Effects of Soil Nitrogen Heterogeneity and Pedicularis Parasitism on Growth, Development, and Root Distribution of Polypogon monspeliensis (Postprint)

Authors: Tian Yuqing, Sui Xiaolin, Zhang Ting, Yanmei Li, Li Airong

Date: 2019-06-17T00:00:00+00:00

Abstract

Soil nutrient heterogeneity is ubiquitous in both agricultural and natural ecosystems, and plants can perceive soil nutrient heterogeneity and adjust root biomass allocation and spatial distribution to acquire more resources. As an important nutrient stress factor, how root hemiparasitic plants affect host plant root spatial distribution and are regulated by nutrient supply levels remains unknown. Understanding the effects of parasitic stress on host growth, development, and root spatial distribution under different nutrient conditions is of great guiding significance for elucidating host adaptation strategies in response to parasitic and nutrient stresses, and consequently for guiding the control of parasitic weeds. This study employed a split-root experiment, by splitting the host roots and controlling nitrogen supply levels on both sides of the root chamber and the degree of parasitic stress, to investigate the effects of nitrogen stress and parasitism by two Pedicularis species with different host dependency levels on the growth, development, and root spatial distribution of the host Polypogon monspeliensis. The results showed that: (1) Both soil nitrogen level and Pedicularis parasitism could significantly affect Polypogon monspeliensis biomass and root-to-shoot ratio, and there was a significant interaction between them, with soil nitrogen level being the main influencing factor. (2) The two Pedicularis species caused different degrees of damage to Polypogon monspeliensis; parasitism by Pedicularis tricolor significantly reduced Polypogon monspeliensis biomass under NPK and 2NPK treatments (shoot: 37.1%, 51.5%; root: 35.6%, 63.6%); whereas parasitism by Pedicularis rex significantly increased Polypogon monspeliensis biomass under NPK treatment (shoot: 29.9%, root: 61.2%). (3) The root growth and spatial distribution of Polypogon monspeliensis were affected by the heterogeneous distribution of nitrogen nutrition and parasitism, demonstrating a clear ability to perceive nutrient spatial distribution and regulate root

growth.

Full Text

Effects of Soil Nitrogen Heterogeneity and Parasitism by *Pedicularis* Species on Growth and Root Spatial Distribution of *Polypogon monspeliensis*

TIAN Yuqing[1,2], SUI Xiaolin[1], ZHANG Ting[1,2], LI Yanmei[1,2], LI Airong[1*]

[1]Yunnan Key Laboratory for Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China

Abstract: Soil nutrient heterogeneity is ubiquitous in both agricultural and natural ecosystems. Plants can perceive soil nutrient heterogeneity and adjust biomass allocation and spatial distribution to optimize resource acquisition. As an important nutrient stress factor, how root hemiparasites affect host root spatial distribution and how this is modulated by nutrient supply levels remain unknown. Understanding the effects of parasitic stress on host growth, development, and root spatial distribution under different nutrient conditions is crucial for elucidating host adaptation strategies to both parasitic and nutrient stress, and for guiding the management of parasitic weeds. Using a split-root experiment, we separated host roots into different compartments and controlled nitrogen supply levels and parasitic stress intensity on each side to examine how nitrogen stress and parasitism by two *Pedicularis* species with different host dependencies affect the growth, development, and root spatial distribution of the host *Polypogon monspeliensis*. The results showed that: (1) Both soil nitrogen levels and *Pedicularis* parasitism significantly affected host biomass and root-to-shoot ratio, with significant interactive effects between the two factors, and soil nitrogen level was the primary influencing factor. (2) The two *Pedicularis* species caused different degrees of damage to *P. monspeliensis*. Under NPK and 2NPK treatments, parasitism by *P. tricolor* significantly reduced host biomass (shoot: 37.1%, 51.5%; root: 35.6%, 63.6%). In contrast, under NPK treatment, parasitism by *P. rex* significantly increased host biomass (shoot: 29.9%, root: 61.2%). (3) Host root growth and spatial distribution were influenced by both heterogeneous nitrogen distribution and parasitism, demonstrating a clear ability to perceive nutrient spatial distribution and regulate root growth accordingly.

Keywords: soil nitrogen level, *Pedicularis*, root hemiparasitic plants, root spatial distribution, split-root pot cultivation

Introduction

Soil nutrients and water are often unevenly distributed in both agricultural and natural ecosystems due to various biotic and abiotic processes, resulting in significant soil environmental heterogeneity (Jackson & Caldwell, 1993). Plants possess the ability to perceive this heterogeneity and adjust nutrient and biomass allocation accordingly to acquire more resources (Benning & Seastedt, 1997; Zhang & Forde 1998; Li et al., 2012b). For example, plants can concentrate more roots in nutrient-enriched zones to improve nutrient uptake efficiency (Wijesinghe et al., 2001; Day et al., 2003; Li, 2014). Additionally, intraspecific and interspecific competition, as well as herbivory, can influence plant root spatial distribution and growth (Schiffers et al., 2011; Wang et al., 2000; Shao et al., 2006). Understanding how plants allocate biomass and alter root spatial distribution in response to biotic and abiotic stresses is essential for elucidating plant adaptation strategies and exploring mechanisms of species interactions and biodiversity maintenance.

Root hemiparasitic plants represent a major group of parasitic plants that possess photosynthetic capacity but typically have underdeveloped root systems with limited ability to acquire nutrients from soil. They must obtain nutrients and water through haustoria attached to host roots for growth and reproduction (Press, 1989; Stewart & Press, 1990; Shamoun, 2009). By extensively exploiting host resources, root hemiparasites often significantly reduce host biomass and quality (Press, 1989; Parker & Riches, 1993). Consequently, many root hemiparasites have become noxious weeds in agricultural and grassland ecosystems, causing substantial losses to crop and livestock production (Press, 1989; Parker & Riches, 1993). For instance, *Striga* species parasitizing sorghum and maize can cause yield reductions of 6-21% (Frost et al., 1997) or even complete crop failure (Hearne, 2009; Smaling et al., 1991), inflicting enormous damage on African agriculture. In China, *Pedicularis* species are widely distributed in subalpine meadows and particularly inhibit the growth of grass hosts, with large-scale infestations seriously threatening local livestock development (Wang et al., 2009; Sui, 2013).

Due to their wide distribution and broad host ranges, root hemiparasites represent a common biotic stress factor in most ecosystems (Press, 1989; Parker & Riches, 1993). Previous research on root hemiparasite-host interactions has primarily focused on aboveground parasitic damage, while little is known about how host plants regulate root spatial distribution to cope with parasitic stress. Although some studies have reported changes in host root investment ratios under parasitic stress (Li et al., 2012a), the responses of parasitized versus unparasitized root zones to *Pedicularis* remain unclear, leaving unanswered whether changes in root investment represent active adaptation or passive stress responses.

Nitrogen is a key nutrient limiting productivity in most grassland ecosystems and plays a crucial role in regulating ecosystem structure and function (Liu et al., 2017). Research shows that soil nitrogen levels significantly affect interactions between root hemiparasites and grass hosts. Aflakpui et al. (2002) found that *Striga hermonthica* caused more severe damage to maize under low soil nitrogen conditions. Gibson & Watkinson (1991) reported that *Rhinanthus minor* caused greater damage to hosts under low nitrogen, while its inhibitory effects decreased under high nitrogen. Recent studies also show that increasing nitrogen levels in grassland ecosystems not only promotes grass growth but also inhibits the growth of the root hemiparasite *Pedicularis kansuensis* (Liu et al., 2017). However, how changes in nitrogen levels alleviate parasitic weed damage by affecting belowground interactions between root hemiparasites and hosts remains unknown.

Given the resource exploitation by parasitic plants and the nutrient-foraging nature of plant roots, we hypothesized that host plants actively regulate root growth when encountering parasitic stress, increasing investment in unparasitized root portions to enhance overall nutrient uptake efficiency. Under high nutrient conditions, increased root investment could acquire more nutrients, partially compensating for losses caused by parasitism, whereas this compensatory effect would be less pronounced under nutrient-poor conditions. Due to heterogeneous soil nutrient distribution, host roots often face dual pressure from local nutrient stress and parasitic stress when root hemiparasites are present. Clarifying how host roots regulate spatial distribution in heterogeneous nutrient environments under parasitic stress, and how soil nutrient changes affect this behavior, is critical for understanding host adaptation strategies to both nutrient heterogeneity and parasitic stress, and for elucidating how fertilization alleviates parasitic damage.

This study selected two *Pedicularis* species with different damage levels to grass hosts from subalpine meadows in northwestern Yunnan, along with a common grass host in their habitat that suffers obvious parasitic damage. Using a split-compartment pot experiment, we separated parasitized and unparasitized host roots, growing half in nutrient-deficient substrate (without nutrient solution) and the other half in nutrient-supplied substrate (with nutrient solution) to investigate the effects of heterogeneous nutrient distribution on root allocation. Additionally, we planted root hemiparasites on the nutrient-supplied side to examine how parasitism by two *Pedicularis* species with different stress levels affects host root-to-shoot ratio and spatial distribution under varying soil nutrient levels. Since grazing activities are frequent in subalpine meadows where *Pedicularis* is abundant, livestock disturbance and manure deposition create more pronounced soil nutrient heterogeneity. Therefore, investigating the effects of *Pedicularis* parasitism on host growth and root spatial distribution under heterogeneous nutrient conditions will help understand how nutrients regulate *Pedicularis*-grass interactions and provide theoretical guidance for targeted nutrient-based management of *Pedicularis* root hemiparasitic weeds.

1.1 Experimental Materials

Two root hemiparasitic *Pedicularis* species were used: *Pedicularis rex* and *P. tricolor*. *P. rex* has lower host dependency and causes milder inhibition of host growth, whereas *P. tricolor* has higher host dependency and causes more severe damage (Li et al., 2012a). The two species also differ in nutrient requirements, with *P. rex* having higher nitrogen demand and *P. tricolor* having higher phosphorus demand (Li et al., 2013b). The host plant was *Polypogon monspeliensis*, a common associated species in the habitats of both *Pedicularis* species.

Seeds of both *Pedicularis* species were collected on September 6, 2016, from the Shangri-La Alpine Botanical Garden in Diqing Prefecture, Yunnan Province (99°38 E, 27°54 N, altitude: 3,370 m). Host *P. monspeliensis* seeds were collected on April 17, 2008, from Kunming Botanical Garden (102°44 E, 25°08 N, altitude: 1,990 m). All seeds were air-dried naturally, stored in paper envelopes at 4°C. The cultivation substrate was a sand-soil mixture (river sand:red soil = 9:1 v/v), thoroughly mixed, bagged, and sterilized in an autoclave at 121°C for 4 h, followed by a second drying treatment at 121°C for 4 h the next day, then cooled naturally before use. The substrate composition followed Sui et al. (2019), with available nitrogen, phosphorus, and potassium contents of 14.3, 2.7, and 62.4 mg · kg ^{1} dry soil weight, respectively.

1.2 Experimental Design

The split-compartment apparatus was modified from Li et al. (2013a). Two square plastic pots (upper width 10 cm, lower width 7 cm, height 8.5 cm) were taped together, with a split-root tube placed between them. The host plant (*P. monspeliensis*) was grown in the split-root tube. Three treatments were established on one side of the pot system: no *Pedicularis* (control, H), *P. rex* (PR+H), or *P. tricolor* (PT+H). To create heterogeneous soil nutrient conditions, nutrient solution was added only to one side of the pot system. In parasitic treatments, *Pedicularis* was planted on the nutrient-supplied side to examine host responses to parasitic stress under varying soil nutrient conditions (Figure 1 [Figure 1: see original paper]).

Based on preliminary results showing significant effects of nitrogen supply on *P. monspeliensis* growth, three nitrogen application levels were established on the nutrient-supplied side: no exogenous nitrogen (nitrogen deficiency, -NPK), standard nitrogen (NPK), and double nitrogen (2NPK) Long Ashton nutrient solution, to examine how nitrogen supply changes affect root spatial distribution regulation. Nutrient composition followed Li et al. (2013a): "NPK"was standard Long Ashton nutrient solution (2 mmol · L ^{1} K SO , 1.5 mmol · L ^{1} MgSO · 7H O, 4 mmol · L ^{1} CaCl , 0.1 mmol · L ^{1} FeEDTA, 4 mmol · L ^{1} (NH) SO , 8 mmol · L ^{1} NaNO , 1.33 mmol · L ^{1} Na HPO , 1.81 mg · L ^{1} MnCl · 4H O, 0.5 mg · L ^{1} ZnSO · 7H O, 0.08 mg · L ^{1} CuSO · 5H O, 0.025 mg · L ^{1} NaMoO · 2H O, 2.86 mg · L ^{1} H BO). "-NPK" indicated nitrogen removal, and "2NPK" indicated doubled nitrogen.

The experiment consisted of 9 treatments with 5 replicates each.

1.4 Host Split-Root Seedling Cultivation

When *P. monspeliensis* radicles reached approximately 2 cm, seedlings were transplanted into disposable nursery pots containing quartz sand (20 seedlings per pot). During the nursery period, 20 mL of standard Long Ashton nutrient solution (NPK) was added weekly to meet seedling growth requirements. Water was applied three times daily to maintain adequate substrate moisture. Nursery pots were repositioned every two days to exclude position effects. After 30 days of cultivation at room temperature, seedlings were ready for split-root transplantation.

1.5 Seedling Transplantation and Cultivation Conditions

When host seedlings developed 8-10 lateral roots (approximately 30 days of nursery growth), they were carefully removed and transplanted into the split-root apparatus (with lateral roots randomly and evenly distributed to both sides). Three *Pedicularis* seedlings were transplanted approximately 2 cm from the split-root tube on the pot side. *Pedicularis* seedlings had radicles about 2 cm long at transplantation and underwent one week of shading to improve survival. After one week, seedlings were thinned to one uniformly vigorous *Pedicularis* per pot. Nutrient solution (-NPK, NPK, or 2NPK) was added twice weekly (15 mL each time) to the side with *Pedicularis*, while an equal volume of tap water was added to the other side, and 2.5 mL of tap water was added to the split-root tube. Substrate moisture was maintained at approximately 10% of dry weight throughout the experiment. Watering was avoided on nutrient addition days to prevent rapid nutrient loss. Pot positions were randomly rearranged after each nutrient addition to exclude position effects. The cultivation experiment was conducted in a glass-controlled greenhouse at Kunming Institute of Botany from October 27, 2017, to February 1, 2018. During this period, minimum temperature was 8.9°C, maximum temperature was 38.3°C, and relative humidity ranged from 21.3% to 99.3%.

1.6 Material Processing and Data Analysis

During cultivation, both *Pedicularis* species grew well initially but later performed poorly and gradually died. *P. tricolor* established parasitic connections with hosts around week 4 after transplantation and died completely by week 7 (parasitism duration approximately 3 weeks, biomass at death <0.0018 g). *P. rex* established parasitic connections around week 4 and died completely by week 10 (parasitism duration approximately 6 weeks, biomass at death <0.0023 g). Host plants were harvested 14 weeks after transplantation. Aboveground and belowground parts were separated, with host roots from each side of the pot system harvested and recorded separately. All plant parts were dried at 85°C for 48 h and weighed. Total root weight was the sum of root dry weights from both sides.

Data were analyzed using SPSS 20.0. One-way ANOVA was used to examine effects of nutrient gradients on biomass and root-to-shoot ratio. Two-way ANOVA was used to examine effects of nutrient gradients and *Pedicularis* parasitism on host biomass and root-to-shoot ratio. t-tests were used to analyze significant differences in root biomass between the two compartments ($P<0.05$). Logarithmic or square root transformations were applied to root biomass data from each side before one-way ANOVA to meet assumptions of normality and homogeneity of variance.

Results

2.1 Effects of Soil Nitrogen Levels and *Pedicularis* Parasitism on *Polypogon monspeliensis* Biomass and Root-to-Shoot Ratio

Two-way ANOVA revealed significant interactive effects between nutrient gradients and *Pedicularis* parasitism on host biomass and root-to-shoot ratio. The sum of squares contributed by nutrient gradients (S) for aboveground biomass, belowground biomass, and root-to-shoot ratio were 7.278, 2.919, and 1.455, respectively, while those contributed by parasitism (S) were 1.03, 0.826, and 0.195, respectively, all showing S > S (Table 1).

2.1.1 Host (*Polypogon monspeliensis*) Biomass Nutrient addition significantly affected aboveground and belowground biomass of unparasitized *P. monspeliensis*, with both increasing significantly as substrate nitrogen levels increased (Figure 2 [Figure 2: see original paper]A). The two *Pedicularis* species showed distinct interspecific differences in their effects on the host under different nutrient conditions. Under nitrogen deficiency (-NPK), *P. tricolor* parasitism had no significant effect on aboveground biomass but showed an increasing trend (22.7% increase), whereas *P. rex* parasitism significantly reduced aboveground biomass to 0.046 g (25.2% decrease). Both species significantly reduced belowground biomass (*P. tricolor*: 65.5% reduction; *P. rex*: 57.1% reduction). Under NPK treatment, *P. tricolor* parasitism significantly reduced aboveground and belowground biomass to 0.657 g and 0.494 g (37.1% and 35.6% reductions, respectively). In contrast, *P. rex* parasitism significantly increased aboveground and belowground biomass to 1.358 g and 1.237 g (29.9% and 61.2% increases, respectively). Under 2NPK treatment, *P. tricolor* parasitism significantly reduced aboveground and belowground biomass to 0.784 g and 0.325 g (51.5% and 63.5% reductions, respectively), while *P. rex* parasitism reduced aboveground and belowground biomass by 13.9% and 9% (to 1.391 g and 0.81 g), respectively, though these differences were not statistically significant.

2.1.2 Host (*Polypogon monspeliensis*) Root-to-Shoot Ratio The root-to-shoot ratio of unparasitized *P. monspeliensis* decreased significantly with increasing soil nitrogen levels (Figure 2B). Effects of *Pedicularis* parasitism on root-to-shoot ratio varied with parasite species and nutrient conditions. Under nitrogen deficiency (-NPK), *P. tricolor* parasitism reduced the root-to-shoot ratio to 0.784 (42% reduction), while *P. rex* parasitism reduced it to 1.12 (17.2% reduction, not statistically significant). Under NPK treatment, *P. tricolor* and *P. rex* parasitism resulted in root-to-shoot ratios of 0.761 and 0.873 (3% and 18.5% increases, respectively), neither statistically significant. Under 2NPK treatment, *P. tricolor* parasitism yielded a root-to-shoot ratio of 0.49 (6.7% reduction), while *P. rex* parasitism yielded 0.534 (1.6% increase), both non-significant (Figure 2B).

2.2 Host Root Biomass Allocation Responses to Nutrient Supply and *Pedicularis* Parasitism in Split-Compartment System

Two-way ANOVA indicated significant interactive effects between *Pedicularis* parasitism and nutrient supply on root biomass in both compartments (Table 2). The sum of squares contributed by nutrient gradients (S) was 0.336 on the unsupplied side and 1.516 on the supplied side, while that contributed by parasitism (S) was 0.125 and 0.261, respectively, showing S > S on both sides, indicating nutrient gradient was the primary factor affecting root biomass in both compartments.

t-test results showed that under nitrogen deficiency (-NPK), *P. tricolor* parasitism resulted in root biomasses of 0.027 g and 0.048 g on the unsupplied and supplied sides, respectively (33% and 2.2% increases compared to unparasitized controls). *P. rex* parasitism yielded 0.021 g on the unsupplied side (3% increase) and 0.03 g on the supplied side (30% decrease), though neither difference was statistically significant. Under NPK treatment, *P. tricolor* parasitism significantly reduced root biomass to 0.146 g and 0.348 g on the unsupplied and supplied sides (45% and 31% reductions, respectively). *P. rex* parasitism significantly increased root biomass to 0.431 g and 0.845 g on the unsupplied and supplied sides (63% and 70% increases, respectively). Under 2NPK treatment, *P. tricolor* parasitism significantly reduced root biomass to 0.104 g and 0.325 g (60% and 53% reductions, respectively). *P. rex* parasitism increased unsupplied-side biomass to 0.324 g (24% increase) and reduced supplied-side biomass to 0.538 g (23% reduction), but neither change was statistically significant (Figure 3 [Figure 3: see original paper], Table 3).

Across all nutrient gradient treatments, host root biomass on the nutrient-supplied side was higher than on the unsupplied side for all cultivation combinations (H, PT+H, and PR+H). Under nitrogen deficiency (-NPK), root biomasses on the supplied side for H, PT+H, and PR+H combinations reached 0.046 g, 0.048 g, and 0.03 g, respectively (132%, 78.6%, and 46.4% higher than unsupplied sides). Under NPK treatment, supplied-side root biomasses were 0.503 g, 0.348 g, and 0.845 g (90.7%, 139%, and 96.1% higher). Under 2NPK treatment, supplied-side biomasses were 0.695 g, 0.325 g, and 0.538 g (165%, 211%, and 66.2% higher) (Figure 3).

Discussion

This study used a split-compartment pot experiment to investigate how the host plant *P. monspeliensis* responds to heterogeneous soil nutrient distribution and *Pedicularis* parasitic stress in terms of root spatial distribution, and how nutrient supply modulates this response. The results demonstrate that host root growth and spatial distribution are simultaneously influenced by nutrient spatial distribution and parasitic stress intensity, representing active regulation of root growth and allocation after integrating information about both nutrient and parasitic stresses.

Under all three nutrient gradients, *P. monspeliensis* root biomass was greater on the nutrient-supplied side than the unsupplied side, indicating root foraging toward nutrient-rich areas. This aligns with previous findings that roots preferentially distribute in nutrient-enriched zones to increase nutrient absorption area and acquisition (Farley & Fitter, 1999; Li et al., 2012b). In the absence of parasitism, increased nitrogen levels significantly enhanced root biomass on the nutrient-supplied side and also increased biomass on the nutrient-poor side. However, further nitrogen elevation continued to increase supplied-side root biomass but did not further increase biomass on the nutrient-poor side, suggesting that *P. monspeliensis* roots possess active nutrient-level recognition and growth regulation capabilities to reduce inefficient investment and avoid resource waste.

The two *Pedicularis* species exhibited significantly different effects on host growth. Although *P. tricolor* parasitism was brief (only 3 weeks), its inhibition of *P. monspeliensis* biomass under NPK and 2NPK treatments was greater than that of *P. rex*, which parasitized for longer (approximately 6 weeks). This is consistent with Li et al. (2012a), who reported that *P. tricolor* has stronger host dependency and causes greater damage than *P. rex*. Under nitrogen deficiency (-NPK), both *P. tricolor* and *P. rex* parasitism reduced the proportion of host root allocation to the side with *Pedicularis*, suggesting that when both root compartments suffer nitrogen stress, *P. monspeliensis* roots tend to avoid parasitic stress. However, as nitrogen supply increased, even when parasitic stress was present on the nutrient-supplied side, *P. monspeliensis* allocated more roots to that side, possibly because parasitic stress in nutrient-rich soil is relatively less severe than nutrient-poor stress. Although this experiment did not examine root distribution responses to parasitic stress under completely uniform nutrient conditions, the host’ s performance under nitrogen deficiency and differential responses to different parasites under other nutrient conditions suggest active root growth responses to parasitic stress.

Interestingly, under nitrogen deficiency (-NPK), *P. rex* parasitism significantly inhibited both aboveground and belowground biomass of *P. monspeliensis*, but under NPK treatment, it significantly increased biomass. *P. rex* has high nitrogen demand and relatively developed roots that compete with hosts for nutrients (Li et al., 2013b). For nitrogen-demanding *P. monspeliensis*, the combination of nitrogen deficiency and parasitic stress clearly suppressed growth. Studies show that plants increase root investment to acquire more nutrients under nutrient scarcity (Fransen et al., 1999; Craine, 2006; Schenk, 2006). Under adequate nitrogen supply, *P. rex* competition for nitrogen reduced substrate nitrogen content, promoting *P. monspeliensis* root growth. Additionally, later death of *P. rex* partially alleviated parasitic pressure, which may explain why *P. rex*-parasitized hosts exceeded unparasized controls under NPK treatment. Cechin et al. (1993, 1994) suggested that appropriate nitrogen supply can reduce or even eliminate parasitic inhibition of host biomass. Generally, adequate nitrogen supply promotes root morphology and mass increase (Forde & Lorenzo, 2001), while excessive nitrogen inhibits root growth (Dong et al., 2001), explaining why additional nitrogen beyond NPK reduced root biomass.

Although parasitism duration was short in this experiment (*P. tricolor*: 3 weeks; *P. rex*: 6 weeks), the effects on *P. monspeliensis* biomass and root development were significant, indicating that even brief parasitic stress can seriously harm host plants. Numerous studies have shown that root hemiparasites can severely damage agriculture and livestock by extensively exploiting host resources, affecting host photosynthesis, and inhibiting host growth (Parker & Riches, 1993; Shamoun, 2009; Sui, 2013). Suetsugu et al. (2012) and Bao et al. (2015) also demonstrated the inhibitory effects of root hemiparasites on grass and legume hosts through parasite removal experiments. However, these studies did not address the effects of parasitic seedlings on host growth. Since some root hemiparasites produce large numbers of seeds with physiological dormancy and seed bank effects (Liu et al., 2011; Sui et al., 2013), numerous seedlings are present throughout the growing season in natural habitats. Due to their small size and difficulty of observation, their ecosystem impacts are often overlooked. This study shows that even brief parasitism by *P. rex* and *P. tricolor* seedlings significantly affects *P. monspeliensis* growth, highlighting the need to consider seedling damage in root hemiparasitic weed management.

Conclusions

1. Both soil nitrogen levels and *Pedicularis* parasitism significantly affect *P. monspeliensis* biomass and root-to-shoot ratio, with significant interactive effects, and soil nitrogen level is the primary influencing factor. Regardless of parasitism, increasing soil nitrogen levels increases host biomass and tends to decrease the root-to-shoot ratio.

2. Short-term parasitism by both *Pedicularis* species can cause obvious damage to *P. monspeliensis*, but the damage degree differs significantly between species and is modulated by soil nitrogen levels in distinct patterns. *P. tricolor* parasitism significantly reduced host biomass under NPK and 2NPK treatments (shoot: 37.1%, 51.5%; root: 35.6%, 63.6%). In contrast, *P. rex* parasitism significantly increased host biomass under NPK treatment (shoot: 29.9%, root: 61.2%).

3. *P. monspeliensis* can sense both soil nitrogen stress and *Pedicularis* parasitic stress and adjust root spatial distribution accordingly. Regardless of parasitic stress, host roots consistently preferentially distribute toward the side with higher soil nitrogen levels. When both compartments suffer nitrogen stress, *P. monspeliensis* tends to increase root investment in the unparasitized compartment when parasitized by either *P. rex* or *P. tricolor*. However, when the parasitized side has high soil nitrogen levels, root avoidance behavior is less pronounced. These changes in root spatial distribution are important for host adaptation to heterogeneous nutrient environments and *Pedicularis* parasitic stress.

References

AFLAKPUI GKS, GREGORY PJ, FROUD-WILLIAMS RJ, 2002. Growth and biomass partitioning of maize during vegetative growth in response to *Striga hermonthica* infection and nitrogen supply[J]. *Exp Agric*, 38:265-276.

BAO GS, SUETSUGU K, WANG XY, et al., 2015. Effects of the hemiparasitic plant *Pedicularis kansuensis* on plant community structure in a degraded grassland[J]. *Ecol Res*, 30:507-515.

BENNING TL, SEASTEDT TR, 1997. Effects of fire, mowing and nitrogen addition on root characteristics in tall-grass prairie[J]. *J Veg Sci*, 8:541-546.

CECHIN I, PRESS MC, 1993. Nitrogen relations of the sorghum *Striga-hermonthica* host-parasite association - germination, attachment and early growth[J]. *New Phytol*, 124:681-687.

CECHIN I, PRESS MC, 1994. Influence of nitrogen on growth and photosynthesis of a C-3 cereal, *Oryza-sativa*, infected with the root hemiparasite *Striga-hermonthica*[J]. *J Exp Bot*, 45:925-930.

CRAINE JM, 2006. Competition for nutrients and optimal root allocation[J]. *Plant Soil*, 285:171-185.

DAY KJ, JOHN EA, HUTCHINGS MJ, 2003. The effects of spatially heterogeneous nutrient supply on yield, intensity of competition and root placement patterns in *Briza media* and *Festuca ovina*[J]. *Funct Ecol*, 17:454-463.

DONG GC, WANG YL, WU H, et al., 2001. Effect of nitrogen supplying levels on the development of roots in rice(*Oryza satival*) [J]. *Jiangsu Agric Res*, (4): 9-13.

FARLEY RA, FITTER AH, 1999. Temporal and spatial variation in soil resources in a deciduous woodland[J]. *J Ecol*, 87:688-696.

FORDE B, LORENZO H, 2001. The nutritional control of root development[J]. *Plant Soil*, 232:51-68.

FRANSEN B, BLIJJENBERG J, DEKROON H, 1999. Root morphological and physiological plasticity of perennial grass species and the exploitation of spatial and temporal heterogeneous nutrient patches[J]. *Plant Soil*, 211:179-189.

FROST DL, GURNEY AL, PRESS MC, et al., 1997. *Striga hermonthica* reduces photosynthesis in sorghum: The importance of stomatal limitations and a potential role for ABA? [J]. *Plant Cell Environ*, 20:483-492.

GIBSON CC, WATKINSON AR, 1991. Host selectivity and the mediation of competition by the root hemiparasite *Rhinanthus-minor*[J]. *Oecologia*, 86:81-87.

HEARNE SJ, 2009. Control - the *Striga* conundrum[J]. *Pest Manag Sci*, 65:603-614.

JACKSON RB, CALDWELL MM, 1993. The scale of nutrient heterogeneity around individual plants and its quantification with geostatistics[J]. *Ecology*, 74:612-614.

LI AR, GUAN KY, STONOR R, et al., 2013a. Direct and indirect influences of arbuscular mycorrhizal fungi on phosphorus uptake by two root hemiparasitic *Pedicularis* species: do the fungal partners matter at low colonization levels? [J]. *Ann Bot*, 112:1089-1098.

LI AR, LI YJ, SMITH SE, et al., 2013b. Nutrient requirements differ in two *Pedicularis* species in the absence of a host plant: Implication for driving forces in the evolution of host preference of root hemiparasitic plants[J]. *Ann Bot*, 112:1099-1106.

LI AR, SMITH FA, SMITH SE, et al., 2012a. Two sympatric root hemiparasitic *Pedicularis* species differ in host dependency and selectivity under phosphorus limitation[J]. *Funct Plant Biol*, 39:784-794.

LI HB, ZHANG FS, SHEN JB, 2012b. Contribution of root proliferation in nutrient-rich soil patches to nutrient uptake and growth of maize[J]. *Pedosphere*, 22:776-784.

LI HB, 2014. The dynamic process of plant responses to heterogeneous nutrient environments and the strategies of root management[D]. Beijing: China Agricultural University.

LIU YY, HU YK, WANG X, et al., 2011. Studies on characteristics of soil seed bank of *Pedicularis verticilata* community[J]. *J Nat Res*, 26(1):48-57.

LIU YY, TAXIPULATI T, GONG YM, et al., 2017. N-P fertilization inhibits growth of root hemiparasite *Pedicularis kansuensis* in natural grassland[J]. *Front Plant Sci*, 8.

MATTHIES D, EGLI P, 1999. Response of a root hemiparasite to elevated CO depends on host type and soil nutrients[J]. *Oecologia*, 120:156-161.

PARKER C, RICHES CR, 1993. *Parasitic weeds of the world: Biology and control*. Parasitic Weeds of the World Biology & Control.

PRESS MC, 1989. Autotrophy and heterotrophy in root hemiparasites[J]. *Trends Ecol Evol*, 4:258-263.

PRESS MC, PHOENIX GK, 2005. Impacts of parasitic plants on natural communities[J]. *New Phytol*, 166:737-751.

SCHENK HJ, 2006. Root competition: Beyond resource depletion[J]. *J Ecol*, 94:725-739.

SCHIFFERS K, TIELBORGER K, TIETJEN B, et al., 2011. Root plasticity buffers competition among plants: Theory meets experimental data[J]. *Ecology*, 92:610-620.

SHAMOUN SF, 2009. Introduction to stem and shoot fungal pathogens and parasitic plants: the values of biological diversity introduction[J]. *Botany*, 87:5-6.

SHAO YQ, ZHAO J, LIU ZL, et al., 2006. Effects of mowing frequency on soil microbial quantity and plant underground biomass in *Leymus chinensis* steppe[J]. *Chin J Ecol*, (10): 1191-1195.

SMALING EMA, STEIN A, SLOOT PHM, 1991. A statistical-analysis of the influence of *Striga-hermonthica* on Maize yields in fertilizer trials in southwestern Kenya[J]. *Plant Soil*, 138:1-8.

STEWART GR, PRESS MC, 1990. The physiology and biochemistry of parasitic angiosperms[J]. *Ann Rev Plant Physiol Plant Mol Biol*, 41:127-151.

SUI XL, 2013. Eco-physiological mechanism for the spatial expansion of *Pedicularis kansuensis* in Bayanbulak grassland of Xinjiang[D]. Beijing: University Chinese Academy Science.

SUI XL, LI AR, GUAN KY, 2013. Impacts of climatic changes as well as seed germination characteristics on the population expansion of *Pedicularis verticillata* [J]. *Ecol Environ Sci*, 22(7): 1099-1104.

SUI XL, ZHANG T, TIAN YQ, et al., 2019. A neglected alliance in battles against parasitic plants: arbuscular mycorrhizal and rhizobial symbioses alleviate damage to a legume host by root hemiparasitic *Pedicularis* species[J]. *New Phytol*, 221:470-481.

SUETSUGU K, TAKEUCHI Y, FUTAI K, et al., 2012. Host selectivity, haustorial anatomy and impact of the invasive parasite *Parentucellia viscosa* on Floodplain vegetative communities in Japan[J]. *Bot J Linn Soc*, 170:69-78.

WANG W, LIANG CZ, LIU ZL, et al., 2000. Analysis of the plant individual behavior during the degradation and restoring succession in steppe community[J]. *Chin J Plant Ecol*, 24(3): 268-274.

WANG WX, SANG GJ, LI L, 2009. Study on the control techniques of poisons grass *Pedicularis* in Xinjiang Bayanbulak prairie[J]. *Grass-Feeding Livestock*, (2): 49-50.

WIJESINGHE DK, JOHN EA, BEURSKENS S, et al., 2001. Root system size and precision in nutrient foraging: Responses to spatial pattern of nutrient supply in six herbaceous species[J]. *J Ecol*, 89:972-983.

ZHANG HM, FORDE BG, 1998. An arabidopsis MADS box gene that controls nutrient-induced changes in root architecture[J]. *Science*, 279:407-409.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv —Machine translation. Verify with original.