Interactions of the Hemiparasitic Species Rhinanthus minor with its Host Plant Community at Two Nutrient Levels

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Ondřej Mudrák
Institute of Botany of the ASCR
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University of South Bohemia in České Budějovice
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Interactions of the Hemiparasitic Species *Rhinanthus minor* with its Host Plant Community at Two Nutrient Levels

Ondřej Mudrák · Jan Lepš

Abstract For root hemiparasites, host plants are both the source of water and nutrients below-ground, but competitors for light above-ground. Hemiparasites can reduce host biomass, and in this way considerably affect the whole plant community. To investigate these effects, we carried out two experiments in an oligotrophic meadow with a native population of *Rhinanthus minor*. In the first experiment, removal of *R. minor* was combined with fertilization in a factorial design, and in the second one, we manipulated *R. minor* density by thinning. The presence of *R. minor* decreased the biomass of its host community, mostly by suppressing grasses. In this way, the species was able to counterbalance the effect of fertilization, which increased community biomass and in particular that of grasses. Neither the presence of *R. minor* nor fertilization affected the total number of species or the Shannon-Wiener diversity index (*H'*) of the host community. However, *H'* of grasses was higher and *H'* of forbs (non-leguminous dicots) was lower in the presence of *R. minor*. Reduction of grasses by *R. minor* favored mainly the dominant forb *Plantago lanceolata*, which partly acquired the role of a competitive dominant. Effects of *R. minor* on community diversity seem to be highly dependent on the relative sensitivities of dominant and subordinate species. Fertilization increased the

O. Mudrák (✉) · J. Lepš
Department of Botany, Faculty of Science, University of South Bohemia, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic
e-mail: ondrej.mudrak@centrum.cz

O. Mudrák
Section of Plant Ecology, Institute of Botany, Academy of Sciences of the Czech Republic, Dukelská 135, CZ-379 82 Třeboň, Czech Republic

J. Lepš
Institute of Entomology, Biology Center, Academy of Sciences of the Czech Republic, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic

O. Mudrák
Institute of Soil Biology, Biology Center, Academy of Sciences of the Czech Republic, Na Sádkách 7, CZ-370 05 České Budějovice, Czech Republic

mortality of seedlings, resulting in a lower number of flowering plants. However, surviving individuals on average produced more flowers. Thinning resulted in lower mortality of *R. minor* plants. This indicates that intraspecific competition in *R. minor* populations results in negative density dependence.

Introduction

Rhinanthus minor L., similarly to other root hemiparasites, can exert a considerable effect on its host plant community and strongly reduce the biomass of its hosts (Ameloot et al. 2005). The lost host biomass is usually not replaced by hemiparasite biomass (Matthies 1995a; Phoenix and Press 2005; Cameron et al. 2008), which results in lower total productivity of the plant community (Ameloot et al. 2005).

The host community simultaneously affects *Rhinanthus minor*. Below ground, the hosts are a (limited) source of water and nutrients, whereas above ground, they are competitors for light (Matthies 1995a). If the hemiparasites occur in high densities, they suppress their hosts and, as a result, can suffer from a lack of nutrients resulting in strong intraspecific competition (van Hulst et al. 1987). Hemiparasites can also attack each other (Prati et al. 1997), an ability that increases with hemiparasite density. This would suggest that the development of a hemiparasite population might be (at least at some stages) controlled by negative density dependence. Conversely, very strong and vigorous hosts are strong competitors for light (Matthies 1995a) resulting in heavy mortality among hemiparasites (van Hulst et al. 1987), which further leads to stronger hosts (positive feedback), which can ultimately lead to complete extinction of the hemiparasite.

Although *R. minor* can parasitize a wide variety of hosts (Gibson and Watkinson 1989), there are large differences in quality among individual host species. Legumes are considered to be the 'best hosts'. Hemiparasites usually produce more biomass, have higher fitness and damage their hosts more when parasitizing leguminous than non-leguminous species (Gibson and Watkinson 1991; Seel and Press 1993; Westbury 2004; Jiang et al. 2008). Another group of preferred hosts are grasses (Gibson and Watkinson 1989; Cameron et al. 2006). *R. minor* is usually less successful in obtaining nutrients from non-leguminous dicots (forbs hereafter), causing little or no biomass reduction in the hosts, and resulting in less biomass and fewer flowers for the hemiparasite (Cameron et al. 2005; Cameron and Seel 2007). However, grasses, forbs, and legumes are very broad and variable groups, and some species do not follow the above rule (Ameloot et al. 2005). Due to selectivity in host use, the presence of hemiparasites can change the competitive balance between preferred and non-preferred hosts (Gibson and Watkinson 1991; Matthies 1996; Cameron et al. 2005).

Rhinanthus species are annual plants. After their death, they leave gaps in the canopy, which are available for colonization by other species (Joshi et al. 2000).

Rhinanthus minor accumulates nutrients in its leaf tissue, providing high quality litter after its death. Due to its relatively short life span, litter often falls before mowing, enriching the immediate vicinity with nutrients (Ameloot et al. 2008). In low productive sub-arctic ecosystems, *R. minor* litter stimulates decomposers, speeding up nutrient cycling (Quested et al. 2003).

Impacts of hemiparasites are host species specific, and in many cases, the dominants are affected most. As a result, presence of a hemiparasite is expected to affect positively the diversity of the plant community, and indeed it has been successfully used to restore species-rich meadows (Pywell et al. 2004; Bullock and Pywell 2005; Westbury et al. 2006; Westbury and Dunnett 2008). However, the effect of *Rhinanthus* species on diversity is not uniform. Increasing, decreasing and no change in diversity have all been observed (Gibson and Watkinson 1992; Pywell et al. 2004; Westbury and Dunnett 2007), and it is not completely clear which factors determine the patterns (Ameloot et al. 2005). Bullock and Pywell (2005) suggested that the effect could be determined by the position of the plot on a productivity gradient with respect to the hump-back model of dependence of species richness on productivity (Grime 1979). Indeed, the effect of hemiparasites on a plant community could be affected by the nutrient status of the environment: the effect of a hemiparasite on hosts (e.g., biomass reduction) decreases with increasing amounts of nutrients in the soil (Gibson and Watkinson 1991; Matthies and Egli 1999; Davies and Graves 2000). Biomass allocation into their own root system is usually very low in hemiparasites (Matthies 1995b, 1997, 1998) and root hemiparasitism enables them to acquire nutrients with limited investment into the root system. However, large root systems are important only under low nutrients. At high nutrient levels, the host plants also invest less in their root system and the advantage of acquiring nutrients from the host disappears. Hemiparasites are therefore more easily excluded in competition for light (with its host). Accordingly, *R. minor* is associated with meadows of relatively low productivity and intensive fertilization can result in its extinction (Matthies 1995a). A considerable part of this evidence comes from pot experiments (Gibson and Watkinson 1991; Matthies and Egli 1999; Davies and Graves 2000).

We were interested in the role and relationships of *Rhinanthus minor* in plant communities, where it regularly appears in the landscape. Consequently, we established two manipulative field experiments in an oligotrophic meadow with a large population of *R. minor*. In the first experiment, we combined removal of *R. minor* with fertilization in a factorial design, and in the second we manipulated the density of *R. minor*. Our objective was to answer the following questions: How does *R. minor* affect its host plant community (all, the total biomass, and biomass of individual species and functional groups, and various characteristics of diversity) and how does this effect vary with changing nutrient level? How is the *R. minor* population affected by nutrient levels in its host community? Are the population dynamics of *R. minor* dependent on its population density?

Methods

The experiments were carried out in an oligotrophic meadow with a large population of *R. minor* in South Bohemia, Czech Republic (48°52′6″ N, 14°14′54″ E,

740 m a.s.l.). Grasses (*Festuca rubra*, *Trisetum flavescens*, *Alopecurus pratensis*) dominate the meadow community; the most common dicots are *Plantago lanceolata* and *Trifolium pratense*. The meadow is mown once a year (after 15th July, most *R. minor* are already dead at this time) and no fertilizer is applied. The meadow has been probably managed in a similar way for a very long time (probably with occasional manuring) and is representative of traditional extensively managed meadows in the area (so-called seminatural meadows).

Experiment 1 – Fertilization and Rhinanthus Removal

The experiment was established in a factorial design with two factors: *Rhinanthus* removal and fertilization. Seedlings of *R. minor* were carefully removed by hand each year at the beginning of the growing season (at the end of April 2005 and 2006). As the seedlings of *R. minor* germinated before the surrounding vegetation started to grow, removal caused only negligible disturbance to the surrounding vegetation. Fertilized plots received (both times in April) 30 g per m^2 (300 kg per ha) of commercial NPK fertilizer (8% N (nitrate and ammonium), 24% P (as P_2O_5) and 11% K (as K_2O)). The experiment was established in eight complete blocks. Each block consisted of the four possible combinations of fertilization and removal and was arranged as four adjacent 2 m×2 m squares positioned in one row, which was perpendicular to the slight slope (less than 5°) and suspected gradient toward the edge of the meadow. Blocks were adjacent to each other. The same treatment combinations never met as side by side neighbors. In each square only the central plot of 1 m×1 m was surveyed to avoid edge effects. The plots were surveyed at the time of peak biomass at the end of June.

In each central 1-m^2 plot we visually estimated the percentage cover of each species. The central 1-m^2 was divided into a grid of 25 subplots each 20 cm×20 cm, and the above-ground biomass was harvested in three of them (the same relative position of subplots in all the plots). The harvested material was sorted into species, dried at 85°C for 24 hours and weighed (*R. minor* was weighed separately for each individual). In 2005 we counted the number of *R. minor* seedlings, and flowering plants and determined the total number of *R. minor* flowers in each subplot, which gave information about the spatial pattern of the species. (In 2006, we counted only total numbers per plot.)

We defined four functional groups of plant species: grasses, other monocots (not presented in the analyses because of their negligible occurrence), legumes and forbs. These functional groups reflect *R. minor* host preferences as used in the literature (see Ameloot et al. 2005).

The whole community, and the grasses and forbs separately, were characterized by their biomass, number of species, and Shannon-Wiener diversity index *H'* calculated on the basis of biomass, using the natural log (log$_e$), and evenness, i.e., *H'*/ log(S).

Legumes were mostly represented by only two species (*Trifolium pratense* and *Trifolium repens*), so we did not calculate any diversity or evenness indices for them.

Multivariate data (i.e., the data on species and functional group composition) were analyzed by redundancy analysis (RDA) using CANOCO 4.5 (ter Braak and Šmilauer 2002), with presence of *R. minor* and fertilization being the categorical

predictors and block as the categorical covariate (centered by species, no standardization by samples, i.e., RDA on the co-variance matrix). When we tested for the effects of individual factors (i.e., fertilization or removal separately), we used the other factor as another covariate. Significance was tested using the Monte Carlo permutation test, with the permutation scheme adjusted to the design of the analysis. In particular, if the three subplots (20 cm × 20 cm plots within one plot) were used, then the split-plot design permutation was used, but when two years were evaluated together, the permutation was adjusted to the repeated measurement design (Lepš and Šmilauer 2003). Principal component analysis (PCA, similarly as for RDA, centered by species, no standardization by samples) was used to illustrate the whole variability in species composition, without direct testing of individual experimental factors. In Figs. 1 and 3 (which evaluate the species composition of the community) only the species that correlate best with the ordination axes are shown.

Univariate community characteristics (like total biomass or diversity) were analyzed using repeated measures Analysis of Variance (ANOVA), where *R. minor* presence and fertilization were fixed factors and block a random factor. Biomass was log-transformed to improve homoscedascity and normality of residuals.

The spatial distribution of *R. minor* was characterized by Lloyd's index

$$L = 1 + \frac{\frac{\text{var}}{\bar{x}} - 1}{\bar{x}}$$

where var is the variance and $\bar{x}$ the mean number of individuals in a subplot (see above). Values larger than one indicate an aggregated spatial pattern, and those smaller than one a tendency to even spacing. The index was chosen because (in contrast to the variance:mean ratio) its value does not change when individuals are randomly removed from a population (Pielou 1969; Lepš 1993) – consequently, its decrease signifies density-dependent mortality. Mortality of *R. minor* was estimated as the proportion of dead plants, i.e., the proportion of the seedlings that died before flowering, and was arcsin-square-root transformed prior to the analyses. The characteristics of *R. minor* population were tested similarly as community characteristics by repeated measures ANOVA.

Experiment 2 – Thinning of the Rhinanthus *Population*

In the second experiment we tested the effect of density-dependence on the *R. minor* population dynamics. At the start of the vegetation season (22nd April 2005), we selected 24 patches where *R. minor* seedlings were abundant and established one 20 cm × 20 cm plot in each patch, and counted the *R. minor* seedlings there. In half of the plots, we thinned the *R. minor* seedlings to one third of their original number. At the height of the vegetation period (at the end of June), we recorded the number of flowering *R. minor* plants and counted the number of flowers of each plant. Above-ground biomass of plots was sorted by species and treated as in experiment 1. We compared the numbers of *R. minor* seedlings and flowering plants (both log-transformed) in thinned and control plots by repeated measures ANOVA. The numbers of *R. minor* flowers (both per plot and per individual) and *R. minor* biomass (both per plot and per individual) in thinned and control plots were compared by
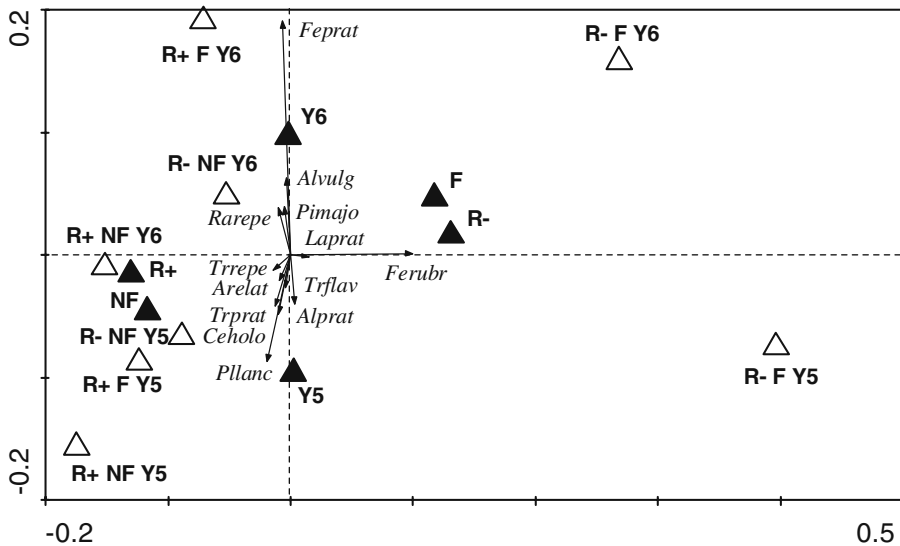
Fig. 1 PCA of species composition (characterized by dry biomass of individual species) with passively projected explanatory variables. Empty triangles represent combination of treatments in individual years; main effects of treatments and year are represented by solid triangles. Scaling was focused on inter-sample distances. Abbreviations are: R+/R– – *Rhinanthus minor* present/absent, F/NF – fertilized/non-fertilized, Y5 and Y6 – individual years 2005 and 2006, Alprat – *Alopecurus pratensis*, Alvulg – *Alchemilla vulgaris* agg., Arelat – *Arrhenatherum elatius*, Ceholo – *Cerastium holosteoides*, Feprat – *Festuca pratensis*, Ferubr – *Festuca rubra*, Laprat – *Lathyrus pratensis*, Pimajo – *Pimpinella major*, Pllanc – *Plantago lanceolata*, Rarepe – *Ranunculus repens*, Trprat – *Trifolium pratense*, Trrepe – *Trifolium repens*, Trflav – *Trisetum flavescens*

t-test. Individual plots (not individual plants) were considered as independent replications in both cases.

We used Statistica 6.1 (StatSoft, Inc. 2002) and Statistica 5.5 (StatSoft, Inc. 1999) for all univariate analyses.

Results

Experiment 1 – Fertilization and Rhinanthus *Removal*

Community changes

Community composition (expressed both in terms of biomass of species and biomass of functional groups) changed significantly between the two study years, and both the main effects, i.e., *Rhinanthus* removal and fertilization, were significant (Table 1). In contrast, none of the interactions were significant: this means that the effect of both manipulated factors were similar in both the observed years. However, the interaction of year and fertilization was marginally significant ($P=0.067$) for the composition of functional groups. (The analysis of community composition characterized by cover of individual species gives similar results; data not shown.) The amount of explained variability (Table 1) reflects also the reduction of dimensionality by the ordination axes; this is why the value is considerably higher

when community composition is expressed in terms of functional groups than when expressed in species composition (where the variability of 59 species had to be reduced into one or two constrained ordination axes). Removal of *R. minor* had a similar effect on community composition as fertilization. In both PCA ordinations (Figs. **1**, and **2**), the first ordination axis corresponds to productivity and the second axis to the effect of year (note that PCA is an indirect ordination, and so the first axis corresponds to the major variability in the community composition, and the environmental variables are only passively projected on the ordination plain). The symbols representing the main effect of *R. minor* removal are in similar positions as the symbols for the main effect of fertilization, determining together the first ordination axis, and thus the dominant variation in community composition. The response of individual species to both treatments can be best demonstrated using the RDA ordination diagram (Fig. **3**). Both fertilization and *Rhinanthus* removal favored mainly grasses, in particular *Festuca rubra*, but not all the species positively affected by fertilization and *Rhinanthus* removal are grasses. In contrast, in the presence of *Rhinanthus,* and in non-fertilized plots, *Plantago lanceolata* was usually dominant (Fig. **3**). Nevertheless, species preferring non-fertilized plots and plots with *Rhinanthus* form a functionally diverse group, which also includes one grass species, *Holcus mollis* (Fig. **3**). However, this species was relatively rare in all the plots.

Both total community biomass (including *Rhinanthus* in the control plots) and the biomass of grasses were lower in the presence of *R. minor* and higher in fertilized plots. The response of legumes was not consistent in either year. Forb biomass was not changed by the presence of *R. minor*, and the response to fertilization was not consistent between years. There was no significant interaction between *R. minor* presence and fertilization (Tables **2**, and **3**).

The number of species was not changed by the treatments (neither in the community as a whole, nor within functional groups). The Shannon-Wiener index (*H'*) for the whole community also did not differ among treatments. However, in the case of grasses, *H'* was higher while for forbs *H'* was lower in the presence of *R. minor*. Fertilization had no significant effect. Evenness of community and evenness

Table 1 Results of an RDA carried out on community biomass and biomass of functional groups

Explanatory variables	Covariables	Community composition			Functional groups		
		Explained variability	F	P	Explained variability	F	P
R	F, B, Y	2.1%	4.55	0.041	5.8%	13.71	0.001
F	R, B, Y	2.2%	4.72	0.030	10.2%	24.29	0.001
R, F	B, Y	4.3%	4.68	0.021	16.2%	19.21	0.001
R×F	R, F, B, Y	0.9%	1.95	0.258	0.6%	1.44	0.311
Y	P	1.4%	6.39	0.001	1.6%	7.67	0.001
Y×R	Y, P	0.2%	1.10	0.280	0.3%	1.69	0.184
Y×F	Y, P	0.4%	1.66	0.067	0.4%	1.97	0.163

Abbreviations: R – presence of *Rhinanthus minor*, F – Fertilization, B – Block, Y – Year, P – Plot.
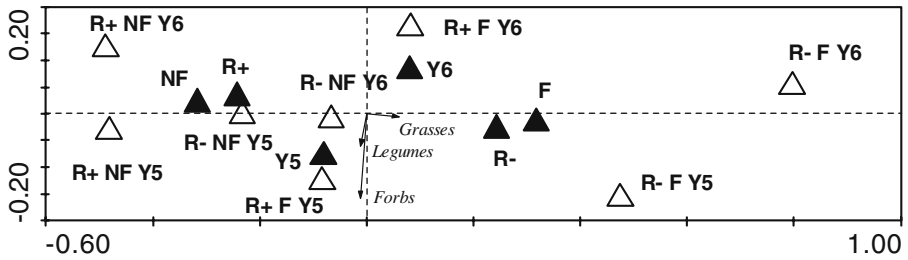
Fig. 2 PCA of functional groups (characterized by dry biomass) with passively projected explanatory variables. Empty triangles represent combination of treatments in individual years, main effects of treatments and year are represented by solid triangles. Abbreviations are: R+/R- – *Rhinanthus minor* present/absent, F/NF – fertilized/non-fertilized, Y5, Y6 – individual years 2005 and 2006

of grasses did not differ between treatments, but the evenness of forbs was lower in the presence of *R. minor* (Tables 2, and 3).

Rhinanthus minor *population size changes*

The total density of *Rhinanthus* plants was considerably higher in the second year (2006). Fertilization affected both the performance of individuals and their mortality, and thus population size. It significantly increased the mortality of *R. minor* (Tables 4, and 5), which resulted in fewer flowering plants in fertilized plots. The difference was more pronounced in 2006. The number of flowering plants increased from 2005 to 2006 in all plots, but the increase was roughly double in fertilized plots, while there were three times more flowering plants in 2006 in non-fertilized

Fig. 3 RDA of species composition (characterized by dry biomass of individual species), where the *Rhinanthus minor* presence and fertilization were used as explanatory variables. Year and block were used as covariables. Focus scaling was symmetric. Abbreviations are: R+/R- – *R. minor* present/absent, F/NF – fertilized/non-fertilized, Alprat – *Alopecurus pratensis*, Cxoval – *Carex ovalis*, Daglom – *Dactylis glomerata*, Ferubr – *Festuca rubra*, Homoll – *Holcus mollis*, Laprat – *Lathyrus pratensis*, Pllanc – *Plantago lanceolata*, Prvulg – *Prunella vulgaris*, Raacri – *Ranunculus acris*, Rarepe – *Ranunculus repens*, Tarude – *Taraxacum* sect. *Ruderalia*, Trflav – *Trisetum flavescens*
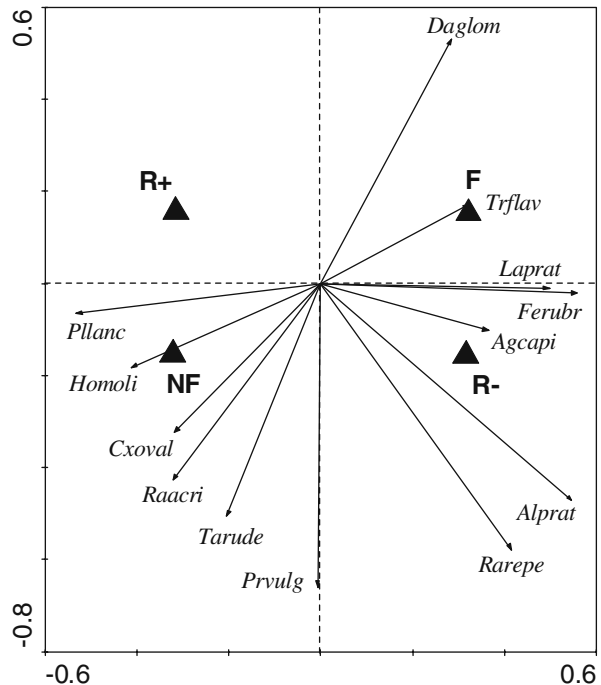
Table 2 Effect of treatments on total biomass (in g/m², including *Rhinanthus*) and diversity indices (number of species, Shannon-Wiener index and evenness) for the total community and its functional groups (grasses, legumes, forbs). Shown are means (bold), upper and lower 95% confidence intervals (above and below the mean respectively)

	2005				2006			
	Fertilized		Non-fertilized		Fertilized		Non-fertilized	
	R. minor present	R. minor removed	R. minor present	R. minor removed	R. minor present	R. minor removed	R. minor present	R. minor removed
Community biomass	388	446	314	323	335	429	279	335
	433	**498**	**350**	**361**	**380**	**487**	**317**	**381**
	483	555	391	402	431	553	360	432
Biomass of grasses	165	281	120	158	180	281	103	176
	200	**350**	**146**	**192**	**223**	**350**	**128**	**219**
	244	435	177	233	278	435	159	272
Biomass of legumes	45	40	44	25	9	15	19	22
	60	**53**	**58**	**33**	**14**	**23**	**29**	**34**
	79	69	77	43	23	37	46	53
Biomass of forbs	100	78	84	86	56	57	64	73
	125	**97**	**104**	**107**	**73**	**73**	**83**	**94**
	156	121	130	134	94	95	108	122
Nr. of species	14.8	14.6	15.4	14.8	14.7	14.5	15.1	14.8
	15.7	**15.5**	**16.3**	**15.7**	**15.7**	**15.4**	**16.1**	**15.8**
	16.6	16.4	17.2	16.6	16.7	16.4	17.0	16.8
Nr. of grass species	6.0	5.8	6.3	6.2	6.2	5.9	6.4	5.8
	6.4	**6.3**	**6.7**	**6.6**	**6.8**	**6.5**	**6.9**	**6.3**
	6.8	6.7	7.1	7.0	7.3	7.0	7.4	6.9
Nr. of forbs species	6.7	6.4	7.0	6.5	6.4	6.1	6.3	6.6
	7.3	**7.0**	**7.6**	**7.1**	**7.1**	**6.8**	**7.0**	**7.3**
	7.9	7.7	8.2	7.7	7.8	7.5	7.8	8.1
Shannon-Wiener	1.79	1.68	1.79	1.82	1.70	1.69	1.75	1.79
	1.89	**1.79**	**1.90**	**1.93**	**1.80**	**1.78**	**1.80**	**1.88**
	2.00	1.89	2.01	2.03	1.90	1.88	1.95	1.98
Shannon-Wiener of grasses	1.07	1.04	1.16	1.08	1.13	1.12	1.22	1.07
	1.20	**1.17**	**1.29**	**1.21**	**1.24**	**1.23**	**1.33**	**1.18**
	1.33	1.30	1.42	1.34	1.35	1.34	1.44	1.30
Shannon-Wiener of forbs	0.80	0.85	0.75	0.85	0.77	0.82	0.61	0.89
	0.93	**0.97**	**0.87**	**0.97**	**0.91**	**0.96**	**0.75**	**1.02**
	1.05	1.10	1.00	1.10	1.05	1.10	0.89	1.16
Evenness	0.65	0.62	0.65	0.67	0.63	0.62	0.64	0.65
	0.69	**0.65**	**0.68**	**0.70**	**0.66**	**0.65**	**0.67**	**0.69**
	0.72	0.69	0.72	0.74	0.69	0.69	0.70	0.72
Evenness of grasses	0.59	0.58	0.62	0.58	0.61	0.61	0.63	0.60
	0.65	**0.64**	**0.68**	**0.64**	**0.66**	**0.67**	**0.69**	**0.66**
	0.71	0.70	0.74	0.70	0.72	0.72	0.75	0.72
Evenness of forbs	0.41	0.44	0.37	0.43	0.41	0.44	0.32	0.45
	0.47	**0.50**	**0.43**	**0.49**	**0.48**	**0.51**	**0.38**	**0.51**
	0.53	0.56	0.49	0.55	0.54	0.57	0.45	0.58

Table 3 Effect of treatments on total biomass and diversity indices (number of species, Shannon-Wiener index and evenness) for the total community and its functional groups (grasses, legumes, forbs). Shown are P values for individual terms in repeated measures ANOVA. Effect d.f. = 1, error d.f. = 7 for all the terms. Block was included as random factor in the model, but is not shown in the table. Significant P values are highlighted

	R. minor presence	Fertilization	R. minor presence × fertilization	Year	Year × R. minor presence	Year × fertilization	Year × R. minor presence × fertilization
Community biomass	**0.012**	**<10⁻³**	0.543	0.269	**0.003**	0.512	0.809
Biomass of grasses	**0.004**	**<10⁻³**	0.977	0.340	0.077	0.191	0.553
Biomass of legumes	0.922	0.569	0.487	**<10⁻³**	**0.007**	**0.005**	0.845
Biomass of forbs	0.775	0.251	0.387	**0.003**	0.308	**0.029**	0.534
Nr. of species	0.372	0.368	0.865	0.911	0.852	0.978	0.571
Nr. of grass species	0.200	0.454	0.689	0.639	0.325	0.402	0.479
Nr. of forbs species	0.483	0.381	0.855	0.300	0.346	0.941	0.147
Shannon-Wiener	0.693	0.260	0.399	0.200	0.532	0.997	0.568
Shannon-Wiener of grasses	**0.022**	0.217	0.572	0.527	0.729	0.532	0.672
Shannon-Wiener of forbs	**0.027**	0.645	0.549	0.310	0.239	0.783	0.443
Evenness	0.905	0.249	0.284	0.163	0.477	0.984	0.518
Evenness of grasses	0.220	0.373	0.693	0.360	0.618	0.771	0.965
Evenness of forbs	**0.023**	0.409	0.556	0.737	0.496	0.565	0.502

plots (significant year by treatment interaction and highly significant effect of year, Tables 4, and 5).

As expected, the number of flowers on *R. minor* plants was positively correlated with its biomass in both years ($r=0.66$, $P<10^{-6}$ – 2005; $r=0.63$, $P<10^{-6}$ – 2006). The number of flowers per individual was in average slightly lower in fertilized plots in the first year, but considerably higher in the second year (significant interaction of time and fertilization). The fertilized plots contained fewer plants, but they were generally larger. As a consequence, fertilization affected neither the total number of flowers, nor total *Rhinanthus* biomass per plot (Tables 4, and 5).

The *R. minor* seedlings occurred in an aggregated pattern at the scale of 20 cm× 20 cm squares. Averaged over all plots, the Lloyd's index for seedlings was higher than 1 (mean=1.28; s.d.=0.26, $t=4.32$, d.f.=14, $P=0.0006$), and the same value was observed also for flowering plants (mean=1.28; s.d.=0.37, $t=3.03$, d.f.=14, $P=0.008$). There was no significant difference among fertilized and not fertilized plots ($F_{1,7}=0.70$, $P=0.432$) and no difference between seedlings and flowering

plants ($F_{1,7}<10^{-4}$, $P=0.998$); the interaction between fertilization and time was also not significant ($F_{1,7}=2.04$, $P=0.196$; repeated measures ANOVA). Lloyd's index was positively correlated with plot biomass ($r=0.55$, $P=0.026$, Fig. 4), i.e., flowering plants grew more aggregated in more productive plots.

Experiment 2 – Thinning of the Rhinanthus *Population*

In patches with a high density of *R. minor* seedlings thinning decreased their mortality. The number of *R. minor* plants declined in control plots, but in thinned plots it remained rather constant (repeated measures ANOVA, significant interaction of thinning with time: $F_{1,22}=8.99$, $P=0.007$; Fig. 5). Nevertheless, the number of plants was lower in thinned plots ($F_{1,22}=10.6$, $P=0.004$). The number of flowers per individual was on average higher in thinned plots (mean=2.8, s.d.=1.33) than in control plots (mean=1.8, s.d.=0.86), but the difference was not significant ($t=1.14$, d.f.=22, $P=0.267$). Biomass per individual in thinned and control was similar (mean thinned=0.132 g, s.d.=0.068, mean control=0.104 g, s.d.=0.053, $t=1.14$, d.f.=22, $P=0.266$). However, the total number of flowers (mean thinned=40.8, s.d.=24.2, mean control=76.0, s.d.=37.4; $t=2.73$, d.f.=22, $P=0.012$, *t*-test) and total *R. minor* biomass (mean thinned=1.76 g, s.d.=0.99, mean control=2.83, s.d.=0.85; $t=2.85$,

Table 4 Effect of fertilization on *Rhinanthus minor*. Shown are mean values of treatments (bold), lower and upper 95% confidence intervals (above and below the mean respectively)

	2005		2006	
	Fertilized	Non-fertilized	Fertilized	Non-fertilized
Nr. of flowering plants	36.7	42.5	93.3	171.5
	63.3	**69.1**	**139.8**	**218.0**
	89.8	95.7	186.2	264.5
Mortality	0.24	0.08	0.49	0.22
	0.38	**0.18**	**0.59**	**0.32**
	0.54	0.30	0.69	0.42
Flowers per individual	3.0	3.2	3.9	2.4
	3.7	**4.0**	**4.6**	**3.1**
	4.4	4.7	5.3	3.8
Flowers per plot	149	162	435	526
	234	**247**	**588**	**678**
	319	332	739	830
Total *R. minor* biomass (g/m^2)	6.7	3.6	24.4	20.7
	13.5	**10.4**	**37.7**	**33.9**
	20.2	17.1	50.9	42.2
Biomass per individual (g)	0.17	0.11	0.14	0.09
	0.26	**0.20**	**0.19**	**0.13**
	0.34	0.29	0.24	0.18

Table 5 Effect of fertilization on *Rhinanthus minor*. Shown are *P* values for individual terms in repeated measures ANOVA. Effect d.f. = 1, error d.f. = 7 for all the terms. Block was included as random factor in the model, but is not shown in the table. Significant *P* values are highlighted

	Fertilization	Year	Interaction
Nr. of flowering plants	**0.019**	**$<10^{-3}$**	**0.019**
Mortality	**0.012**	**$<10^{-3}$**	0.820
Flowers per individual	**0.025**	0.907	**0.033**
Flowers per plot	0.815	**$<10^{-3}$**	0.769
Total *R. minor* biomass (g/m^2)	0.329	**0.004**	0.928
Biomass per individual (g)	0.262	**0.046**	0.996

d.f.=22, *P*=0.009, *t*-test) was higher in control plots. Community biomass did not explain significantly any measured characteristics of *R. minor*.

Discussion

The effect of a hemiparasite on its host community can be investigated using two experimental manipulations: sowing it into a stand with no hemiparasite, or removal from its natural growth. Bullock and Pywell (2005) suggested that the sowing approach is better because it causes smaller disturbance to the studied community. However, absence of any species in a community (including hemiparasites) can have various causes, and it is difficult to say a priori which one is the case. If the absence is caused by dispersal limitation (Zobel 1997), then sowing might be a better solution. However, the absence of a species can be caused also by internal factors at the community level ("habitat and community filter"); if this is the case, we would study very unnatural interactions in the sowing experiment, even if *Rhinanthus* germinates and temporarily grows in the locality. Preliminary data (Blažek 2009)

Fig. 4 Relationship between Lloyd's index for *Rhinanthus minor* individuals and community biomass (*r*=0.55, *P*=0.026)
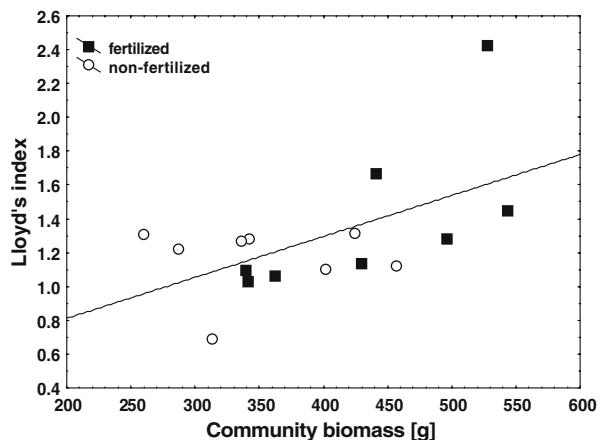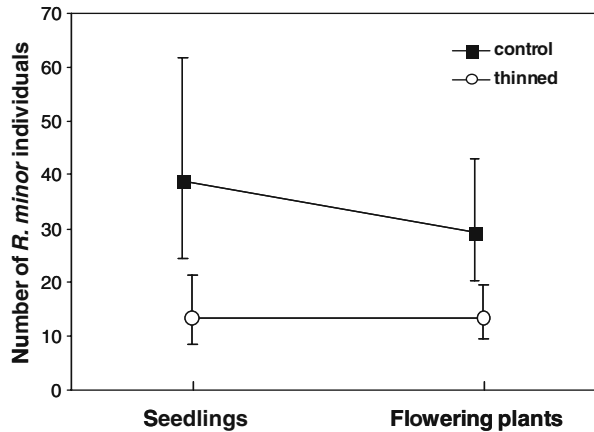
Fig. 5 Numbers of *Rhinanthus minor* individuals in thinned and control plots (20 cm × 20 cm) at the seedling and flowering stages (results of repeated measures ANOVA, significant interaction between the effects of thinning and time: $F_{1,22}=8.99$, $P=0.007$). Means and 95% confidence intervals (based on the between plot MS, backtransformed from log) are shown

suggest that it is often hard to ascribe *Rhinanthus minor* absence in a locality solely to dispersal limitation. Plant removal causes some disturbance to the community; however, we can be sure that we study the effect of a hemiparasite in its natural community. Moreover, in our opinion, careful removal of *R. minor* seedlings at the beginning of the growing season causes only negligible disturbance to surrounding vegetation. The differences between sowing and removal are not only methodological ones. By removal in a community where the hemiparasite was present for a long time (our case), we demonstrate which species were probably suffering most from the parasite effect in the community, and we see their potential for recovery. It has been shown (re-analysis of Mizianty 1975 data by Bullock and Pywell 2005) that the reaction to removal and additional sowing are different. In our case, mainly *Festuca rubra* exhibited a fast and pronounced increase after hemiparasite removal. Interestingly, the reaction was more pronounced when nutrients were added.

Similarly as in other studies (Rabotnov 1959; Mizianty 1975; Davies et al. 1997; Joshi et al. 2000; Ameloot et al. 2005; Cameron et al. 2005), the presence of *R. minor* reduced the biomass of grasses, which resulted in lower total biomass of the community. Nevertheless, our results show that not all the grass species are supported by *Rhinanthus* removal, and similarly, not all the forbs are more abundant in plots with *Rhinanthus* present (Fig. 3). As expected, the main effect of fertilization was to increase grass biomass and total biomass. Consequently, the effect of *R. minor* partially counteracted the effect of fertilization. This fact supports the possibility of using *R. minor* for restoration purposes (Bullock and Pywell 2005). High amounts of soil nutrients causing high productivity of a few dominant species (often grasses) is an important restriction in the restoration of species rich meadows (Walker et al. 2004).

The response to *R. minor* removal was fast and detectable within the first year. We can expect that the long-term effect can be even stronger, because the effect of hemiparasites on their hosts persists also after hemiparasite death (Seel and Press 1996). However, the between-year variation in both the host community and the hemiparasite population makes it difficult to see a trend in a two-year experiment. According to our field experience, manifold increases and subsequent declines in

Rhinanthus population size (often synchronized over larger areas) are quite common. And similarly, in our permanent experimental meadow plots not far from this experimental site, we documented large variation in biomass of individual species (Lepš 2004), probably caused by weather; the variation was particularly pronounced in fertilized plots.

Suppression of dominant species by parasitism should increase diversity of the community (Gibson and Watkinson 1992). Two years are a rather short time for species to become extinct or newly establish themselves, but we expected changes in the proportion of species and corresponding changes in diversity expressed as *H'*. Although we observed a reduction of the dominant grass *Festuca rubra*, a reduction of total grass biomass and total community biomass in the presence of *Rhinanthus*, total diversity did not increase. However, there were diversity changes within functional groups. The diversity of grasses was higher while that of the forbs was lower when *R. minor* was present. The legumes, usually considered most sensitive to parasitism (Phoenix and Press 2005), did not show a clear response to *R. minor* removal. This was probably because legumes were mostly represented by just two species, *Trifolium pratense* (more abundant) and *T. repens*; *T. pratense* is known to be tolerant to parasitism and benefits from the presence of *R. minor* (Rabotnov 1959, Ameloot et al. 2005; Bullock and Pywell 2005).

Grasses are the preferred hosts of hemiparasites (Gibson and Watkinson 1989) as they do not possess an effective defense against parasitism (Cameron et al. 2006; Cameron and Seel 2007). Indeed, the dominant grass species, *Festuca rubra*, was harmed most by parasitism. The most abundant forb species, *Plantago lanceolata*, however, is known to be a very poor host with efficient defense mechanisms (Rümer et al. 2007; Cameron et al. 2008). Accordingly, the reduction of grass biomass in the presence of *R. minor* favored this species (see Figs. **1**, **2**, and **3**), which further reduced evenness of the forbs. This also indicates that when a dominant species is suppressed, another species (even from another functional group) can acquire its role in the community, suppressing again competitively inferior species. Westbury and Dunnett (2007) also observed high cover and biomass in one species (*Leucanthemum vulgare*) resistant to parasitism by *R. minor*. The final effect of *R. minor* on total diversity might be rather idiosyncratic; we suggest that it 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.

Diversity will increase when strong dominants are suppressed most (which is often the case, because they might be expected to have the longest roots) and, as a consequence, subordinate species are released from competition. However, it need not always be the case. If the lost grass biomass is replaced by a strong resistant co-dominant (such as *Plantago lanceolata* in our case), the subordinate species remain suppressed. Such mechanisms could be responsible for the unchanged or lower diversity in the presence of *Rhinanthus* spp. reported by some authors (e.g., Gibson and Watkinson 1992; Westbury and Dunnett 2007). Although pot experiments demonstrated a smaller reduction of host biomass at higher nutrient levels (Gibson and Watkinson 1991; Matthies and Egli 1999; Davies and Graves 2000), in our study the effect of *R. minor* on the host community was independent of the nutrient levels.

We observed significant effects of nutrient levels on the population dynamics of *R. minor*. In particular, *R. minor* mortality was higher in the fertilized treatment resulting in a lower number of flowering individuals. The seedlings were probably outcompeted by the denser host vegetation (de Hullu 1985). Plants in the fertilized plots had, however, more flowers per individual, and consequently, the total number of flowers per plot remained the same at both nutrient levels. Very similar results, i.e., a lower number of larger plants in more productive vegetation, was also observed by van Hulst et al. (1987), whose research was conducted in the grasslands of Canada. The similar results from both studies, despite the large geographical distance between the studied sites, suggest that this pattern is rather general. It seems that the negative effect of higher nutrients was more pronounced at the seedling level. The individuals that managed to survive then took advantage of better fed hosts and grew larger.

Although we did not observe a reduced total performance of *R. minor* under higher nutrients, the species is usually associated with unproductive grasslands (Matthies 1995a). We expect that further increases in productivity will probably increase seedling mortality even more and *R. minor* could be competitively excluded from the community. Thus, it is likely that the effect of *R. minor* on a community will weaken with increasing productivity.

Smith (2000) published a mathematical model predicting an increase in hemiparasitic plant abundance as productivity of the system increases. Neither our, nor other published studies (de Hullu 1985; van Hulst et al. 1987) on *Rhinanthus* spp. support such a prediction. The non-realistic prediction is probably a consequence of the omission of above-ground competition between host and hemiparasite in the model (see Fibich et al. 2010, this issue).

Most Central European root hemiparasitic plants are annuals, and can produce many seeds (Westbury 2004 reports around ten seeds per capsule for *Rhinanthus minor*). This suggests that these species should be able to increase their populations exponentially, unless regulated by negative density dependence. Our thinning experiment demonstrated clear density dependence, particularly at the seedling stage. The seeds are probably washed into small depressions, where they form very dense patches (up to 143 individuals per 400 cm^2). There, the seedlings can shade each other, resulting in strong intraspecific competition for light within patches. However, negative density dependence (both higher mortality and slightly lower number of flowers per individual) is very probably also caused by below-ground interactions: either increased competition for host roots and the resources inside the host, or increased reciprocal root parasitism.

Negative density dependence was already demonstrated by van Hulst et al. (1987). de Hullu et al. (1985) found no density-dependent mortality in the case of *R. angustifolius*, but the authors discussed that the density was possibly not high enough. Matthies (2003) found positive density dependence among *R. alectolor- ophus* seedlings in a freshly sown plant community on ex-arable land. Seedlings of the hemiparasite emerged earlier than those of the potential host species and the reciprocal parasitism among seedlings probably led to a better distribution of resources among parasite individuals, similar to that among ramets of clonal plants. Such an interaction was shown by Prati et al. (1997). It seems that the mortality of *R. minor* is strongly affected by density of its individuals and vegetation biomass.

Rhinanthus minor individuals grew aggregated on a spatial scale of 20 cm × 20 cm quadrats, which fit the patches of high population density well. Density dependent mortality was probably important mainly in those patches. This suggests that the species dynamics are, at least partially, controlled by negative density dependence. Because of the aggregated pattern, local density within patches (which is important for intraspecific competition) is much higher than the overall density of the species in the locality. Aggregation of flowering plants of *R. minor* increased with community biomass. This indicates that in some places the seedlings had a higher probability of survival, presumably in patches with lower productivity among the dense tussocks. This is in concordance with Westbury et al. (2006), who found that gaps created in sod increased the survival of *R. minor* seedlings. Whereas thinning experiment 2 demonstrated the negative density dependence in survival, the value of Lloyd's index in experiment 1 remained constant, suggesting a density-independent mortality. The thinning experiment was carried out in selected high-density patches, where the mortality is really caused by intraspecific competition of neighbors. The experiment 1 plots cover these patches proportionally to their representation in the whole locality, and so the power to detect the possible density dependence was much lower. It seems that the dynamics of this species starts to be governed by negative density dependence only when local density in patches exceeds some (relatively high) limit.

In accordance with our expectation, *R. minor* in a semi-natural meadow decreased the productivity of the whole community and suppressed the dominant grass, so that its removal affected both total biomass and species composition in a similar manner as fertilization. The effect on community diversity is rather complicated; it depends on the relative sensitivities of dominant and subordinate species, and so can be highly idiosyncratic. Fertilization increased the mortality of *R. minor* (probably due to competition of the host vegetation), nevertheless, the survivors grew bigger and produced more flowers. The final response of both the hemiparasite and its host community to increased nutrients is interplay of below- and above-ground interactions, which are differently affected by nutrient amendments.

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