# Effects of soil factors on Pedicularis kansuensis invasion in alpine grassland

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## Abstract

Root hemiparasitic Pedicularis kansuensis is an invasive native species in China and has been expanding in the Bayanbulak Grassland of Xinjiang Uygur Autonomous Region over the past decade, threatening the local livestock industry. To understand why this damaging species expands in some areas but not in others, we compared soil water content, soil nutrient status, and plant community structures between heavily infected and non-infected sites. We hypothesized that soil nutrient levels and plant species composition would be more beneficial to the spatial expansion of P. kansuensis in heavily infected areas than in non-infected ones. Quadrat  $(1 \text{ m} \times 1 \text{ m})$  surveys were carried out in the Bayanbulak Grassland. Species number, percent vegetation cover, aboveground plant biomass, plant height, and relative frequency were recorded. Three topsoil samples were taken at 0–10 cm depth from each quadrat and their nutrient statuses were determined. The results showed that (1) P. kansuensis invasion reduces the species richness of the community compared to the uninvaded area (3.07%), but increases the canopy cover overall (16.99%); (2) soil water content (SW) and soil nutrient content are the main factors that determine invasion by P. kansuensis, and SW plays the more important role; (3) SW controls P. kansuensis leaf stoichiometry, decreases the Ratio of leaf nitrogen (LN) to leaf phosphorus (LP), and affects LP (P < 0.005). Finally, after combining the results, we found that the soil factor accounted for 46.50% and plants accounted for 22.50% of P. kansuensis invasions in the Bayanbulak Grassland.

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# Abstracts

Root hemiparasitic *Pedicularis kansuensis* is an invasive native species in China and has been expanding in the Bayanbulak Grassland of Xinjiang Uygur Autonomous Region over the past decade, threatening the local livestock industry. To understand why this damaging species expands in some areas but not in others, we compared soil water content, soil nutrient status, and plant community structures between heavily infected and non-infected sites. We hypothesized that soil nutrient levels and plant species composition would be more beneficial to the spatial expansion of P. kansuensis in heavily infected areas than in non-infected ones. Quadrat  $(1 \text{ m} \times 1 \text{ m})$  surveys were carried out in the Bayanbulak Grassland. Species number, percent vegetation cover, aboveground plant biomass, plant height, and relative frequency were recorded. Three topsoil samples were taken at 0–10 cm depth from each quadrat and their nutrient statuses were determined. The results showed that (1)P. kansuensis invasion reduces the species richness of the community compared to the uninvaded area (3.07%), but increases the canopy cover overall (16.99%); (2) soil water content (SW) and soil nutrient content are the main factors that determine invasion by P. kansuensis , and SW plays the more important role; (3) SW controls P. kansuensis leaf stoichiometry, decreases the Ratio of leaf nitrogen (LN) to leaf phosphorus (LP), and affects LP (P < 0.005). Finally, after combining the results, we found that the soil factor accounted for 46.50% and plants accounted for 22.50% of P. kansuensis invasions in the Bayanbulak Grassland.

Keywords: root hemiparasitic plant; alpine grassland; Pedicularis kansuensis; native species

# Introduction

Biological invasion is a serious global environmental issue and is a major problem associated with global change (Drenovsky and Batten, 2007). In 1958, Elton (1958) defined biological invasion as being when a species expanded into a new area where its offspring could reproduce, spread, and sustain themselves. Theoretically, invasive species include invasive non-native plants and invasive native plants, but both of them have the common characteristic that they can spread and cause harm. However, current research on invasive ecology is mainly focused on invasive alien species (Ai Zemin et al., 2017) and little attention had been paid to invasive native species, especially, invasive native species that have parasitic properties.

Parasitic plants are special plants that complete their life cycle by obtaining nutrients from other living plants using parasitic organs called haustoria. There are about 270–275 genera of known parasitic plants. They contain nearly 4500 species and account for about 1% of the total number of angiosperm species (260 000) (Press and Phoenix, 2005). About half of the parasitic plant species are root hemiparasitic plants (Joel et al., 2013). They retain a certain photosynthetic capacity, but still need to obtain water, mineral nutrients, and some carbohydrate supplies from their host plants via haustoria (Tëšitel et al., 2011). Aboveground, they compete with their host plants for light, but belowground, they depend on their host plant and obtain nutrients and water from the soil via the roots of their host (Cameron et al., 2008; Demey et al., 2013). Therefore, the relationship between hemiparasitic plants and the soil is more important and complicated than for other types of plants.

In general, as a significant component of the soil environment, soil water content (SW) plays a decisive role in the composition and distribution of plant communities (Li et al., 2003) and it has been shown that the addition of water will increase the occurrence of invasive plants (Blumenthal et al., 2008). In contrast, drought decreases stomatal conductance, and thus, reduces the aboveground biomass of plants (Knapp et al., 2002; Loydi et al., 2018). However, under natural conditions, grasslands are relatively dry or in dry periods, which means that the occurrence of hemiparasitic plants has little impact on the vegetation community in which they are located. However, they do reduce the resources available for community growth and development (Ameloot et al., 2005). Previous research has also found that under drought conditions, the hemiparasitic *Rhinanthus minor* had little impact on the host and community species, which meant that it could not develop an advantage by changing species diversity, but in higher soil moisture content areas, such as in mesic grasslands, *R. minor* can effectively improve the diversity of community species (Těšitel et al., 2018).

Apart from SW, soil nutrients are essential nutrients for plant life, and soil organic carbon (SOC), nitrogen (N) and phosphorus (P) are the main nutrient indexes for soil. Soil organic carbon is also a vital factor in the formation of soil structure, which directly affects soil fertility, water holding capacity, soil erosion resistance and soil bulk density (Sterner and Elser, 2002; Yu et al., 2010). There is a general consensus that resources play an important role in determining the success or not of invasive species (Harpole, 2006). For example, non-native plants invasions have been correlated with changes in soil nutrients (Eller and

Oliveira, 2017). Furthermore, studies also have confirmed that soil nutrients have important effects on the growth and development of the genus *Pedicularis* (Li and Guan, 2006; Decleer et al., 2013). For example, in areas where soil fertility and species richness are low, *Pedicularis canadensis* densities can reach a maximum (Hedberg et al., 2005). In contrast, the growth and development of the host plants are significantly affected by hemiparasitic plants in poor fertility soil and under good light conditions (Press and Phoenix, 2005; Borowicz and Armstrong, 2012). This could be related to macroelements, such as N, P, K, Ca, and Mg, in root hemiparasitic plants and microelements, such as Zn and B, that are mainly obtained from their host plants (Cameron and Seel, 2007).

Pedicularis kansuensis is an annual or biennial root hemiparasite plant that belongs to the genus Pedicularis, , an endemic species to China, and it is mainly distributed in southwest and western China (Zhong, 1963). In recent years, it has occurred in Qinghai and Gansu Province, especially in the Bayanbulak alpine grassland of Xinjiang Uygur Autonomous Region. Its appearance has seriously affected the yield and quality of forage grass and threatens the local livestock industry (Li et al., 2006; Sui et al., 2015; Bao et al., 2015). Similar to other invasive plants, it has the characteristics of a greater ripening rate and strong adaptability, and often spreads rapidly in grassland ecosystems in the form of a cluster distribution within a short period of time (Liu et al., 2008; Sui et al., 2016). According to the incomplete statistics, its hazard area is up to  $2.33 \times 10^4$ ha and it is spreading at a rate of  $3.3 \times 10^3$  ha year<sup>-1</sup> (Song, 2006; Liu et al., 2008). Therefore, clarifying the expansion factors associated with *P. kansuensis* in the Bayanbulak alpine grassland ecosystem in Xinjiang is important.

According to a field survey report, *P. kansuensis* is generally distributed in cold and high-altitude areas, it has a greater requirement for moist conditions (Wang et al., 2007), and is a native species in the Bayanbulak alpine grassland. Our previous study indicated that the nearer the water source, the higher the *P. kansuensis* density and the greater its distribution area (Liu et al., 2011). Our previous research also found that the *P. kansuensis* became extinct when 90 kg N ha<sup>-1</sup> year<sup>-1</sup> of nitrogen was added (Liu et al., 2017). Therefore, it is important to identify the roles played by SW and nutrient contents in the rapid spread of *P. kansuensis*. To investigate these factors, we set up eight sampling sites each in infected and non-infected areas of the Bayanbulak alpine grassland based on different constructive species, investigated plant community structure, species diversity, SW, and soil nutrient differences between the heavily infected and non-infected sites, and addressed the following questions: (1) how does the community structure change after *P. kansuensis* invasion; (2) what kind of ecological environment is beneficial to *P. kansuensis*; and (3) is it water or nutrients that determine invasion by *P. kansuensis* ?

# Materials and methods

## 2.1 Study area

The study was carried out in the Bayanbulak Grassland, which is the second largest grassland in China and is located in the Bayanbulak Basin of the Tianshan Mountains, Xinjiang Uygur Autonomous Region, northwest China. It covers an area of approximately 23 000 km<sup>2</sup> and contains around 36 plant species belonging to 26 genera and 16 families (Gong et al., 2010).

# 2.2 Site selection and data collection

In late July 2019, eight *P. kansuensis* infected sites and eight non-infected sites were set up based on the different dominant and constructive species in Bayanbulak alpine grassland. A total of three 1 m x 1 m quadrats were placed in each site. The elevation and spatial geographical coordinates at each site were recorded using a GPS (GPSMAP(r)60CSx, Garmin, Olathe, Kansas, USA). The species compositions and other detailed information are shown in Table 1. Plant species composition, canopy cover, and species number and abundance were recorded in each quadrat. Five to ten individual fresh and mature plant leaves from each species in each quadrat were collected and then separately stored in labeled paper bags.

All the plant samples were collected and brought back to the laboratory and dried at 105oC for 30 min to avoid losses due to respiration and decomposition. Then, the plant samples were dried at 65oC for 48 h,

weighed and ground with ball mill, and stored in sample bags for N, P, and K determination. In addition, 0–10 cm surface soil samples were obtained using a hand auger from the infected and non-infected sites with five replicates taken at each site. One part of the soil subsample was used to measure SW, and the other was evenly mixed and stored at 4oC for the measurement of SOC, N, P, and K.

Plant community composition was measured as species richness (number of plant species); plant abundance was the number of individuals per square meter; and plant canopy cover was defined as the proportion of the ground occupied by a perpendicular projection of the aerial parts of individuals.

# 2.3 Soil water content and nutrient analysis

The soil subsamples were weighed and oven-dried at 105degC for 24 h to a constant weight for soil water determination. Soil N was determined by the modified semi-micro-Kjeldahl method (Liao, 1981) and soil P was determined by NaOH digestion followed by quantification using colorimetry (Smith and Bain, 1982). The SOC was quantified using a modified Walkley-Black chromic acid wet oxidation method (Wang et al., 2012) and the soil K concentration was determined by the atomic absorption method (Bao, 2000).

#### 2.4 Plant tissue nutrient analysis

Dried leaves were milled (Retsch MM 400, Retsch GmbH and Co. KG, Haan, Germany) and passed through a 1 mm sieve. Leaf P concentration was measured using the molybdate/stannous chloride method (John, 1970; Bao, 2000) after digesting a  $\sim 0.1$  g sample in H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub>. Leaf N and carbon concentrations were determined using an elemental analyzer NA1500 (Carlo Erba, Thermo Fisher Scientific, Waltham, MA, USA) and leaf K concentration was determined by the atomic absorption method (Bao, 2000). All concentrations were calculated on a dry mass basis.

#### 2.5 Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze species richness, total coverage, the importance value (IV) of shared species, and the IV of unique species between infected sites and non-infected sites. The data were checked for normality and the significance level was 0.05. The linear or nonlinear relationships were analyzed based on a regression analysis between the soil nutrient and leaf nutrient (C, N, P, K) concentrations and SW and leaf nutrient concentrations (N, P, N:P, N:K, and P:K ratio). The above statistical analyses were carried out using SPSS 21 (PASW statistics 21.0; IBM Corporation, Armonk, NY, USA) and the graphs were constructed using SigmaPlot 12.5 (SyStat Software Inc., San Jose, CA, USA). The variation in leaf N, P, and the N:P ratio was partitioned between two explanatory variable groups (soil water content and soil nutrient content) using a partial regression analysis (PCA) with a redundancy analysis (RDA) (Chen et al., 2016). The PCA and RDA were carried out using CANOCO 5.0 (Microcomputer Power, Ithaca, NY, USA).

The response ratios (RR) for invasion by *P. kansuensis* were calculated according to Hedges et al. (1999):

 $RR = \ln(Xt \ / \ Xc \ )$ 

where Xt represents the soil nutrient value for the *P. kansuensis* infected sites and Xc is the soil nutrient value for the control group (non-infected sites).

# Results

#### 3.1 Effects of P. kansuensis invasion on community structure

Pedicularis kansuensis invasion slightly reduced the species richness of the community compared to the noninvaded area (-3.07%, Fig. 1A), but it increased overall canopy cover (+16.99%, Fig. 1B) (excluding *P. kansuensis*). Our study found that *P. kansuensis* invasion significantly reduced the species importance of the common species in the community compared to the non-invaded areas (-44.22%, Fig. 1C). In addition, there was also a significant decrease in the importance value of the unique species (-31.26%, Fig. 1D). That is to say, *P. kansuensis* invasion increased community coverage, but decreased the species diversity of the community and the importance of the other species. As a result, *P. kansuensis* invasion affected the ability of the community to maintain a stable ecosystem. In other words, only the communities with low diversity were conducive to the invasion of P. kansuensis .

# 3.2 Effects of soil water content and soil nutrient content on invasion by P. kansuensis

The RR value represents the response rate for P. kansuensisinvasion shown by the environment. The results showed that the response rate was significant for soil organic carbon (SOC), soil nitrogen (SN), and soil phosphate (SP); extremely significant for SW; but not significant for soil potassium (SK). Our results indicated that SW and soil nutrient content were the main factors determining invasion by P. kansuensis and that SW plays the most important role (Fig. 2).

# 3.3 Effects of P. kansuensis on the stoichiometry of its community

The unitary linear recursive analysis results for the stoichiometry between soil nutrients (SN, SP, and SK) and the leaves (LN, LP, and LK) showed that there was no linear or nonlinear relationship between the stoichiometry of the paired factors (SN and LN, Fig. 3A; SP and LP, Fig. 3B; SN:SP and LN:LP, Fig. 3D; and SN:SK and LN:LK, Fig. 3E) except for SK. Indeed, SK had a significant impact on the amount of LK (P < 0.05, Fig. 3C and Fig. 3F). There was a negative correlation between SN and LN and SP and LP. However, SK and LK had a significant and positively correlated relationship. Figure 4 shows that SW controlled the leaf stoichiometry of P. kansuensis rather than soil nutrients. There were no statistically significant relationships between LN, LK, or LN:LK and SW, respectively (P [?] 0.05, Figs. 4A, C, E). It mainly affected LP (Figs. 4B, D, and F). However, SW greatly affected LP ( $R^2 = 0.88$ , P = 0.0047, Fig. 4B), LN:LK ( $R^2 = 0.88$ , P = 0.0053, Fig. 4D) and LN:LP ( $R^2 = 0.77$ , P = 0.0266, Fig. 4F). The SW decreased LN:LP and this was probably due to the fact that the nitrogen increase in leaves was lower than that for phosphorus in leaves as SW rose and this led to a decrease in the LN:LP.

The second axis explains 28.39% of the variation, which meant that the studied factors explained the stoichiometry well. Soil K had a large impact on LN:LP, whereas SN had a considerable influence on LN:LP and LN:LK (Fig. 5A). Figure 5B shows the variance decomposition of the stoichiometry for plant leaves. Leaf stoichiometry is mainly affected by the combined action of soil and plants. The soil accounted for 46.50%, which meant that the main factor affecting soil was SW (Fig. 5B). Plants accounted for 22.50% and was the other major factor affecting community disturbance and competition by *P. kansuensis* (Fig. 5B).

## Discussion

# 4.1 Effects of species diversity on invasion by P. kansuensis

Species richness in the area invaded by P. kansuensis was lower than that of the non-invaded area after removing the *P. kansuensis* population, which agreed with previous reports on how greater species diversity or species richness can effectively resist invasion by invasive species (Marvier and Smith, 1997), but the difference between the invaded and non-invaded sites was small. In contrast, the invaded area had a greater canopy cover, which means that the communities with low species diversities are more conducive to invasion by P. kansuensis. It is possible that species-rich communities are more likely to contain species with stronger competitive abilities, which allow them to effectively discourage invasive species (El-Barougy et al., 2020). The results are also related to the root hemiparasitic characteristics of P. kansuensis. Another reason might be that a community with higher species diversity has a stronger ability to compete for light, which makes it unfavorable for *P. kansuensis* survival and growth. Some researchers believe that invasion is a probabilistic process and that a community that contains native species will have a higher plant diversity level. They suggest that this improves the ability of a community to efficiently resist invasive plants (Tilman, 1999; Fargione et al., 2003; Fargione and Tilman, 2005). A further reason could be that communities with high species diversities reduce the likelihood of successful invasions by filling niche spaces with different resident species that jointly and efficiently use local resources (HilleRisLambers et al., 2012; El-Barougy et al., 2020). For example, a field study on *Rhinanthus alectorolophus* showed that the presence of *R. alectorolophus* increased total aboveground biomass in low diversity plots, but had no effect on total aboveground biomass in high diversity plots (Joshi et al., 2000).

Some researchers have reported that it was nutrition that played a decisive role in the success of alien species (Driscoll and Strong, 2018). For example, invasive plants often show a superior competitive ability at acquiring and using nutrients than natives, even in nutrient deficient areas (Funk and Vitousek, 2007). For example, it has been reported that the established species were not easily attacked by hemiparasitic plants in nutrient-rich habitats, even though the established species were the superior hosts. However, the reverse was true in nutrient-poor areas (Marvier and Smith, 1997). In our study, P. kansuensis was more prone to emerge in areas with high SWs and organic matter contents. Therefore, our results confirmed the above studies. Other studies have found that water stress can reduce aboveground biomass and primary productivity (Knapp et al. 2002; Yahdjian and Sala, 2006). Generally, root hemiparasitic plants obtain nutrients, such as water and inorganic materials, from their host. Therefore, regions with higher SWs are more favorable to *P. kansuensis* growth. Other researchers think that nutrient allocation strategies are used. They have suggested that invasive plants might develop different biomass allocation strategies than native species in order to ensure a successful invasion (Press and Phoenix, 2005; Demey et al., 2013). For example, studies have found that under the interaction of temperature increase and nitrogen addition treatment (W x N), an invasive population of *Plantago virginica* (PV-In) and the native population (PV-Na) had different biomass allocation strategies when in competition with the local competitor P. asiatica (Luo et al., 2020).

We found that there was a significant positive correlation between SK and LK, and SP:SK and LP:LK. Soil water content was significantly negatively correlated with LP:LK and extremely significant positively and significantly positively correlated with LP and LN:LP, respectively. This could be related to the fact that the N:P ratios in plant organs can be regulated by internal nutrient transfer (Zhang et al., 2016). In other words, the plant itself has stoichiometric plasticity and can adjust its elemental balance under environmental pressure (Sterner and Elser, 2002). Furthermore, soil nutrients are widely used to manipulate resource availability to reduce invasion by exotic species in fragile ecosystems because they play a key role in the success of invasive species (Perry et al., 2004). Meanwhile, it has reported that increasing nutrient supply modulated the dominant plant defensive tactics from tolerance to induced resistance (Burghardt, 2016). For example, Bromus tectorum invasion was controlled by regulating the nutrient ratio in California (Walker et al., 2017), but in the wetlands, carbon addition combined with repeated biomass harvesting was used to reduce invasion by *Phalaris arundinacea* (Perry et al., 2004). The root hemiparasitic characteristics shown by *P. kansuensis* means that it mainly obtains water and other inorganic materials from its host, which is why sites with high SOC were used in this study. Our results also agreed with previous research that showed that invasion by root hemiparasitic plants was mainly affected by soil nutrient levels (Borowicz and Armstrong, 2012).

# 4.3 Plants and soil act together to promote plant invasion

Previous studies have found that root hemiparasitic plants usually occur in soils with poor nutrient levels (Press and Phoenix, 2005), and this can be attributed to their root hemiparasitism. Their aboveground parts are usually at a disadvantage when competing with their host plants for light and space (Tëšitel et al., 2011), but belowground, they obtain nutrients from their host root via haustoria (Joel et al., 2013; Sui et al., 2015). Plants will invest more nutrients into root growth when nutrient conditions are poor (Yu et al., 2010) and increased numbers of roots increase the initiation of haustoria. We expected to find that soil nutrient levels were lower in *P. kansuensis* invasive areas, but the results of a previous nutrient addition test showed that the *P. kansuensis* invasive areas when 9 m<sup>-2</sup> of nitrogen was added. Furthermore, the nutrient contents in the *P. kansuensis* invaded area were higher than in the non-invaded area according to the in-situ soil sampling results, although the difference was not significant. Our results showed that there may be a certain amount, it is not conducive to *P. kansuensis* growth, but when it is below a certain amount, it may favor its establishment and growth. Previous studies confirmed that water loss can drastically reduce or even stop photosynthesis and growth in plants, but excess water can also inhibit soil respiration and affect the normal growth of plants (Fay and Schultz, 2009; Ferrante et al., 2014). Other research has shown

that the abundance and occurrence of a species are mainly influenced by interspecies interactions at small scales, but in large areas, it is soil properties that are the major factors (Wiegand et al., 2012; Driscoll and Strong, 2018). Furthermore, another reason for the better growth in nutrient poor areas is that intraspecies competition is greater under low N conditions. However, further studies are required to confirm to what extent SOC in soil is conducive to *P. kansuensis* invasion. It has also been reported that nutrient abundance is more conducive to invasive plants than native plants when soil fertility is high (Broadbent et al., 2017).

Overall, our results demonstrate that both plant and soil factors drive P. kansuensis invasions in the Bayanbulak Grassland, and that SW plays the decisive role. This explains why there are more P. kansuensis distributed near the sides of water flows in the Bayanbulak Grassland.

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Table 1. Altitude, longitude, latitude, and species compositions of eight infected and non-infected sites.

sitos	Altitudo (m)	Latituda (N)	Longitudo (E)	Dominant plant
	Annual (III)		Longitude (E)	species
$1_{t}$	2570	42.8686212°	82.8883191°	Pk, Kc, Gp, Pb, Cs, Ol, Tt, Ce, Pv, En, Ta, Ga
1 <sub>c</sub>				$\begin{array}{l} Kc, \ Cs, \ St, \ Pv, \\ Ta, \ Pa, \ Gt, \ Ol, \\ Ps \end{array}$
$2_{t}$	2489	42.899457°	83.680126°	Pk, En, Tt, Pm, Pl, Ol, Ko, Gt
$2_{\rm c}$				En, Pb, Tt, Pl, Pm
$3_{t}$	2417	42.835368°	84.425209°	Pk, Ol, Pm, Pc, Au, Pl, Sp, Ac, Sa, Pa.
3 <sub>c</sub>				Ac, Sp, Ol, Pm, Au, Sc, Pl, Pb, Ko, Ll, Pc
$4_t$	2528	42.797188°	84.538411°	Pk, Ol, Ea, Pa, Pm, Pl, Mn, Ge, Po, En, Gt
$4_{\rm c}$				Ko, Pb, Pm, Aa, As, Pa, Mn, Ll, Lt, Ac
$5_{t}$	2403	42.687566°	84.456462°	$\begin{array}{l} Pk, \ Ll, \ Ea, \ Cs, \\ Gb, \ Gt, \ Pm, \ Ta, \\ Ge, \ Rp, \ Sa, \ Sp, \\ Pa  Ol \end{array}$
5 <sub>c</sub>				Sp, Mn, Ll, As, Pa, Cs, Ea, Au, Pb
$6_{ m t}$	2410	42.718905°	83.990367°	Pk, Gb, Tp, Pa, Pl, Pm, Fo, Sp, Pb, Pa, Cs, Ll, Ea
6 <sub>c</sub>				Fo, Cs, Pb, Kc, Ol, Ll, Pa

sites	Altitude (m)	Latitude (N)	Longitude (E)	Dominant plant species
7 <sub>t</sub>	2464	42.876408°	83.718601°	$\begin{array}{c} Pk, \ Gt, \ Gb, \ Ce, \\ Pm, \ Tt, \ Sc, \ As, \\ At \\ Sr, \ At \\ Ll \ Ol \ Ac \end{array}$
8 <sub>*</sub>	2578	42.722323°	83.704058°	<i>Sp</i> , <i>At</i> , <i>Lt</i> , <i>Ot</i> , <i>As</i> , <i>Pm</i> , <i>Au</i> , <i>Ss</i> , <i>Gm</i> , <i>Pa</i> , <i>Pb</i> , <i>Cs</i> , <i>Ea</i> <i>Pk</i> , <i>Sp</i> , <i>Ko</i> , <i>Cs</i> .
8 <sub>c</sub>				$\begin{array}{c} Pa, \ Ol, \ Pm, \ Au, \\ En, \ Er, \ Ll \\ Sp, \ At, \ As, \ Aa, \end{array}$
				$ \begin{array}{l} Ko, \ Ac, \ Pa, \ Ll, \\ Tt, \ Pl, \ Ol \end{array} $

Notes: t, infected sites, c, non-infected sites. Pk ,Pedicularis kansuensis . Kc , Kobresia capillifolia. Gp , Geranium pretense. Pb , Potentilla bifurca .Cs , Carex stenocarpa . Ll , Leontopodium leontopodioides . Ol , Oxytropis lapponica . Tt ,Taraxacum tianschanicum . Pa , Potentilla anserine .Pm, Potentilla multifidi
Ce, Cirsium esculentum .Au , Androsace umbellate . Pv , Polygonum viviparum . At , Aneurolepidium tianschanicum . Fo ,Festuca ovina . Ea , Erigeron aurantiacus . Sp ,Stipa purpurea . En , Elymus nutans . Gt ,Gentianella turkestanorum . St , Salix tianschanica .Ta , Thalictrum alpinum . As , Astragalus alpinus . Ac , Asropyron crisfatum . Sa ,Saussurea alpine . Gm , Gentiana macrophylla .Ga , Gentiana algida . Aa , Aster altaicus .Ko , Koeleria cristata . Po , Poe annua .Er , Erigeron acer Lin. Gb , Gentianopsis barbata . Tp , Triglochin palustre . Ge , Galium aparine L. Ps , Primula sibirica . Ss , Stipa subsessiliflora . Rp , Ranunculus pulchellus . Lappula tianschanica , Lt . Plantago minuta, Pl . Schultzia ,Sc . Polemonium coeruleum , Pc . Saxifragasp stolonifera , Sa . Polemonium coeruleum , Pc .



Fig. 1 Differences in richness, total coverage, and the importance values (IVs) of the shared species and

unique species between the *P. kansuensis* invaded (*P.K* .) and non-invaded (CK) sites. The red line in the box diagram is the mean. Significant differences are reported from one-way analysis of variance as ns, P[?]0.05; \*P < 0.05; \*P < 0.01.



Fig. 2 Response rates of the studied factors when *P. kansuensis* invades a site. SOC, soil organic carbon content in sample sites; SN, soil total nitrogen content; SP, soil total phosphorus content; SK, soil total potassium content; SW, soil water content. Significant differences are reported from one-way analysis of variance as \*P < 0.05; \*\*P < 0.01.



Fig. 3 Soil nutrients-leaf stoichiometry relationships. LN, Leaf nitrogen content; LP, Leaf phosphorus content; LK, Leaf potassium content; LN:LP, Ratio of leaf nitrogen content to leaf phosphorus content; LN:LK, Ratio of leaf nitrogen content to leaf potassium content; LP:LK, Ratio of leaf phosphorus content to leaf potassium content.



**Fig. 4** Correlations between SW and leaf stoichiometry. SW, soil water content; LN, Leaf nitrogen content; LP, Leaf phosphorus content; LK, Leaf potassium content; LN:LP, Ratio of leaf nitrogen content to leaf phosphorus content; LN:LK, Ratio of leaf nitrogen content to leaf potassium content; LP:LK, Ratio of leaf nitrogen content to leaf potassium content; LP:LK, Ratio of leaf nitrogen content.



Fig. 5 Redundancy analysis (RDA) of leaf stoichiometry and RDA of the soils and plants.

A: Variations in soil and leaf stoichiometry and *P. kansuensis* coverage. Richness was partitioned among two explanatory variable groups (soil and plants) using a partial regression analysis with an RDA. B: Variationpartitioning analysis produced four fractions: single effect of soil, single effect of plants, joint effects of soil and plants, and unexplained variation.  $\Delta$ SW, The value of soil water content from treatment minus control;  $\Delta$ SN, The value of soil nitrogen content from treatment minus control;  $\Delta$ SN, The value of soil potassium content from treatment minus control;  $\Delta$ SK, The value of soil potassium content from treatment minus control;  $\Delta$ SR, The value of soil potassium content from treatment minus control;  $\Delta$ SK, The value of soil potassium content from treatment minus control; LN, Leaf nitrogen content; LP, Leaf phosphorus content; LK, Leaf potassium content; LN:LP, Ratio of leaf nitrogen content to leaf phosphorus content; CP, Coverage of *P. kansuensis*.







