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Parasites as ecosystem modulators: foliar pathogens suppress top-down effects of large herbivores

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Summary

- Parasites can catalyze or inhibit interactions between their hosts and other species, but the ecosystem-level effects of such interaction modifications are poorly understood.
- We conducted a large-scale field experiment in temperate grasslands of China to understand how foliar fungal pathogens influenced top-down effects of cattle on plant diversity and productivity.
- When foliar pathogens were suppressed, cattle grazing strongly reduced biomass of the dominant grass, *Leymus chinensis*, generating competitive release that significantly increased community-level species richness and evenness. In the absence of grazing, pathogen attack on *L. chinensis* had no measurable effect on host biomass. However, pathogens disrupted top-down effects of herbivory by inhibiting grazing effects on plant biomass and species richness. Mechanistically, fungal pathogens were linked to increased alkaloid and reduced nitrogen levels in leaf tissue, which appeared to deter cattle grazing on *L. chinensis*.
- In conclusion, foliar pathogens can suppress top-down effects of large herbivores on grassland community composition and ecosystem function by modifying the strength of their host's interactions with dominant consumers. Parasites may act as modulators of ecosystem function when their direct effects on host abundance are overshadowed by powerful influences on host traits that modify their interactions with competitors, herbivores, or predators.

Introduction

Ecologists have made tremendous advances in identifying and explaining primary drivers of ecological processes like competition, herbivory, predation, and parasitism. However, elucidating how these processes interact to assemble communities remains an advancing frontier. For example, it is well established that competitively dominant species can suppress diversity within the same trophic level via competitive exclusion (Connell, 1961; Menge, 1976). Moreover, it has been demonstrated that top-down effects of both herbivores and predators can increase diversity at lower trophic levels by suppressing the abundance of competitive dominants (Paine, 1969; Olff & Ritchie, 1998). However, integrating additional processes complicates predictions of system-level outcomes, particularly when indirect effects in the form of interaction modifications are involved. Interaction modifications are trait-mediated indirect interactions that differ from density-mediated interactions in that they arise when one species changes the interaction strength between two other species rather than simply propagating chains of density effects (Wootton, 1994). Most work on interaction modifications has focused on predator–prey and herbivore-plant systems (e.g. Werner & Peacor, 2003; Ohgushi, 2005). However, parasitism, a cryptic and ubiquitous ecological process, is strongly linked to interaction modifications and may play an underappreciated role in modulating ecosystem processes (Hudson *et al.*, 2006).

Parasites can have a wide range of interactions at all trophic levels (Hatcher *et al.*, 2006), with powerful ramifying effects on communities and ecosystems (Dobson & Crawley, 1994; Hudson *et al.*, 2006; Holdo *et al.*, 2009; Allan *et al.*, 2010). Such effects sometimes arise from direct impacts of pathogens on host abundance, particularly when novel host–pathogen relationships arise from species introductions (Dobson & Crawley, 1994; Hudson *et al.*, 2006; Parker & Gilbert, 2018). However, evolutionarily, host–parasite relationships may tend toward weaker direct interactions that reduce impacts on hosts, allowing both host and parasite to persist (Levin & Pimentel, 1981; Anderson & May, 1982; Ricklefs, 2015). This phenomenon has been empirically demonstrated by long-term observations of novel host–parasite relationships wherein initially powerful pathogen effects that dramatically

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suppress host abundance are moderated over time to weak interactions via host resistance and/or reduced pathogen virulence (e.g. Myxoma virus in Britain and Australia; May & Anderson, 1983).

While an evolutionary trend may drive pathogens toward modest direct effects on host abundance, sublethal effects can still sufficiently influence the host to catalyze other ecological processes. For example, in predator–prey systems, sublethal effects of pathogens on their hosts can increase host susceptibility to predators, potentially enhancing top-down effects of predation (Hudson *et al.*, 1992). This outcome is not driven by strong direct impacts of the pathogen on host abundance but rather by weakening the host and thereby modifying the interaction strength between predator and prey (Hatcher *et al.*, 2006). More generally, pathogens can cause a wide range of interaction modifications within and among trophic levels (Hatcher *et al.*, 2006). Yet, how such interactions manifest at the community and ecosystem levels is not fully understood, particularly for plant pathogens (Hatcher *et al.*, 2006; Hudson *et al.*, 2006). Theoretically, the overall influence of a pathogen on system-level processes should be predicted by the strength of its direct effects on host abundance relative to indirect effects on other species arising from host interaction modifications. If direct effects of pathogens on their host tend to be weaker than the interaction modifications generated with other species, pathogens may commonly act primarily as ecosystem modulators.

In grassland ecosystems, large herbivores can alter plant community composition, diversity, and productivity through a variety of mechanisms (Milchunas *et al.*, 1988; Augustine & McNaughton, 1998; Bakker *et al.*, 2006; Liu *et al.*, 2015; Koerner *et al.*, 2018). Importantly, when large herbivores selectively consume dominant plant species, they can greatly increase plant diversity via competitive release (Olff & Ritchie, 1998), but how plant pathogens might influence these interactions is poorly understood. Plant fungal pathogens can exert profound influences on plant communities by directly suppressing growth, inhibiting reproduction, and/or increasing mortality rates of their host plants (Mitchell, 2003; Borer *et al.*, 2007; Mordecai, 2011; Creissen *et al.*, 2016). When they suppress biomass of community dominants, pathogens can increase plant diversity by preventing competitive exclusion, much like large herbivores (Bradley *et al.*, 2008; Allan *et al.*, 2010; Bagchi *et al.*, 2014; Bever *et al.*, 2015). However, plant pathogens can also strongly modify host interactions with other organisms, including consumers (Hatcher *et al.*, 1995; Stout *et al.*, 2006). In contrast to the pattern seen in predator–prey systems, plant pathogens commonly strengthen host resistance to consumer attack rather than weakening it, specifically by altering the quality (e.g. reduced nutrient concentration and/or increased defense compounds) of host plants for herbivores (Thaler *et al.*, 2010; Mason *et al.*, 2014; Eberl *et al.*, 2020; see also De Vos *et al.*, 2005; Stout *et al.*, 2006). Such interaction modifications could inhibit rather than catalyze top-down effects on lower trophic levels. However, few studies have considered the ecosystem-level effects of plant pathogens.

We conducted a large-scale, *in situ* experiment to assess how large herbivores (cattle; *Bos taurus*) and foliar fungal pathogens affect plant community structure and function (e.g. diversity and productivity) in semiarid grasslands of northeastern China over 3 yr. We monitored shifts in plant nutrients and secondary compounds along with cattle foraging behavior to consider underlying mechanisms. In this system, the perennial grass *Leymus chinensis* is the dominant plant, which constitutes 60–90% of total canopy cover (Zhong *et al.*, 2021). Cattle, which are the dominant vertebrate herbivores, primarily forage on *L. chinensis* but casually feed on other grasses and forbs (Zhong *et al.*, 2022), which can increase grassland plant diversity (Liu *et al.*, 2015). *Leymus chinensis* is commonly heavily infected by foliar fungal pathogens (mainly rusts and leaf spots) with much higher levels of pathogen attack than seen for other grasses and forbs in the system (Lu, 2015). We experimentally manipulated the abundance of cattle and foliar fungal pathogens in a full factorial experiment to evaluate their independent and interactive effects on the dominant *L. chinensis* and subordinate grasses and forbs. We made two key predictions. First, we predicted that cattle would have strong top-down effects that would directly reduce *L. chinensis* biomass and indirectly increase plant diversity due to selective foraging of cattle on *L. chinensis*. Second, we predicted that *L. chinensis* foliar pathogens, which are mainly specialists with well-adapted host–pathogen relationships, would have minimal direct effects on host biomass but relatively strong indirect effects by modifying *L. chinensis* interactions with cattle and thereby weakening top-down effects of cattle on plant biomass and diversity.

Materials and Methods

Study site and background

This study was conducted at the Songnen Grassland Ecosystem National Observation and Research Station of Northeast Normal University, Jilin Province, China (44°35′30″N, 123°30′30″E). The site is located in the eastern region of the Eurasian steppe, which has a semiarid continental monsoon climate. Annual mean temperature ranges from 4.6°C to 6.4°C, and annual precipitation is between 280 and 400 mm, with over 80% falling during the growing season from June to August based on the average from 2012 to 2021 (Zhong *et al.*, 2021). The plant community is dominated by native taxa, in particular the perennial grass *L. chinensis*. Other common native plant species include the grasses *Calamagrostis epigejos* and *Phragmites australis*, and the forbs *Artemisia scoparia*, *Kalimeris integrifolia*, *Lespedeza davurica*, and *Melilotus suaveolens* (Zhong *et al.*, 2017).

The plant community hosts many foliar fungal pathogens, with 30–60% of all plant individuals being infected. The dominant pathogens are from the phyla of Ascomycetes, Basidiomycetes, and Deuteromycotina, including pathogens belonging to *Erysiphe*, *Phyllosticta*, and *Puccinia* (Lu, 2015, also see Supporting Information Table S1). Domestic cattle (*Bos taurus*) are the main vertebrate herbivores in this system. Invertebrate herbivores such as grasshoppers are common (Zhong *et al.*, 2014), but their densities are relatively low in the cattle-grazed areas, where they have limited effects on plant biomass (Zhu *et al.*, 2019). The area has a long history of low-intensity livestock grazing and mowing; however, it was fenced to exclude grazing in 2005 when it became a research site (see

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Fig. S1 for more details about study system components). The study site was also never applied to any fertilizers and insecticides before the beginning of the experiments.

Experimental design

In June 2018, we established six blocks (120–250 m spacing), each including a pair of 50 × 50 m fenced plots separated by *c.* 20 m. One of the enclosure plots per block was randomly assigned to cattle grazing, and the other served as an ungrazed control. Within each plot, we randomly established two 5 × 5 m subplots separated by *c.* 15 m. One of the subplots within each plot was randomly assigned to foliar pathogen suppression via fungicide treatment, while the other was left unmanipulated as a control with natural pathogen infection. Thus, we had four experimental treatments in a fully crossed 2 × 2 split-plot design, that is, cattle only, cattle and foliar pathogens, foliar pathogens only, and no cattle and no foliar pathogens (Fig. S2).

From 2019 to 2021, the plots were grazed by Simmental cattle (body weight 305.0 ± 6 kg, mean ± SE) at a light-to-moderate intensity level (< 40% of plant aboveground biomass consumed by cattle), as recommended by local governments. A total of 30 mature cows were assigned to the six grazed plots, with five cows enclosed in each plot. Plots were grazed from June to August during the first 2 wk of each month from 06:00 to 08:00 h and 16:00 to 18:00 h each day, which is similar to local grazing practices (Zhong *et al.*, 2021). In subplots assigned to pathogen suppression, we sprayed the foliar fungicide Yangcai (Syngenta Crop Protection Inc., Nantong, Jiangsu, China), a combination of Azoxystrobin (7.0%) and Propiconazole (11.7%), every 2 wk from May to August 2019 to 2021. Both Azoxystrobin and Propiconazole are broad spectrum systemic fungicides used widely in agriculture to prevent foliar fungal diseases, and the combination has been used to effectively suppress these diseases in previous experiments, with no detectable nontarget effects on mycorrhizal fungi or plant growth (Borer *et al.*, 2015; Seabloom *et al.*, 2017). Each time we applied the fungicide, we sprayed an equivalent amount of water on non-fungicide subplots to account for any potential effect of irrigation (Bagchi *et al.*, 2014).

Foliar pathogen prevalence

To examine the effects of treatments on foliar fungal pathogens, we quantified infection levels associated with *L. chinensis*, forbs, and other grasses, using standard plant pathology techniques (Mitchell, 2003) in three randomly located 0.5 × 0.5 m quadrats per subplot (Fig. S2). Sampling was conducted in August (peak of the growing season) 2018, 1 yr before treatments began, and again in a second set of quadrats (with different locations compared with the three quadrats in 2018 in the subplots) in August 2021, after 3 yr of treatment. Disease severity, defined as the percentage of leaf area visibly covered by fungal lesions, was measured in the field for each disease type observed (i.e. rusts, fungus-caused leaf spots, powdery mildew, smuts, and blight diseases; Table S1) on each host species per quadrat. To do so, we collected five leaves from each of five randomly selected individuals per host species and quadrat. If there were < 5 individuals or 25 leaves in a quadrat, we examined all the leaves available. With the aid of cards with digitized images of known disease severity, we visually estimated the percentage of leaf area per disease type for each leaf sample.

In the laboratory, we identified fungal pathogen taxa associated with observed disease types in leaf samples (Table S1) using an Olympus light microscope (Olympus Corp., Tokyo, Japan). The disease severity index (V_i) represented the average percentage of leaf area of the specific host species infected by the disease i per quadrat. For the dominant grass, *L. chinensis*, we used this index to represent infection levels for each of the two primary foliar pathogens observed, rusts, and fungus-caused leaf spots. To estimate infection levels for forbs and other grasses across plant taxa in each group, we calculated the pathogen load (l), which weighted the disease severity index for each disease (V_i) and associated host species by species biomass (a_i) in the quadrat (see below), across all diseases (S), after Mitchell *et al.* (2002):

$$l = \frac{\sum_{i=1}^{S} a_i V_i}{\sum_{i=1}^{S} a_i}.$$

Plant community properties

After inspecting the leaves for pathogens in the field, we measured plant community properties in the three 0.5 × 0.5 m quadrats established per subplot in August of 2018 (pretreatment) and 2021 (post-treatment year three), respectively. To represent species richness per quadrat, we identified and counted all plant species. All living plant material in the quadrat was then harvested to 1 cm above ground level and sorted into *L. chinensis*, forbs, and other grasses. Plants were dried at 70°C for 48 h and weighed to determine aboveground biomass per group and quadrat. Pielou's evenness index was calculated as $E = (-\sum p_i \log_e p_i)/\log_e S$, where p_i is the abundance (percentage of total biomass) of species i in the quadrat and S is the number of species.

Plant nutrients and secondary compounds

Given that foliar pathogens have the potential to affect herbivore foraging activities by altering plant nutrients and chemical compounds (Thaler *et al.*, 2010; Mason *et al.*, 2014), we measured these plant traits in conjunction with post-treatment biomass sampling in August 2021. Dried leaf samples from material collected in each quadrat for *L. chinensis*, forbs, and other grasses, respectively, were ground to a fine powder by a Wiley ball-mill and analyzed for total nitrogen (N) and carbon (C) concentration with a Vario MAX cube Elemental Analyzer (Elementar GmbH, Hanau, Germany). For secondary compounds, finely ground plant powder materials were weighed, homogenized with extraction, and then centrifuged. We determined concentrations of alkaloids, total phenols, and cellulose per sample using a Microplate Reader (Michy

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Biomedical Technology Co. Ltd, Suzhou, China), and the analytes were quantified according to absorbance at the relevant peak (at 416, 760, and 620 nm, respectively).

Cattle foraging activities

To evaluate how foliar pathogen suppression affected cattle foraging activities, we measured grazing frequency on plants within each 5 × 5 m subplot in the six grazed plots in August of 2021. Within each subplot, we randomly established a 2-m transect consisting of 10 adjacent 0.2 × 0.2 m quadrats. In each quadrat, *L. chinensis*, forbs, and other grasses, respectively, were scored for visual evidence of cattle grazing (i.e. biomass removal). The proportion of quadrats per transect with evidence of grazing represented the grazing frequency for each plant group and subplot.

Statistical analyses

We used generalized linear mixed-effects models (GLMMs) in SAS v.9.4 (SAS Institute, 2013) to examine the effects of cattle grazing and foliar pathogen suppression on response variables representing pathogen infection levels, plant community properties, plant nutrient and secondary compounds, and cattle foraging. Pretreatment (2018) and post-treatment data (2021) were analyzed separately. With one exception, models contained cattle grazing (grazed or ungrazed), pathogens (present or suppressed), and their interaction as fixed factors, while random factors included block and plot within block to account for the split-plot design, and subplot within plot to account for covariance among embedded quadrats. Grazing frequencies, which were measured in grazed plots only, were analyzed with a simpler model that contained pathogens as a fixed factor and block as a random factor. For each response variable, we specified the error distribution that best fit the data, using a negative binomial distribution for count data, a beta distribution for variables representing proportions, and a lognormal distribution for remaining metrics with positive skewness. In cases where the cattle × pathogen interaction was significant ($P < 0.05$), we tested for *post hoc* differences among treatment means using multiple comparisons wherein the *P*-value was adjusted for the number of comparisons via the Bonferroni method.

Because we did not find significant pretreatment differences in pathogen prevalence or plant community properties among grazing and pathogen treatments (Table S2), we focus presentation on post-treatment results, giving test statistics and *P*-values for individual effects in most cases. However, to summarize multiple nonsignificant tests, we indicate the minimum observed *P*-value and reference Table S3 for full test statistics. Means are presented as least squares means with associated standard errors, as obtained from GLMMs to properly account for error distributions and the split-plot design.

Results

Effectiveness of the foliar pathogen suppression treatment

Three years of foliar fungicide application (2019–2021) led to strong suppression of the primary foliar pathogens infecting the dominant grass, *L. chinensis*, with a 72% reduction in disease severity for rusts ($F_{1,10} = 170.69$, $P < 0.001$) and a 76% reduction for leaf spots ($F_{1,10} = 226.39$, $P < 0.001$; Fig. S3A,B). The fungicide application similarly led to a 56% albeit marginally significant ($F_{1,10} = 4.86$, $P = 0.052$; Fig. S3C) reduction in the foliar pathogen load for forbs, and a 63% reduction for other grasses ($F_{1,10} = 18.44$, $P = 0.002$; Fig. S3D). In no case did the degree of pathogen suppression vary significantly with cattle grazing (cattle × pathogens: $P > 0.1$; Table S3). Rust disease severity for *L. chinensis* was marginally lower in grazed vs ungrazed subplots ($F_{1,5} = 4.39$, $P = 0.09$), but this difference was small compared with the effect of pathogen suppression (Fig. S3A). In remaining cases, pathogen infection levels did not vary by grazing treatment ($P > 0.3$; Table S3).

Effects of grazing and foliar pathogens on plant community properties

Cattle grazing reduced biomass of the dominant grass, *L. chinensis*, when tested across pathogen treatments ($F_{1,5} = 26.11$, $P = 0.004$), while foliar fungal pathogens increased this metric when tested across grazing treatments ($F_{1,10} = 7.73$, $P = 0.02$; Fig. 1a). However, cattle and pathogens interacted significantly, with each of their effects conditioned by the status of the other ($F_{1,10} = 27.09$, $P < 0.001$). Specifically, *L. chinensis* biomass declined by 56% in grazed vs ungrazed subplots when pathogens were suppressed but did not significantly differ between grazing treatments when pathogens were intact (Fig. 1a). Conversely, *L. chinensis* biomass increased by 15% in subplots with pathogens intact vs suppressed when grazed by cattle but was not significantly affected by pathogens when ungrazed (Fig. 1a). Forb biomass increased by 79% in grazed relative to ungrazed subplots ($P = 0.031$), with no significant effect of pathogens either independently ($F_{1,10} = 0.16$, $P = 0.69$) or interactively ($F_{1,10} = 2.96$, $P = 0.12$; Fig. 1b). Biomass of other grasses was not significantly affected by grazing or pathogens ($P > 0.2$; Table S3; Fig. 1c).

Treatment effects on plant biomass translated to shifts in diversity. Cattle grazing significantly increased species richness when tested across pathogen treatments ($F_{1,5} = 16.99$, $P = 0.009$), whereas pathogens did not affect this variable overall ($F_{1,10} = 0.82$, $P = 0.39$; Fig. 2a). However, these factors once again interacted ($F_{1,10} = 5.04$, $P = 0.049$), with the strength of the grazing effect weakening due to pathogens. Specifically, species richness increased by 75% in grazed compared with ungrazed subplots when foliar pathogens were suppressed but did not differ significantly between grazing treatments when foliar pathogens were intact (Fig. 2a). In addition, Pielou's evenness index increased by 15% due to grazing ($F_{1,5} = 12.06$, $P = 0.018$), although this metric was not sensitive to pathogens ($P > 0.6$; Table S3; Fig. 2b).

Effects of grazing and foliar pathogens on plant nutrients and secondary compounds

Foliar fungal pathogens significantly reduced nitrogen (N) concentration in *L. chinensis* leaves ($F_{1,10} = 31.5$, $P < 0.001$), with a decline of 19% in subplots with pathogens intact vs suppressed (Fig. 3a). Carbon (C) concentration was marginally higher with

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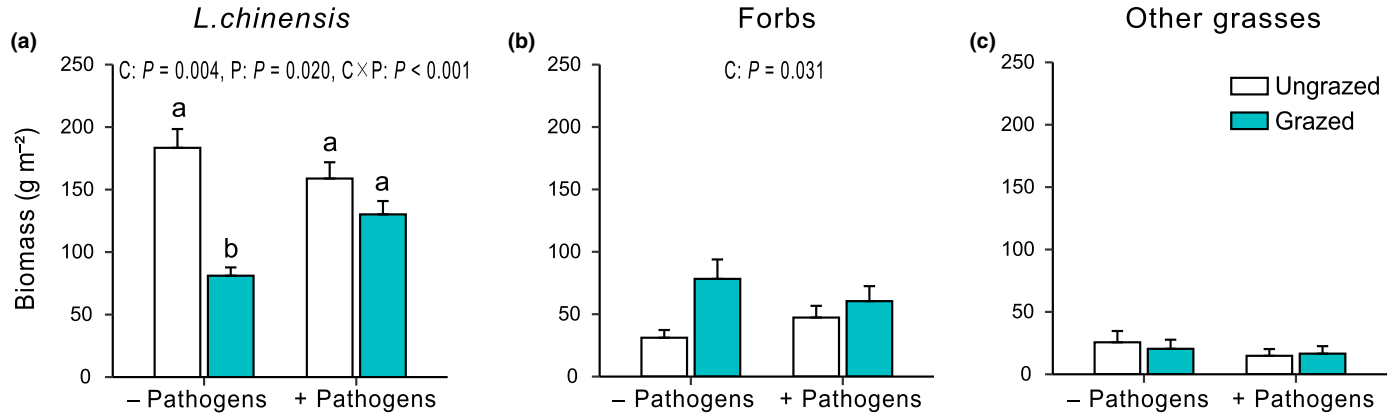


Fig. 1 Combined effects of 3-yr cattle grazing and pathogen suppression treatments on aboveground biomass of (a) *Leymus chinensis*, (b) forbs, and (c) other grasses measured in 5 × 5 m subplots in August 2021. Foliar pathogens were suppressed with fungicide (– Pathogens) or left intact (+ Pathogens). Significant ($P < 0.05$) treatment effects (C, cattle grazing; P, pathogens) are indicated for each panel as relevant. Significant interactions between grazing and pathogens were evaluated with *post hoc* comparisons; means that do not share letters are significantly different. Error bars represent +1SE.

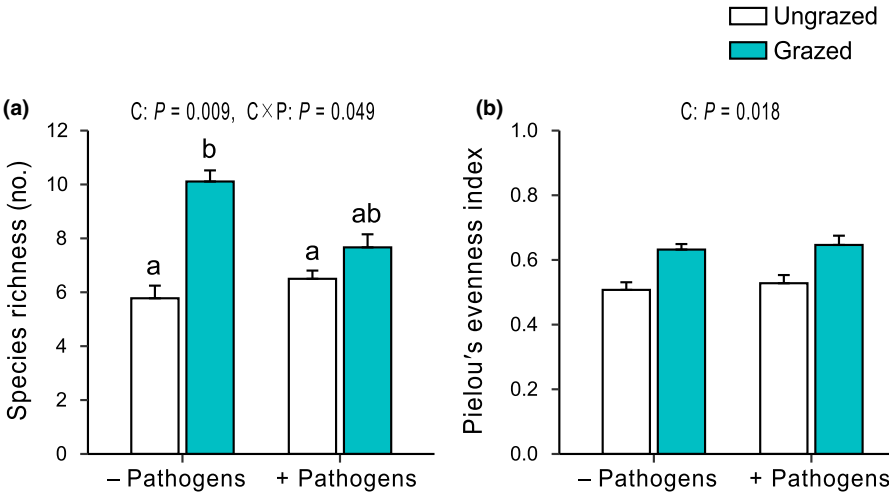


Fig. 2 Combined effects of 3-yr cattle grazing and pathogen suppression treatments on (a) species richness, and (b) Pielou's evenness index, measured in 5 × 5 m subplots in August 2021. Foliar pathogens were suppressed with fungicide (– Pathogens) or left intact (+ Pathogens). Significant ($P < 0.05$) treatment effects (C, cattle grazing; P, pathogens) are indicated for each panel as relevant. Significant interactions between grazing and pathogens were evaluated with *post hoc* comparisons; means that do not share letters are significantly different. Error bars represent +1SE.

pathogens intact ($F_{1,10} = 4.17$, $P = 0.069$), although the difference between treatment means was small (< 1%; Fig. 3b). Cattle grazing did not significantly affect the concentration of these nutrients in *L. chinensis* leaves, whether independently or interactively ($P > 0.15$; Table S3). For forbs and grasses, nutrient concentration was not sensitive to treatments ($P > 0.18$; Table S3; Fig. S4).

Foliar pathogens significantly increased alkaloid concentration in *L. chinensis* leaves ($F_{1,10} = 27.01$, $P < 0.001$), with an increase of 45% with pathogens intact vs suppressed (Fig. 3c). Neither cattle grazing nor its interaction with pathogens affected this plant secondary compound ($P > 0.14$; Table S3). Treatment effects were not significant for total phenol or cellulose concentration in *L. chinensis* leaves ($P > 0.1$; Table S3; Fig. S5), or for any of the secondary compounds measured in leaves of forbs or other grasses ($P > 0.1$; Table S3; Fig. S6).

Effects of foliar pathogens on cattle foraging activities

In the cattle-grazed plots, foliar pathogens significantly reduced cattle foraging on *L. chinensis* ($F_{1,10} = 7.56$, $P = 0.04$), with a 42% decline in grazing frequency in subplots with pathogens intact vs suppressed (Fig. 4a). By contrast, pathogens did not significantly affect grazing frequency on forbs ($F_{1,10} = 1.09$, $P = 0.35$; Fig. 4b) or other grasses ($F_{1,10} = 0.63$, $P = 0.46$; Fig. 4c).

Discussion

Pathogens may play powerful roles in modulating ecosystem functions, but both pathogens and the interactions they generate are cryptic and difficult to observe. In grasslands of northeastern China, we show that cattle grazing strongly suppressed the dominant plant, *L. chinensis*, driving competitive release that increased plant richness and evenness – but only when foliar pathogens were suppressed. Foliar pathogens had no measurable direct impacts on the abundance of their host, *L. chinensis*, but they negated the strong top-down effects of grazing by modifying host traits (secondary compounds and N concentrations) in ways that reduced consumption by cattle (Fig. 5). In sum, the pathogens acted primarily as ecosystem modulators because they initiated

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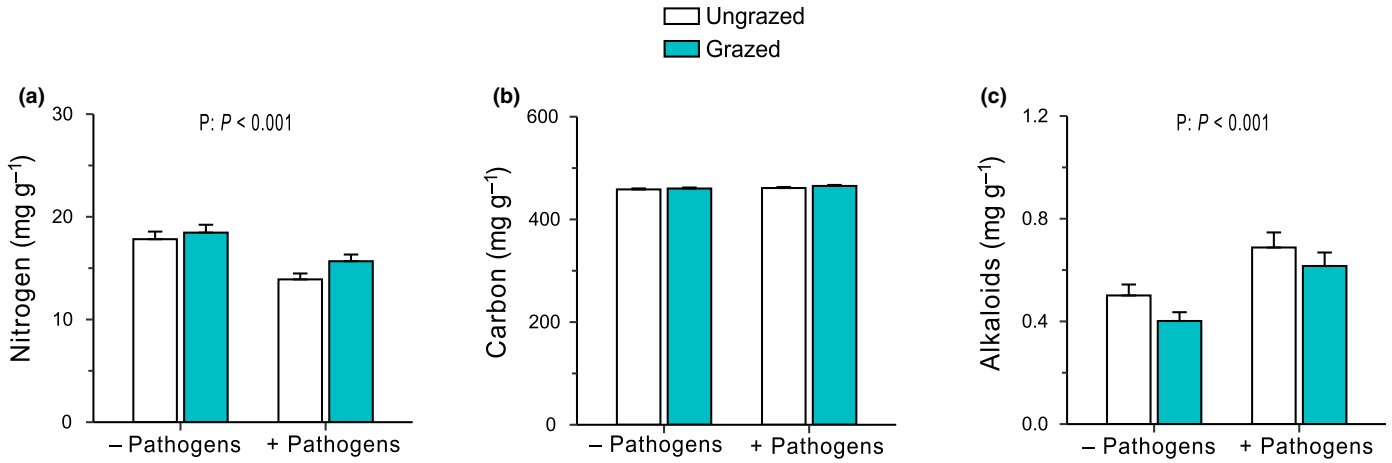


Fig. 3 Combined effects of 3-yr cattle grazing and pathogen suppression treatments on the concentration of (a) nitrogen, (b) carbon, and (c) alkaloids in *Leymus chinensis* leaves based on dry mass. All variables were measured in the 5 × 5 m subplots in August 2021. Foliar pathogens were suppressed with fungicide (– Pathogens) or left intact (+ Pathogens). Significant ($P < 0.05$) treatment effects (C, cattle grazing; P, pathogens) are indicated for each panel as relevant. Error bars represent +1SE.

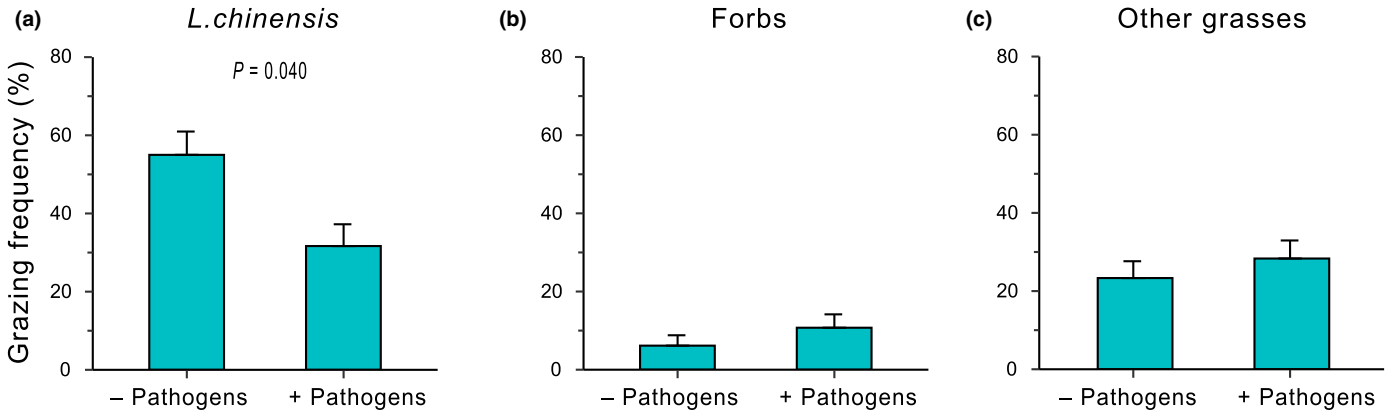


Fig. 4 Effects of the pathogen suppression treatment on frequency of cattle grazing on (a) *Leymus chinensis*, (b) forbs, and (c) other grasses in the six grazed plots. All variables were measured in the 5 × 5 m subplots in August 2021. Foliar pathogens were suppressed with fungicide (– Pathogens) or left intact (+ Pathogens). Significant ($P < 0.05$) pathogens (P) effects are indicated for each panel as relevant. Error bars represent +1SE.

strong trait-mediated interactions that suppressed herbivory and its effects on plant diversity and function while having weak direct effects on host abundance. As we discuss below, there is evidence that foliar pathogens may act as ecosystem modulators more commonly than currently recognized. Moreover, ecosystem modulation by pathogens may occur across trophic levels with divergent outcomes.

Mechanistically, the pathogen-driven interaction modification between *L. chinensis* and cattle was associated with reduced N concentration and increased alkaloid levels in leaves of pathogen-infected plants. Both lower leaf N concentrations (Loranger *et al.*, 2012) and particularly higher alkaloid levels (Clay & Schardl, 2002; Mithöfer & Boland, 2012; Matsuura & Fett-Neto, 2015) are known to inhibit herbivory. Hence, the observed interaction modifications appeared to arise from plastic responses of the plants induced by the pathogens. However, fungal endophytes, which are symbionts, can directly produce alkaloids that serve the same purpose (Clay & Schardl, 2002), so it is possible that fungal pathogens may also directly produce alkaloids. Existing studies suggested that some necrotrophic pathogen (e.g. *Claviceps purpurea*) could produce alkaloids, but not for most biotrophic pathogens (Tente *et al.*, 2021). While it is of interest to know the specific source of alkaloid production, either mechanism generates the same interaction modification and system-level outcome. We note that although our fungicide treatment could have impacted fungi associated with soil or litter addition to foliar fungi (see also Seabloom *et al.*, 2017), our results show a clear linkage between marked suppression of the latter, shifts in *L. chinensis* leaf chemistry, and increased herbivory on this dominant plant. Given that foliar pathogen attacks frequently alter plant chemistry in these ways, foliar pathogens may commonly weaken top-down effects of herbivory by both large mammals and invertebrates (see Hatcher *et al.*, 1995, 2006; Stout *et al.*, 2006). However, at other trophic levels, pathogens may play divergent roles in modulating system processes (Hatcher *et al.*, 2006; Hudson *et al.*, 2006). Notably, pathogen attack on herbivores has been shown to weaken the host and increase its susceptibility to predation (e.g. Hudson *et al.*, 1992) – a

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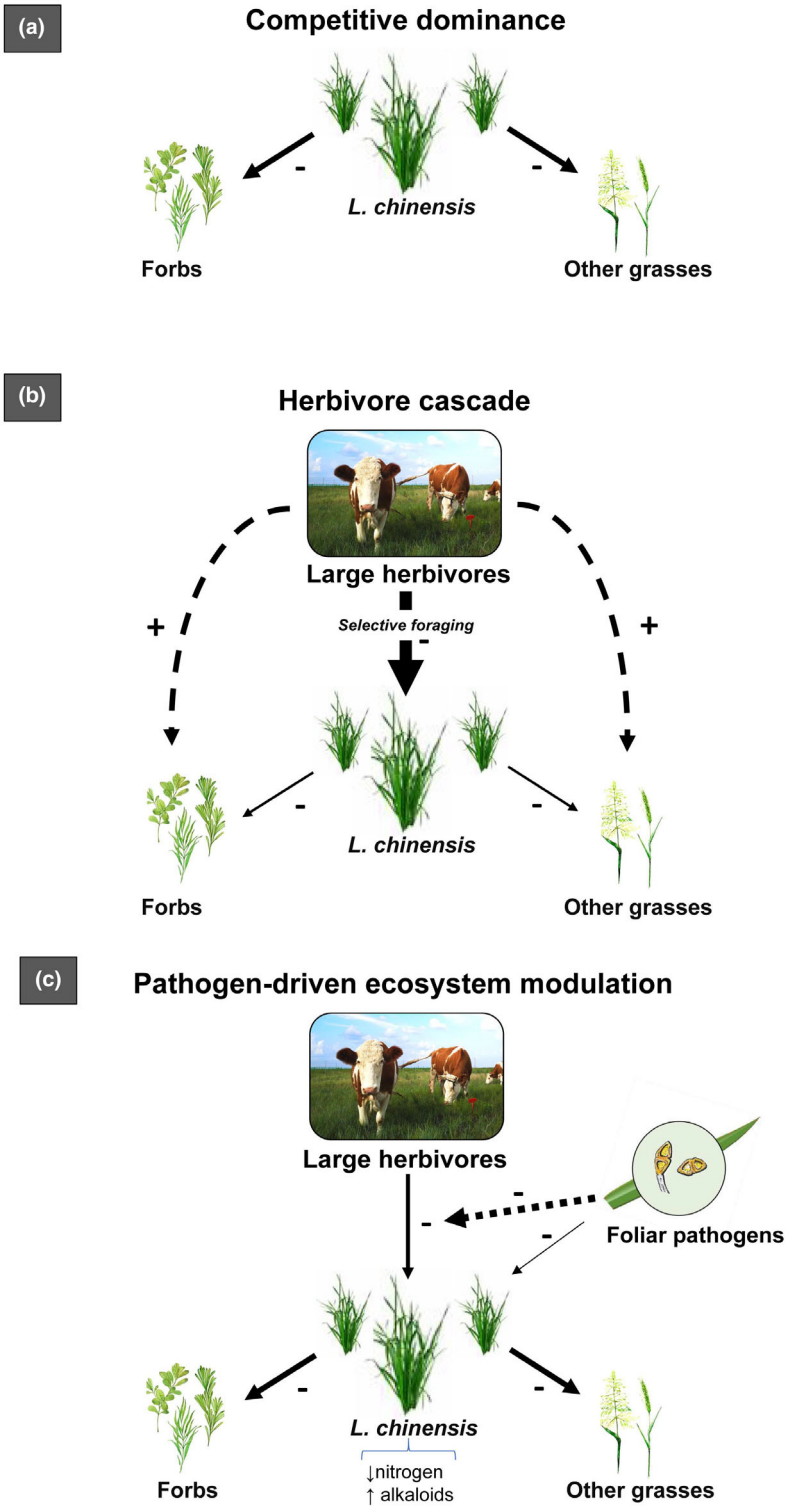


Fig. 5 Role of foliar pathogens as ecosystem modulators in semiarid grasslands of northeastern China. When cattle are absent and foliar pathogens suppressed (a), system dynamics are driven by competitive dominance of *Leymus chinensis*, which strongly suppresses forbs. When cattle are present and pathogens suppressed (b), system dynamics are driven by a herbivore cascade, as cattle preferentially feed on *L. chinensis*, thereby suppressing its biomass and releasing other plants from competitive dominance, which increases plant species and functional diversity. When foliar pathogens are intact (c), they generate a powerful interaction modification that dissipates the herbivore cascade by altering *L. chinensis* leaf traits (reducing nitrogen and increasing chemical defenses) in ways that render the plant less palatable to cattle. Given that the foliar pathogens have no measurable effect on *L. chinensis* biomass, the pathogens serve primarily as powerful modulators of other interactions in this system, with negligible density-driven effects. Solid arrows indicate density-driven direct effects. Dashed arrows linking cattle to plants represent indirect density-mediated effects. The dotted line connecting pathogens to *L. chinensis* indicates an interaction modification. Line weights reflect interaction strength/effect size, and signs indicate negative vs positive effects.

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modulation that would tend to strengthen top-down effects. Of course, pathogen attacks on predators would be expected to weaken the predator and hence attenuate top-down effects (e.g. Wilmers *et al.*, 2006). In sum, pathogens may play critical and complex roles as modulators of primary ecological functions like primary productivity, competition, herbivory, and predation.

Our case study highlights the need to recognize the roles that ecosystem modulators play in governing ecological networks. Ecological networks are comprised of nodes or species and linkages that directly connect organisms to one another via the density-mediated interactions that regulate energy flow through ecological systems, with the nature and strength of these interactions defined by species' roles as herbivores, competitors, predators, etc. (Wootton, 1994). For example, spiders that aggressively consume grasshoppers have a strong negative linkage to grasshoppers via density effects (Zhong *et al.*, 2017). However, many species also alter traits of other organisms in ways that can modify interactions between species, for example, spiders not only consume grasshoppers but also 'scare' them into modified foraging behaviors that can drive functional shifts in primary productivity (Schmitz, 1998). Hence, the scaffolding or circuitry of ecological networks is defined by the energy flows transmitted through species by density-mediated interactions, but trait-mediated interactions modulate the strength of these energy flows much in the way that modulators in electronic networks alter the amplitude and frequency of electromagnetic waves. Importantly, trait effects are cryptic. As such, standard ecological models, which are built on density effects, will fail to capture system dynamics when trait effects drive powerful system-level outcomes (Golubski *et al.*, 2016). While most species likely initiate trait-mediated interactions to some degree, species that initiate trait effects that are much stronger than their density effects will generate predominantly hidden interactions. Moreover, if such species strongly influence key system-level interactions, they could play profound roles as ecosystem modulators.

Hudson *et al.* (2006) speculated on the various roles that pathogens might play in food webs and ecosystems, highlighting the paucity of empirical system-level studies (see also Mitchell, 2003; Hatcher *et al.*, 2006). By conducting a large-scale, replicated, *in situ* experiment, we show that pathogens can have powerful but cryptic effects as modulators of ecosystem processes. Further, we postulate that if host–pathogen evolution tends toward reduced virulence, then many pathogens, particularly specialized pathogens, may serve as potent but underrecognized ecosystem modulators. Advancing ecology will require that interaction modifications and ecosystem modulators are better incorporated into ecological frameworks and network models.

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Competing interests

None declared.

Author contributions

TL, ZZ and DW designed the research, DEP, TL, YKO and ZZ wrote the manuscript; YKO and TL analyzed data; ACR and DW revised the manuscript; TL, WL, HZ and YL performed the research. All authors edited the manuscript.

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Data availability

All observational and experimental data have been deposited via Dryad (https://datadryad.org/) at doi: 10.5061/dryad.cz8w9gj6x.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Cattle, foliar fungal pathogens, plants, and their interspecific interactions in the study ecosystem.

Fig. S2 Design of the field experiment of this study.

Fig. S3 Effects of cattle grazing and pathogen suppression treatments on pathogen infection levels.

Fig. S4 Combined effects of cattle grazing and pathogen suppression treatments on the concentration of total nitrogen and carbon in leaves of forbs and other grasses.

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Fig. S5 Combined effects of cattle grazing and pathogen suppression treatments on the concentration of total phenols and cellulose in *Leymus chinensis* leaves.

Fig. S6 Combined effects of cattle grazing and pathogen suppression treatments on the concentration of alkaloids, total phenols and cellulose in leaves of forbs and other grasses.

Table S1 Plant community composition and the common diseases and associated pathogen taxa found in this study.

Table S2 Generalized linear mixed-effects model results for pretreatment differences in response variables representing foliar fungal pathogen prevalence, plant biomass, and community diversity, as measured in 2018.

Table S3 Generalized linear mixed-effects model results for effects of grazing and pathogen suppression on response variables representing foliar fungal pathogen prevalence, plant biomass, community diversity, leaf composition, and cattle grazing frequency, as measured in 2021.

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