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Hemiparasitic plant impacts animal and plant communities across four trophic levels

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Abstract. Understanding the impact of species on community structure is a fundamental question in ecology. There is a growing body of evidence that suggests that both subdominant species and parasites can have disproportionately large effects on other organisms. Here we report those impacts for a species that is both subdominant and parasitic, the hemiparasite *Rhinanthus minor*. While the impact of parasitic angiosperms on their hosts and, to a lesser degree, coexisting plant species, has been well characterized, much less is known about their effects on higher trophic levels. We experimentally manipulated field densities of the hemiparasite *Rhinanthus minor* in a species-rich grassland, comparing the plant and invertebrate communities in plots where it was removed, present at natural densities, or present at enhanced densities. Plots with natural and enhanced densities of *R. minor* had lower plant biomass than plots without the hemiparasite, but enhanced densities almost doubled the abundance of invertebrates within the plots across all trophic levels, with effects evident in herbivores, predators, and detritivores. The hemiparasite *R. minor*, despite being a subdominant and transient component within plant communities that it inhabits, has profound effects on four different trophic levels. These effects persist beyond the life of the hemiparasite, emphasizing its role as a keystone species in grassland communities.

Key words: abundance; diversity; grassland; hemiparasite; herbivore; indirect interaction; keystone species; *Rhinanthus minor*; species richness.

INTRODUCTION

Fundamental to the science of ecology is not only an understanding of the components of ecosystems, which define their structure, but also how structure varies over time and the mechanisms that drive such changes. The latter often involve complex indirect and nonlinear interactions between organisms at different trophic levels and the abiotic environment (Ripple et al. 2001, Bozec et al. 2013, Angelini and Silliman 2014, Zhao et al. 2014). In an endeavor to identify general principles that seek to explain structure–function relationships, top predators (Pace et al. 1999) and dominant plant species (Ellison et al. 2005, Hartley and Mitchell 2005) have commonly been ascribed key roles. However, there is also strong evidence to show that subordinate members of the community, including pathogens and parasites, can exert disproportionately large effects (e.g., Declerck et al. 2013, Fisher et al. 2013, Angelini and Silliman 2014) and, indeed, are thought to be significant factors in understanding, for example, invasibility, where their

absence allows exotic species to flourish (Young et al. 2010). Here, we report the results of a field study on an organism which is both subordinate and also parasitic, the root-hemiparasitic angiosperm *Rhinanthus minor*, and show that it has impacts on the abundance of plant and animal species across four trophic levels.

Hemiparasitic plants (widespread components of many ecosystems) are a distinctive functional group that are photosynthetic, but also gain water and nutrients from host plants via haustoria that penetrate host plant roots (Press and Phoenix 2005). The direct impacts that root-attaching hemiparasites exert on their host plants are often highly disproportionate to their size: the removal of water and nutrients reduces the photosynthetic rate, biomass, and competitive ability of their host (Marvier 1998, Jiang et al. 2003, Pywell et al. 2004, Press and Phoenix 2005, Cameron et al. 2008), and typically decreases the overall biomass of the infected community (Ameloot et al. 2005). Main host species are usually rapidly growing community dominants, and reductions in their performance caused by hemiparasites result in well-documented indirect effects on plant community structure (Pennings and Callaway 1992, Davies et al. 1997, Pywell et al. 2004), often enhancing plant species richness.

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The direct and indirect impacts that hemiparasites have on other trophic levels within the community are poorly understood, but are likely to be substantial given their impact on plant species composition, primary productivity, and the quality and nutrient content of vegetation (Quested et al. 2003, Bardgett et al. 2006, Ameloot et al. 2008, Watson 2009, Jiang et al. 2010, Fisher et al. 2013). These parameters are all key drivers of herbivore abundance and species composition (Siemann 1998, Langelotto and Denno 2004, Haddad et al. 2009), and further indirect impacts would be expected due to hemiparasite-induced changes in vegetation structure and hence the microclimate within the community (Morris 2000, Woodcock et al. 2009). Furthermore, as consumers of plant resources, hemiparasites potentially compete with invertebrate herbivores exploiting the same plant (Puustinen and Mutikainen 2001, Puustinen et al. 2001, Bass 2004, Bass et al. 2011). Despite this, herbivorous insects from three different guilds exhibited preference for consuming infected over uninfected plants, and in one case showed improved performance on parasitized plants (Ewald et al. 2011). At higher trophic levels, the indirect impacts of parasitic plants, via changes to plant and herbivore communities, may influence secondary consumer species. The high concentrations of foliar nutrients found in many parasites could make them attractive food plants in themselves (Press and Phoenix 2005, Watson 2009), and have a direct positive impact on herbivore abundance. The nutrient-rich litter of hemiparasites and their effects on nutrient cycling rates (Quested et al. 2003, Bardgett et al. 2006) suggest impacts on detritivores may also occur. Thus, we anticipated impacts of hemiparasite presence on animals from several trophic levels.

The annual root hemiparasite *Rhinanthus minor*, a xylem feeder, is a frequent component of grasslands across Europe and North America (Westbury 2004), and as one of the most widely studied hemiparasites is an excellent model species for examining the ecosystem impacts of hemiparasites. The suppression of host photosynthesis by *R. minor*, coupled with its inefficient use of parasitized resources, high transpiration rates (Jiang et al. 2003), and ability to achieve relatively high densities within a sward, means that its impact on host plants and plant community composition can be substantial. For example, because grasses are often favored hosts and preferentially parasitized, *R. minor* has been used to decrease grass dominance and so enhance the diversity of species-poor grasslands (Bullcock and Pywell 2004, Westbury and Dunnett 2008). *R. minor* has also been shown to impact on belowground processes in model grassland ecosystems, including the rate of nitrogen cycling (Bardgett et al. 2006). Such profound effects on producer communities would be expected to drive significant changes in consumer communities (Haddad et al. 2009), but little is known of the indirect impacts of *R. minor* on animals, and

effects on herbivore and predator communities have not yet been tested in natural grasslands (Bass et al. 2011, Ewald et al. 2011).

Using a field manipulation experiment, we tested the hypothesis that the presence of *R. minor* would impact on both plant and invertebrate communities in a species-rich grassland and that these impacts would be proportional to its density within the community. We manipulated *R. minor* density to levels both above and below the natural densities in the community and predicted specifically that (1) *R. minor* would alter the composition and biomass of the plant community and decrease the dominance of grasses, and so would have strong indirect effects on grass-feeding herbivores, and (2) indirect impacts of *R. minor* would be evident at other trophic levels.

MATERIALS AND METHODS

Experimental design

Four 1 × 1 m study plots were marked out in each of 13 replicate blocks distributed around the field site (Castle Hill National Nature Reserve, Sussex, UK; 50°50'49" N, 0°3'17" W) in September 2006, in an area of species-rich calcareous grassland on ex-arable land. Three of the four plots in each block were naturally infected with *R. minor* and the fourth plot had no natural *R. minor* colonization in the 2006 growing season (the absent treatment). The three infected plots were randomly assigned to one of three *R. minor* density treatments for the following season: removed (in which all emerging *R. minor* seedlings were manually removed), present (*R. minor* present at un-manipulated densities and allowed to seed and germinate naturally), and enhanced (*R. minor* density experimentally enhanced, though still within the range observed both in the wild and at this site). In a survey at the field site in July 2006, 25 randomly selected 1-m² plots of grassland were monitored for *R. minor* density: values ranged from 0 to 640 plants/m², with a mean value of 156 plants/m². The densities resulting from our experimental manipulations one year later (Table 1) were thus well within the natural range at this study site, with our enhanced treatment density only half the observed maximum density.

Enhanced density plots were sown in October and December 2006 with 2 g (i.e., ~700) *R. minor* seeds collected from the field site in September 2006. *R. minor* density and percent cover were recorded for each plot in June 2007. As this was a seminatural community with a population of *R. minor*, seedlings did occasionally emerge in the removed and absent treatment plots, but were uprooted as soon as they were identified. The use of both absent and removed treatments allowed us to account for the natural patchy distribution of the hemiparasite and ensured that any effects of our treatments could not be ascribed to fundamental a priori differences in community structure between locations where *R. minor* did and did not occur.

TABLE 1. *Rhinanthus minor* density, total plant cover, *R. minor* cover (as a percentage of total plant cover), plant species richness, diversity, and sward height in the four treatments (Castle Hill National Nature Reserve, Sussex, UK) in July 2007.

Treatment	<i>R. minor</i> density (plants/m ²)	Total plant cover (%)	<i>R. minor</i> cover (%)	Plant species richness	Plant diversity	Sward height (cm)
Absent	0	166.0 (6.29)	0	27.7 (1.72)	2.6 (0.06)	18.2 (2.73)
Removed	0	169.9 (8.02)	0	29.0 (0.81)	2.8 (0.04)	17.7 (2.47)
Present	152.9 (14.32)	151.1 (6.30)	8.4 (0.91)	30.1 (1.18)	2.8 (0.08)	12.8 (0.89)
Enhanced	311.0 (22.8)	146.2 (4.55)	14.3 (0.92)	28.8 (0.95)	2.9 (0.03)	12.9 (0.75)

Notes: Plant diversity was calculated as Shannon's *H* index. Means for the 13 plots are shown with SE in parentheses. Total plant cover was recorded as the sum of the cover of individual species (per quadrat), which, because of the over-layering of different species in this vegetation type, can have a value of greater than 100%.

Plant and invertebrate sampling

Plant and invertebrate communities were censused in all plots in May, July, and late August/early September 2007. The July sampling date was at the height of the growing season, coinciding with the maximum abundance of vegetation and invertebrates. For this reason, we focus here on the effects of the *R. minor* density treatment in July, although results from analyses of data collected in August/September are also presented. Within each 1-m² plot, rooted *R. minor* plants in the central 50 × 50 cm were counted at the end of April and June 2007. All other measurements on vegetation and invertebrates were within the central 80 × 80 cm of each plot to reduce any edge effects. The percent cover, defined as the proportion of the ground occupied by a perpendicular projection of the aerial parts of individuals of the species under consideration, was estimated by eye for each species in four 20 × 20 cm quadrats per plot. Total plant cover per quadrat was recorded as the sum of the cover of individual species, which because of the over-layering of different species in this vegetation type, can have a value of greater than 100%. Quadrats were randomly selected from a 4 × 4 grid with only one quadrat per row and column, and the mean cover from all four quadrats was used in the statistical analyses. Vegetation height was measured using a sward stick as described in Stewart et al. (2001). Plant species richness was expressed as the total number of species per plot and the diversity of the plant community was calculated as the Shannon index, *H* (Magurran 2004).

Invertebrate communities were sampled using a Vortis suction sampler (Brook et al. 2008), in warm dry weather (19–26°C) between 11:00 and 15:00, with all four treatment plots per block sampled within 10 min. Nine 10-s samples per plot were bulked for sorting. Due to the selectivity of the Vortis sampler, some invertebrate groups were assessed by direct hand counts (molluscs and lepidopteran larvae within an 80 × 80 cm quadrat and grasshoppers using a 50 × 50 × 50 cm cage placed over the plot). These additional counts were made on separate days to the Vortis sampling to allow plots and invertebrate populations to recover. Samples were frozen, and individuals sorted into orders with particular groups further sorted to lower taxa. Among the invertebrate groups sampled, a number could be assigned to particular trophic levels (herbivore, preda-

tor, and detritivore). Herbivores were the sap-feeding Hemiptera, Acrididae (grasshoppers), Lepidoptera larvae, and Curculionidae (weevils). Predators were Araneae and Opiliones (spiders and harvestmen) and Hymenoptera: Aculeata (wasps only) and Hymenoptera: Parasitica (parasitic wasps). Detritivores were Isopoda (woodlice) and Arthropleona (one order of springtails). A number of other invertebrate groups present in significant numbers within the community could not be assigned to particular trophic levels. These included groups that contained both herbivores and detritivores; Gastropoda (slugs and snails) and Neelipleona and Symphypleona (two orders of springtails), as well as Diptera (flies), Acari (mites), Staphylinidae (rove beetles), and Formicidae (ants).

Statistical analyses.—Results from the field experiments were analyzed using mixed-effects models in R v.2.14.1 (R Development Core Team 2011). In all analyses, treatment was fitted as the categorical explanatory variable and block was fitted as a random effect to control for variation in species abundance and diversity between blocks. Linear mixed models (LMM; nlme library; Pinheiro et al. 2015) were used for continuous response variables. In cases where residuals were non-normal (arachnid diversity and vegetation height), Box-Cox plots were used to select an appropriate power transformation. For response variables in the form of counts, generalized linear mixed models (GLMM; lme4 library; Bates et al. 2014) with Poisson errors were used. Where overdispersion occurred, this was accounted for by including individual-level random effects in the model (Maindonald and Braun 2010). In all cases, model simplification proceeded by backward deletion of nonsignificant terms until further removals led to significant ($P < 0.05$) increases in deviance, assessed using log-likelihood (*L*) tests for LMM and χ^2 values for GLMM. Post hoc multiple comparisons (glht function in multcomp library; Hothorn et al. 2008) were used to test for differences between treatments.

RESULTS

The percent cover of *R. minor* and the richness, diversity, and height of the plant community in each of the four density treatments in July 2007 are given in Table 1. As expected, manipulation of *R. minor* density had pronounced effects within the plant community.

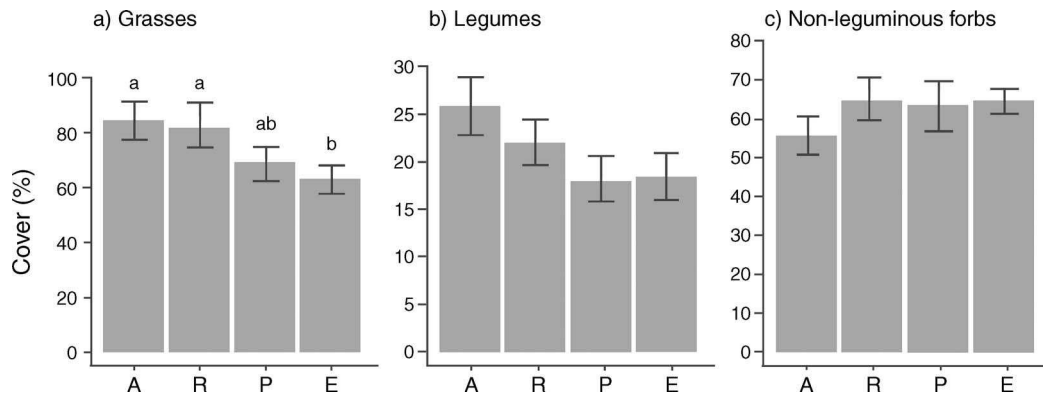


FIG. 1. Percent cover of (a) grasses, (b) legumes, and (c) non-leguminous forbs in each of the four density treatments at Castle Hill National Nature Reserve, Sussex, UK; *Rhinanthus minor* absent (A), removed (R), present (P), and enhanced (E), in July 2007 (shown as means \pm SE). Different lowercase letters denote significantly different ($P < 0.05$) results, based on post hoc multiple comparisons.

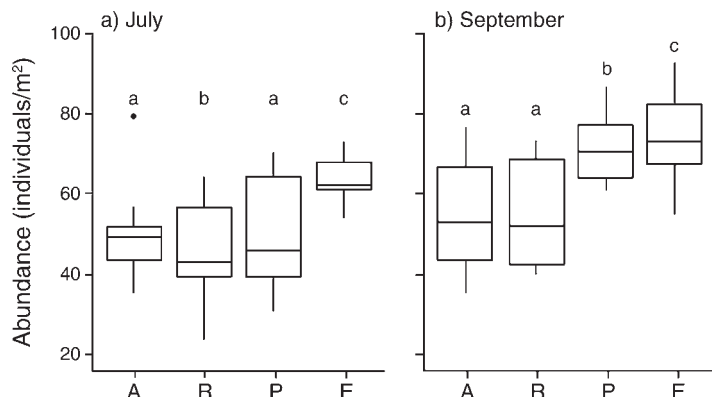
Grass cover varied significantly between treatments, with 16% and 24% lower cover in the *R. minor* present and enhanced treatments, respectively, compared with the removal treatment ($L_3 = 11.07$, $P = 0.01$; Fig. 1a). In contrast, the percent cover of forbs did not vary significantly between treatment (legumes, $L_3 = 5.85$, $P = 0.12$; non-leguminous forbs, $L_3 = 4.14$, $P = 0.25$; Fig. 1b, c). Across the community as a whole, plant diversity in July was higher in the enhanced treatment than in the removal treatment ($L_3 = 10.71$, $P = 0.01$), though this was not accompanied by a difference in overall species richness ($L_3 = 2.82$, $P = 0.42$). The physical structure of the community was also affected by *R. minor* presence, with a 27% lower sward height in the *R. minor* present treatment compared with the removal treatment ($L_3 = 9.77$, $P = 0.02$; Table 1).

The hemiparasite also had a large impact on the invertebrate community in July, with total invertebrate abundance increasing by 39% in the enhanced treatment compared with the removal treatment ($\chi^2_3 = 16.87$, $P = 0.0008$; Fig. 2a). Within this overall result, there were pronounced effects on a broad range of invertebrate groups spanning multiple trophic levels. Within the herbivores, the most common group was the sap-feeding

Hemiptera, which increased in abundance by 39% in the present treatment and 130% in the enhanced treatment compared with the removal treatment ($\chi^2_3 = 233.75$, $P < 0.0001$; Fig. 3a). Similar effects were observed for lepidopteran larvae and Curculionidae, which increased in abundance by 217% and 188%, respectively, in the enhanced treatments compared with the removal treatment (Lepidoptera, $\chi^2_3 = 25.75$, $P < 0.0001$; Fig. 3b; Curculionidae, $\chi^2_3 = 50.89$, $P < 0.0001$; Fig. 3c). In contrast, acridid abundance decreased in the presence of *R. minor*, with a reduction of 119% in the enhanced treatment compared with the removal treatment ($\chi^2_3 = 49.3$, $P < 0.0001$; Fig. 3d). This decline may reflect the decrease in the cover of grasses, their primary food plant group, as described previously.

Strikingly, *R. minor* also affected the abundance of predators within the community in July. Araneae and Opiliones increased in abundance by 142% in the enhanced treatment compared with the removal treatment ($\chi^2_3 = 101.78$, $P < 0.0001$; Fig. 3e). Similarly, numbers of Hymenoptera were 180% higher in the enhanced treatment than in the removal treatment ($\chi^2_3 = 96.93$, $P < 0.0001$; Fig. 3f).

FIG. 2. Total invertebrate abundance (individuals/m²) in (a) July and (b) September 2007 in each of the four *R. minor* density treatments (see Fig. 1). Horizontal lines represent median values, the top and bottom lines of each box represent the first and third quartiles, and whiskers represent approximately two standard deviations around the interquartile range (diamonds denote outliers). Different lowercase letters denote significantly different ($P < 0.05$) results, based on post hoc multiple comparisons.



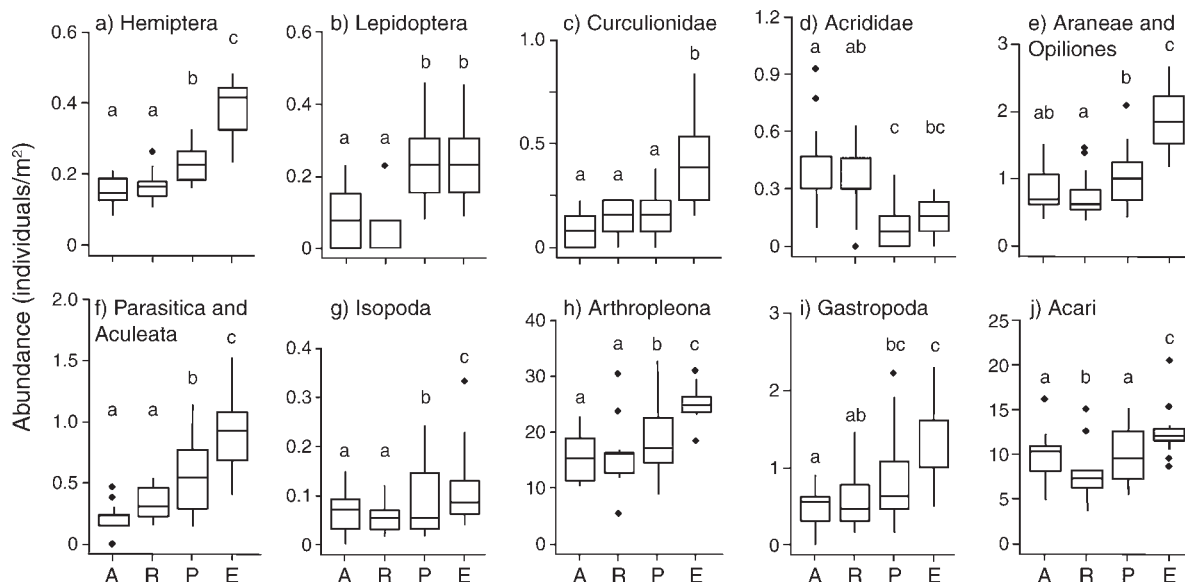


FIG. 3. Abundance of the major invertebrate groups (individuals/m²) in each of the four *R. minor* density treatments (see Fig. 1) in July 2007; invertebrates (a) Hemiptera, (b) Lepidoptera, (c) Curculionidae, (d) Acrididae, (e) Araneae and Opiliones, (f) Parasitica and Aculeata (wasps), (g) Isopoda, (h) Arthropleona, (i) Gastropoda, and (j) Acari. Horizontal lines represent median values, the top and bottom lines of each box represent the first and third quartiles, and whiskers represent approximately two standard deviations around the interquartile range (diamonds denote outliers). Different lowercase letters denote significantly different ($P < 0.05$) results, based on post hoc multiple comparisons.

The density of *R. minor* also had a major impact on the abundance of detritivore groups within the community in July. The abundance of Isopoda increased by 116% in the enhanced treatment compared with the removal treatment ($\chi^2_3 = 10.41$, $P = 0.02$; Fig. 3g). The abundance of Arthropleona showed a similar response to *R. minor* density, with an increase of 58% in the enhanced vs. removal treatment ($\chi^2_3 = 24.06$, $P < 0.0001$; Fig. 3h).

In addition to the pronounced effects on key herbivore, predator, and detritivore groups, the density of *R. minor* also affected other invertebrate groups feeding at multiple trophic levels. Numbers of gastropods and Acari increased by 103% and 57%, respectively, in the enhanced treatment compared to the removal treatment (gastropods, $\chi^2_3 = 40.13$, $P < 0.0001$; Fig. 3i; Acari, $\chi^2_3 = 15.68$, $P = 0.001$; Fig. 3j). The abundance of Formicidae was also found to vary significantly between treatments; however, these differences were strongly driven by a small number of high counts. When the data were reanalyzed with these outliers omitted, there was no significant effect of treatment on formicid abundance ($\chi^2_3 = 6.46$, $P = 0.09$; data not shown). Similarly, there was no significant effect of *R. minor* density on the abundance of Diptera ($\chi^2_3 = 4.59$, $P = 0.20$), Staphylinidae ($\chi^2_3 = 3.20$, $P = 0.36$), or Neelipleona and Symphyleona ($\chi^2_3 = 6.71$, $P = 0.08$; data not shown).

In addition to causing large changes in the abundance of many invertebrate groups, the density of *R. minor* also affected invertebrate species richness and diversity in July, with increases in the species richness of

Hemiptera ($\chi^2_3 = 17.79$, $P = 0.0005$; Fig. 4a) and family richness of Araneae and Opiliones ($\chi^2_3 = 109.0$, $P < 0.0001$; Fig. 4b), and in the species diversity of Hemiptera ($L_3 = 18.44$, $P < 0.0005$; Fig. 4c) in the enhanced treatment compared with the removal treatment.

Importantly, differences in plant cover and in the abundance of key invertebrate groups were still apparent in late August–early September 2007, when *R. minor* had senesced and was no longer a component of the community (Fig. 2b; Table 2). However, the effects of *R. minor* density on plant diversity and invertebrate richness and diversity observed in July were no longer apparent later in the season (data not shown).

DISCUSSION

We have demonstrated that the manipulation of a single subdominant plant species causes substantial changes in the abundance and diversity of organisms across four trophic levels in individual plots in a complex grassland community. The changes induced in the plant community were consistent with previous studies of the impact of *R. minor* (Gibson and Watkinson 1992, Davies et al. 1997, Pywell et al. 2004, Ameloot et al. 2005), particularly in the suppression of grasses. However, the striking enhancement of invertebrate abundance has not been recorded previously, certainly not across four trophic levels.

Hemiparasite effects on plants.—Changes in the plant community were generally consistent with previous studies in terms of the overall responses of grasses,

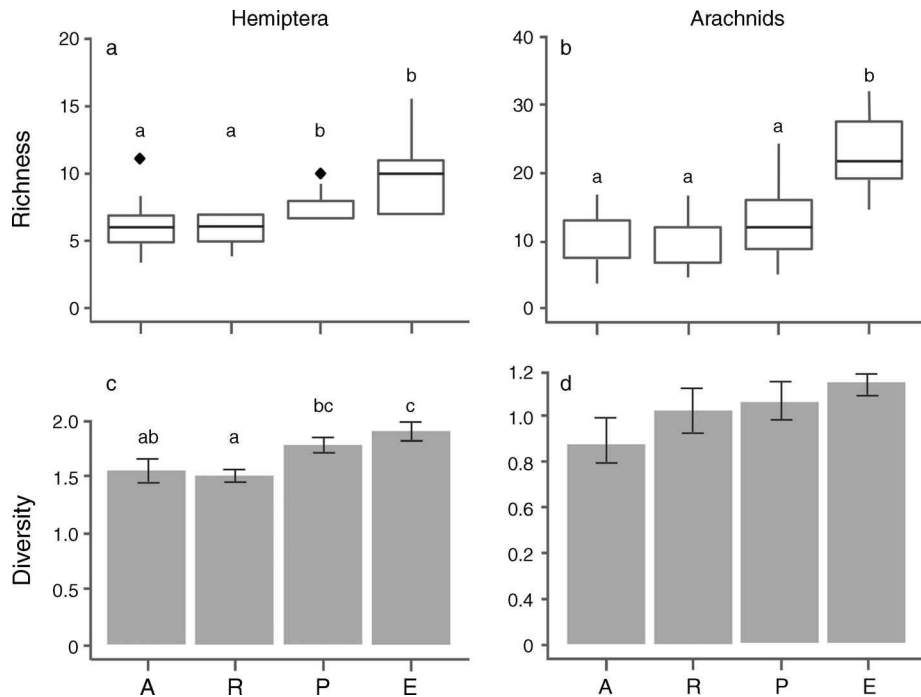


FIG. 4. Richness and diversity (untransformed) of (a, c) Hemiptera and (b, d) arachnids (Araneae and Opiliones) in each of the four *R. minor* density treatments (see Fig. 1) in July 2007. For (a, b) richness plots, horizontal lines represent median values, the top and bottom lines of each box represent the first and third quartiles, and whiskers represent approximately two standard deviations around the interquartile range (diamonds denote outliers). For (c, d) diversity plots, means are shown \pm SE. Hemiptera richness was calculated as the number of species and arachnid richness as the number of families. For both groups, diversity was calculated as Shannon's H index. Different lowercase letters denote significantly different ($P < 0.05$) results, based on post hoc multiple comparisons.

legumes, and non-leguminous forbs to the presence of *Rhinanthus*. However, there have been few other studies to show these changes on such a fine scale, within a single season, and in an ecosystem where *R. minor* is already present rather than newly introduced (but see Mudrák and Lepš 2010). Furthermore, our finding that the impact of *R. minor* on the plant community lasts until the end of the growing season, i.e., well beyond its senescence as a short-lived annual, is also significant (Joshi et al. 2000). In mimicking the natural movement of *R. minor* through a plant community (Cameron et al. 2009), our experiment has demonstrated the potential for short-term "legacy" effects on both the plant and animal communities. There were no significant impacts on plant species richness; we would not have expected species to be lost or gained over this time scale in an established perennial-dominated grassland (though plant diversity in July did differ significantly between enhanced and removal plots).

Hemiparasite effects on invertebrates.—We found strong positive effects of *R. minor* presence on the herbivore community even though few herbivores are known to feed on *R. minor* directly (Westbury 2004). The most abundant group sampled in our study was the Hemiptera, known to be highly selective feeders responding to changes in both plant quality (Prestidge

and McNeill 1983) and sward structure (Morris 2000, Hartley et al. 2003, Woodcock et al. 2007, 2009). In this context, the positive response of hemipteran abundance, species richness, and diversity to *R. minor* presence was unexpected, given that most species are specialist feeders on grasses, which collectively showed an overall decline in biomass. Instead, our evidence suggests that these effects of *R. minor* were indirect, although we cannot tell whether they operated through changes to plant community composition or plant quality, or through the effect that changes in sward height will have had on the abiotic environment. However, evidence from a recent study indicates that plant quality could be responsible. When given a choice in a greenhouse study, Hemiptera, Aphididae, and Orthoptera chose to feed on parasitized grass plants preferentially over unparasitized plants (Ewald et al. 2011), suggesting that parasitized grasses are more attractive to a wide range of herbivorous insects, irrespective of their feeding guild, since both sap-feeders and leaf-chewers exhibit this preference. This was despite reductions in grass nitrogen content caused by the hemiparasite when at peak biomass (Ewald et al. 2011).

Remarkably, hemiparasite presence also influenced both abundance and richness of predators. These increases could simply reflect a response to the increased

TABLE 2. Impact of *R. minor* density treatment on invertebrate and plant communities in August–September 2007.

Response	Statistic	<i>P</i>	Change (enhanced vs. removed)
Plant cover			
Grasses	$L_3 = 30.39$	<0.0001	–41.1%
Legumes	$L_3 = 2.36$	0.50	
Non-leguminous forbs	$L_3 = 16.26$	0.001	+24.7%
Total invertebrate abundance	$\chi_3^2 = 32.62$	<0.0001	+35.5%
Herbivore abundance			
Hemiptera	$\chi_3^2 = 59.65$	<0.0001	+39.3%
Lepidoptera	$\chi_3^2 = 14.21$	0.003	+112.5%
Curculionidae	$\chi_3^2 = 42.47$	<0.0001	+164.5%
Acrididae	$\chi_3^2 = 4.32$	0.23	
Predator abundance			
Araneae and Opiliones	$\chi_3^2 = 79.5$	<0.0001	+55.3%
Hymenoptera	$\chi_3^2 = 53.32$	<0.0001	+220.0%
Detritivore abundance			
Arthropleona	$\chi_3^2 = 44.39$	<0.0001	+68.4%
Isopoda	$\chi_3^2 = 3.32$	0.35	
Abundance of other invertebrate groups			
Mollusca	$\chi_3^2 = 4.82$	0.19	
Neelipleona and Symphypleona	$\chi_3^2 = 3.46$	0.33	
Diptera	$\chi_3^2 = 2.82$	0.42	
Acari	$\chi_3^2 = 17.84$	0.0005	+33.4%
Staphylinidae	$\chi_3^2 = 0.35$	0.95	
Formicidae	$\chi_3^2 = 1.49$	0.68	

Notes: For statistics, log-likelihood ratios (*L*) are provided for linear mixed-model analyses and χ^2 values are provided for generalized linear mixed-model analyses. Impacts were significant at $P < 0.05$. Cells left blank indicate no change.

numbers of prey, but may also reflect changes in sward structure, which are known to influence predator behavior and efficiency (Langellotto and Denno 2004, Woodcock et al. 2007, Woodcock and Pywell 2009). It is also possible that the reduction in sward height and total plant cover caused by *R. minor* improved the microclimate for invertebrates; a more open community may have increased light and higher temperatures, for example. We have some evidence for these effects in that our measures of photosynthetically active radiation at the soil surface demonstrated a 400% increase in *R. minor* enhanced plots compared with the absent plots, in both July (absent, $124.5 \pm 22.2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; enhanced, $514.1 \pm 69.3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and September (absent, $137.8 \pm 29.0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; enhanced, $513.8 \pm 84.9 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). There is a well-established inverse relationship between plant biomass and light intensity reaching the soil surface in grasslands (e.g., Veer and Kooijman 1997).

While we expected to see the diversity of the invertebrate community responding positively to enhanced diversity of the plant community caused by the hemiparasite, we did not expect to see such a striking increase in the overall abundance of invertebrates, given that total plant cover was reduced. Improved sampling efficiency in the shorter vegetation of infected field plots could not have contributed more than an insignificant

proportion of this increase (see Brook et al. 2008: Fig. 3). In natural grasslands, parasitic plants, including *R. minor*, have a large impact on nutrient cycling, and can enhance the nitrogen content of vegetation overall (Quested et al. 2003, Ameloot et al. 2008, Fisher et al. 2013). Their readily decomposed, nutrient-rich litter (Quested et al. 2003) may contribute to the increase in abundance of detritivores we observed in field plots infected with *R. minor*. *R. minor* presence also impacts the soil microbial community, increasing rates of litter decomposition and nitrogen mineralization (Bardgett et al. 2006), effects which might be expected to feed through to increases in plant quality and herbivore abundance, though the increases we observed seem too rapid for this mechanism. More likely, the changes in the relative cover of grasses and forbs may have had an impact on herbivore preference and behavior, as could the microclimate effects noted previously.

As in all plot-based experiments, there are also issues relating to the spatial scale of our treatments in relation to invertebrate dispersal ability. This problem is more acute when physical barriers are used to delineate experimental plots (Moise and Henry 2010), but it is the case that we cannot tell from this study whether invertebrates were attracted to directly parasitized plants or to unparasitized neighbors of those plants. We do not know whether the observed increases in invertebrates in

infected plots were due to intrinsic increases in population growth, although this seems unlikely given that the majority of the invertebrates in our study grassland were univoltine, and so had limited ability to increase numerically in response to the treatments over the time scale of our measurements, or were a result of the greater attractiveness of the vegetation compared to neighboring (less heavily infected) vegetation, and therefore reflect increased patchiness in invertebrate abundance without an overall increase in numbers. It is also possible that differences in survival of invertebrates between infected and control plots were an explanation for our findings.

Whatever the proximate causes of the elevated numbers of invertebrates observed where hemiparasites were present, it is clear that the invertebrates themselves will also be impacting on the plant community, possibly contributing to reduced plant biomass and alterations in the competitive interactions between plant species. In the experiments reported here, we did not control for the effect of invertebrates on the plant community, but clearly there is the possibility of synergistic effects, with any negative effects of the hemiparasite on plant biomass being amplified by the herbivores that are attracted to infected plants.

Overall, our findings demonstrate that parasitic plants can have dramatic and lasting impacts on abundance, richness, and diversity across multiple trophic levels within seminatural grassland communities. Although for a small number of herbivore species these facilitative effects may be direct, we suggest that the majority are indirect via changes in the plant community and the abiotic environment. The consequences of this large increase in invertebrate abundance for ecosystem structure and function are unknown, but are likely to be substantial (Watson 2009).

Our study provides a clear demonstration of the importance of indirect interactions as major structuring forces in ecology and the strong cascading effects of these interactions across trophic levels (Pace et al. 1999). Further, our results illustrate that keystone species such as *R. minor* can have both negative and positive impacts on other species; such facilitative effects mean their loss can cause reductions in diversity, secondary extinctions, or even community collapse (Ebenman and Jonsson 2005). The importance of keystone species has led some ecologists to suggest that they warrant special conservation efforts (Mills et al. 1993; but see Bruno et al. 2003). Conversely, the impacts they have on the structure of ecological communities may make them potential tools for the conservation of other species, as is the case for *R. minor* (Pywell et al. 2004, Westbury and Dunnett 2008). Conservation efforts with hemiparasites have focused on their benefits for plant communities thus far (e.g., Declerck et al. 2013), but our study shows that hemiparasites are a hitherto unexplored but potentially widespread and potent factor influencing invertebrate abundance.

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LITERATURE CITED

- Ameloot, E., K. Verheyen, and M. Hermy. 2005. Meta-analysis of standing crop production by *Rhinanthus* spp. and its effect on vegetation structure. *Folia Geobotanica* 40:289–310.
- Ameloot, E., G. Verlinden, P. Boeck, K. Verheyen, and M. Hermy. 2008. Impact of hemiparasitic *Rhinanthus angustifolius* and *R. minor* on nitrogen availability in grasslands. *Plant and Soil* 311:255–268.
- Angelini, C., and B. R. Silliman. 2014. Secondary foundation species as drivers of trophic and functional diversity: evidence from a tree–epiphyte system. *Ecology* 95:185–196.
- Bardgett, R. D., R. S. Smith, R. S. Shiel, S. Peacock, J. M. Simkin, H. Quirk, and P. J. Hobbs. 2006. Parasitic plants indirectly regulate below-ground properties in grassland communities. *Nature* 439:969–972.
- Bass, K. A. 2004. Resource partitioning in the multi-species interaction between a host plant, a parasitic angiosperm and an insect herbivore. Dissertation. University of Sussex, Brighton, UK.
- Bass, K. A., E. A. John, N. C. Ewald, and S. E. Hartley. 2011. Insect herbivore mortality is increased by competition with a hemiparasitic plant. *Functional Ecology* 6:1228–1233.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: linear mixed-effects models using Eigen and S4. R package version 1.1-7. <http://CRAN.R-project.org/package=lme4>
- Bozec, Y. M., L. Yakob, S. Bejarano, and P. J. Mumby. 2013. Reciprocal facilitation and non-linearity maintain habitat engineering on coral reefs. *Oikos* 122:428–440.
- Brook, A. J., B. A. Woodcock, M. Sinka, and A. J. Vanbergen. 2008. Experimental verification of suction sampler capture efficiency in grasslands of different vegetation height and structure. *Journal of Applied Ecology* 45:1357–1363.
- Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* 18:119–125.
- Bullock, J. M., and R. F. Pywell. 2004. *Rhinanthus*: a tool for restoring diverse grassland? *Folia Geobotanica* 40:273–288.
- Cameron, D. D., J. W. Geniez, L. J. Seel, and I. J. Irving. 2008. Suppression of host photosynthesis by the parasitic plant *Rhinanthus minor*. *Annals of Botany* 101:573–578.
- Cameron, D. D., A. White, and J. Antonovics. 2009. Parasite–grass–forb interactions and rock–paper–scissor dynamics: predicting the effects of the parasitic plant *Rhinanthus minor* on host plant communities. *Journal of Ecology* 97:1311–1319.
- Davies, D. M., J. D. Graves, C. O. Elias, and P. J. Williams. 1997. The impact of *Rhinanthus* spp. on sward productivity and composition: implications for the restoration of species-rich grasslands. *Biological Conservation* 82:87–93.
- Declerck, K., D. Bonte, and R. Van Diggelen. 2013. The hemiparasite *Pedicularis palustris*: ‘Ecosystem engineer’ for fen-meadow restoration. *Journal for Nature Conservation* 21:65–71.
- Ebenman, B., and T. Jonsson. 2005. Using community analysis to identify fragile systems and keystone species. *Trends in Ecology and Evolution* 20:568–575.
- Ellison, A. M., et al. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3: 479–486.
- Ewald, N. C., E. A. John, and S. E. Hartley. 2011. Responses of invertebrate herbivores to sharing a host plant with a hemiparasite: impacts on preference and performance differ with feeding guild. *Ecological Entomology* 36:596–604.

- Fisher, J. P., G. K. Phoenix, D. Z. Childs, M. C. Press, S. W. Smith, M. G. Pilkington, and D. D. Cameron. 2013. Parasitic plant litter input: a novel indirect mechanism influencing plant community structure. *New Phytologist* 198:222–231.
- Gibson, C. C., and A. R. Watkinson. 1992. The role of the hemiparasitic annual *Rhinanthus minor* in determining grassland community structure. *Oecologia* 89:62–68.
- Haddad, N. M., G. M. Crutsinger, K. Gross, J. Haarstad, J. M. H. Knops, and D. Tilman. 2009. Plant species loss decreases arthropod diversity and shifts trophic structure. *Ecology Letters* 12:1029–1039.
- Hartley, S. E., S. M. Gardner, and R. J. Mitchell. 2003. Indirect effects of grazing and nutrient addition on the Hemiptera community of heather moorlands. *Journal of Applied Ecology* 40:793–803.
- Hartley, S. E., and R. J. Mitchell. 2005. Manipulation of nutrient and grazing levels on heather moorlands: changes in *Calluna* dominance and consequences for community composition. *Journal of Ecology* 93:990–1004.
- Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. *Biometric Journal* 50:346–363.
- Jiang, F., W. D. Jeschke, and W. Hartung. 2003. Water flows in the parasitic association *Rhinanthus minor*/*Hordeum vulgare*. *Journal of Experimental Botany* 54:1985–1993.
- Jiang, F., W. D. Jeschke, W. Hartung, and D. D. Cameron. 2010. Interactions between *Rhinanthus minor* and its hosts: a review of water, mineral nutrient and hormone flows and exchanges in the hemiparasitic association. *Folia Geobotanica* 45:369–385.
- Joshi, J., D. Matthies, and B. Schmid. 2000. Root hemiparasites and plant diversity in experimental grassland communities. *Journal of Ecology* 88:634–644.
- Langelotto, G. A., and D. F. Denno. 2004. Responses of invertebrate natural enemies to complex structured habitats: a meta-analytical synthesis. *Oecologia* 139:1–10.
- Magurran, A. E. 2004. *Measuring biological diversity*. Blackwell Science, Oxford, UK.
- Maindonald, J., and W. J. Braun. 2010. *Data analysis and graphics using R*. Third edition. Cambridge University Press, New York, New York, USA.
- Marvier, M. A. 1998. Parasite impacts on host communities: plant parasitism in a California coastal prairie. *Ecology* 79:2616–2623.
- Mills, L. S., M. E. Soulé, and D. F. Doak. 1993. The keystone-species concept in ecology and conservation. *BioScience* 43:219–224.
- Moise, E. R. D., and H. A. L. Henry. 2010. Like moths to a street lamp: exaggerated animal densities in plot-level global change field experiments. *Oikos* 119:791–795.
- Morris, M. G. 2000. The effects of structure and its dynamics on the ecology and conservation of arthropods in British grasslands. *Biological Conservation* 95:129–142.
- Mudrák, O., and J. Lepš. 2010. Interactions of the hemiparasitic species *Rhinanthus minor* with its host plant community at two nutrient levels. *Folia Geobotanica* 45:407–424.
- Pace, M. L., J. J. Cole, S. R. Carpenter, and J. F. Kitchell. 1999. Trophic cascades revealed in diverse ecosystems. *Trends in Ecology and Evolution* 14:483–488.
- Pennings, S. C., and R. M. Callaway. 1992. Impact of a parasitic plant on the structure and dynamics of salt marsh vegetation. *Ecology* 77:1410–1419.
- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2015. nlme: linear and nonlinear mixed effects models. R package version 3.1-120. <http://CRAN.R-project.org/package=nlme>
- Press, M. C., and G. K. Phoenix. 2005. Impacts of parasitic plants on natural communities. *New Phytologist* 166:737–751.
- Prestidge, R. A., and S. McNeill. 1983. The role of nitrogen in the ecology of grassland Auchenorrhyncha. Pages 257–281 in J. A. Lee, S. McNeill, and I. H. Rorison, editors. *Nitrogen as an ecological factor*. Blackwell, Oxford, UK.
- Puustinen, S., O. Jarvinen, and K. Tiilikkala. 2001. Asymmetric competition between a hemiparasitic plant and a cyst nematode on a shared host plant. *Ecoscience* 8:51–57.
- Puustinen, S., and P. Mutikainen. 2001. Host–parasite–herbivore interactions: implications of host cyanogenesis. *Ecology* 82:2059–2071.
- Pywell, R. F., J. M. Bullock, K. J. Walker, S. J. Coulson, S. J. Gregory, and M. J. Stevenson. 2004. Facilitating grassland diversification using the hemiparasitic plant *Rhinanthus minor*. *Journal of Applied Ecology* 41:880–887.
- Qustedt, H. M., J. H. C. Cornelissen, M. C. Press, T. V. Callaghan, R. Aerts, F. Trosien, P. Riemann, D. Gwynn-Jones, A. Kondratchuk, and S. E. Jonasson. 2003. Decomposition of sub-arctic plants with differing nitrogen economies: a functional role for hemiparasites. *Ecology* 84:3209–3221.
- R Development Core Team. 2011. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>
- Ripple, W. J., E. J. Larsen, R. A. Renkin, and D. W. Smith. 2001. Trophic cascades among wolves, elk and aspen on Yellowstone National Park's northern range. *Biological Conservation* 102:227–234.
- Siemann, E. 1998. Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. *Ecology* 79:2057–2070.
- Stewart, K. E. J., N. A. D. Bourn, and J. A. Thomas. 2001. An evaluation of three quick methods commonly used to assess sward height in ecology. *Journal of Applied Ecology* 38:1148–1154.
- Veer, M. A. C., and A. M. Kooijman. 1997. Effects of grass encroachment on vegetation and soil in Dutch dry dune grasslands. *Plant and Soil* 192:119–128.
- Watson, D. M. 2009. Parasitic plants as facilitators: more Dryad than Dracula? *Journal of Ecology* 97:1151–1159.
- Westbury, D. B. 2004. Biological flora of the British Isles *Rhinanthus minor* L. *Journal of Ecology* 92:906–927.
- Westbury, D. B., and N. P. Dunnett. 2008. The promotion of grassland forb abundance: a chemical or biological solution? *Basic and Applied Ecology* 9:653–662.
- Woodcock, B. A., S. G. Potts, T. Tscheulin, E. Pilgrim, A. J. Ramsey, J. Harrison-Cripps, V. K. Brown, and J. R. Tallowin. 2009. Responses of invertebrate trophic level, feeding guild and body size to the management of improved grassland field margins. *Journal of Applied Ecology* 46:920–929.
- Woodcock, B. A., S. G. Potts, D. B. Westbury, A. J. Ramsey, M. Lambert, S. J. Harris, and V. K. Brown. 2007. The importance of sward architectural complexity in structuring predatory and phytophagous invertebrate assemblages. *Ecological Entomology* 32:302–311.
- Woodcock, B. A., and R. F. Pywell. 2009. Effects of vegetation structure and floristic diversity on detritivore, herbivore and predatory invertebrates within calcareous grasslands. *Biodiversity and Conservation* 19:81–95.
- Young, J. C., M. Van Kleunen, and W. Dawson. 2010. The role of enemy release, tolerance and resistance in plant invasions: linking damage to performance. *Ecology Letters* 13:937–946.
- Zhao, C., X. W. Wu, J. N. Griffin, X. Q. Xi, and S. C. Sun. 2014. Territorial ants depress plant growth through cascading non-trophic effects in an alpine meadow. *Oikos* 123:481–487.