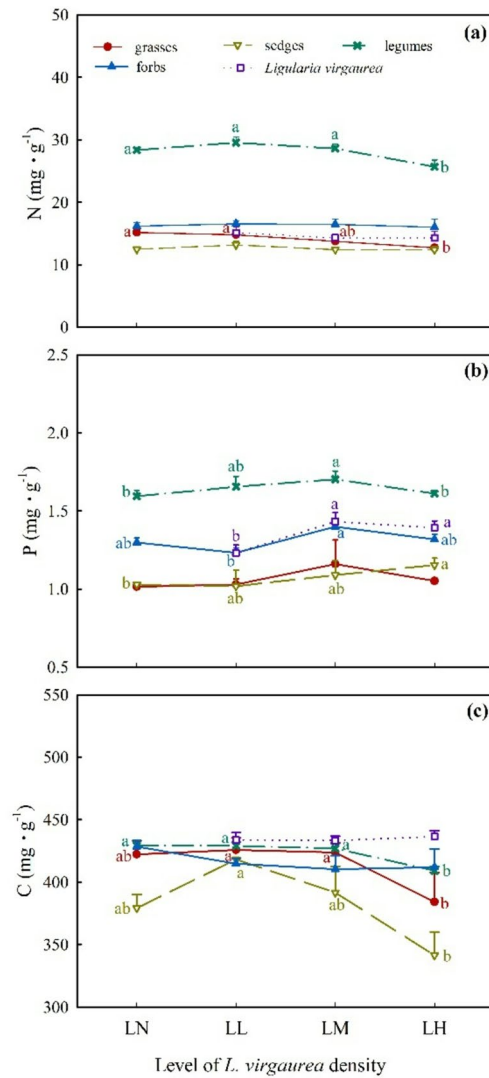
With increasing *L. virgaurea* density, the total aboveground biomass in the alpine meadow increased significantly (Fig. 2). However, when the biomass of *L. virgaurea* was excluded, the total aboveground biomass of other plant functional groups declined gradually with *L. virgaurea* density. Specifically, as *L. virgaurea* density increased, the biomass of forbs and legumes showed a decreasing trend; in contrast, sedge biomass increased, and grasses biomass showed an initial decline followed by an increase.

The nitrogen content in aboveground tissues of grasses and legumes decreased with increasing *L. virgaurea* density (Fig. 3a). In high-density *L. virgaurea* (LH) conditions, the nitrogen content of grasses and legumes was significantly lower than that in the no *L. virgaurea* (LN) and low-density *L. virgaurea* (LL) conditions ( $P < 0.05$ ). However, *L. virgaurea* density had no significant effect on the nitrogen content of sedges, forbs, or *L. virgaurea* itself ( $P > 0.05$ ). In the LH community, the nitrogen content of *L. virgaurea* was higher than that of both grasses and sedges. The phosphorus content in plant tissues of grasses, legumes, forbs, and *L. virgaurea* initially increased and then decreased with increasing *L. virgaurea* density (Fig. 3b). The highest phosphorous content values for all sample types was observed in the moderate-density *L. virgaurea* (LM) community. In contrast, phosphorus content in sedge tissues increased gradually with increasing *L. virgaurea* density. In the LH community, phosphorus content in *L. virgaurea* was higher than that in forbs, grasses, and sedges. As *L. virgaurea* density increased, the organic carbon content of plants in the other four functional groups decreased (Fig. 3c). *Ligularia virgaurea* had higher organic carbon content than the other four functional groups, and the difference in organic carbon content widened as *L. virgaurea* density increased. Overall, both the functional group and *L. virgaurea* density significantly affected plant nitrogen, phosphorus, and organic carbon contents. Additionally, the interaction effect on carbon, nitrogen, and phosphorous contents between functional groups and *L. virgaurea* density was significant (Table 1). Phosphorus content was significantly higher in the LH and LM treatments than that in the LN and LL treatments. Nitrogen and carbon contents were significantly lower in the LH treatment than that in the LN, LL and LM treatments (Tukey's HSD,  $P < 0.05$ , Table 1; Fig. 3).

As *L. virgaurea* density increased, the C: N ratio in aboveground tissues of grasses gradually increased (Fig. 4a). Under LH conditions, the C: N ratio of grasses was significantly higher than that under LN conditions ( $P < 0.05$ ). Conversely, the C: N ratio of sedges decreased as *L. virgaurea* density increased, being significantly lower in LH conditions relative to LN and LL conditions ( $P < 0.05$ ). There were no significant differences in plant C: N ratios for legumes, forbs, and *L. virgaurea* as *L. virgaurea* density changed. Thus, while *L. virgaurea* density had no significant effect on plant C: N, the interaction between functional group and *L. virgaurea* density was significant (Table 1). Except for legumes, the C: P ratio of the other groups, i.e., grasses, sedges, forbs, and *L. virgaurea*, decreased as *L. virgaurea* density increased (Fig. 4b). Significant differences in the C: P ratio were observed between LH and LN conditions among grasses and sedges. Additionally, the C: P ratio of forbs in LM conditions was significantly lower than that in LN and LL conditions, and the C: P of *L. virgaurea* in LM and LH conditions was significantly lower than that in LL conditions ( $P < 0.05$ ). Therefore, both functional group and *L. virgaurea* density significantly affected plant C: P, and their interaction was significant (Table 1). As *L. virgaurea* density increased, the N: P ratio of grasses, forbs, and *L. virgaurea* first decreased and then increased (Fig. 4c). In contrast, sedges and legumes showed a gradual decrease in N: P ratio as *L. virgaurea* density increased. Both *L. virgaurea* density and functional group significantly affected plant N: P, but their interaction was not significant ( $P > 0.05$ , Table 1). The difference of C: N ratio was significant between LH and LM treatments. C: P and N: P



**Fig. 2.** Aboveground biomass of different functional groups in the community with different levels of *Ligularia virgaurea*.



**Fig. 3.** The changes of nitrogen (a), phosphorus (b) and carbon (c) contents of different functional groups with the increasing of levels of *Ligularia virgaurea*. Different lowercase letters represent significant differences ( $P < 0.05$ ) among different density of *L. virgaurea*. Different colors of lowercase letters represent different functional groups. Red: grasses; Yellow: sedges; Green: legumes; Blue: forbs; Purple: *Ligularia virgaurea*.

ratios were significantly lower in the LM and LH treatments than that in the LN and LL treatments (Tukey's HSD,  $P < 0.05$ , Table 1; Fig. 4).

### Stoichiometry of aboveground and belowground tissues under different *L. virgaurea* densities

As *L. virgaurea* density increased, the nitrogen content of community plants decreased, resulting in a general increase in C: N ratios (Fig. 5a, b). In LM and LH conditions, nitrogen content was significantly lower and C: N ratios were significantly higher than in LN conditions ( $P < 0.05$ ). Conversely, plant phosphorus content increased with *L. virgaurea* density, with the highest phosphorous contents observed in LM conditions, significantly exceeding those in LN and LL conditions (Fig. 5c). Consequently, plant C: P ratios decreased as *L. virgaurea* density increased, and C: P in LH and LM conditions was significantly lower than that in LN and LL conditions ( $P < 0.05$ , Fig. 5d). Plant organic carbon content did not significantly differ for any of the plant groups among LN, LL, and LM conditions ( $P > 0.05$ , Fig. 5e). However, in LH conditions, plant carbon content was significantly lower than that in the three lower *L. virgaurea* densities. Among N: P ratios, there was a declining trend as *L. virgaurea* density increased. The N: P ratios of plants in LH and LM conditions were significantly lower than those in LN and LL conditions ( $P < 0.05$ , Fig. 5f).

The changes in nitrogen content and C: N ratio of roots as *L. virgaurea* density increased showed no consistent pattern (Fig. 6a, b). In LH conditions, root nitrogen content in the 0–10 cm soil layer was the highest and significantly higher than that in LL conditions. Correspondingly, the C: N ratio in LH conditions was the lowest and significantly different from that under LM conditions ( $P < 0.05$ ). There was no significant difference in root

Treatments		df	N		P		C		C: N		C: P		N: P		
			F	P	F	P	F	P	F	P	F	P	F	P	
Two-way ANOVA	<i>L. virgaurea</i> density (D)	3	18.42	<0.001	17.26	<0.001	25.38	<0.001	1.19	0.32	20.08	<0.001	29.52	<0.001	
	Functional group (G)	4	1212.55	<0.001	249.97	<0.001	48.82	<0.001	378.72	<0.001	81.16	<0.001	130.26	<0.001	
	D×G	11	4.28	<0.001	2.26	0.023	7.72	<0.001	4.39	<0.001	3.39	0.001	1.74	0.089	
Treatments		<i>P</i> value													
		N		P		C		C: N		C: P		N: P			
Tukey's HSD	LN-LL	0.829		1		0.058		0.258		0.605		0.967			
	LN-LM	0.001		<0.001		0.921		0.004		0.001		<0.001			
	LN-LH	<0.001		0.004		<0.001		0.079		<0.001		<0.001			
	LL-LM	0.011		<0.001		0.175		0.286		<0.001		<0.001			
	LL-LH	<0.001		0.002		<0.001		0.921		<0.001		<0.001			
	LM-LH	0.001		0.042		<0.001		0.652		0.727		0.923			

**Table 1.** Two-way ANOVA results of *Ligularia virgaurea* density (D) and functional group (G) and post-hoc tukey's HSD results of *L. virgaurea* density on nitrogen (N), phosphorus (P) and carbon (C) contents, and C: N, C: P and N: P ratios of plants. Note: LN: No *L. virgaurea*; LL: Low density of *L. virgaurea*; LM: Moderate density of *L. virgaurea*; LH: High density of *L. virgaurea*.

nitrogen and C: N ratios in the 10–20 cm soil layer across the different *L. virgaurea* densities ( $P > 0.05$ ). Root phosphorus content in the 0–10 cm soil layer increased with *L. virgaurea* density, while C: P ratios decreased gradually (Fig. 6c, d). This difference between LN conditions and each of the *L. virgaurea*-containing conditions was significant. In LN conditions, root phosphorus content in the 0–10 cm soil layer was significantly lower, and the C: P ratio was significantly higher than that in the 10–20 cm layer ( $P < 0.05$ ). The highest root organic carbon content in both the 0–10 cm and 10–20 cm soil layers was observed under LM conditions (Fig. 6e). Roots under LN and LL conditions in the 0–10 cm layer and under LH conditions in the 10–20 cm layer had significantly lower organic carbon content than those under LM conditions. For N: P ratios, LN conditions had a significantly higher value than those of LL and LM conditions in the 0–10 cm root layer (Fig. 6f). However, there was no significant difference in N: P ratios of the 10–20 cm root layer across the different *L. virgaurea* density levels ( $P > 0.05$ ).

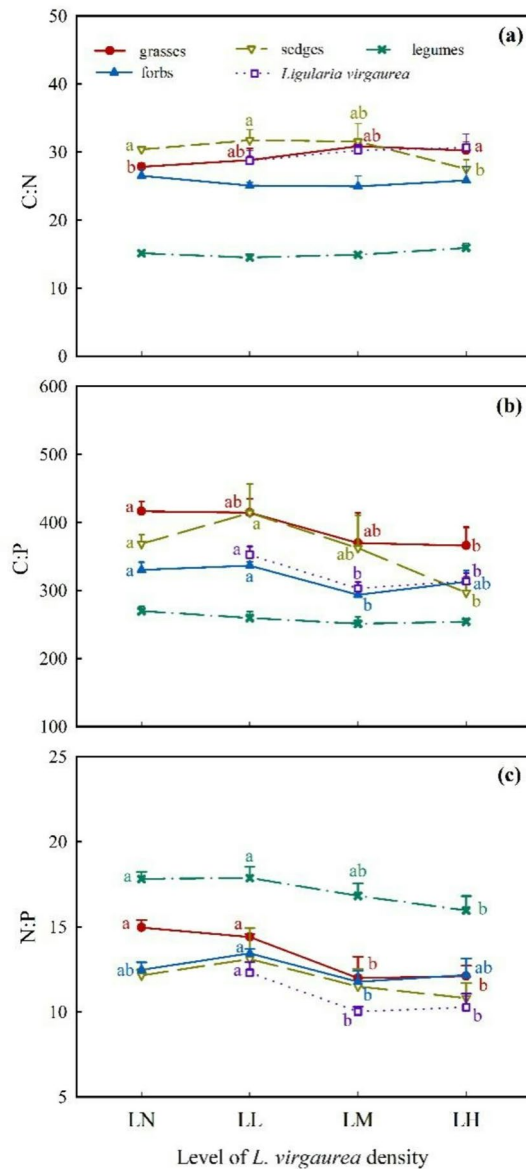
### Correlations in soil nutrient contents and plant stoichiometry during the spread of *L. virgaurea*

Soil total nitrogen (TN) and total phosphorus (TP) contents showed no significant change as *L. virgaurea* density increased ( $P > 0.05$ , Table 2). However, ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) content decreased significantly ( $P < 0.05$ ), while available phosphorus (AP) content increased with *L. virgaurea* density. Soil nitrate-N ( $\text{NO}_3^-\text{-N}$ ) was highest under LL conditions ( $15.17 \mu\text{g}\cdot\text{g}^{-1}$ ) but was sharply lower under both LM and LH conditions. Additionally, total organic carbon (TOC), microbial biomass nitrogen (MBN), microbial biomass carbon (MBC), and microbial biomass phosphorus (MBP) contents increased significantly with *L. virgaurea* density ( $P < 0.05$ , Table 2).

Mantel test results showed significant negative correlations both between  $\text{NH}_4^+\text{-N}$  and TOC and between  $\text{NO}_3^-\text{-N}$  and TP, MBN, and MBC ( $P < 0.05$ , Fig. 7). MBC, MBP, and MBN were significantly positively correlated with each other. Additionally, MBC exhibited significant positive correlations with TOC, AP, and TP, while MBP had significant positive correlations with TN only. MBN exhibited significant positive correlations with both TP and AP ( $P < 0.05$ ). For plant stoichiometry, nitrogen content and C: N ratio in plants had highly significant correlations with soil AP ( $P < 0.01$ ) and significant correlations with soil TOC and MBC ( $P < 0.05$ ). Plant phosphorus and C: P exhibited significant correlations with soil MBC and highly significant correlations with both  $\text{NH}_4^+\text{-N}$  and TOC ( $P < 0.01$ ). Plant carbon content exhibited a significant correlation with soil MBN and highly significant correlations with both soil AP and MBC ( $P < 0.01$ ). Plant N: P exhibited highly significant correlations with both soil  $\text{NH}_4^+\text{-N}$  and soil AP ( $P < 0.01$ ) and significant correlations with soil TOC, MBN, and MBC ( $P < 0.05$ , Fig. 7).

### Discussion

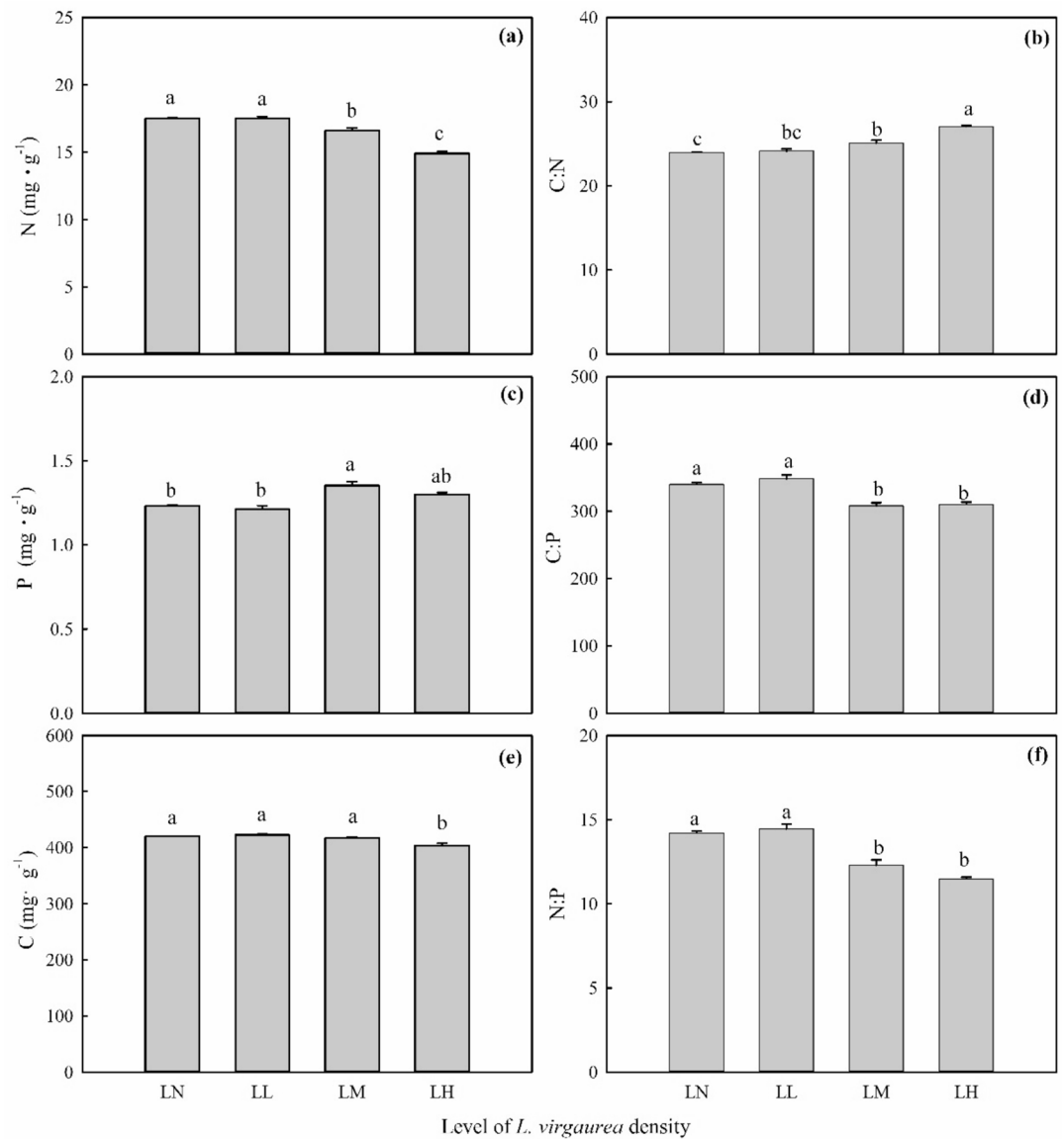
The present study comprehensively examined the ecological consequences of *L. virgaurea* invasion in a typical alpine meadow of the Qinghai–Tibetan Plateau. Our findings revealed several important insights into the intricate interactions between invasive species and native plant communities, as well as their implications for nutrient cycling and ecosystem functioning. Studies have suggested that overgrazing provides a competitive advantage to *L. virgaurea* by altering plant community structure, which is closely related to the soil environment<sup>29</sup>. In our study, the aboveground biomass of different functional groups changed as *L. virgaurea* density increased. The total biomass of other plant functional groups declined gradually as *L. virgaurea* density increased, primarily owing to the observed decrease in biomass of legumes and forbs. As a species reported to possess allelopathic properties<sup>28</sup>, *L. virgaurea* is likely to displace high-quality forage and affects the seed germination and plant establishment of cooccurring species<sup>33</sup>. Thus, legumes and forbs may spatially compete with *L. virgaurea*, resulting in their lower dominance after *L. virgaurea* invasion. Previous studies have shown that volatile and aqueous leaf and root extracts of *L. virgaurea* influence the germination and growth of native forb species<sup>28,34</sup>. Our present results are



**Fig. 4.** The changes of C: N (a), C: P (b) and N: P (c) ratios of different functional groups with the increasing of levels of *Ligularia virgaurea*. Different lowercase letters represent significant differences ( $P < 0.05$ ) among different density of *L. virgaurea*. Different colors of lowercase letters represent different functional groups. Red: grasses; Yellow: sedges; Green: legumes; Blue: forbs; Purple: *Ligularia virgaurea*.

consistent with these previous findings. While plants with different life strategies compete for limited resources (i.e., water, light, and nutrients), they may also gain facilitative shelter from neighboring plants against severe climatic events in alpine grasslands<sup>12,16</sup>; this phenomenon could explain the observed increase in sedge biomass associated with *L. virgaurea* density. Although grasses suffered some observable competitive suppression effects, it is possible that they receive some similar benefit from the aggressive spread of *L. virgaurea*.

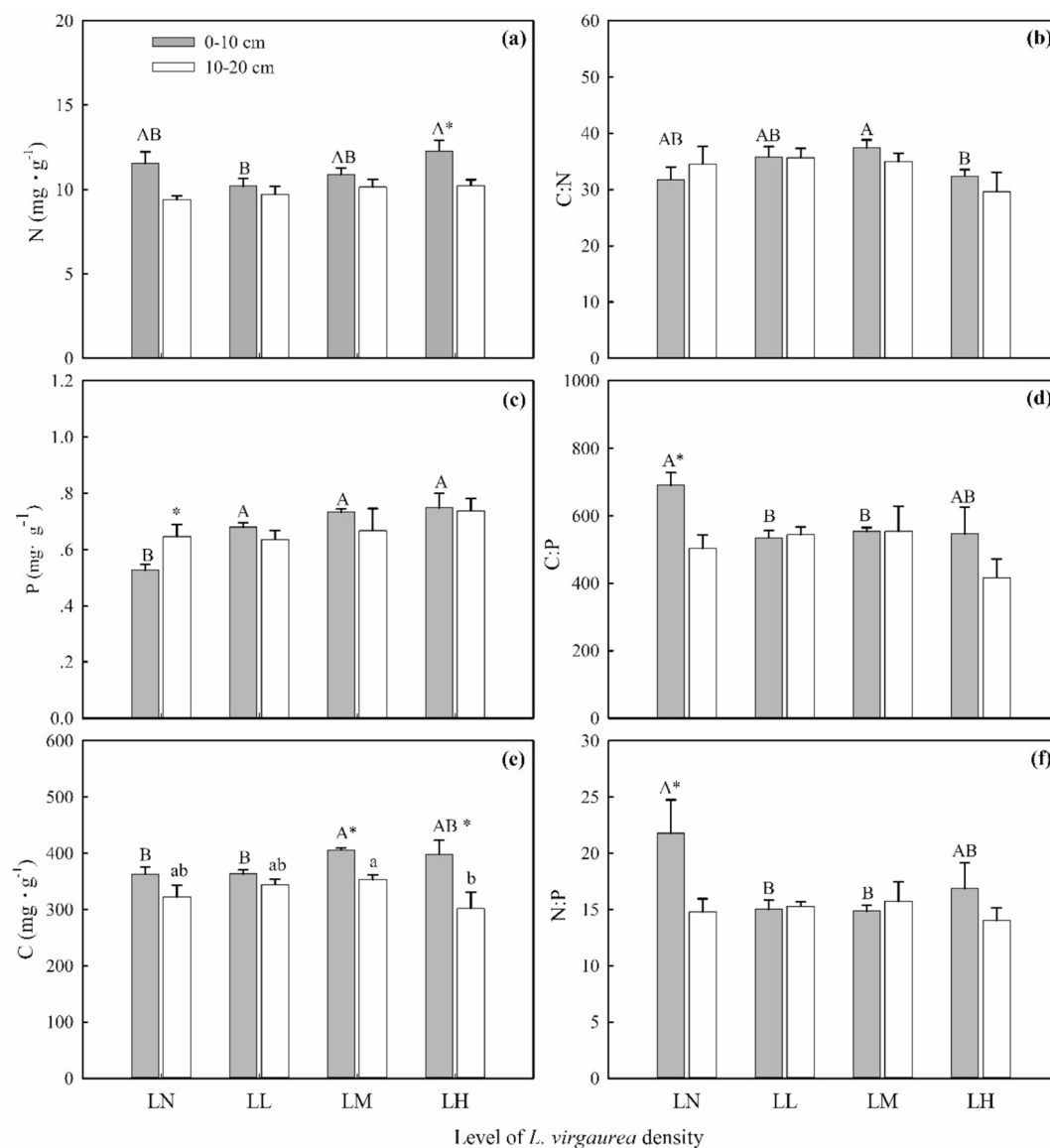
It is well known that carbon, nitrogen, and phosphorus are the principal chemical elements and the stoichiometric C: N:P ratios of plant have been widely used as indicators of nutrient limitations on plant growth<sup>8,35,36</sup>. The pathway of carbon assimilation differs from the absorption of nitrogen and phosphorus, as carbon is obtained from atmospheric CO<sub>2</sub> through photosynthesis<sup>37,38</sup>. In this study, the carbon contents of grasses, sedges, and legumes were significantly lower under the highest *L. virgaurea* density (i.e., LH) relative to the other density levels (Fig. 3c). This might be owing to limited sunlight reaching plants under high *L. virgaurea* density, which reduces the photosynthetic rate. Additionally, photosynthetic capacity is strongly correlated with nitrogen allocation to the photosynthetic apparatus<sup>39</sup>, so the lower photosynthetic efficiency of grasses and legumes would result in reduced nitrogen absorption. However, the nitrogen content of *L. virgaurea* remained unchanged across its density levels (Fig. 3a). This could be explained by the stronger capacity for nitrogen absorption of *L. virgaurea* compared to grasses and legumes. This aligns with the observed decline in soil inorganic nitrogen content (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N, Table 2) as *L. virgaurea* density increased. Thus, *L. virgaurea*



**Fig. 5.** Nitrogen (a), phosphorus (c) and carbon (e) contents, and C: N (b), C: P (d) and N: P (f) ratios of plants in the community with different levels of *Ligularia virgaurea*. Different lowercase letters represent significant differences ( $P < 0.05$ ) among different density of *L. virgaurea*.

can spread rapidly by more efficiently absorbing nitrogen relative to other species in the community. Our results are consistent with Suter<sup>40</sup> et al., who found that low nitrogen input promotes weed (*Senecio jacobaea*) invasion by reducing competition from desirable species. In association with changes in carbon and nitrogen contents, the C: N ratio increased in grasses and decreased in sedges as *L. virgaurea* density increased (Fig. 4a). Many studies have found that sedges have greater root biomass and root-to-shoot ratios than grasses<sup>41,42</sup>. Therefore, we suggest that sedges have a greater ability to take up nitrogen from belowground compared to grasses under the same conditions associated with inefficient photosynthesis. This is consistent with the observed change in biomass (Fig. 2), indicating that sedges might be more competitive than grasses under *L. virgaurea* invasion. While our study focused on stoichiometric outcomes and did not measure root depth or water-use efficiency, these factors could be significant contributors to the observed competitive suppression. Differences in root architecture or water competition may have further limited the nitrogen uptake capacity of grasses relative to the more deeply rooted *L. virgaurea* or sedges<sup>42,43</sup>.

Except for nitrogen, the phosphorus content of an organism is largely associated with the allocation of phosphorus to ribosomal RNA, which is related to its growth rate<sup>7</sup>. Thus, a change in N: P ratio is thought to alter the competitiveness of a species in a manner that depends on their growth rate and life history<sup>8,35</sup>. In this study, the plant phosphorus contents of most functional groups increased firstly and then declined with *L. virgaurea* density increasing (Fig. 3b). This may be attributed to the high biomass production and persistent litterfall of *L. virgaurea* that stimulate microbial activity, particularly phosphorus-solubilizing bacteria (PSB)

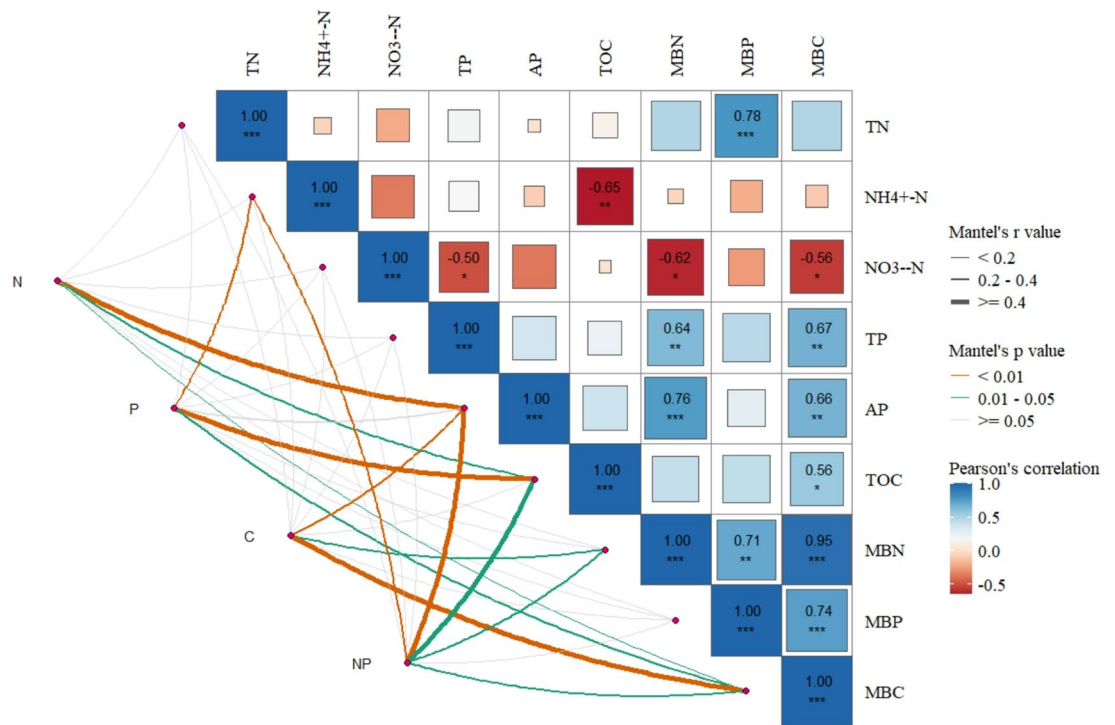


**Fig. 6.** Nitrogen (a), phosphorus (c) and carbon (e) contents, and C: N (b), C: P (d) and N: P (f) ratios of plant roots in the community with different levels of *Ligularia virgaurea*. Different lowercase letters represent significant differences ( $P < 0.05$ ) among different density of *L. virgaurea* in 10–20 cm soil. Different capital letters represent significant differences ( $P < 0.05$ ) among different density of *L. virgaurea* in 0–10 cm soil.

Level of <i>L. virgaurea</i> density	TN ( $\text{mg}\cdot\text{g}^{-1}$ )	$\text{NH}_4^+\text{-N}$ ( $\mu\text{g}\cdot\text{g}^{-1}$ )	$\text{NO}_3^-\text{-N}$ ( $\mu\text{g}\cdot\text{g}^{-1}$ )	TP ( $\text{mg}\cdot\text{g}^{-1}$ )	AP ( $\mu\text{g}\cdot\text{g}^{-1}$ )	TOC ( $\text{mg}\cdot\text{g}^{-1}$ )	MBN ( $\mu\text{g}\cdot\text{g}^{-1}$ )	MBP ( $\mu\text{g}\cdot\text{g}^{-1}$ )	MBC ( $\mu\text{g}\cdot\text{g}^{-1}$ )
LN	5.74 ± 0.09	3.61 ± 0.09a	8.44 ± 1.17bc	0.98 ± 0.03	5.24 ± 0.52b	56.91 ± 0.96c	241.41 ± 14.16c	16.55 ± 0.59b	610.69 ± 26.93c
LL	5.57 ± 0.10	2.56 ± 0.17b	15.17 ± 3.14a	0.95 ± 0.05	5.20 ± 0.15b	94.05 ± 0.93b	214.83 ± 11.64c	14.50 ± 0.27b	574.10 ± 25.77c
LM	5.88 ± 0.21	2.84 ± 0.30b	9.74 ± 1.22b	1.01 ± 0.02	5.42 ± 0.16b	99.60 ± 1.17b	276.49 ± 9.13b	24.30 ± 3.04a	754.38 ± 21.52b
LH	5.87 ± 2.77	2.77 ± 0.27b	6.03 ± 1.76c	1.05 ± 0.03	6.63 ± 0.18a	104.53 ± 0.99a	315.43 ± 5.81a	22.51 ± 0.55a	869.21 ± 22.11a

**Table 2.** The nutrient contents of soil in the community with different levels of *Ligularia virgaurea*. Note: Different lowercase letters represent significant differences ( $P < 0.05$ ) among different density of *L. virgaurea*.

and fungi, which secrete phosphatases to hydrolyze organic phosphorus<sup>44</sup>. This enhanced mineralization would directly increase soil phosphorus availability. Consequently, phosphorus acquisition of neighboring plants would increase within a certain range of *L. virgaurea* densities. As an increasingly dominant plant in alpine grasslands, much like *Stellera*<sup>45</sup>, *L. virgaurea* might create islands of fertility by this microbial activation, thus promoting its own continued expansion. However, when *L. virgaurea* density reaches a certain level, the phosphorus



**Fig. 7.** Mantel's results of soil nutrients on the stoichiometry traits of plants in the *Ligularia virgaurea* spread community. N: plant nitrogen content and C: N in aboveground biomass and roots; P: plant phosphorus content and C: P in aboveground biomass and roots; C: plant carbon content in aboveground biomass and roots; NP: plant N: P in aboveground biomass and roots; TN: soil total nitrogen; NH<sub>4</sub><sup>+</sup>-N: soil ammonium nitrogen; NO<sub>3</sub><sup>-</sup>-N: soil nitrate nitrogen; TP: soil total phosphorus; AP: soil available phosphorus; TOC: soil total organic carbon; MBC: microbial biomass carbon; MBP: microbial biomass phosphorus; MBN: microbial biomass nitrogen.

concentration in some plants (such as grasses, legumes, and forbs) appeared to decline owing to increased phosphorus demand. The different stoichiometric responses among functional groups likely reflect their distinct ecological strategies. For example, the sustained biomass and lower C: P and N: P ratios in sedges (Figs. 2 and 4) are consistent with a resource-conservative strategy and efficient belowground nutrient foraging, traits well-documented for alpine *Kobresia* species<sup>41,43</sup>. However, the competitive ability of forbs was weaker under the highest *L. virgaurea* density compared to the other densities, according to the value of N: P (Fig. 4c). This may relate with the similar lifestyle between forbs and *L. virgaurea*. The over intensity of *L. virgaurea* influences the ability of forbs to capture resources around. On the other hand, grasses suffer stronger nitrogen limitations induced by the spread of *L. virgaurea* but initiatively enhance their competition in this community by adjusting the value of N: P. Our observation that plants adjusted C: N, C: P and N: P ratios to maintain competitiveness under invasion supports the idea that plant functional groups differentially regulate stoichiometry in response to environmental change<sup>46</sup>.

At the community level, the carbon content in 0–10-cm deep roots increased with *L. virgaurea* density (Fig. 6e). This is related to the greater amount of litter falling back to the rhizosphere soil under higher *L. virgaurea* density, which is consistent with the findings of previous studies<sup>45,47</sup>. However, the nitrogen content of plant aboveground tissues declined significantly with increasing *L. virgaurea* density, thus increasing the C: N ratio (Fig. 5a, b). This may be attributed to the substantial increase in *L. virgaurea* plants, which absorb more nitrogen to support their comparatively high aboveground biomass growth. Consequently, nitrogen availability for other species (such as grasses and legumes) was more limited, causing a decline in community plant nitrogen concentration. The lower nitrogen absorption capacity of other species compared to *L. virgaurea* was also reflected in root nitrogen concentration (Fig. 6a, b). In comparison with native species, *L. virgaurea* demanded more nitrogen and thus absorbed more nitrogen, as indicated by its greater root biomass and root biomass nitrogen stock<sup>47</sup>. Similar to the findings of *Calluna vulgaris* in N-saturated heathlands, our study suggests that nitrogen limitation can persist even under conditions of high nitrogen availability, possibly due to efficient N uptake by invasive species or microbial N immobilization<sup>48</sup>. On the other hand, the significant increase in plant C: N ratios predict the production of poorer quality litter that may decompose more slowly, potentially leading to a longer-term accumulation of soil organic carbon and a reduction in nitrogen mineralization rates<sup>49–51</sup>. In contrast with nitrogen, phosphorus content in aboveground tissues and 0–10-cm deep roots increased with *L. virgaurea* density. This directly reduced C: P and N: P ratios in both aboveground and belowground tissues (Figs. 5c, d and f and 6c, d and f). In particular, aboveground N: P ratios were between 14 and 16 in LN

and LL conditions, indicating co-limitation of nitrogen and phosphorus<sup>52</sup>. As *L. virgaurea* density increased, communities gradually became more nitrogen-limited ( $N:P < 14$ ). Thus, the increased density of *L. virgaurea* may have increased the phosphorus uptake of surrounding plants while also exacerbating nitrogen limitation of this plant community. This phenomenon has also been reported in alpine meadows in Gansu province, China, where soil nitrogen limitation was identified as a dynamic factor controlling *L. virgaurea* expansion<sup>33</sup>. Simultaneously, the widespread decrease in C:P and N:P ratios (Figs. 5d and f and 6d and f) suggests a relative enrichment of phosphorus in plant tissues and litter, which could accelerate phosphorus cycling. This decoupling of nitrogen and phosphorus cycles—towards greater nitrogen limitation and phosphorus availability—may create a positive feedback that stabilizes the dominance of *L. virgaurea* and other phosphorus-efficient species, thereby altering long-term nutrient cycling trajectories in these alpine meadows.

Plants can dramatically modify the composition, biomass, and activity of soil microbial communities, largely through rhizodeposition<sup>53</sup>. Microbial biomass and activity in soils associated with *L. virgaurea*, as observed among many other invasive plants, were higher compared to native species<sup>45,54</sup>. In our study, microbial biomass carbon, nitrogen, and phosphorus each increased significantly as *L. virgaurea* density increased (Table 2). This was related to the greater aboveground litter input and belowground root biomass compared to soils associated with other species<sup>47</sup>, suggesting that *L. virgaurea* benefits microbial communities reliant on C-rich exudates. The significant increase in soil microbial biomass phosphorus (MBP) under higher *L. virgaurea* density (Table 2) indicates a strengthened microbial capacity for phosphorus transformation. This suggests that the high biomass and root exudates of *L. virgaurea* likely stimulate the microbial community, particularly phosphorus-solubilizing microorganisms, which are known to secrete phosphatases to hydrolyze organic phosphorus<sup>44</sup>. The significant increases in microbial biomass carbon (MBC) and nitrogen (MBN) (Table 2) hold broader ecological significance beyond indicating greater microbial abundance. They signal a shift in the belowground ecosystem towards a state of enhanced nutrient retention and accelerated turnover within the microbial loop. This ‘primed’ microbial community likely plays a crucial role in mineralizing nutrients that support *L. virgaurea*’s dominance. While our data cannot confirm a permanent microbial regime shift, the establishment of this self-reinforcing feedback—where the invader promotes a microbial community that in turn facilitates its nutrition—suggests a stable, alternative state for the invaded meadow that could be resistant to reversion.

The increase in microbial biomass nutrients indirectly altered soil element concentrations. Specifically, total organic carbon (TOC) in soil increased with *L. virgaurea* density (Table 2). This is consistent with previous studies<sup>45,55</sup>. The rise in total organic carbon highlights the capacity of *L. virgaurea* to augment soil carbon stocks through its high biomass production and recalcitrant litter—which together serve as a feedback mechanism that stabilizes its dominance in invaded grasslands<sup>26,55</sup>. On the other hand, soil ammonium-N ( $\text{NH}_4^+\text{-N}$ ) decreased significantly in *L. virgaurea*-invaded communities compared to non-invaded controls, indicating intensified competition for bioavailable nitrogen as *L. virgaurea* established its dominance. This aligns with the documented efficient nitrogen acquisition of invasive plants that suppresses native competitors by limiting critical nutrients<sup>56,57</sup>. However, nitrate-N ( $\text{NO}_3^-\text{-N}$ ) was highest under LL conditions but declined sharply under higher-density conditions (Table 2), likely reflecting a shift in plant–microbe interactions. Early invasion stages may temporarily elevate nitrate-N owing to disrupted microbial nitrification, while higher densities drive rapid plant uptake, as observed in the alteration of nitrogen cycling caused by other invasive species<sup>57</sup>. Soil available phosphorus (AP) increased progressively with *L. virgaurea* density, indicating enhanced phosphorus mobilization occurred in invaded soils. Plant-derived organic inputs and microbial activity jointly drive the transformation of phosphorus from its recalcitrant to bioavailable forms, thus enhancing phosphorus accessibility without altering total phosphorus pools<sup>58</sup>. Meanwhile, high-P litter from *L. virgaurea* decomposes rapidly, thus releasing phosphorus back into the soil and sustaining available phosphorus levels<sup>59</sup>. This phenomenon also explains the observed increases in plant phosphorus and soil available phosphorus contents, which occurred alongside negligible change in total soil phosphorus. Similar to *Hieracium* invasion in New Zealand grasslands<sup>60</sup>, *L. virgaurea* may reduce soil mineral N and alter microbial biomass, potentially creating a nutrient environment that favors its persistence over native species.

According to the results, we found that significant correlations exist between plant stoichiometric traits and soil nutrient dynamics in *L. virgaurea* communities. The significant correlations between plant N-related metrics (nitrogen content, C:N) and soil available phosphorus ( $P < 0.01$ ), organic carbon ( $P < 0.01$ ), and microbial biomass carbon ( $P < 0.05$ ) reflect the adaptive plasticity of *L. virgaurea* to alleviate N limitation through root exudation to enhance phosphatase or microbial activity, a strategy documented in other invasive species that exploit nutrient imbalances<sup>26,61</sup>. Notably, the linkage between plant N:P ratios and both soil AP and ammonium-N indicates a co-limitation scenario, in which *L. virgaurea* adjusts its N:P homeostasis to exploit transient pulses in nutrient availability—a trait linked to invasion success in fluctuating environments<sup>62</sup>. The “microbial priming” strategy mirrors mechanisms observed in other allelopathic invasive species that reengineer soil communities to favor their own mutualistic microbes<sup>29,45</sup>. In general, the success of *L. virgaurea* can be partially attributed to its distinct stoichiometric signature compared to the native species it displaces. Our data show that *L. virgaurea* maintains higher tissue concentrations of nitrogen and phosphorus than key native functional groups under high invasion pressure. This superior nutrient acquisition ability, potentially driven by more efficient uptake or stronger associations with soil microbes, allows it to outcompete natives like forbs and legumes that suffer from nutrient suppression, thereby facilitating its dominance. The competitive dynamics observed are likely driven by the combined effects of shading and allelopathy. The declines in nitrogen content and photosynthetic capacity (inferred from higher C:N) in grasses and legumes are classic responses to light limitation under shading. In contrast, the specific vulnerability of forbs and the significant alteration of the soil microbial biomass (Table 2) provide indirect support for the role of allelopathy, a mechanism previously confirmed for *L. virgaurea* in controlled bioassays<sup>28,34</sup>. A synthesis of our results with this literature suggests that shading may be the dominant mechanism driving reduced nitrogen status across the community, while allelopathy may be more critical for

specific plant-soil feedback and the suppression of certain competitors like forbs. Future studies employing factorial designs (e.g., using shade cloth to mimic light reduction and activated carbon to adsorb allelochemicals in the soil) are needed to quantitatively disentangle these intertwined mechanisms, and studies that directly link stoichiometric shifts with a wider suite of plant functional traits, such as SLA and root: shoot ratio, would provide even deeper mechanistic insights into the strategies plants employ during invasion.

## Conclusions

In summary, *Ligularia virgaurea* invasion disrupts biogeochemical cycling of carbon, nitrogen, and phosphorus in alpine meadows. It achieves dominance through a superior nutrient acquisition strategy, characterized by efficient nitrogen uptake and the promotion of microbially-mediated phosphorus conversion, which collectively suppress native competitors. Different plant functional groups exhibit distinct stoichiometric responses: grasses face intensified nitrogen limitation, while sedges maintain competitiveness through efficient nitrogen and phosphorus use. At the ecosystem level, the invasion enhances carbon storage and microbial biomass but exacerbates nitrogen limitation, creating a feedback loop that likely stabilizes the invaded state. These findings underscore the critical role of plant-soil-microbe interactions in driving invasion success and altering ecosystem stoichiometry.

Building on our findings, a critical next step is to test the specific hypothesis that climate warming exacerbates the positive plant-microbe feedback underpinning *L. virgaurea*'s dominance. A future multi-factorial experiment, coupling climate manipulation (e.g., warming and altered precipitation) with detailed profiling of the rhizosphere microbiome (e.g., metagenomics and enzyme activities), would elucidate whether the invader's stoichiometric advantages are stable under future climates. This approach could identify key microbial functional groups that are sensitive to climate change, revealing novel targets for ecological management aimed at disrupting the core mechanism of invasion. Beyond plant-soil feedbacks, investigating how such stoichiometrically-driven changes in plant quality influence herbivory and food web dynamics represents a critical next step for understanding the comprehensive ecosystem-level impact of *L. virgaurea* invasion.

## Data availability

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

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## Author contributions

Meiling Song and Yuqin Wang conceived the ideas and designed methodology. Meiling Song and Hongsheng Wang received the funding. Yuqin wang, Rui Zhou and Bing Li performed field and laboratory work. Meiling Song, Chunping Zhang and Rui Zhou performed data processing and statistical analysis. All authors contributed critically to the drafts and gave final approval for publication.

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## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

**Correspondence** and requests for materials should be addressed to M.S. or Y.W.

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