

Article

Effects of Hemiparasites in Grassland Restorations Are Not Universal

Anna Scheidel ^{1,2} and Victoria Borowicz ^{1,*}¹ School of Biological Sciences, Illinois State University, Normal, IL 61790, USA² Champaign County Forest Preserve District, 109 Lake of the Woods Rd., Mahomet, IL 61853, USA

* Correspondence: vaborow@ilstu.edu; Tel.: +1-309-438-5208

Abstract: Root hemiparasites infiltrate the vascular tissue of host roots to acquire water and nutrients, which often reduces host growth. Hemiparasites are postulated to be keystone species in grassland communities if they suppress dominant species and increase plant community biodiversity, and ecosystem engineers if they increase nutrient accessibility for surrounding species. We examined keystone effects by evaluating species richness and evenness in 1 m² plots in a recent prairie restoration where *Castilleja sessiliflora* was naturally present or absent, and in a longer-established prairie restoration with or without *Pedicularis canadensis*. We examined ecosystem engineer effects by determining nitrate and phosphate concentrations under, 25 cm from, and 50 cm from hemiparasites, and in the center of hemiparasite-free plots. On the *C. sessiliflora* site, plots with the hemiparasites had higher species richness due to more forbs and higher floristic quality, consistent with the keystone species hypothesis. Soil phosphate levels were also greater in plots with *C. sessiliflora* present, consistent with the hypothesis of ecosystem engineering by this hemiparasite. In contrast, plots with/without *P. canadensis* showed no associations of any community metrics with the hemiparasite, and no correspondence between the presence of hemiparasites and soil nutrients. Although hemiparasites can increase grassland community heterogeneity, the effect is not universal, and the direction and strength of effects likely depends on local conditions.

Keywords: *Castilleja*; grassland management; nutrients; *Pedicularis*; species richness; tallgrass prairie



Citation: Scheidel, A.; Borowicz, V. Effects of Hemiparasites in Grassland Restorations Are Not Universal. *Diversity* **2024**, *16*, 102. <https://doi.org/10.3390/d16020102>

Academic Editor: Xiaowei Guo

Received: 15 December 2023

Revised: 23 January 2024

Accepted: 30 January 2024

Published: 3 February 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Numerous anthropogenic effects threaten grasslands globally [1]. In the US, prairie grasslands are a critically endangered ecoregion [1–3]. Once a dominant ecosystem from North Dakota to Texas [4], less than 0.01% of high-quality tallgrass prairies remains in many parts of its previous extent, often in isolated remnants [5]. As habitat for many threatened animal species [5] and as significant providers of ecosystem services, including carbon sequestration, pollinator habitat, and freshwater filtration [6], the protection and restoration of prairie ecosystems is urgent.

Prairies persist as a result of disturbance, with a combination of fire and grazing [7] commonly used in management. In combination with the deliberate choice of seed, these practices aim to produce spatial heterogeneity and a resilient, species-rich community [8]. Within this broad framework of management, additional measures that promote local diversity of species typical of undisturbed habitat can increase success of restoration efforts and promote ecosystem functioning [9]. Inclusion of root hemiparasites in restorations may be one such measure. These green plants form vascular bridges to the roots of neighbors to extract minerals, water, and some organic compounds, usually causing reduced growth and reproduction of the host [10,11]. Hemiparasites generally constitute a relatively minor portion of a community's biomass but when their theft of resources more strongly reduces growth of competitive dominant species compared to competitive subordinates, they can have positive effects on species diversity. Species that have disproportionate effects on

community diversity are considered keystone species [12,13]. Consistent with the Keystone Species Hypothesis, diversity was higher in the presence of hemiparasites in some grassland studies [14–18] but not in all studies [17–19].

In addition to reducing the growth of hosts through parasitism, hemiparasites can change physical conditions in the environment and change the accessibility of resources for other organisms, i.e., they exert ecosystem-engineering effects [11,20,21]. Due to high transpiration rates needed to uptake resources from the host xylem, hemiparasites tend to produce nutrient-rich leaves [22,23]. Consistent with the Ecosystem Engineer Hypothesis, the deposition of nutrient-rich litter by hemiparasites can produce a patchy pattern of soil nutrient availability and promote nutrient-processing bacteria, expediting the cycling of nutrients in litter [24]. These ecosystem engineering effects of root hemiparasite leaf litter have been studied primarily in sub-arctic systems [25,26], which have limited nutrients in their soils [27,28] and in mesocosms with annual *Rhinanthus* spp. [24]. Perennial root hemiparasites are common constituents of prairie grasslands, including tallgrass prairies, which are characterized by a rich soil organic matter content. The Ecosystem Engineer Hypothesis has not been tested with hemiparasites in this ecosystem.

Thus, hemiparasites can affect prairie productivity and increase species diversity at a local scale via several pathways but decrease in diversity or no significant affect are also potential outcomes. The extant studies on a limited range of species and ecosystems point toward positive effects of root hemiparasites on species diversity. However, studies of more species in a broader range of habitats are needed to better predict when hemiparasites will produce a positive effect on diversity and to understand the mechanisms producing this effect. Practitioners have expressed great interest in using hemiparasites in prairie restoration but incomplete knowledge of the impact of hemiparasites in prairie ecosystems could result in wasted effort or counterproductive results. In this observational study conducted on restored prairies, we tested whether the presence of hemiparasites was associated with (1) greater species richness or evenness, as predicted by the Keystone Species Hypothesis, and (2) higher nutrient concentrations in the soil, as predicted by the Ecosystem Engineer Hypothesis. Within the context of ecological theory, our goals are to broaden the base of knowledge needed to predict the direction and magnitude of hemiparasite effects, and to suggest avenues for future study relevant for management and restoration of a threatened ecosystem.

2. Materials and Methods

2.1. Study Species

We studied *Castilleja sessiliflora* Pursh. and *Pedicularis canadensis* L. because they share similar prairie habitat and prior research on *P. canadensis* suggested that it would be a likely keystone species [29–32]. The genus *Castilleja* consists of about 200 species commonly known as Paintbrushes. A generalist hemiparasite [33], *C. sessiliflora* (downy painted cup) is a low-growing perennial that flourishes under full sun in low-nutrient soils from Illinois west through the Plains states of the US and Southern Canadian provinces [34]. The genus *Pedicularis*, referred to as lousewort or betony, is geographically widespread with about 400 species [35] that tend to be generalist hemiparasites [36]. *Pedicularis canadensis* L. is commonly found in mesic and dry black soil prairies located in Western Canadian provinces and most of the US [34]. A clonal species, *P. canadensis*, frequently forms a diffusely expanding ring, especially conspicuous in the spring due to spikes of yellow flowers pollinated by bumble bees.

2.2. Field Sites

Observations were conducted at the 1500-ha Nachusa Grasslands [37], a nature preserve mainly comprised of remnant and restored prairies and woodlands owned and managed by The Nature Conservancy in Lee and Ogle Counties, IL, USA. *Castilleja sessiliflora* grows on the sandy knobs of Senger Tract, a dry, upland site (41.900937, −89.370542). Formerly under row crop cultivation, Senger Tract was planted with seeds from 217 early-

to late-successional species sourced from dry and dry-mesic units of the preserve in November 2015. We studied *P. canadensis* at Eight Oaks, a mesic tallgrass prairie (41.884242, −89.355778). This site on poorly drained silty loam was first planted in the 1990s. Compared to Senger Tract, Eight Oaks was seeded with a less diverse forb seed mix and a higher concentration of tallgrasses (E. Bach, personal comm.). *Pedicularis canadensis* was subsequently planted in the established restoration 2006–2008 as part of an experiment. Since 2014, bison have roamed parts of Nachusa Grasslands and graze Eight Oaks but not Senger Tract. Both sites were burned as part of management in spring 2019.

2.3. Vegetation Surveys

We established plots in late May 2019 at each site when the target hemiparasite was in full bloom. At each site, we established 20 1 m² plots: 10 with hemiparasites and 10 hemiparasite-free control plots, with each of the latter located in the nearest hemiparasite-free area \geq five meters from a parasite-present plot. In all hemiparasite plots, the target hemiparasite was the only hemiparasite species present. For plots with the hemiparasite, no hemiparasites occurred within 0.5 m of the plot in at least 2 cardinal directions so that soil nutrient levels could be measured at different distances from the hemiparasite without input from additional hemiparasite individuals. The control plots had no hemiparasites within the plot and in the surrounding 0.5 m.

We tested the Keystone Species Hypothesis using vegetation survey data of *C. sessiliflora* taken between 14 and 29 June, and *P. canadensis* data taken between 11 July and 5 August. In each 1 m² plot, we determined the cover of each species except for the hemiparasites using a modified Daubenmire [38] scale: 0, 0–1%, 1–5%, 6–25%, 26–50%, 51–75%, 76–95%, and 95%, with the midpoints of each category as estimates of cover. Hemiparasite cover was estimated directly to the nearest 10%. From these data, we determined the total number of species in each plot (richness) and calculated the evenness by dividing Shannon's H by the natural log of species richness. We also recorded the coefficient of conservatism (C value) for each native species using the values from Flora of the Chicago Region [33], with non-native species assigned a value of zero. C values are assigned by expert botanists to species of a regional flora and range from 0 to 10. A C value of 0 indicates a ruderal species typical of sites degraded by human activity whereas species with high C values are more likely to appear in high-quality remnant ecosystems. C values have also been demonstrated to reflect life history traits and mycorrhizal responsiveness of tallgrass prairie species [39]. Using the C values, we calculated the Floristic Quality Index (FQI) for each plot, which was obtained by multiplying the mean C value of a plot by the square root of the plot's species richness [40,41]. The FQI reflects a site's ecological integrity and here we use it to examine whether local effects of hemiparasites on measures of diversity are in accord with enhanced quality. In addition to community metrics, we inspected the data to determine which species might have contributed significantly to community response to parasitism. Because other studies have demonstrated the effects of *P. canadensis* on the performance of *Andropogon gerardii* [29–31] and *Solidago canadensis* [29], we focused on these species.

All statistical analyses were conducted using SAS 9.4. PROC GLM was used to test the effects of site, hemiparasite (present/absent), and their interaction on total cover, a measure of productivity. Because each hemiparasite species occurred at a unique site, the effects of the hemiparasite species and site cannot be separated. We selected the site based on the species and therefore regarded site as a fixed effect, as was hemiparasite presence vs. absence. Multivariate analysis of variance (MANOVA) was used to test the effects of site, hemiparasite, and their interaction on evenness and species richness and we examined standardized canonical coefficients to determine which of the multiple variables contributed to each effect. For *C. sessiliflora*, we analyzed the number of species in each functional group (graminoids, legumes, and non-legume forbs) in plots as response variables. Because there were many zero values for legumes in *P. canadensis* plots, legume species were included with other forbs in each plot in similar analyses for *P. canadensis*. To

examine how a community changed, we analyzed the floristic quality index for each plot as a response variable and hemiparasite presence/absence as the main effect.

2.4. Soil Nutrients

To test the effects of hemiparasites on soil minerals, we charged new 2.5 cm × 10 cm cation and anion exchange resin strips (Membranes International Inc., Ringwood, NJ, USA) using the Kellogg LTER protocol (<https://lter.kbs.msu.edu/protocols/105>; accessed on 10 May 2018) with one modification: during the five-hour charging process, the sodium bicarbonate bath was changed after 2.5 h. A small hole was punched at the top of each strip through which a colored zip tie was looped to make retrieval easier.

Based on the timing of senescence, we inserted ion exchange resin strips into the ground at each plot using a 4 cm wide beekeeping prybar to minimize soil disturbance. This occurred 31 August for the *C. sessiliflora* site and 19 October for *P. canadensis*. One cation strip and two anion strips constituted one ‘set’ of strips. We placed one set in the center of each control plot. In plots with hemiparasites, we placed two sets under the crown of the hemiparasite on opposite sides of the rosette, two sets 25 cm away from the crown, and two sets 50 cm away from the crown. When possible, the more distant sets were in line with the crown set. We pushed leaf litter aside to insert strips, then replaced the litter, leaving only the top edges of strips and zip ties visible for eventual retrieval.

The strips remained in the ground for five weeks. Upon removal, the strips were placed in labeled plastic bags and refrigerated for two to three weeks until they were extracted following the Kellogg protocol. From each set of ion strips, we used the cation strip for ammonium extraction, one anion strip for nitrate extraction, and the second anion strip for phosphate extraction. Extracts remained frozen until January 2020, and then analyzed at The Morton Arboretum in Lisle, IL using microplate protocols adapted from Sims et al. [42], Hood-Nowotny et al. [43], and Hedley et al. [44].

To test the Ecosystem Engineer Hypothesis, we first evaluated whether distance from the hemiparasite diluted any effect of hemiparasite litter on phosphate or nitrate concentrations in plots with hemiparasites. Log-transformed values for nitrate and phosphate were analyzed with a multivariate repeated-measures design, with distance from the crown (0, 25, 50 cm) as the within-subjects repeated factor and the hemiparasite (=site) as the between-subjects factor. We then tested the effects of individual hemiparasite species on soil minerals by analyzing for nitrate and phosphate ($\mu\text{g}/\text{cm}^2$) extracted from ionic strips from under the crown of hemiparasites (distance = 0) vs. from the center of hemiparasite-free plots. Nine of the forty strips for ammonium analysis at the Senger Tract were dislodged by animals. Due to this loss of replicates and failure to meet statistical model assumptions, we conducted more limited statistical analysis of ammonium concentrations. We conducted Kruskal–Wallis tests to evaluate the effects of hemiparasite presence/absence on ammonia concentrations in separate analyses for the two species.

3. Results

3.1. Vegetation Surveys

Mean total cover, our measure of aboveground growth, was significantly greater at Senger Tract, which was the *C. sessiliflora* site evaluated in June ($113.6\% \pm 6.4\%$), than at Eight Oaks Prairie, the *P. canadensis* site evaluated a month later ($85.1\% \pm 6.4\%$, $F_{[1,36]} = 9.89$, $p = 0.003$). The presence vs. absence of hemiparasites did not alter this difference between sites (interaction: $F_{[1,36]} = 0.0$, $p = 0.967$) and as a main effect, hemiparasites did not affect total cover ($F_{[1,36]} = 0.33$, $p = 0.567$).

In addition to differences in the total cover of these 1 m² plots, the two sites differed in the diversity of the plant community (Pillai’s Trace: $F_{[2,35]} = 31.46$, $p < 0.0001$), mainly due to differences in the numbers of species rather than evenness (standardized canonical coefficients: richness = 1.53, evenness = 0.38). Plots on the upland, more recently the restored Senger Tract, averaged 23.4 (± 0.8) species, with a total of 99 species in the 20 plots, compared to an average of 14.9 (± 0.8) species with a total of 64 species in the 20 plots at the more

mesic Eight Oaks site. Hemiparasites were marginally associated with significantly altered diversity (Pillai's Trace: $F_{[2,35]} = 3.28$, $p = 0.049$), primarily through effects on species richness (standardized canonical coefficients: richness = 1.58, evenness = -0.52). However, the impact of hemiparasites depended on site (Pillai's Trace: $F_{[2,35]} = 6.91$, $p = 0.003$), again affected most by species richness (standardized canonical coefficients: richness = 1.34, evenness = 0.61). Species richness was greater in the presence of *C. sessiliflora* at the Senger Tract ($p = 0.002$) but was not affected significantly by *P. canadensis* at Eight Oaks ($p = 0.921$; Figure 1).

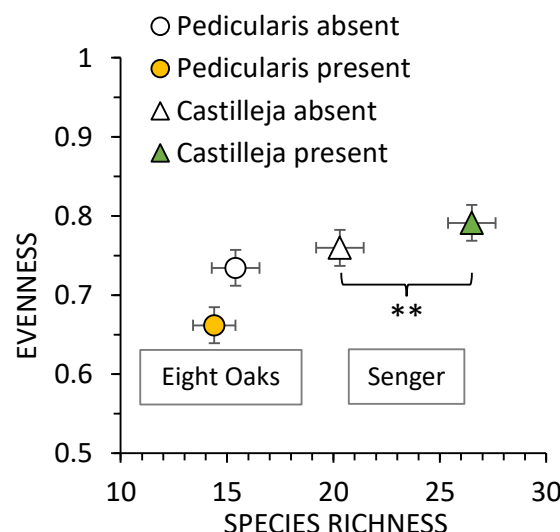


Figure 1. Mean (\pm SE) evenness and richness of plant species in 1 m² plots with/without *Castilleja sessiliflora* (Senger Tract) and *Pedicularis canadensis* (Eight Oaks Prairie) at Nachusa Grasslands, Franklin Grove, IL, USA, in 2019. $N = 10$ per treatment at each site. ** $p = 0.002$.

The relationship between *C. sessiliflora* and species richness differed among functional groups (Pillai's Trace: $F_{[3,16]} = 3.51$, $p = 0.0397$), with forbs contributing most to the hemiparasite effect (standardized canonical coefficients: forbs = 1.139, graminoids = 0.467, legumes = 0.116). Compared to plots without *C. sessiliflora*, plots with the hemiparasite had 5.3 more species of forbs present ($p = 0.0064$; Figure 2). The FQI for plots with *C. sessiliflora* (median = 28.3, range = 25.0–33.6) was greater and thus more consistent with high-quality native prairie than FQI for plots without the hemiparasite (median = 21.4, range = 17.7–31.0; Kruskal–Wallis test $\chi^2 = 7.0$, $df = 1$, $p = 0.008$). Inspection of the cover data showed that four forb species with coefficient of conservatism values (C values) of 9 or 10, though never abundant, were found only in plots with *C. sessiliflora*: *Arnoglossum atriplicifolium* (pale Indian plantain, four plots), *Coreopsis palmata* (prairie coreopsis, five plots), *Gentiana alba* (cream gentian, two plots), and *Helianthus pauciflorus* (prairie sunflower, three plots). *Hieracium longipilum* (hairy hawkweed, C value = 7) was found in four plots, all with *C. sessiliflora*. *Amorpha canescens* (leadplant, C value = 10), a legume, occurred in one plot without *C. sessiliflora* and in seven plots with the hemiparasite.

The presence of *P. canadensis* at Eight Oaks Prairie was not significantly associated with the total numbers of species of graminoids and herbaceous dicots (Pillai's trace: $F_{[2,17]} = 0.30$, $p = 0.747$). The mean FQI also did not differ between plots with *P. canadensis* (14.0 ± 0.7) and those without the hemiparasite (14.3 ± 0.7 ; $p = 0.895$). Inspection of cover data suggested no notable associations between *P. canadensis* and species with especially high or low CC values. *Solidago canadensis* tended to occur in plots without *P. canadensis* (6 of 10; cover range = 0–37.5%) compared to plots with the hemiparasite (3 of 10; cover range = 0–15%), but the difference in association was not significant (Fisher's exact test = 0.37, $p > 0.05$). Among grasses, *Andropogon gerardii* did not stand out as differing in frequency (in eight plots vs. nine plots) or % cover (median 15% vs. 3.5% cover) when *P. canadensis* was absent vs. present.

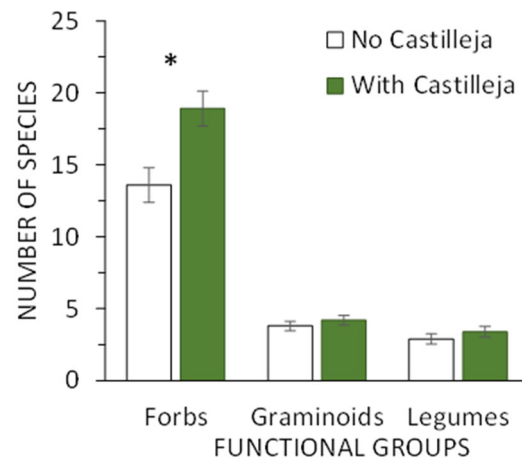


Figure 2. Mean (\pm SE) number of plant species in three functional groups in 1 m² plots with *Castilleja sessiliflora* naturally absent or present. $N = 10$ for each treatment. Plots with *C. sessiliflora* had significantly more forb species. * $p = 0.0064$.

3.2. Soil Nutrients

Concentrations of NO₃ and PO₄ absorbed by ionic membranes inserted in plots with hemiparasites differed between sites (Pillai's trace: $F_{[2,17]} = 42.18$, $p < 0.0001$), with phosphate higher at the Senger Tract (Figure 3). However, there was no evidence of a dilution effect because distance from the hemiparasites did not significantly affect nutrient levels (Pillai's trace: $F_{[4,15]} = 0.22$, $p = 0.9229$; Figure 3). Thus, any spatial effect of nutrient input from the hemiparasite litter was not detectable against background levels. This lack of a distance effect occurred at both sites (Pillai's trace: $F_{[4,15]} = 2.35$, $p = 0.1016$).

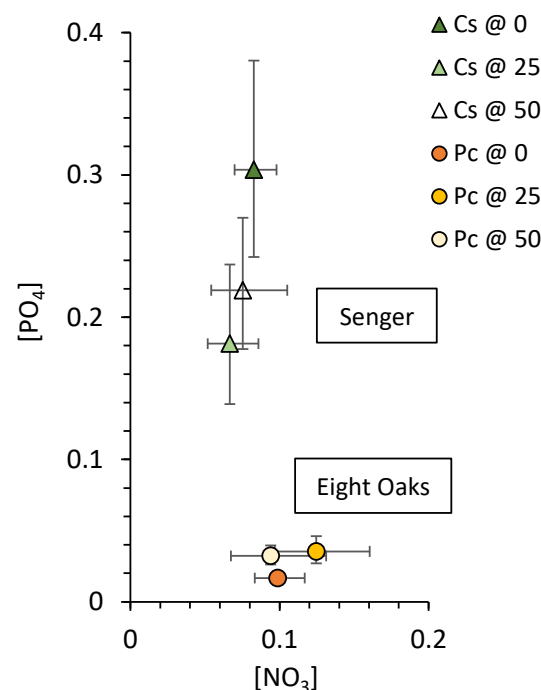


Figure 3. Mean nutrients absorbed ($\mu\text{g}/\text{cm}^2$) by ionic strips from soil at the edge (0 cm), 25 cm, and 50 cm from the canopy of hemiparasites within hemiparasite-occupied plots. *Castilleja sessiliflora* (Cs) and *P. canadensis* (Pc) occurred in different sites and sites differed significantly ($p < 0.0001$). Means with upper and lower standard error bars were back-transformed.

Analysis of NO₃ and PO₄ absorbed by ionic membranes directly under hemiparasites or in the middle of hemiparasite-free plots yielded a slightly different outcome. While

the hemiparasite effect (presence vs. absence) itself was not significant (Pillai's trace: $F_{[2,35]} = 0.48$, $p = 0.6221$), site was significant ($F_{[2,35]} = 39.52$, $p < 0.0001$) and interacted with hemiparasite ($F_{[2,35]} = 4.09$, $p = 0.0254$). This significant interaction was due to the response of PO_4 (standardized canonical coefficients: $\text{PO}_4 = 1.794$, $\text{NO}_3 = -0.034$). A multivariate test of the effect of hemiparasite presence on nutrient response yielded a marginally significant effect of *C. sessiliflora* ($F_{[2,35]} = 3.28$, $p = 0.0493$) with $[\text{PO}_4]$ greater in plots with this hemiparasite (Figure 4). Phosphate concentrations were lower at Eight Oaks and *P. canadensis* did not affect these nutrients ($F_{[2,35]} = 1.28$, $p = 0.2896$).

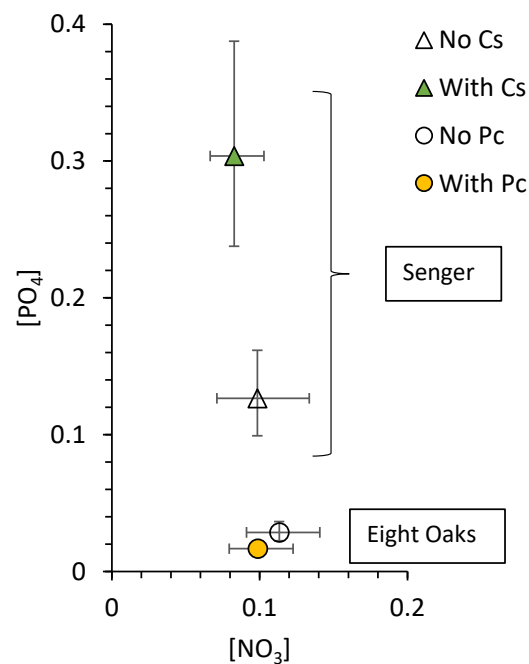


Figure 4. Mean (\pm SE) phosphate and nitrate concentrations of plant species in 1 m² plots with/without *Castilleja sessiliflora* (Senger Tract) and *Pedicularis canadensis* (Eight Oaks Prairie) at Nachusa Grasslands, Franklin Grove, IL, USA in 2019. $N = 10$ per treatment at each site. Multivariate analysis of a significant site \times hemiparasite interaction indicated that *C. sessiliflora* altered concentrations of soil nutrients ($p = 0.0493$).

Ammonium concentrations under *C. sessiliflora* did not differ from concentrations in hemiparasite-free plots (Table 1a). Similarly, *P. canadensis* presence did not significantly alter ammonium concentrations (Table 1b); however, there was a trend toward a greater concentration in the plots without the hemiparasite.

Table 1. Median, range, sample size, and Kruskal–Wallis tests of differences in ammonium concentration in the center of hemiparasite-control plots vs. under the crowns of hemiparasites. (a) *Castilleja sessiliflora*, sampled at the Senger Tract, and (b) *Pedicularis canadensis*, sampled at the Eight Oaks Prairie at Nachusa Grasslands, Franklin Grove, IL, USA.

(a) <i>C. sessiliflora</i>	Median ($\mu\text{g}/\text{cm}^2$)	Range	<i>n</i>
Absent	0.073	0.068–0.107	9
Present	0.083	0.069–0.148	6
$X^2 = 1.690$, $\text{df} = 1$, $p = 0.184$			
(b) <i>P. canadensis</i>	Median ($\mu\text{g}/\text{cm}^2$)	Range	<i>n</i>
Absent	0.070	0.068–0.084	10
Present	0.069	0.051–0.073	10
$X^2 = 3.451$, $\text{df} = 1$, $p = 0.063$			

4. Discussion

Numerous studies have demonstrated a positive association between biodiversity and ecosystem functioning [9]. Maintaining a diverse community may be vital for the ecosystem persistence of grasslands increasingly stressed by climate change and other anthropogenic effects [9]. We studied how root hemiparasites influence the surrounding plant community by conducting field observations related to two hypotheses: hemiparasitism alters plant diversity by affecting competitive interactions among other species (Keystone Hypothesis), and nutrient-rich leaf litter from hemiparasites alters nutrient availability for other species (Ecosystem Engineer Hypothesis). Previous studies that predominantly examined annual hemiparasites in the genus *Rhinanthus* have demonstrated the significant effects of root hemiparasites on community structure, with diversity often higher where the hemiparasite is present [17,23,24,45,46]. The mechanism producing these effects is usually inferred to be direct parasitism of dominant species. Like much of the past field research on hemiparasites in communities, ours was not manipulative and therefore we cannot definitively ascribe cause. Through vegetation analysis and nutrient data from the soil, we found some support for each hypothesis, but significant effects were associated with only one hemiparasite species.

4.1. Keystone Species Hypothesis

Compared to nearby plots without *C. sessiliflora*, plots with this hemiparasite averaged more forb species, had greater species richness, and exhibited a higher FQI indicative of a higher-quality plant community [40]. Several species with high C values occurred only in plots with hemiparasites. Three hypotheses for the putative association of *C. sessiliflora* and higher C values of surrounding species are (1) these species are resistant to *C. sessiliflora* and benefit from the suppression of more dominant species, as predicted by the keystone species hypothesis, (2) these species are susceptible to, yet tolerant of parasitism and especially valuable hosts for *C. sessiliflora*, and (3) both the hemiparasite and rarer species are responding to a third factor and are thus indirectly correlated. Controlled tests are needed to distinguish susceptibility, resistance, and tolerance to hemiparasites. There is a distinct lack of studies that have examined the growth responses of putative hosts with *C. sessiliflora*, and more generally, the knowledge of host ranges for most hemiparasites remains largely anecdotal. Such basic natural history information is needed so that managers can most effectively include hemiparasites in seed mixes.

Regardless of the cause of a higher FQI with *C. sessiliflora*, this very young restoration was originally seeded with 217 species and is likely still on a trajectory toward fewer species. Greater species richness in the presence of *C. sessiliflora* suggests that hemiparasites serve as biotic filters. From a management perspective, hemiparasites might be most effective in retaining higher-quality species when used as a biotic filter early in restoration rather than when added to suppress dominant species in a well-established community. This hypothesis could be tested on sites where parcels of land are restored at various intervals.

We failed to detect any significant effect of *P. canadensis* on measures of species diversity. Thus, *P. canadensis* does not necessarily serve as a keystone species in prairie communities. Our result stands in contrast to a positive association between *P. canadensis* and species richness [29], Shannon's H, and, weakly, FQI [32] on one reconstructed prairie, and higher FQI in an observational study of five sites in Illinois [31]. The mechanism underlying these results was not clear from associated greenhouse experiments examining effects of this hemiparasite on the growth of putative hosts. *Pedicularis canadensis* marginally reduced growth, especially of the shoot, in solitary *Andropogon gerardii* [30] but promoted shoot growth of this same grass and suppressed the shoot growth of an annual legume, *Chamaecrista fasciculata*, in a mesocosm study of six species [31]. Variation in effects on host species among sites may be common in hemiparasites. Davies et al. [47] compared *Rhinanthus* spp. studies and concluded that hemiparasites exhibit conditional preference for host species with the dominant functional group more strongly affected, as predicted for the Keystone Hypothesis. In our work reported here, we detected no difference in cover for

the most common species, *Andropogon gerardii*, nor differences in functional groups in plots with vs. without *P. canadensis*. Unlike the studies in which *P. canadensis* had a significant effect [29,31,32], Eight Oaks Prairie was regularly grazed by the bison herd. While there was no large-scale disturbance, grazing and nutrient redistribution contributed to variance, which could have made the impact of *P. canadensis* difficult to detect. Grazing of the host can also reduce growth of *P. canadensis* [48] and thus its impact on the community.

Root hemiparasites typically reduce productivity [47], and studies of *Rhinanthus* spp. have shown that graminoids and legumes often experience greater effects [49]. Thus, it was surprising that neither *C. sessiliflora* nor *P. canadensis* presence was associated with reduced total cover. While there indeed may have been no effect on growth of other species in the community through parasitism, cover at midsummer may not adequately reflect total biomass by the end of the season, particularly for C4 grasses. Alternatively, the negative effects of hemiparasites through nutrient extraction may be compensated through enhanced availability of nutrients provided through hemiparasite litter and through culture of nutrient-processing bacteria [50,51].

4.2. Ecosystem Engineer Hypothesis

Hemiparasites can offset negative effects of parasitism by means of nutrient input through nutrient-rich leaf litter [23,25] and its effects on nutrient-processing bacteria [24]. If hemiparasites act as ecosystem engineers, we predicted that soil nutrients would be more concentrated immediately surrounding the crown of the hemiparasites compared to further from the hemiparasite or compared to plots with no hemiparasites. In plots with hemiparasites, there were no measurable differences in soil nutrient levels with distance from the hemiparasite's crown. The dilution effect in the soil could be too variable to detect at a scale of centimeters from the hemiparasites, particularly on sites that are routinely burned where mineral ash may be redistributed by rain. Also, the deposition of litter in previous years could produce a legacy effect.

Because each species of hemiparasite was studied at unique sites, we could not isolate effects of site from the species of hemiparasite, and these two factors may have interacted. Significantly higher soil phosphate levels in plots with *C. sessiliflora* compared to parasite-free plots nearby suggest this hemiparasite is altering soil nutrient concentrations. The soil of Senger Tract was sandier, thus more susceptible to nutrient loss compared to the ion-trapping clay of Eight Oaks [52,53]. Senger Tract is also a more recently restored site, and so more recently disturbed. Disturbed sites tend to have fewer large soil aggregates known to anchor macro- and micronutrients in place. Sandier soil composition and smaller aggregate size may have increased the impact of *C. sessiliflora* litter. The summer and fall of 2019 had a higher-than-average rainfall, resulting in occasional flooding in poorly drained soils of Eight Oaks, potentially blunting the impact of *P. canadensis* by redistributing nutrients. Furthermore, bison grazing at Eight oaks may alter plant-soil feedbacks, as has been demonstrated for insect herbivory of grasses, where more intense herbivory reduced the strength of plant-soil feedbacks and plant performance [54].

4.3. Prairie Management

Prairie restoration and management rely on the same processes that were at work prior to European settlement. All prairies require disturbance to halt succession and ensure vegetative heterogeneity [55,56]. Although disturbance by fire and grazing have long been used to restore and maintain grasslands, parasitic plants also produce heterogeneity [32,50]. Hemiparasites have been included in restored prairies, and at times are highly sought after when they decrease the growth of dominant grasses [10,57]. Due to the relatively inexpensive cost of adding them to a seed mix, the use of root hemiparasites in ecological restoration can be appealing to land managers as an adjunct to other land management.

In Europe, researchers have advocated the use of *Rhinanthus* species to increase plant diversity [14,17,47,58]. Similarly, *Pedicularis palustris*, an endangered species, can increase community diversity in fen-meadows where *Carex acuta* dominates [15,16]. *Pedicularis*

kansuensis has also been considered a potential tool for increasing the diversity of grasslands in China [16]. However, *P. kansuensis* also reduces productivity [16], which suggests that managers should seriously examine their motivation and all potential outcomes before adding a hemiparasite, even when it is a native species.

The impact of hemiparasites on community diversity appears to be context-dependent, with physical factors such as light, moisture, and nutrient availability, and biotic factors such as the mycorrhizal dependency of host plants, persistence of a seed bank, and extant species determining the direction and strength of interactions within a plant community [15,18,21,59]. Our research indicates that hemiparasites do not necessarily increase species diversity and the work of others show that sometimes hemiparasites are associated with reduced diversity [19]. Even when they increase diversity, hemiparasites potentially provide opportunities for invasion by exotic or less desirable species [21,60,61]. This may be particularly true in disturbed habitats where conditions favor invasion. Better knowledge of host range for hemiparasites and resistance to parasitism by desired species would enhance the potential for using hemiparasites to reduce troublesome species. In our study, *C. sessiliflora* had the greatest impact on community metrics, and this was on a very recent restoration, suggesting that hemiparasites could have significant value as biotic filters for directing the trajectory of community development early in the process of restoration.

Many private citizens are passionate about and engage in prairie restoration. Collaboration among scientists, practitioners, and citizen scientists offer opportunities to test hypotheses of relevance for the management of prairies. Together, they can conduct empirical work concerning host–hemiparasite relations and competition, nutrient processing, and above-/belowground interactions needed for integrating hemiparasites into the conceptual foundation for ecological restoration. Such advances are greatly needed to meet the challenges of the future [21,62].

Author Contributions: Conceptualization and methodology, A.S. and V.B.; investigation, A.S.; formal analysis, V.B.; data curation, V.B.; original draft preparation, A.S.; writing—review and editing, V.B.; funding acquisition, A.S. and V.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by grants from the Friends of Nachusa Grasslands, the Beta Lambda Chapter of the Phi Sigma Biological Honors Society, and the Graduate School of Illinois State University.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data will be deposited with ISURd, hosted by Digital Commons.

Acknowledgments: We thank M. Midgley at Morton Arboretum for expertise in nutrient analyses and the use of facilities for soil and tissue analyses, S. Juliano for help in statistical analysis, and both for insightful discussion. T. Martin, J. Howard, K. Cazzato, J. Edmundson, E. Berry, and A. Morgan assisted in the field and lab. We are indebted to the staff of Nachusa Grasslands, especially E. Bach, B. Kleiman, C. Considine, for sharing their knowledge of site history, prairies, and restoration.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Hoekstra, J.M.; Boucher, T.M.; Ricketts, T.H.; Roberts, C. Confronting a biome crisis: Global disparities of habitat loss and protection. *Ecol. Lett.* **2005**, *8*, 23–29. [\[CrossRef\]](#)
2. Noss, R.F.; La Roe, E.T., III; Scott, J.M. *Endangered Ecosystems of the United States: A Preliminary Assessment of Loss and Degradation. Biological Report 28*; U.S. Department of Interior, National Biological Service: Washington, DC, USA 1995.
3. Samson, F.B.; Knopf, F.; Ostlie, W.R. Great Plains Ecosystems: Past, present, and future. *Wildlife Soc. B* **2004**, *32*, 6–15. [\[CrossRef\]](#)
4. Anderson, R.C. Evolution and origin of the Central Grassland of North America: Climate, fire, and mammalian grazers. *J. Torrey Bot. Soc.* **2006**, *133*, 626–647. [\[CrossRef\]](#)
5. Samson, F.B.; Knopf, F.L. Prairie conservation in North America. *BioScience* **1994**, *44*, 418–421. [\[CrossRef\]](#)
6. Zhao, Y.; Liu, Z.; Wu, J. Grassland ecosystem services: A systematic review of research advances and future directions. *Landscape Ecol.* **2020**, *35*, 793–814. [\[CrossRef\]](#)

7. Fuhlendorf, S.D.; Engle, D.M. Application of the fire-grazing interaction to restore a shifting mosaic on tallgrass prairie. *J. Appl. Ecol.* **2004**, *41*, 604–614. [\[CrossRef\]](#)
8. Towne, E.G.; Hartnett, D.C.; Cochran, R.C. Vegetation trends in tallgrass prairie from bison and cattle grazing. *Ecol. Appl.* **2005**, *15*, 1550–1559. [\[CrossRef\]](#)
9. Hong, P.; Schmid, B.; De Laender, F.; Eisenhauer, N.; Craven, D.; De Boeck, H.J.; Hautier, Y.; Petchey, O.L.; Reich, P.B.; Steudel, B.; et al. Biodiversity promotes ecosystem functioning despite environmental change. *Ecol. Lett.* **2022**, *25*, 555–569. [\[CrossRef\]](#)
10. Cameron, D.D.; Hwangbo, J.; Keith, A.M.; Geniez, J.; Kraushaar, D.; Rowntree, J.; Seel, W.E. Interactions between the hemiparasitic angiosperm *Rhinanthus minor* and its hosts: From the cell to the ecosystem. *Folia Geobot.* **2005**, *40*, 217–229. [\[CrossRef\]](#)
11. Press, M.C.; Phoenix, G.K. Impacts of parasitic plants on natural communities. *New Phytol.* **2005**, *166*, 737–751. [\[CrossRef\]](#)
12. Paine, R.T. Food web complexity and species diversity. *Am. Nat.* **1966**, *100*, 65–75. [\[CrossRef\]](#)
13. Davic, R. Linking keystone species and functional groups: A new operational definition of the keystone species concept. *Conserv. Ecol.* **2003**, *7*, r11. [\[CrossRef\]](#)
14. Bullock, J.M.; Pywell, R.F. *Rhinanthus*: A tool for restoring diverse grassland? *Folia Geobot.* **2005**, *40*, 273–288. [\[CrossRef\]](#)
15. Decler, K.; Bonte, D.; Van Diggelen, R. The hemiparasite *Pedicularis palustris*: ‘Ecosystem engineer’ for fen-meadow restoration. *J. Nat. Conserv.* **2013**, *21*, 65–71. [\[CrossRef\]](#)
16. Bao, G.; Suetsugu, K.; Wang, H.; Yao, X.; Liu, L.; Ou, J.; Li, C. Effects of the hemiparasitic plant *Pedicularis kansuensis* on plant community structure in a degraded grassland. *Ecol. Res.* **2015**, *30*, 507–515. [\[CrossRef\]](#)
17. Fibich, P.; Lepš, J.; Chytrý, M.; Těšitel, J. Root hemiparasitic plants are associated with high diversity in temperate grasslands. *J. Veg. Sci.* **2017**, *28*, 184–191. [\[CrossRef\]](#)
18. Těšitel, J.; Mládek, J.; Horník, J.; Těšitelová, T.; Adamec, V.; Tichý, L. Suppressing competitive dominants and community restoration with native parasitic plants using the hemiparasitic *Rhinanthus alectorolophus* and the dominant grass *Calamagrostis epigejos*. *J. Appl. Ecol.* **2017**, *54*, 1487–1495. [\[CrossRef\]](#)
19. Gibson, C.C.; Watkinson, A.R. The role of the hemiparasitic annual *Rhinanthus minor* in determining grassland community structure. *Oecologia* **1992**, *89*, 62–68. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Jones, C.G.; Lawton, J.H.; Shachak, M. Organisms as ecosystem engineers. *Oikos* **1994**, *69*, 373–386. [\[CrossRef\]](#)
21. Chaudron, C.; Mazalová, M.; Kuras, T.; Malenovský, I.; Mládek, J. Introducing ecosystem engineers for grassland biodiversity conservation: A review of the effects of hemiparasitic *Rhinanthus* species on plant and animal communities at multiple trophic levels. *Perspect. Plant Ecol.* **2021**, *52*, 125633. [\[CrossRef\]](#)
22. Quested, H.M. Parasitic plants—Impacts on nutrient cycling. *Plant Soil* **2008**, *311*, 269–272. [\[CrossRef\]](#)
23. Fisher, J.B.; Phoenix, G.K.; Childs, D.Z.; Press, M.C.; Smith, S.W.; Pilkington, M.G.; Cameron, D.D. Parasitic plant litter input: A novel indirect mechanism influencing plant community structure. *New Phytol.* **2013**, *198*, 222–231. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Bardgett, R.D.; Smith, R.S.; Shiel, R.S.; Peacock, S.; Simkin, J.M.; Quirk, H.; Hobbs, P.J. Parasitic plants indirectly regulate below-ground properties in grassland communities. *Nature* **2006**, *439*, 969–972. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Quested, H.M.; Press, M.C.; Callaghan, T.V.; Cornelissen, J.H.C. The hemiparasitic angiosperm *Bartsia alpina* has the potential to accelerate decomposition in sub-arctic communities. *Oecologia* **2002**, *130*, 88–95. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Quested, H.M.; Press, M.C.; Callaghan, T.V. Litter of the hemiparasite *Bartsia alpina* enhances plant growth: Evidence for a functional role in nutrient cycling. *Oecologia* **2003**, *135*, 606–614. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Berendse, F.; Jonasson, S. Nutrient use and nutrient cycling in northern ecosystems. In *Arctic Ecosystems in a Changing Climate, an Ecophysiological Perspective*; Chapin, F.S., Jefferies, R.L., Reynolds, J.F., Shaver, G.S., Svoboda, J., Eds.; Academic Press: San Diego, CA, USA, 1992; pp. 337–356.
28. Callaghan, T.V.; Jonasson, S. Arctic terrestrial ecosystems and environmental change. *Philos. Trans. R. Soc. Lond.* **1995**, *352*, 259–276. [\[CrossRef\]](#)
29. Hedberg, A.M.; Borowicz, V.A.; Armstrong, J.E. Interactions between a hemiparasitic plant, *Pedicularis canadensis* L. (Orobanchaceae), and members of a tallgrass prairie community. *J. Torrey Bot. Soc.* **2005**, *132*, 401–410. [\[CrossRef\]](#)
30. Borowicz, V.A.; Armstrong, J.E. Resource limitation and the role of a hemiparasite on a restored prairie. *Oecologia* **2012**, *169*, 783–792. [\[CrossRef\]](#)
31. DiGiovanni, J.P.; Wysocki, W.P.; Burke, S.V.; Duvall, M.R.; Barber, N.A. The role of hemiparasitic plants: Influencing tallgrass prairie quality, diversity, and structure. *Restor. Ecol.* **2017**, *25*, 405–413. [\[CrossRef\]](#)
32. Borowicz, V.A.; Walder, M.R.; Armstrong, J.E. Coming undone: Hemiparasite presence and effects in a prairie grassland diminish over time. *Oecologia* **2019**, *190*, 679–688. [\[CrossRef\]](#)
33. Wilhelm, G.; Rericha, L. *Flora of the Chicago Region: A Floristic and Ecological Synthesis*; Indiana Academy of Science: Indianapolis, IN, USA, 2017.
34. USDA, NRCS. The PLANTS Database. Available online: <http://plants.usda.gov> (accessed on 14 June 2021).
35. Schneider, M.J.; Stermitz, F.R. Uptake of host plant alkaloids by root parasitic *Pedicularis* species. *Phytochemistry* **1990**, *29*, 1811–1814. [\[CrossRef\]](#)
36. Piehl, M.A. The parasitic behavior of *Dasistoma macrophylla*. *Rhodora* **1962**, *64*, 331–336.
37. Bach, E.M.; Kleiman, B.P. Twenty years of tallgrass prairie restoration in northern Illinois, USA. *Ecol. Solut. Evid.* **2021**, *2*, e12101. [\[CrossRef\]](#)
38. Daubenmire, R. A canopy coverage method of vegetation analysis. *Northwest Sci.* **1959**, *33*, 43–64.

39. Bauer, J.T.; Koziol, L.; Bever, J.D. Ecology of Floristic Quality Assessment: Testing for correlations between coefficients of conservatism, species traits and mycorrhizal responsiveness. *AoB Plants* **2018**, *10*, plx073. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Taft, J.B.; Wilhelm, G.S.; Ladd, D.M.; Masters, L.A. Floristic quality assessment for vegetation in Illinois, a method for assessing vegetation integrity. *Erigenia* **1997**, *15*, 3–95.
41. Spyreas, G. Floristic Quality Assessment: A critique, a defense, and a primer. *Ecosphere* **2019**, *10*, e02825. [\[CrossRef\]](#)
42. Sims, G.K.; Ellsworth, T.R.; Mulvaney, R.L. Microscale determination of inorganic nitrogen in water and soil extracts. *Commun. Soil Sci. Plant Anal.* **1995**, *26*, 303–316. [\[CrossRef\]](#)
43. Hood-Nowotny, R.; Hinko-Najera Umana, N.; Inselbacher, E.; Oswald-Lachouani, P.; Wanek, W. Alternative methods for measuring inorganic, organic, and total dissolved nitrogen in soil. *Nutr. Manag. Soil Plant Anal.* **2010**, *74*, 1018–1027. [\[CrossRef\]](#)
44. Hedley, M.J.; Stewart, J.W.; Chauhan, B. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Sci. Soc. Am. J.* **1982**, *46*, 970–976. [\[CrossRef\]](#)
45. Matthies, D. Parasitic and competitive interactions between the hemiparasites *Rhinanthus serotinus* and *Odontites rubra* and their host *Medicago sativa*. *J. Ecol.* **1995**, *83*, 245–251. [\[CrossRef\]](#)
46. Watson, D.M. Parasitic plants as facilitators: More dryad than Dracula? *J. Ecol.* **2009**, *97*, 1151–1159. [\[CrossRef\]](#)
47. Davies, D.M.; Graves, J.D.; Elias, C.O.; Williams, P.J. The impact of *Rhinanthus* spp. on sward productivity and composition: Implications for the restoration of species-rich grasslands. *Biol. Conserv.* **1997**, *82*, 87–93. [\[CrossRef\]](#)
48. Van Hovel, M.D.; Evans, B.A.; Borowicz, V.A. Hemiparasite-host plant interactions and the impact of herbivory: A field experiment. *Botany* **2001**, *89*, 537–544. [\[CrossRef\]](#)
49. Ameloot, E.; Verheyen, K.; Hermy, H. Meta-analysis of standing crop production by *Rhinanthus* spp. and its effect on vegetation structure. *Folia Geobot.* **2005**, *40*, 289–310. [\[CrossRef\]](#)
50. Spasojevic, M.; Suding, K. Contrasting effects of hemiparasites on ecosystem processes: Can positive litter effects offset the negative effects of parasitism? *Oecologia* **2011**, *165*, 193–200. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Demey, A.; Ameloot, E.; Staelens, J.; De Schrijver, A.; Verstraeten, G.; Boeckx, P.; Hermy, M.; Verheyen, K. Effects of two contrasting hemiparasitic plant species on biomass production and nitrogen availability. *Oecologia* **2013**, *173*, 293–303. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Bach, E.M.; Baer, S.G.; Meyer, C.K.; Six, J. Soil texture affects soil microbial and structural recovery during grassland restoration. *Soil. Biol. Biochem.* **2010**, *42*, 2182–2191. [\[CrossRef\]](#)
53. Baer, S.G.; Meyer, C.K.; Bach, E.M.; Klopff, R.P.; Six, J. Contrasting ecosystem recovery on two soil textures: Implications for carbon mitigation and grassland conservation. *Ecosphere* **2010**, *1*, 1–22. [\[CrossRef\]](#)
54. Heinze, J.; Simons, N.K.; Seibold, S.; Wacker, A.; Weithoff, G.; Gossner, M.M.; Prati, D.; Bezemer, T.M.; Joshi, J. The relative importance of plant-soil feedbacks for plant-species performance increases with decreasing intensity of herbivory. *Oecologia* **2019**, *190*, 651–664. [\[CrossRef\]](#)
55. Hamman, S.T.; Dunwiddie, P.W.; Nuckols, J.L.; McKinley, M. Fire as a restoration tool in Pacific Northwest prairies and oak woodlands: Challenges, successes, and future directions. *Northwest Sci.* **2011**, *85*, 317–328. [\[CrossRef\]](#)
56. Rook, E.J.; Fischer, D.G.; Seyferth, R.D.; Kirsch, J.L.; LeRoy, C.J.; Hamman, S. Responses of prairie vegetation to fire, herbicide, and invasive species legacy. *Northwest Sci.* **2011**, *85*, 288–302. [\[CrossRef\]](#)
57. Henderson, R.A. Are there keystone plant species driving diversity in Midwest prairies. In *Promoting prairie, Proceedings of the 18th North American Prairie Conference*; Fore, S., Ed.; Truman State University: Kirksville, MO, USA, 2003; pp. 63–66.
58. Pywell, R.F.; Bullock, J.M.; Walker, K.J.; Coulson, S.J.; Gregory, S.J.; Stevenson, M.J. Facilitating grassland diversification using the hemiparasitic plant *Rhinanthus minor*. *J. Appl. Ecol.* **2004**, *41*, 880–887. [\[CrossRef\]](#)
59. Stein, C.; Rißmann, C.; Hempel, S.; Renker, C.; Buscot, F.; Prati, D.; Auge, H. Interactive effects of mycorrhizae and a root hemiparasite on plant community productivity and diversity. *Oecologia* **2009**, *159*, 191–205. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Joshi, J.; Matthies, D.; Schmid, B. Root hemiparasites and plant diversity in experimental grassland communities. *J. Ecol.* **2000**, *88*, 634–644. [\[CrossRef\]](#)
61. Walder, M.; Armstrong, J.E.; Borowicz, V.A. Limiting similarity, biotic resistance, nutrient supply, or enemies? What accounts for the invasion success of an exotic legume? *Biol. Invasions* **2019**, *21*, 435–449. [\[CrossRef\]](#)
62. Perring, M.P.; Standish, R.J.; Price, J.N.; Craig, M.D.; Erickson, T.E.; Ruthrof, K.X.; Whiteley, A.S.; Valentine, L.E.; Hobbs, R.J. Advances in restoration ecology: Rising to the challenges of the coming decades. *Ecosphere* **2015**, *6*, 131. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.