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## Effects of the hemiparasitic plant *Pedicularis kansuensis* on plant community structure in a degraded grassland

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**Abstract** Species-rich grasslands have high conservation value because they support diverse floral and faunal assemblages. Alpine grassland is an important and characteristic ecosystem of the Qinghai-Tibet Plateau, but ca. 30 % has been severely degraded by the combined effects of climate change, human activity, overgrazing and rodent damage. One potential method to restore grassland diversity involves using hemiparasitic plants to modify the competitive relationships among neighboring species. A possible candidate is *Pedicularis kansuensis*, a hemiparasitic plant found in grasslands throughout the Qinghai-Tibet Plateau, but more information on its host associations and its effects on surrounding vegetation is required to assess its suitability. Thus, we examined host associations of *P. kansuensis* by direct root excavation at a selected site on the Qinghai-Tibet Plateau and found that haustoria formation is non-randomly distributed among host species, with preferential bias for members of Poaceae, Rosaceae and

Fabaceae. We also found that aboveground biomass of grasses and legumes was higher in quadrats from which the parasite had been removed than in intact controls, while the biomass of sedges and forbs was unaffected by parasite removal. However, removal significantly decreased plant species richness and Shannon–Wiener diversity. These results suggest that *P. kansuensis* modifies the competitive balance in grassland communities of the Qinghai-Tibet Plateau, allowing for colonization by subordinate species and thereby enhancing species diversity and contributing to restoration of these degraded grasslands.

**Keywords** Host selectivity · Haustoria · Host range · Community structure · Orobanchaceae

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### Introduction

Parasitic plants, which comprise more than 20 families, 270 genera and more than 1 % of all angiosperm species, are important components of numerous natural and semi-natural communities (Press 1998). They can be classified as root or shoot parasites depending where connections are formed with host plants and as hemiparasites or holoparasites depending on whether they possess or lack functional chloroplasts, respectively (Musselman and Press 1995). Root hemiparasites retain the capacity for photosynthesis, but are partially dependent upon hosts for the acquisition of water, mineral nutrients and carbon, which they obtain through connections made by specialized organs called haustoria (Irving and Cameron 2009).

It has been suggested that in semi-natural communities, root hemiparasites capable of interacting with diverse host species may act as ecosystem engineers, modifying community structure and function (Pywell et al. 2004; Ameloot et al. 2005; Decler et al. 2013). Although root hemiparasitic plants generally have a wide host range, they damage some species (preferred hosts) more than others (Press and Phoenix 2005). Consequently, they may be capable of altering the

competitive balance among surrounding plants by decreasing the competitive advantage of dominant species, allowing subordinate species the opportunity to colonize the habitat (Gibson and Watkinson 1992; Marvier 1998; Ameloot et al. 2005; Hedberg et al. 2005; Press and Phoenix 2005; Hellström et al. 2011; Decler et al. 2013). In doing so, root hemiparasitic plants may alter plant community structure and increase diversity.

The ecological role of species in the root-hemiparasitic genus *Rhinanthus* has been well characterized (Gibson and Watkinson 1991, 1992; Davies et al. 1997). Species of *Rhinanthus* prefer poaceous hosts and have been shown to reduce host competitive advantage, releasing forb species from competitive exclusion and thereby enhancing grassland diversity (Westbury 2004; Ameloot et al. 2005; Bardgett et al. 2006). The introduction of *Rhinanthus* spp. has been effectively used to restore low-diversity pastures to high-diversity meadows in Europe (Pywell et al. 2004; Westbury 2004; Bullock and Pywell 2005). However, little is known about the effects of other hemiparasites on the structure and productivity of natural plant communities, including whether they increase species diversity by limiting competitive dominants and thereby facilitate coexistence (Press and Phoenix 2005). If root hemiparasites prefer subordinate species as hosts, they could reduce the abundance of subordinate species, enhancing the competitive advantage of dominant species, and reducing the overall diversity of the plant community (Gibson and Watkinson 1989). Thus, rigorous assessment of hemiparasitic species' host preferences is required to predict their effects on specific ecosystems.

Species-rich grasslands have high conservation value because they support diverse floral and faunal assemblages. The Qinghai-Tibet Plateau, sometimes called the Earth's "third pole", is an important eco-region (Wang and Cheng 2001) in which alpine grasslands are a characteristic landscape. Combined effects of climate change, human activity, overgrazing and rodent damage, however, have severely degraded almost 30 % of alpine grasslands in the plateau (Yang et al. 2004; Shang and Long 2007). Consequently, grassland restoration is vital to conserve biodiversity in the Qinghai-Tibet Plateau.

*Pedicularis* (Orobanchaceae) is one of the largest genera of flowering plants, comprising 800 species mainly distributed in montane and alpine zones of the northern hemisphere (Mill 2001; Wang et al. 2003; Ree 2005). There are an estimated 364 species of *Pedicularis* in China alone (Yang et al. 2010; Yu et al. 2011), most of which inhabit degraded or semi-natural grasslands with a consistent assemblage of co-occurring plants (Zhou et al. 2003; Qiu et al. 2006; Zhe et al. 2010). Many *Pedicularis* species can exploit a wide range of hosts (Piehl 1963; Musselman and Mann 1977; Hedberg et al. 2005; Ren et al. 2010), but little is known about their selectivity and degree of host preference. The focal species in this study, *Pedicularis kansuensis*, is distributed throughout the Qinghai-Tibet Plateau and is a

candidate for use in grassland restoration programs in this region; yet, as a representative species of degraded grasslands (Song 2006; Bao and Wang 2011), it may have host preferences for subordinate species and thus contribute to reductions in grassland diversity.

More information on the host range, preferences and effects of *P. kansuensis* on degraded plant communities is essential both for understanding its ecological role and for rigorously assessing its potential use in the restoration of species-rich grasslands. Thus, we examined the host selectivity and effects on community structure and productivity of *P. kansuensis* at a selected grassland site on the Qinghai-Tibet Plateau. Specifically, we examined the host range by directly observing root systems, quantified host selectivity by comparing numbers of haustorial connections to each host species with root abundance and investigated the effects of *P. kansuensis* removal on plant community structure and productivity.

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## Materials and methods

### Study species and site

*P. kansuensis* Maxim. is an annual or biennial herb that is endemic to northwestern China and typically grows to 20–45 cm in height. The stems are usually straight with a terminal inflorescence that is typically 25–30 cm long, with numerous purple–pink to purple–red, or sometimes white, flowers (Yang et al. 1998). It invades semi-natural or degraded grasslands and has been used as an indicator species of degraded pastures (Qiu et al. 2006; Zhe et al. 2010). Like other root or stem parasites, *P. kansuensis* absorbs nutrients and water from its host via haustoria.

Field studies were carried out at a grassland site in Haiyan County, Haibei Tibetan Autonomous Prefecture, Qinghai Province, China (37°03'44.82"N, 100°42'34.40"E) between May and September of both 2012 and 2013. According to data acquired from the weather bureau of Qinghai Province, the average annual rainfall from 2000 to 2012 in this region was 286.4 mm, most of which (180.5 mm) fell between June and August. The mean annual average temperature was –1.9 °C, with monthly means ranging from –16.4 °C in January to 11.8 °C in August. Because of intense grazing, vegetative cover at the site during the study period was less than 60 %. Therefore, according to the cover scale of Zhou et al. (2005) (non-degraded >85 %, lightly degraded 70–85 %, moderately degraded 50–70 %, heavily degraded <50 %), the grassland was classified as moderately degraded. The vegetation of this alpine grassland was composed primarily of *Kobresia capillifolia* (Cyperaceae), *Stipa purpurea* (Poaceae), *Potentilla anserina* (Rosaceae) and *P. kansuensis* (patchily distributed among the co-occurring plants). During the observational periods, *P. kansuensis* was mostly in the flowering developmental stage.

## Examination of haustorial connections

To investigate haustorial connections, seven samples (20 × 20 × 20 cm) of the top soil layer and associated vegetation (each including two or three *P. kansuensis* plants) were manually removed with a shovel from randomly selected points in the site. The samples were immediately placed in an icebox and taken to the laboratory, where they were immersed in a water bath at room temperature for 24 h. Samples were then individually washed and carefully brushed to remove the rhizosphere soil without damaging the haustorial connections. Haustoria were detected by visual observation, as they are easily distinguished from rhizobial nodules of legume species by their color and shape. Host species were then identified and recorded using shoot and root morphological features and the number of haustoria formed on each host plant was counted. The roots of each species in each sample were separated, oven-dried at 65 °C to constant weight and weighed to the nearest mg to calculate the relative belowground biomass of each species (Gibson and Watkinson 1989). The resulting values were then multiplied by the total number of haustoria found in the samples to obtain the expected distribution of haustoria for each recorded species in the case of no host preferences.

## Effects of *P. kansuensis* on the grassland community

To investigate effects of *P. kansuensis* on grassland community structure, we established 10 experimental plots (each 4 × 4 m, spaced at least 100 m apart) in early September 2012 before *P. kansuensis* seeds were released. Each plot had similar densities of *P. kansuensis* with a homogeneous distribution of *P. kansuensis* and other plants. Each plot was divided into four 2- × 2-m quadrats, from two of which *P. kansuensis* plants were carefully removed with forceps to create 20 “naturally parasitized” and “parasite-removed” quadrats each. Germinating *P. kansuensis* seedlings were removed weekly from “parasite-removed” quadrats during the following summer until the experiment finished (late June–mid August 2013). At this time, when most annual and perennial species (including *P. kansuensis*) were in the reproductive developmental stage, the cover, frequency and aboveground biomass of all species were determined in a sub-quadrat (0.5 × 0.5 m) at the center of each quadrat. Sub-quadrats were taken from the center to minimize the confounding effects of seed dispersal into the cleared areas by leaving a 0.75-m buffer zone. The confounding effects of variation in soil fertility and species composition, as well as patchiness of *P. kansuensis* distribution, were minimized by treating the 4- × 4-m plots as blocks. The aboveground biomass of each plant was oven-dried at 65 °C to constant weight and weighed. Species diversity responses to removal and control treatments were determined by calculating the richness index (*RI*), the Shannon–Wiener diversity index

(*H*) and the evenness index (*EI*) in the corresponding quadrats and blocks using the following formulae (Whittaker 1972; Ren et al. 2013):

$$\text{Richness index (RI)} = S, \quad (1)$$

$$\text{Shannon-Wiener diversity index (H)} = - \sum_{i=1}^S (P_i \ln P_i), \quad (2)$$

$$\text{Evenness index (EI)} = H / \ln S, \quad (3)$$

where  $P_i$  is the importance of the  $i$ th species, calculated from its mean relative cover, relative frequency and relative biomass, and  $S$  is the total number of species in a quadrat.

## Statistical analyses

The Chi square ( $\chi^2$ ) test was used to evaluate host selectivity of *P. kansuensis*. Specifically, it determined post-attachment selectivity (the suitability of hosts after infection), which does not necessarily indicate the degree to which *P. kansuensis* actively chooses among potentially available hosts prior to infection (Suetsugu et al. 2008). Using ANOVA, the impact of *P. kansuensis* on the grassland community was assessed by comparing aboveground biomass and diversity indices between naturally parasitized and parasite-removed quadrats, with presence/removal of *P. kansuensis* as a fixed factor and block as a random factor. We then assessed the effects of *P. kansuensis* on biomass and diversity indices (*H*, *EI* and *RI*) of different functional groups (sedges, grasses, legumes and non-leguminous forbs; hereafter, forbs). To do so, we used MANOVA and ANOVA to test the significance of differences in multiple and individual response variables, respectively, between parasitized and parasite-removed quadrats. MANOVA was run after using Principal Component Analysis (PCA) to reduce the number of species' biomass and cover variables, where the model included parasite removal and block as main effects and a removal × block interaction. In addition, the independent samples *t* test was used to assess the effects of *P. kansuensis* on individual principal components. Prior to this analysis, biomass was log-transformed and cover values were arcsine square-root-transformed to achieve homoscedasticity and normality. All of the analyses were performed with Statistical Product and Service Solution (SPSS) software version 19.0 (SPSS China, Shanghai, China).

## Results

### Haustorial connections

Direct examination of the samples revealed haustorial connections between *Pedicularis kansuensis* and plants of eighteen species and eight families, with significant

differences between observed and expected frequencies of haustoria among both host species and families ( $\chi^2$  test,  $P < 0.001$ ; Tables 1, 2). The  $\chi^2$  values and differences between observed and expected values revealed that *Astragalus adsurgens*, *Potentilla anserina* and *Stipa purpurea* were the most preferred host species, while *Saussurea pulchra* and *Kobresia humilis* were the least preferred (Table 1). The preferred host families appeared to be Fabaceae, Rosaceae and Poaceae; Cyperaceae and Asteraceae were least preferred (Table 2). It should be noted, however, that too few haustoria were detected in some species or families to confidently infer the significance of differences between observed and expected frequencies.

#### Effects of *P. kansuensis* on the grassland community

Total aboveground biomass was significantly higher in parasite-removed quadrats than in naturally parasitized quadrats at the end of the experiment (ANOVA;  $F_{1,9} = 144.28$ ,  $P < 0.001$ ). The aboveground biomass of the four functional groups also differed between treatments (Table S1, MANOVA;  $F_{4,17} = 193.98$ ,  $P < 0.001$ ) and were significantly affected by an interaction between *P. kansuensis* removal and block ( $F_{36,80} = 2.43$ ,  $P = 0.001$ ). Similarly, in parasite-removed quadrats, there was significantly higher total aboveground biomass of grasses and legumes ( $F_{1,9} = 44.40$ ,  $P = 0.02$  and  $F_{1,9} = 122.16$ ,  $P < 0.001$ , respectively), but removal had no significant effect on either forb or sedge biomass (Fig. 1; Table S2).

The PCA on species biomass extracted eight principal components (PCs) that explained 92.2 % of the varia-

tion (Table S3). *Artemisia capillaris*, *Dracocephalum heterophyllum* and *Artemisia frigida* had negative loadings on PC1, while *Elymus nutans*, *Leymus secalinus*, *Astragalus adsurgens* and *Oxytropis kansuensis* had positive loadings on PC1. Therefore, PC1 reflected the range of host preference or avoidance of *P. kansuensis*. *A. frigida* (Asteraceae) and *Leontopodium nanum* (Asteraceae) had negative loadings on PC2, while *Gentianopsis paludosa* (Gentianaceae), *Swertia musсотii* (Gentianaceae) and *Gentiana macrophylla* (Gentianaceae) had positive loadings on PC2. Thus, PC2 mainly reflected the biomass change of species in Gentianaceae and Asteraceae (see detailed results in Fig. S1; Table 1; Tables S3, S4). As in the biomass analysis, the PCA explained 77.2 % of the variation in cover (Table S3). *A. capillaris*, *D. heterophyllum* and *Tibetia himalaica* had negative loadings on PC1, but *O. kansuensis*, *Stipa purpurea* and *L. secalinus* had positive loadings on PC1. Therefore, PC1 explained the differences in species composition between parasitized and parasite-removed plots. *Saussurea pulchra* (Asteraceae) loaded positively on PC2, while *L. nanum* (Asteraceae) and *Lancea tibetica* (Asteraceae) loaded negatively on PC2, indicating that PC2 reflects the change in cover between species of Asteraceae (see detailed results in Tables S5; Fig. S2). The PCs extracted from both the species biomass and the cover data differed between the parasite removal and control treatments ( $F_{8,13} = 83.56$  and  $F_{9,12} = 131.97$ ,  $P < 0.001$  for both; Tables S6, S7), and the impact of removal varied spatially among blocks ( $F_{72,160} = 5.52$  and  $F_{81,180} = 2.24$ ,  $P < 0.001$  for both). The PCs extracted from the species biomass data were significantly higher in parasite-removed plots except for PC4; the first three PCs of cover were also significantly higher in

**Table 1** Observed and expected numbers of haustoria formed between *Pedicularis kansuensis* and each host species

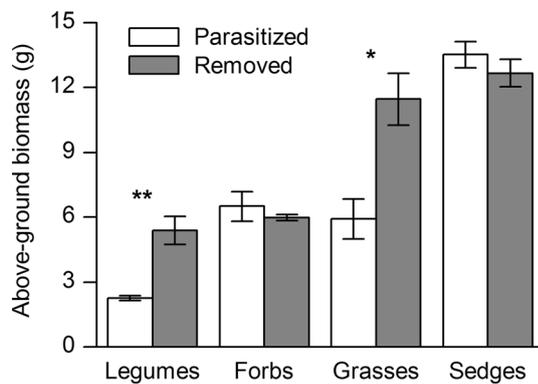
Species	Family	Habit	Number of haustoria		$\chi^2$
			Observed	Expected	
<i>Potentilla anserina</i>	Rosaceae	Perennial	252	159.0	<b>54.4</b>
<i>Kobresia capillifolia</i>	Cyperaceae	Perennial	229	199.4	4.4
<i>Astragalus adsurgens</i>	Fabaceae	Perennial	100	20.1	<b>317.6</b>
<i>Medicago ruthenic</i>	Fabaceae	Perennial	84	44.6	<b>34.8</b>
<i>Elymus nutans</i>	Poaceae	Perennial	64	70.3	0.6
<i>Stipa purpurea</i>	Poaceae	Perennial	64	12.5	<b>212.2</b>
<i>Kobresia humilis</i>	Cyperaceae	Perennial	59	209.3	<b>107.9</b>
<i>Leymus secalinus</i>	Poaceae	Perennial	46	33.3	4.8
<i>Oxytropis kansuensis</i>	Fabaceae	Perennial	43	40.0	0.2
<i>Potentilla multifida</i>	Rosaceae	Perennial	40	59.4	6.3
<i>Taraxacum dissectum</i>	Asteraceae	Perennial	37	11.9	<b>52.9</b>
<i>Plantago depressa</i>	Plantaginaceae	Annual and biennial	24	10.0	19.6
<i>Ixeridium gramineum</i>	Asteraceae	Perennial	20	4.0	<b>64.0</b>
<i>Leontopodium nanum</i>	Asteraceae	Perennial	19	12.8	3.0
<i>Gentianopsis paludosa</i>	Gentianaceae	Perennial	14	18.8	1.2
<i>Lancea tibetica</i>	Scrophulariaceae	Annual	9	10.3	0.2
<i>Gentiana macrophylla</i>	Gentianaceae	Perennial	6	15.6	5.9
<i>Saussurea pulchra</i>	Asteraceae	Perennial	5	183.7	<b>173.8</b>
Total			1115	1115	1064.0

Significant Chi squared values ( $\chi^2 > 27.6$ ; d.f. = 17,  $P < 0.001$ ) are marked in bold

**Table 2** Observed and expected numbers of haustoria formed between *P. kansuensis* and each host family

Family	Number of haustoria		$\chi^2$
	Observed	Expected	
Rosaceae	292	218.4	<b>24.8</b>
Cyperaceae	288	408.7	<b>35.6</b>
Fabaceae	227	104.7	<b>142.9</b>
Poaceae	174	116.1	<b>28.9</b>
Asteraceae	81	212.4	<b>81.3</b>
Gentianaceae	20	34.4	6.0
Plantaginaceae	24	10.0	<b>19.6</b>
Scrophulariaceae	9	10.3	0.2
Total	1115	1115	339.3

Significant Chi squared values ( $\chi^2 > 14.1$ ; d.f. = 7,  $P < 0.001$ ) are marked in bold



**Fig. 1** ANOVA of four plant functional groups' biomass in naturally parasitized quadrats (parasitized, open bars) and in those from which *Pedicularis kansuensis* was removed (removed, black bars). Bars and error bars indicate mean  $\pm$  standard errors, respectively; asterisks indicate significant differences between treatments (\*\* $P < 0.001$ , \* $P < 0.05$ )

parasite-removed plots, whereas PC4 was significantly higher in parasitized plots (Table S8).

Both the cover and biomass of several dominant species (e.g. *E. nutans*, *S. purpurea* and *K. humilis*) were higher in parasite removal plots. Conversely, two subordinate species (*Gernium pylzowianum* and *L. nanum*) were more abundant in naturally parasitized quadrats, and some annual forbs (e.g. *A. capillaries* and *D. heterophyllum*) were only found when the parasites were present (Table 3). Accordingly, species diversity indices were significantly higher in naturally parasitized quadrats (ANOVA:  $F_{1,9} = 32.29$ ,  $P < 0.001$ ; MANOVA:  $F_{3,18} = 64.6$ ,  $P < 0.001$ ; Table S9) and were also affected by a significant interaction ( $F_{27,60} = 2.00$ ,  $P = 0.013$ ) between *P. kansuensis* removal and block. Specifically, *H* and *RI* were significantly higher in naturally parasitized quadrats than in controls ( $F_{1,9} = 35.62$  and  $F_{1,9} = 26.86$ , respectively;  $P < 0.001$  for both; Table S10; Fig. 2b, c), but *EI* did not differ significantly between them (Fig. 2a;  $F_{1,9} = 1.49$ ,  $P = 0.25$ ).

## Discussion

### Host associations of *P. kansuensis*

Careful and extensive root excavation is generally required to assess the host range and selectivity of root parasites in natural ecosystems. Consequently, most relevant studies have focused on the performance of individual parasites in pot experiments using selected host species (e.g. Malcolm 1966; Chuang and Heckard 1971; Gibson and Watkinson 1989; Ren et al. 2010). However, this approach can result in inaccurate predictions of host utilization patterns in natural systems (Marvier and Smith 1997), and does not provide sufficient information on host selection of a parasite to assess its suitability for ecosystem engineering or restoration. Furthermore, no previous studies have quantitatively examined host associations of root hemiparasites on the Qinghai-Tibet Plateau.

Direct examination of sampled root systems revealed haustorial connections between *P. kansuensis* and plants representing eighteen species and eight families (Tables 1, 2), with diverse life histories and growth habits, including two annual or annual-biennial species and sixteen herbaceous perennials. Thus, *P. kansuensis* is not restricted to particular taxonomic groups or to plants with specific life histories or growth forms. Despite the numerous sympatric plants with which *P. kansuensis* formed connections, frequencies of haustoria significantly differed among host species. Comparison of expected and observed numbers of haustoria revealed that three species [*Stipa purpurea* (Poaceae), *Potentilla anserina* (Rosaceae) and *Astragalus adsurgens* (Fabaceae)] were preferentially parasitized. These results are consistent with previous findings that *Pedicularis* spp. display considerable host selectivity and commonly parasitize members of Poaceae, Rosaceae and Fabaceae (Piehl 1963; Gauslaa and Odasz 1990; Ren et al. 2010). However, some species attacked by parasites may not be suitable hosts because attached haustoria may not penetrate their vascular tissues (e.g., Cameron et al. 2006; Cameron and Seel 2007; Suetsugu et al. 2012). Thus, further studies combining careful root excavations with haustorial histology are required to completely define the host range and preferences of *P. kansuensis*.

Numerous factors may influence the host preferences of a parasitic plant. For instance, grasses often form abundant, horizontally distributed, fibrous rootlets in response to soil compaction, which may increase risks of encounter and attack by root hemiparasitic plants (Press and Phoenix 2005; Li et al. 2011). This suggests that root architecture may partially explain the observed preference of *P. kansuensis* for Poaceae. In contrast, high preference for legumes (Fabaceae) may be linked to characteristics such as nitrogen fixation, which imparts high nutritional value (Gibson and Watkinson 1991) and/or the lack of an exodermis, which is the first line of physical defense in many roots. The lack of an exoder-

**Table 3** Biomass and cover of each species in naturally parasitized (Parasitized) quadrats and in quadrats from which parasites were removed (Removed)

Species	Cover (%)		Biomass (g)	
	Parasitized	Removed	Parasitized	Removed
<i>Potentilla anserina</i>	2.1 ± 0.51	2.4 ± 0.68	0.65 ± 0.05	1.12 ± 0.04
<i>Kobresia capillifolia</i>	1.7 ± 0.30	2.2 ± 0.26	4.17 ± 0.43	5.24 ± 0.44
<i>Astragalus adsurgens</i>	2.2 ± 0.37	3.4 ± 0.25	1.12 ± 0.13	2.20 ± 0.30
<i>Medicago ruthenic</i>	2.9 ± 0.40	4.8 ± 0.37	0.54 ± 0.11	1.53 ± 0.11
<i>Elymus nutans</i>	3.3 ± 0.37	6.0 ± 0.32	1.63 ± 0.16	3.58 ± 0.12
<i>Stipa purpurea</i>	5.2 ± 0.40	8.0 ± 0.32	2.50 ± 0.13	5.12 ± 0.27
<i>Kobresia humilis</i>	4.9 ± 0.40	5.6 ± 0.25	3.37 ± 0.10	4.45 ± 0.34
<i>Leymus secalinus</i>	1.9 ± 0.33	3.7 ± 0.44	1.41 ± 0.11	3.47 ± 0.28
<i>Oxytropis kansuensis</i>	1.4 ± 0.51	2.4 ± 0.28	0.60 ± 0.11	2.57 ± 0.21
<i>Potentilla multifida</i>	1.0 ± 0.27	1.5 ± 0.22	0.88 ± 0.02	0.98 ± 0.05
<i>Taraxacum dissectum</i>	0.5 ± 0.22	0.6 ± 0.19	0.19 ± 0.08	0.35 ± 0.04
<i>Plantago depressa</i>	0.4 ± 0.25	0.5 ± 0.23	0.07 ± 0.04	0.21 ± 0.04
<i>Ixeridium gramineum</i>	0.4 ± 0.19	0.3 ± 0.12	0.09 ± 0.04	0.16 ± 0.07
<i>Leontopodium nanum</i>	1.8 ± 0.37	1.6 ± 0.25	0.23 ± 0.01	0.20 ± 0.02
<i>Gentianopsis paludosa</i>	1.0 ± 0.32	0.9 ± 0.25	0.22 ± 0.06	0.19 ± 0.06
<i>Lancea tibetica</i>	1.0 ± 0.51	0.9 ± 0.19	0.19 ± 0.06	0.42 ± 0.04
<i>Gentiana macrophylla</i>	0.8 ± 0.37	0.9 ± 0.33	0.20 ± 0.10	0.42 ± 0.11
<i>Saussurea pulchra</i>	0.3 ± 0.20	0.4 ± 0.18	0.06 ± 0.01	0.18 ± 0.08
<i>Tibetia himalaica</i>	0.8 ± 0.26	0	0.08 ± 0.03	0
<i>Artemisia capillaris</i>	1.9 ± 0.25	0	0.05 ± 0.01	0
<i>Artemisia frigida</i>	1.4 ± 0.43	1.5 ± 0.22	0.49 ± 0.04	0.52 ± 0.04
<i>Swertia mussotii</i>	0.9 ± 0.40	0.8 ± 0.12	0.32 ± 0.09	0.44 ± 0.04
<i>Pedicularis kansuensis</i>	10.2 ± 1.72	0	7.51 ± 0.20	0
<i>Ranunculus tanguticus</i>	2.1 ± 0.46	2.3 ± 0.51	0.56 ± 0.02	0.78 ± 0.04
<i>Geranium pylzowianum</i>	1.3 ± 0.30	1.7 ± 0.16	0.82 ± 0.08	0.89 ± 0.08
<i>Dracocephalum heterophyllum</i>	0.7 ± 0.30	0	0.05 ± 0.02	0

mis makes roots susceptible to invasion by haustoria (Cameron et al. 2005; Rümer et al. 2007; Jiang et al. 2008). Histological examinations of host–parasite interfaces also indicate that defense mechanisms such as induced lignification and encapsulation may prevent root parasites from penetrating the stele of some potential hosts (Cameron et al. 2006; Suetsugu et al. 2012).

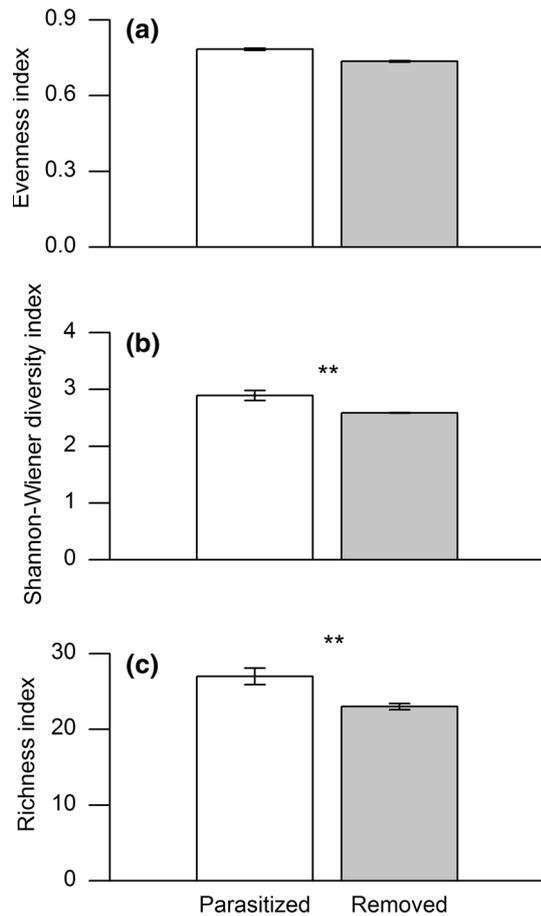
Overall, our findings show that although *P. kansuensis* can form haustorial connections with diverse hosts, including both monocot and dicot species, it selectively attacks some species more frequently than others. Selectivity may provide a mechanism by which *P. kansuensis* depresses the performance of preferred hosts, with important consequences on both the structure and productivity of sympatric plant communities. These results suggest *P. kansuensis* could be used in grassland restoration programs.

#### Effects of *Pedicularis kansuensis* on grassland communities

Hemiparasitic plants, comprising ca. 3,000 known species, are among the most ubiquitous parasites in both managed and natural ecosystems (Kuijt 1969; Press and Graves 1995). Compared with animal parasites (Lafferty et al. 2006, 2008; Sato et al. 2011, 2012), however, little is known about their effects on the structure and productivity of surrounding plant communities or their overall ecosystem contribution (Bardgett et al. 2006). Our results

suggest that the selectivity and resulting effects of *P. kansuensis* on host plants may be strong enough to modify competitive relationships and consequently affect species diversity. Conversely, removal of *P. kansuensis* significantly increased total aboveground biomass of co-occurring plants, suggesting that its presence may reduce productivity. Previous studies have also found that parasitic plants generally accumulate less biomass than the associated reduction in host growth, resulting in an overall community loss of productivity. For instance, Decler et al. (2013) found that the introduction of the hemiparasite *P. palustris* reduced total biomass of fen meadow plant communities from 670 g m<sup>-2</sup> in 1994 to 460 g m<sup>-2</sup> in 2000. Similarly, Borowicz and Armstrong (2012) indicated that removal of the hemiparasite significantly increased total aboveground biomass on a restored tallgrass prairie. These effects may result both from metabolic costs of resource acquisition in the parasite, which prevents compensation for hosts' losses (Davies et al. 1997; Marvier 1998; Ameloot et al. 2005; Huang et al. 2011) and from other physiological changes that impair host performance (Watling and Press 2001).

Parasite removal had different effects on the biomass of the four host plant functional groups; it significantly increased grass and legume biomass, but reduced that of sedges and forbs, relative to the control (Fig. 1). Parasite removal also significantly reduced indices of species diversity (*H*) and richness (*RI*) (Fig. 2b, c). The strong preference *P. kansuensis* showed for grasses and legumes suggests that the differences in community structure and



**Fig. 2** ANOVA of species diversity in naturally parasitized (Parasitized) and parasite-removed (Removed) plots. **a** Evenness Index ( $EI$ ), **b** Shannon–Wiener Diversity ( $H$ ), **c** Richness Index ( $RI$ ). Bars and error bars indicate mean  $\pm$  standard errors, respectively; asterisks indicate significant differences between treatments (\*\* $P < 0.001$ )

diversity resulting from the experimental treatments were probably caused, at least in part, by shifts in the competitive balance among species in the community. The competitive capabilities of the dominant legumes and grasses are affected differently by host preferences, allowing subordinate species to colonize. These shifts are consistent with reported reductions in diversity following removal of *Rhinanthus minor* from British grasslands (Gibson and Watkinson 1992). *R. minor* may also increase plant diversity in lightly fertilized model grassland communities (Bardgett et al. 2006). Similarly, cover of *P. canadensis* positively correlated with species richness in a tallgrass prairie community (Hedberg et al. 2005), and *Castilleja* spp. was shown to increase the evenness of montane meadow communities (Reed 2012).

Despite the important role of direct parasitism, numerous other factors may affect the competitive capability of neighboring plants. Notably, hemiparasitic plants may significantly enhance nutrient availability and cycling in the litter layer (Fisher et al. 2013), as they typically have relatively high concentrations of foliar

nutrients and are often present in nutrient-limited communities (Pate 1995; Quedsted et al. 2003a, b; Spasojevic and Suding 2011). Increased litter nutrient content may also shift the competitive balance among species by differentially enhancing the growth of plants most capable of accessing nutrients from the parasite litter (Pennings and Callaway 1996). Thus, further research is required to determine whether *P. kansuensis* can also influence plant community structure by factors other than direct parasitism, such as inputs of nutrient-rich litter.

In summary, our findings indicate that *P. kansuensis* decreases the biomass of the dominant grasses and legumes and increases that of forbs and sedges, enhancing grassland diversity by reducing the competitive advantage of grasses and legumes, its preferred hosts. Although the presence of *P. kansuensis* is usually considered an indicator of a degraded, species-poor grassland (Song 2006; Bao and Wang 2011), there are several possible explanations for its high abundance in such areas. For instance, like other hemiparasitic forbs (e.g. Těšitel et al. 2011, 2013), *P. kansuensis* is probably highly sensitive to competition for light, but intense grazing in some degraded grasslands may provide suitable gaps for *P. kansuensis* seedlings to establish and form colonies (Press and Phoenix 2005; Van Hovelst et al. 2011). In addition, the host preference for grasses and legumes is likely to significantly affect habitat preference of *P. kansuensis*, and species-poor homogeneous patches dominated by grasses and legumes may provide a greater abundance of suitable hosts. Yet, as the abundance of preferred hosts diminishes as a result of parasitism by *P. kansuensis*, reductions in fitness of these dominants could facilitate the colonization of subordinate species in these species-poor, degraded grasslands. Our results clearly indicate that *P. kansuensis* has the potential to facilitate colonization of subordinate forb species in such grasslands. To determine optimal management regimes for using *P. kansuensis* in grassland restoration programs, however, further research is required, including long-term studies of the effects of seed introductions in areas that lack *P. kansuensis*.

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