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The effects of hemiparasitic plant removal on community structure and seedling establishment in semi-natural grasslands

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Nomenclature
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Abstract

Question: Hemiparasitic plants can profoundly affect the structure of the community in which they occur, mainly due to parasitic suppression of hosts. As a consequence, non-host species have the opportunity to colonize resulting gaps. In contrast to most grassland species, hemiparasites are generally short-lived and can reach high densities; as a consequence, vegetation gaps are left after their death. These gaps form microsites more suitable for seed germination and therefore might increase recruitment of other species. Which species suffer or profit from parasitism and is there a positive effect on seedling establishment?

Location: Semi-natural grasslands in northern Belgium.

Methods: We selected two hemiparasitic plant species from contrasting vegetation types: Rhinanthus angustifolius growing in mesotrophic grassland and Pedicularis sylvatica growing in oligotrophic heath-grassland. A weeding experiment was set up at six sites in which the hemiparasite was repeatedly removed in half of the plots during three growing seasons. The abundance change of individual species was compared between weeded and control plots. After the second growing season, seeds of up to ten species were added. The number of seedlings in the third year was then compared between weeded and control plots.

Results: Rhinanthus removal significantly affected the abundance of species relative to control plots, both positively and negatively, and decreased the species evenness. Pedicularis removal only increased the abundance of some species. Only Juncaceae (but no other graminoid families) increased after Rhinanthus and Pedicularis weeding, and there was considerable variation within functional groups. Moreover, based on our observations, we propose as a new hypothesis that species with persistent clonal spread are more vulnerable to parasitism. Once attached, resources are potentially drawn from a whole network of interconnected ramets. Finally, only half of the sown species successfully established seedlings; hemiparasite removal had a significantly negative effect on seedling number for two of these species.

Conclusions: Effects of hemiparasites on species differ considerably, also within functional groups. Persistent clonal spread emerges as an important plant trait determining vulnerability to hemiparasites. Finally, our results suggest that hemiparasitic plants might have a limited positive effect on seedling establishment in these semi-natural grasslands where chances for successful establishment were shown to be low.
Introduction

Hemiparasitic plants – carbon autotrophic plants that depend on host plants for water and mineral provisioning – can profoundly affect plant community structure and diversity (Press & Phoenix 2005). The net effect on the plant community results from both parasitism and litter pathways (Spasojevic & Suding 2011). Due to differences in host quality, competitive relationships in the plant community may change (Gibson & Watkinson 1989, 1991; Musselman & Press 1995; Matthies 1996; Cameron 2004). In addition, the input of often nutrient-rich and rapidly decomposing hemiparasite litter (Seel & Press 1993; Pate 1995; Quested et al. 2002) potentially increases nutrient cycling and availability (e.g. Bardgett et al. 2006; Ameloot et al. 2008; Fisher et al. 2013; Demey et al. 2014), and thus may stimulate primary production of both host and non-host species, depending on how nutrients are re-distributed between species (Press 1998).

To study possible effects of hemiparasitic plants on community structure, pot experiments have been conducted to examine parasite-host interactions between hemiparasitic plants and a selection of hosts (e.g. a legume, a grass and a forb). For the well-studied *Rhinanthus minor*, host quality is reported to decrease from legumes over grasses to non-leguminous forbs (e.g. Gibson & Watkinson 1991; Cameron et al. 2006), and was shown to be determined by differential resistance of the host species rather than host selectivity (Cameron et al. 2006). For *Pedicularis* spp., in contrast, host quality appeared to be the opposite (forb > grass > legume; Hedberg et al. 2005; Ren et al. 2010). However, only two or three host species were included in both studies. Both observational and experimental field studies using artificial species assemblages (Joshi et al. 2000) or (semi-)natural plant communities (Ameloot et al. 2005; Press & Phoenix 2005) found that *Rhinanthus* suppressed total, grass and legumes biomass. In contrast, effects on non-leguminous forbs were variable (Davies et al. 1997; Ameloot et al. 2006a,b; Mudrak & Leps 2010).

While pot experiments allow the inclusion of few species and lack the representativeness to infer in situ effects on species composition, field studies generally do not consider the effects of hemiparasitic plants on individual species, and focus on functional groups instead (mostly graminoids, legumes and other forbs; but see Mudrak & Leps 2010). Since hemiparasitic plants have been reported to differently affect members within groups such as grasses and forbs (Gibson & Watkinson 1991; Cameron et al. 2006; Mudrak & Leps 2010), more species-level field studies are needed to better understand and predict possible effects of hemiparasitic plants on community composition.

Hemiparasitic plants can also influence the community composition by increasing establishment opportunities for co-occurring and colonizing species. Gaps appear in the vegetation after the death of the hemiparasite. As a result, suitable microsites (e.g. more light, bare ground) for germination and seedling establishment are created (Joshi et al. 2000; Ameloot et al. 2006b). This expectation can be tested with a seed addition experiment. Reviews on seed addition studies showed that seed limitation – the availability of seeds – is very common in (semi-)natural vegetation (Turnbull et al. 2000). Yet, establishment limitation – the availability of suitable microsites – is the real seed-to-seedling bottleneck for many species, and above-ground disturbance (e.g. trampling, mowing) generally has a significantly positive effect on seedling emergence (Clark et al. 2007). Limited knowledge on the effects of the presence of a hemiparasite on seedling establishment of sown species is available. For instance, Pywell et al. (2004) found that the frequency of six out of ten sown forb species was positively correlated with *R. minor* abundance in the previous year.

Here, we studied how hemiparasites influence other species in the community depending on ‘life stage’. The central question is: how do hemiparasites influence species in the established phase (part I) and species in the establishment phase (part II)? We set up a removal experiment in which contrasting hemiparasites, *i.e. Rhinanthus angustifolius* C.C. Gmel. and *Pedicularis sylvatica* L. (hereafter referred to as *Rhinanthus* and *Pedicularis*) were either repeatedly weeded or not during 3 yr. In the first part, we aimed to identify species that are suppressed by parasitism and, on the other hand, which non-host species are favoured by parasitic suppression of host species. Therefore, abundance of individual species was assessed. We expected to find considerable variation in species’ response to hemiparasite weeding, not clearly linked to functional groups. In the second part, we determined the number of established seedlings after seed addition of up to ten species in both parasitized and weeded plots. We expected to find more seedlings in parasitized plots compared to control plots as a result of decreased establishment limitation.

Methods

Study systems

Two grassland hemiparasitic plant species differing in life-history traits and growing in contrasting environments were selected for this study: (1) the annual *Rhinanthus angustifolius* C.C. Gmel. (Orobanchaceae), which typically grows in mesotrophic grasslands belonging to the *Molinio-Arrhenatheretea* class (sensu Zuidhoff et al. 1996); and (2) the biennial *Pedicularis sylvatica* L. (Orobanchaceae), which thrives in relatively oligotrophic heath-grasslands belong-
ing to the Nardetea class (sensu Swertz et al. 1996). Within the study area, Rhinanthus flowers in June, turning fields yellow if abundant. Pedicularis seedlings start to grow in May and produce only leaves the first year, which die off in winter. In the second year, the plant re-sprouts from the taproot and produces several lying branches. Pedicularis produces conspicuous purple flowers from late May, and plants die off following seed maturity in July. Unlike Rhinanthus, Pedicularis plants are generally distributed in patches, so we restricted the studied communities to those patches. For both hemiparasites, three sites were chosen in nature reserves in northern Belgium, within both species’ native ranges. For Rhinanthus, these sites were: Achter Schoonhoven (R1, 50°58’58" N 4°51’39" E), Doode Bemde (R2, 50°48’57" N 4°38’54" E) and Papendel (R3, 51°0’33" N 4°46’31" E); and for Pedicularis, Hooiput (P1, 51°18’53" N 5°7’34" E), Langdonken (P2, 51°1’30" N 4°52’5" E) and Walenbos (P3, 50°55’48" N 4°53’14" E). The long-term (1961–1990) mean annual temperature is 9.5 °C and annual precipitation amounts to 821 mm for these sites (Royal Meteorological Institute of Belgium). The soils at Rhinanthus sites have a silt loam (R1), silt (R2) or sandy loam (R3) texture and an average pH-H₂O of 5.2 ± 0.1; the soils at Pedicularis sites are more acidic (4.6 ± 0.2) and more sandy with a loamy sand (P1, P2) or sandy clay loam (P3) texture. Rhinanthus sites have a relatively high local species richness (on average 18 ± 1 species 0.25 m⁻²) and are characterized by a herbaceous vegetation of perennial graminoids (e.g. Agrostis capillaris, Anthoxanthum odoratum, Festuca rubra, Juncus acutiflorus, Luzula multiflora, Scirpus sylvaticus), legurnes (e.g. Lathyrus pratensis, Lotus uliginosus, Trifolium pratense) and many non-leguminous forbs (e.g. Ajuga reptans, Centaurea jacea, Dactylorhiza maculata, Leucanthemum vulgare, Lychmis flos-cuculi, Plantago lanceolata, Prunella vulgaris, Ranunculus acris, Succisa pratensis). Pedicularis sites contain fewer species (on average 12 ± 1 0.25 m⁻²) and have a greater share of (dwarf) shrubs such as Calluna vulgaris, Erica tetralix and Salix aurita; grasses include Agrostis canina, Carex pilulifera, Danthonia decumbens, Juncus acutiflorus, Luzula multiflora and Molinia caerulea. The most abundant forb species are Polygala serpyllifolia and Potentilla erecta.

Hemiparasite removal experiment

At all sites, three blocks of ca. 2 m × 2 m were randomly selected within a 20 m × 20 m area in April 2009, i.e. when most Rhinanthus and Pedicularis individuals have emerged. Within each block, four plots of 0.5 m × 0.5 m were chosen through stratified random selection: only patches with a minimum of 20% hemiparasite cover and a similar species composition across the blocks could be included. The rather small plot size is justifiable because we evaluate within-plot abundance ratios between treatments using temporally paired plots, rather than comparing treatment effects at one moment in time. Between 26 May 2009 and 2 June 2009, both hemiparasites were weeded by hand in two randomly chosen plots among the four plots in each block, leaving the other two as untreated controls: the number of plants removed was on average 444 ± 38 m⁻² for Rhinanthus and 184 ± 13 m⁻² for Pedicularis. Additionally, a buffer zone of 10 cm surrounding the plot was kept free of hemiparasites to avoid edge effects. Weeding was repeated between 16 and 23 June 2009); thereafter a monthly check was done and weeding was repeated whenever necessary between April and October until 2011.

In 2009, 2010 and 2011, we estimated species cover in all plots (after removal of hemiparasites from the weeding treatments). In 2009 and 2011, directly after species cover estimations, the above-ground biomass of all species was clipped to 2 cm above the soil surface between mid-July and early September, i.e. mimicking the mowing regime of these grasslands. Samples were identified to the species level and sorted (Poaceae, Cyperaceae and Juncaceae were kept as groups) before they were oven-dried (48 h at 70 °C) and weighed. Rhinanthus sites R1 and R2 were additionally mown in September to correspond with the management regime; samples were oven-dried and weighed but not sorted. For the population P1, data on above-ground biomass were unavailable for 2009 and were therefore estimated from cover estimates in 2009 based on regressions between cover estimates and above-ground biomass at the same site in 2011 (R² > 0.4).

Sowing experiment

In November 2010, i.e. the weeding treatment had been applied for nearly two growing seasons creating variation in the community structure, a sowing treatment was applied to one control and one weeded plot within each block. Hence, we created a full factorial design. Sown species were selected from representative species of these communities (Swertz et al. 1996; Zuidhoff et al. 1996; App. S1). For Rhinanthus sites, we selected Lotus uliginosus, Ranunculus acris, Lychmis flos-cuculi, Succisa pratensis, Heracleum spondylium, Bellis perennis, Lythrum salicaria, Lychnis europaea, Eupatorium cannabinum and Valeriana repens. For Pedicularis sites, we selected Lotus uliginosus, Potentilla erecta, Erica tetralix, Succisa pratensis, Achillea millefolium, Hieracium umbellatum, Hieracium pilosella, Plantago lanceolata and Hypochaeris radicata. For species already present at the sites, seeds were collected in the field. On the rare occasion that adult plants of the same species produced seeds in the control plots, we removed them to avoid noise in our data. Seeds from other species – not present or insufficiently

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Hemiparasitic plants affect community composition

A. Demey et al.

abundant for seed collection – were acquired from Ecoflora, a nursery providing seeds acquired from regional populations (http://www.ecoflora.be). Seedlings of the sown species were counted in all seed addition plots during the summer and autumn of 2011. The maximum number of seedlings of both surveys was used for further analysis to represent a maximal seedling establishment estimate. *Hietericum* spp. seedlings were identified to the genus level.

At the same time seeds were added in the field, a germination trial in open ground was performed at the lab (outside) to test viability of the seeds under field-realistic conditions. Seeds were added (50 seeds, three replicates) to 5-cm deep layers of compost in plastic trays that were dug in the ground. Different composts were used for the *Rhinanthus* and *Pedicularis* seed mixtures so that the pH was similar to the soil pH at *Rhinanthus* sites (5.2) and *Pedicularis* sites (4.6). As none of the collected seeds of *Erica tetralix* germinated in open ground, seedlings observed in field plots were considered to result from established individuals and therefore excluded from the analyses.

**Data analysis**

We used R 2.12.1 (R Foundation for Statistical Computing, Vienna, AT) for data analysis. Data for *Rhinanthus* and *Pedicularis* sites were analysed separately. To visualize the divergence between weeded (hemiparasites removed) and control plots between the start (2009) and the end (2011) of our experiment, we performed a distance-based redundancy analysis (dbRDA) on the biomass data (excluding the hemiparasites) using the vegan package in R. A factor variable with levels ‘2009 control’, ‘2009 weeded’, ‘2011 control’ and ‘2011 weeded’ was used as explanatory variable, and ‘block’ was used as covariable. Effects of hemiparasite removal on the vegetation were evaluated by the difference in year-to-year change between control and weeded plots, thereby using 2009 as reference year. This eliminates a lot of noise from our data due to initial differences between treatments and the rather small plot size.

The effect of hemiparasite removal on plant community diversity (species richness, Shannon diversity, species evenness, excluding the hemiparasite) was tested as the weeding × year interaction in a mixed effects model (*lme4* package) ~ weeding * year + (1|plot) + (−1 + year|location), with a random intercept for plot (36 levels) to account for the repeated measurements within a plot, and a random year effect for location (three levels) to account for the spatial correlation of measurements. The difference in hemiparasite abundance between the years in control plots was tested using Tukey’s honestly significant difference post-hoc test after fitting the model ~ year + (1|plot), with a random intercept for plot to account for the repeated estimations within the same plots. We analysed the effect of hemiparasite weeding on individual species’ biomass response ratio using the model ln(mass20XX/mass2009) ~ weeding + (1|block), in which massx is the above-ground biomass of an individual species in year x, and including random intercepts for block (nine levels, three at each location). The random effect was added because biomass measurements within the same block are considered non-independent replicates. Using proportional rather than absolute abundance changes allows for evaluation of effects between rare and abundant species. The log-transformation was necessary to retain a normal distribution. The mean weeding effect and 95% confidence intervals per species was calculated using Markov Chain Monte Carlo (MCMC) methods in the language R package (v 1.2, http://cran.r-project.org/web/packages/languageR/index.html), excluding species with frequencies <25% (nine out of 36 plots). Because we measured above-ground biomass for graminoids only at the family level (*Poaceae, Cyperaceae* and *Juncaceae*), cover data were used in a separate analysis to evaluate weeding effects on individual graminoid species. As the hemiparasite abundance in 2011 was low (Fig. 1), we could not detect weeding effects on 2011:2009

Fig. 1. Hemiparasite abundance in control plots and the effect of hemiparasite removal on total biomass at *Rhinanthus* sites (R1, R2, R3) and *Pedicularis* sites (P1, P2, P3): average above-ground biomass (±SE) of the hemiparasite in non-weeded plots (n = 6 per site) in 2009 [III], 2010 [II] and 2011 [I] (left y-axis), and the mean effect (±SE) of hemiparasite weeding on the above-ground biomass ratio, ln(biomass2009/biomass2010), of the potential host community (total minus hemiparasite; points and lines, right y-axis). For P1 (no data for 2009), the ln(biomass2011/biomass2010) ratio is used.
cover ratios; therefore 2010 cover data were used instead by substituting the dependent variable in the model above with ln(cover2010/cover2009). Spearman correlations ($r_s$) between weeding effects from cover- and biomass-based models in 2011 ($r_s = 0.45$) and between weeding effects from 2010 and 2011 cover-based models ($r_s = 0.74$) justified this approach. We also tested the effect of hemiparasite removal on the group of species with persistent clonal spread, and the group of species not clonally spreading or with non-persistent clonal growth organs (see ‘clonal integration hypothesis’ in the Discussion). Species with persistent clonal spread were defined as species forming long-lived clonal growth organs (>2 yr) that spread faster than 1 cm·yr$^{-1}$. We derived this information from the CLOPLA3 database (http://clopla.butbn.cas.cz, 6 Aug 2014; Klimesová & de Bello 2009; App. S2). To account for the fact that records from the same species are not independent, a random intercept for species was added to the model: ln(mass$_{2011}$/mass$_{2009}$) ~ weeding + (1|block) + (1|species). Biomass of graminoids was assigned to the individual species proportional to cover estimates. Finally, we tested the weeding × clonal type interaction in a model on all species: ln(mass$_{2011}$/mass$_{2009}$) ~ weeding * clonal-type + (1|block) + (1|species) to test the difference between weeding effects on clonal types.

Similar to effects on species abundances, we analysed the effect of hemiparasite weeding on the number of germinated seeds of individual species using the model $N_{seedlings} ~ weeding + (1|block)$, in which $N_{seedlings}$ is the number of seedlings observed in 2011, weeding the independent fixed factor with levels ‘control’ and ‘weeded’, and including random intercepts for block. A Poisson distribution was used for this count data. We checked for overdispersion, a common issue when modelling ecological count data using a Poisson distribution. Since overdispersion was too high for Pedicularis sites, we changed to a nested random structure ($llocation$|block), which solved this. Model fits were checked by graphical inspection for normality and homogeneity of residuals.

**Results**

**Hemiparasite abundance and overall community effects**

Hemiparasite above-ground biomass showed considerable inter-annual variation (Fig 1). Post-hoc comparison using Tukey’s HSD test revealed that Rhinanthus above-ground biomass in control plots was highest in 2009 (16.5 ± 1.9 g m$^{-2}$), declined by 34% in 2010 ($Z = 2.5$, $P < 0.005$) and 71% in 2011 ($Z = 5.4$, $P < 0.001$) relative to 2009. The concurrent effect of Rhinanthus removal on the total above-ground mass response ratio $\ln(\text{mass}_{\text{year}}/\text{mass}_{\text{2009}})$ was significant in 2010 ($+0.24$, $+27%$; $\chi^2 = 13.1$, $P < 0.001$), but not in 2011 ($+0.03$, $+3%$; $\chi^2 = 0.2$, $P = 0.7$).

Post-hoc comparison using Tukey’s HSD test revealed that Pedicularis above-ground biomass in control plots was highest in 2009 (16.5 ± 1.9 g m$^{-2}$), declined by 34% in 2010 ($Z = 2.5$, $P = 0.03$) and 71% in 2011 ($Z = 5.4$, $P < 0.001$) relative to 2009. The concurrent effect of Pedicularis removal on the total above-ground mass response ratio $\ln(\text{mass}_{\text{year}}/\text{mass}_{\text{2009}})$ was insignificant in both 2010 ($+0.13$, $+14%$; $\chi^2 = 0.9$, $P = 0.4$) and 2011 ($+0.15$, $+16%$; $\chi^2 = 1.1$, $P = 0.3$).

The change in plant community composition between 2009 and 2011 differs between parasitized controls and weeded plots, as shown by the divergence of treatments in the ordination diagram (Fig. 2). This effect is most obvious for Rhinanthus sites, where this change is associated with a decrease in Shannon diversity ($-0.10$, $F = 5.4$, $P = 0.03$) and species evenness ($-0.04$, $F = 9.39$, $P = 0.005$) in weeded plots relative to parasitized controls between 2009 and 2010 (App. S3). Between 2009 and 2011, there was no significant effect on diversity indices. Weeding of Pedicularis did not change diversity of the remaining community.

**Effects on species abundances**

The effects of Rhinanthus removal on individual species’ above-ground mass response ratios $\ln(\text{mass}_{2011}/\text{mass}_{2009})$ ranged from $-1.08$ to $+1.36$, indicating that the 2011:2009 biomass ratio in weeded plots was between 34% and 390% of that in control plots (Fig. 3a). Weeding had a positive effect on the performance of Achillea ptarmica ($+1.36$, $+290%$; $P_{MCMC} = 0.04$) and a marginally significant effect on Juncaceae ($+0.31$, $+36%$; $P_{MCMC} = 0.06$) relative to unweeded plots, and reduced the performance of Rumex acetosa ($-0.98$, $-62%$; $P_{MCMC} = 0.04$) and Dactylorhiza maculata ($-0.59$, $-45%$; $P_{MCMC} = 0.04$). Unexpectedly, weeding did not increase the performance of Poaceae as a group ($-0.12$, $-11%$; $P_{MCMC} = 0.43$), or that of the legumes Lotus uliginosus ($+0.05$, $+5%$; $P_{MCMC} = 0.89$) and Vicia cracca ($-0.61$, $-46%$; $P_{MCMC} = 0.33$). When using cover response ratios $\ln(\text{cover}_{2011}/\text{cover}_{2009})$ for individual graminoid species, weeding had a positive effect on the performance of Agrostis capillaris ($+0.70$, $+101%$; $P_{MCMC} = 0.005$) and Juncus acutiflorus ($+0.41$, $+51%$; $P_{MCMC} = 0.001$) relative to unweeded plots. For other graminoids (Anthoxanthum odoratum, Holcus lanatus, Carex spp., Luzula multiflora and Scirpus sylvaticus), weeding had no effect.

The effect of Pedicularis removal on individual species’ above-ground mass response ratios ranged from $-0.24$ to $+1.73$, meaning that the above-ground mass ratio in weeded plots was between 79% and 564% of that in control plots (Fig. 3b). Weeding had a positive effect on the performance of Erica tetralix ($+1.73$, $464%$;
Hemiparasitic plants affect community composition

A. Demey et al.

Effects on seed germination

Four out of ten species (Valeriana repens, Eupatorium cannabinum, Lycopeus europaeus and Lythrum salicaria) did not germinate at all at Rhinanthus sites, and another species (Bellis perennis) germinated only in one of 18 plots. The remaining five species germinated in at least ten of 18 plots. The effect of Rhinanthus removal on the number of seedlings in the year after seed addition was negative for Lychnis flos-cuculi (−0.4; $P_{\text{MCMC}} = 0.01$) and marginally significant, for Succisa pratensis (−0.52; $P_{\text{MCMC}} = 0.09$), while weeding had no effect on the establishment of Lotus uliginosus, Ranunculus acris and Heracleum sphondyllum (Fig. 4a). Weeding had a significant effect on the total number of seedlings (−0.15; $P_{\text{MCMC}} = 0.04$).

At Pedicularis sites, two out of the seven considered species (Achillea millefolium and Plantago lanceolata) did not germinate in the field, while Lotus uliginosus germinated only in three out of 18 plots. The remaining species germinated in at least seven of 18 plots. The effect of Pedicularis removal on the number of seedlings in the year after seed addition was negative for Hieracium spp. (−1.31; $P_{\text{MCMC}} = 0.01$) and Succisa pratensis (−0.78; $P_{\text{MCMC}} = 0.01$), while weeding had no effect on Hypochaeris radicata and Potentilla erecta (Fig. 4b). There was no effect on total number of seedlings (−0.03; $P_{\text{MCMC}} = 0.71$).

Discussion

Winners and losers

At Rhinanthus sites, we identified both winner and loser species as a result of hemiparasite weeding. At Pedicularis sites we also identified winner species, but none of the other species declined in biomass following hemiparasite removal. Species that increase their above-ground biomass after hemiparasite removal were most likely severely parasitized before: resource allocation to the shoot is decreased by hemiparasitic infection (Graves 1995), and is expected to recover after removal of the hemiparasite. Species that show no or negative effects are most likely unfavourable hosts. At Rhinanthus sites, where the total above-ground biomass is relatively high (362 ± 72 g·m$^{-2}$ in control plots in 2011), loser species might be outcompeted by one or more winner species due to competition for light. Released from parasitic infection, the winner species may allocate relatively more resources to their shoots, which is a key factor determining light competition (Pan et al. 2011). The concurrent decrease in species evenness (App. S3) indicates that it is indeed the more dominant species that increase (and the rare species that decrease) after hemiparasite removal. The more open vegetation structure and low total above-ground biomass at Pedicularis sites (176 ± 22 g·m$^{-2}$ in control plots in 2011), in contrast,
Fig. 3. The effects of hemiparasite weeding (±95% confidence bounds) on species’ proportional biomass change, ln(biomass$_{2011}$/biomass$_{2009}$), occurring in Rhinanthus sites (a) and Pedicularis sites (b). Positive effects indicate that weeding increased the 2011:2009 biomass ratio, and vice versa. Only species that were present in 25% of the plots in both years are shown. Significance levels: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, (*) $P < 0.1$. Species were present at 1—, 2— or 3— of 3 locations. For the species-specific analysis on graminoids (greyed-out area), the ln(cover$_{2010}$/cover$_{2009}$) ratio was used. Species with persistent clonal spread form long-lived (>2 yr) clonal growth organs that spread at least 1 cm·yr$^{-1}$. See Appendix S2 for more detail.
suggests that competition for light is probably less important there. In combination with the insignificant increase in above-ground biomass following Pedicularis removal, this could explain why none of the species were negatively affected by the weeding treatment. Hautier et al. (2009) support this idea by showing that a fertilizer-induced biomass increase in herbaceous vegetation invoked biodiversity loss, unless light limitation was removed with lamps under the canopy. In a weeding experiment with Rhinanthus minor, Mudrak & Leps (2010) found Ranunculus repens was associated with plots lacking Rhinanthus, while R. acris was intermediate between weeded and control plots. The ranking of the effects of Rhinanthus weeding on both Ranunculus species (Fig. 3a) in the present study showed the same trend, although weeding effects were not significant. The family Orchidaceae was reported to be strictly avoided by Rhinanthoidae (Weber 1976). Therefore, removal of Rhinanthus is not likely to favour orchids. Our results were even more dramatic and showed a significant decrease in abundance of the only orchid, Dactylorhiza maculata, after weeding of Rhinanthus. This suggests that this species is particularly sensitive to competition for light, which was likely increased following the removal of Rhinanthus.

Variable effects within graminoids

Juncaceae clearly won after hemiparasite removal in both studied vegetation types. Poaceae and Cyperaceae, on the other hand, showed no significant effects as a group. However, at the species level, there was considerable variation in the cover of these taxonomic groups. Within Juncaceae, Juncus acutiflorus – but not Luzula multiflora – showed a significant response to hemiparasite removal in both vegetation types. Within Poaceae at Pedicularis sites, Agrostis canina – although only marginally significant – responded positively to weeding, while Molinia caerulea did not. Within Poaceae at Rhinanthus sites, Agrostis capillaris is positively affected by weeding, while Anthoxanthum odoratum and Holcus lanatus are not. In another removal experiment, with Rhinanthus minor (Mudrak & Leps 2010), Agrostis capillaris was associated with Rhinanthus removal, while Holcus mollis was associated with parasitized plots. Similarly, Hautier et al. (2010) showed that Rhinanthus alectorolophus performed less well when grown with A. odoratum and H. lanatus than expected based on the absolute growth rates of these grasses. In the same study, R. alectorolophus growing with A. capillaris performed – as with most other studied grasses – as expected from its absolute growth rate. Hautier et al. (2010) suggested that grasses might differ in their resistance to parasitism. Our results corroborate these findings. Moreover, we agree with the suggestion postulated by Mudrak & Leps (2010) that the net effect on diversity depends on the relative sensitivity of individual dominants, co-dominants and subordinate species in the community, and on the ability of resistant or tolerant species to take advantage when the sensitive dominants are suppressed.

Defence mechanisms

Species differ in their ability and strategies to prevent the formation of successful parasite–host connections (haustoria). Species showing a strong defensive response such as Plantago lanceolata (P. lanceolata cells surrounding invading
We found that species with persistent clonal spread increased in abundance after removal of hemiparasites relative to species without persistent clonal spread (Fig. 3). *Rhinanthus* removal had a negative effect on species without persistent clonal spread ($-0.31$, $-27\%$; $P_{MCMC} = 0.03$), while the effect on species with persistent clonal spread was not significant ($+0.16$, $+17\%$; $P_{MCMC} = 0.23$). There was a significant difference in weeding effect between species with and without persistent clonal spread ($P_{MCMC} = 0.02$). *Pedicularis* removal had a positive effect on species with persistent clonal spread ($+1.22$, $+239\%$; $P_{MCMC} < 0.001$), while the effect on species without persistent clonal spread was not significant ($+0.06$, $+6\%$; $P_{MCMC} = 0.72$). There was a significant difference in weeding effect between species with and without persistent clonal spread ($P_{MCMC} < 0.001$). This could be explained by a stronger parasite-induced suppression of clonally integrated ramets, as we hypothesize. Alternatively, plants that reproduce clonally might simply colonize the space that becomes available after hemiparasite removal more quickly due to their growth strategy. Although this ‘clonal integration hypothesis’ needs broader testing, our results suggest – for the first time – that persistent clonal spread might indeed be a key trait determining species’ susceptibility to hemiparasitic infection.

It is worth mentioning that two of the host species of *Pedicularis* with persistent clonal spread (*Erica tetralix* and *Calluna vulgaris*) are also known for symbiosis with ericoid mycorrhizal fungi. Mycotrophy has been suggested to be another potentially important nutrient source for *Pedicularis* species in addition to parasitism (Li & Guan 2008; Li et al. 2013). If so, the effect of *Pedicularis* removal on species with persistent clonal spread could be confounded with the (type of) mycorrhizal associations.

**Germination success**

Not all sown species were able to germinate in the field. In the more productive *Rhinanthus* sites, a smaller proportion of species germinated compared to the more open vegetation at *Pedicularis* sites. It should be noted, however, that the composition of the sown seed mixture was different in the two vegetation types (only two species were common to both applied seed mixtures). *Rhinanthus* and *Pedicularis* removal had significantly negative effects on the germination success of two out of five and two out of four species, respectively (Fig. 4). However, only *Rhinanthus* removal significantly decreased the total number of seedlings across species. For the species that germinated successfully in the field, our results corroborated the hypothesis that hemiparasitic plants can enhance the germination success of certain other species. Hautier et al. (2009) found that seed mortality increased with light limitation. However, our
hemiparasite removal experiment does not allow us to distinguish between effects on light availability and effects on the availability of bare ground patches (‘gap’ formation). Interestingly, Pywell et al. (2004) found that the frequency of \textit{Rhinanthus minor} in the previous year explained variation in richness and frequency of sown species, whereas \textit{R. minor} frequency in the current year did not. This could explain why we see a negative effect of weeding on the number of seedlings of sown species despite the population collapse of both hemiparasitic species in the year of germination.

\section*{Temporal variation of hemiparasite abundance}

There was considerable inter-annual variation in the abundance of \textit{Rhinanthus} and \textit{Pedicularis}. Both hemiparasites performed best in 2009, declined by ca. 30\% in 2010 and ca. 73\% in 2011 relative to 2009. The considerable temporal and spatial variation of \textit{Rhinanthus} and \textit{Pedicularis} abundances is probably related to their short-lived life strategy, in combination with short-lived seeds (Petru 2005; Ameloot et al. 2006b). Ameloot et al. (2006b) concluded that early spring drought was the main reason for population collapse of the annual \textit{R. angustifolius}. This could explain the collapse of \textit{Rhinanthus} in 2011 relative to previous years: the spring of 2011 was the third driest spring on record (since 1833) in central Belgium (71 mm rainfall compared to 188 mm long-term average; Royal Meteorological Institute of Belgium). The spring in 2010 was also remarkably dry, with no precipitation events between 8 and 29 April, when \textit{Rhinanthus} seedlings and \textit{Pedicularis} plants emerged. We conclude that spring drought is likely the main reason for the year-to-year difference in the hemiparasite abundances we observed.

The low abundance of hemiparasites in 2011 raises the question of whether we can use biomass data of this year to evaluate the effect of hemiparasites on species abundances and germination success. While it is likely that effects are less pronounced due to the low hemiparasite abundance in unweeded plots, we did find significant effects. These are likely to be related to the much stronger hemiparasite abundance in 2010 (and 2009): Ameloot et al. (2006b) found that grass and forb cover were related to \textit{Rhinanthus} cover in the previous year (rather than in the same year) in similar grassland types as in the present study, indicating that vegetation responses lag behind hemiparasite abundance changes.

\section*{Future directions}

In accordance with previous studies, our results suggest that hemiparasites are important mediators of vegetation structure by changing the relative abundances of host and non-host species, as well as creating opportunities for seedling establishment. In line with the results of Mudrak & Leps (2010), we showed that more species-level field studies are required because we found several contrasting effects of hemiparasites on members of the same functional group or family. This implies that, depending on species composition, effects on functional group composition and species diversity can be adverse. For the first time we found support for a new hypothesis that determines vulnerability to parasite attacks, in addition to the lack of a sound defensive response: species with persistent clonal spread are more vulnerable to parasite attacks because parasites can potentially drain resources from a whole network of interconnected ramets. To find evidence for this hypothesis, future research could compare the impact of hemiparasites on a species when the connections between individual ramets are experimentally interrupted or not.

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\section*{References}


Hemiparasitic plants affect community composition

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11


Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Number of seeds added per field plot (0.5 × 0.5 m²), average germination percentage in open ground determined in a separate trial (mean ± 95% confidence interval of 3 replicates) and the number of viable seeds added per field plot (the product of added seeds and percentage germination in open ground) for seed mixtures used in both vegetation types. Seeds from underlined species were collected in the field, others acquired from Ecoflora (http://www.ecoflora.be). The seed mixtures have two species in common (shaded).

**Appendix S2.** Plant species categories used to test the ‘clonal integration hypothesis’: are plants with persistent clonal spread that form networks of interconnected ramets more vulnerable to parasitism?

**Appendix S3.** Changes in plant community diversity caused by weeding of the hemiparasite since 2009.