Hemiparasite-density effects on grassland plant diversity, composition and biomass

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Abstract

Hemiparasitic plants are considered as ecosystem engineers because they can modify the interactions between hosts and other organisms. Thereby, they may affect vegetation structure, community dynamics and facilitate coexistence as they are able to reduce interspecific competition by parasitizing selectively on competitive species and promote subordinate ones. In agri-environmental schemes, introducing the hemiparasite Rhinanthus has therefore been suggested as a low-cost method to increase grassland plant diversity, which is still subject to debate.

The majority of previous studies simply compared sites with and without hemiparasites. However, as hemiparasite effects are most likely density-dependent, we present a novel approach assessing the effect of Rhinanthus alectorolophus density on grassland plant diversity, yield and biomass quality. Moreover, we investigated whether functional plant composition and community mean traits are affected, which has been largely neglected in previous studies.

The relationship between species richness and relative Rhinanthus biomass followed an optimum curve with highest values at 31% relative Rhinanthus biomass. At this Rhinanthus-biomass level, species richness was increased by 12% and yield decreased by 26% compared with plots without Rhinanthus. At relative Rhinanthus biomass > 60%, species richness was even lower than in plots without Rhinanthus. Overall, the biomass of grasses and the cumulative cover of legumes decreased linearly with increasing relative Rhinanthus biomass. Community mean trait analysis revealed that an increasing Rhinanthus density shifts the community composition towards smaller plant species. Biomass quality was not affected by increasing relative Rhinanthus biomass.
In summary, our results of increased plant diversity – in line with a slightly lower yield but similar biomass quality – indicate that *Rhinanthus* is a suitable biological tool for grassland restoration.

**Keywords**

Biomass quality; community mean traits; Near Infrared Spectroscopy; *Rhinanthus alectorolophus*; yield
Introduction

In Central Europe, land-use intensification has led to homogenous grassland communities with only few highly competitive species and a loss of the overall diversity and ecosystem functions (Wesche et al., 2012; Allan et al., 2014; Soliveres et al., 2016; Gossner et al., 2016).

To reverse this trend, low intensity farming is currently promoted by agri-environmental schemes (e.g. Kleijn et al., 2009) to restore diverse and highly valuable grassland ecosystems. However, high residual soil fertility is a key factor limiting restoration success (Pywell et al., 2007, Klaus et al. 2011). Various attempts to overcome this problem have been suggested: Nutrient removal by frequent mowing or grazing tends to be slow and, particularly in the case of phosphorus, ineffective (Bullock and Pywell, 2005), whereas the more straightforward removal of top-soil is effective but costly (Hölzel & Otte 2003, Mudrák et al., 2014). An alternative and cost-effective tool for grassland restoration counteracting the inhibitive effects of dominant species is the introduction of native parasitic plants into these ecosystems (Davies et al., 1997; Smith et al., 2003; Mudrák et al., 2014).

The genus Rhinanthus, a member of the large parasitic Orobanchaceae family, is the most common root hemiparasitic genus throughout temperate Europe (Ameloot et al., 2005). Hemiparasites are photosynthetically active and consume water and nutrients from their hosts (Watson, 2009; Westwood et al., 2010), but also a considerable amount of assimilates and secondary compounds may be taken up (Adler, 2000; Tĕšitel et al., 2010). Rhinanthus spp. are generalists, parasitizing many different species (Gibson and Watkinson, 1991). Their roots form haustoria on any root they encounter (Cameron and Seel 2007). Rhinanthus is therefore more often successfully parasitizing hosts with a diffuse and wide spreading root system, such as grasses, than the ones with e.g. taproot during their whole lifecycle (Mudrák et al. 2016). Moreover, forbs with high nutrient contents, e.g. legumes, or forbs without
adequate defence mechanisms preventing penetration and access to the xylem have been suggested to be better-suited hosts (Seel and Press, 1993; Matthies, 1996; Ameloot et al., 2005; Cameron et al., 2006). They are able to reduce interspecific competition by parasitizing competitive species or by exerting density-dependent control of dominant plants, thereby facilitating coexistence and promoting the stability of natural communities (Phoenix and Press, 2005; Těšitel et al., 2015a). Moreover, it has been suggested that in particular annual hemiparasites can create gaps after senescence, which in turn promotes the establishment of other species (Joshi et al., 2000; Bullock and Pywell, 2005; but see also Davies et al., 1997). *Rhinanthus* spp. have been recommended as tool for grassland restoration as they are a natural part of European grasslands. In addition, because they are annuals, their density can be controlled easily by mowing before seed ripening (Magda et al., 2004; Bullock and Pywell, 2005; Westbury et al., 2006; Mudrák et al., 2014). Today, *Rhinanthus* spp. are already part of seed mixtures for biodiversity promoting areas (BPA) in Switzerland. However, whether effects on plant diversity are positive (e.g. Bardgett et al., 2006; Fibich et al., 2016) or negative (Gibson and Watkinson, 1992) is still unclear (see also Ameloot et al., 2005 for a meta-analysis).

Similarly, while most studies found *Rhinanthus* to reduce community biomass (Ameloot et al., 2005, 2006), some studies found a biomass increase (Joshi et al., 2000). The intuitive expectation of many farmers is a reduction of yield by the presence of hemiparasites, which makes *Rhinanthus* very unpopular for them (Magda et al., 2004; Ameloot et al., 2006). Related to this, it is often presumed that *Rhinanthus* reduces the nutritional value of the yield with severe losses in fodder quality. Such fodder quality decreases might be driven by changes in the functional plant composition of infested grasslands and should then be reflected by changes in nitrogen, mineral and fibre contents in plant biomass. However, as
this has not been tested systematically, it remains dubious whether hemiparasites indeed affect biomass quality.

Moreover, the functional role of hemiparasites in grasslands is still unclear (see Quested et al., 2003). As they are known for their low nutrient and water-use efficiency (Phoenix and Press, 2005) one can assume that they may affect the abiotic environment, which might be reflected by changes in mean environmental indicator values of the vegetation, e.g. for moisture and nutrient availability (e.g. Landolt, 2010). In addition, whether hemiparasites affect plant community structure and composition, indicated by changes in functional groups and community mean traits was also largely neglected in previous studies (but see Demey et al., 2015; Mudrák et al., 2016). In particular, one can assume changes in resource related community mean traits such as specific leaf area and leaf dry matter content as well as competition relates ones such as mean plant height or dispersal and colonization related ones such as seed mass.

So far, studies investigating hemiparasite effects on grasslands used an 'all or nothing' approach, comparing sites with and without hemiparasites (e.g. Gibson and Watkinson, 1992; Stein et al., 2009; Mudrák and Lepš, 2010). However, this approach may not be fully informative as it does not take the variation in *Rhinanthus* density into account, although hemiparasite effects are most likely density-dependent. Thus, it is unclear whether there is a *Rhinanthus*-density-grassland plant diversity optimum. In addition, different sites may not be fully comparable as they might differ in environmental conditions and not only the presence of the hemiparasite. It has been shown that the abiotic site conditions such as water and nutrient availability have profound interactive effects on hemiparasite and host biomass and fitness (Těšitel et al., 2015b). Thus, in studies showing decreases in productivity in the presence of *Rhinanthus* (e.g. Davies et al., 1997) it remains unclear whether *Rhinanthus*
drives this decrease or simply prefers low-productive conditions (Ameloot et al., 2005; Bullock and Pywell, 2005).

Here, we present results from an alternative approach testing whether varying Rhinanthus alectorolophus densities affect the plant diversity, plant community composition, productivity and biomass quality along Rhinanthus-density gradients within sites. Studying density effects within a site instead of between sites ensures similar environmental conditions among the replicates. Furthermore, to more explicitly explain plant community patterns and test for individual species responses along the Rhinanthus gradient, we used hierarchical Huisman-Olff-Fresco (HOF) models, a novel approach for modelling species response curves.

We therefore conducted a multi-site study, investigating 47 sites in three distinct regions in Switzerland allowing us to draw more general conclusions than investigating only a single region. This may further allow us to draw recommendations on the suitability of Rhinanthus in grassland restoration. In particular, we tested whether and how increasing Rhinanthus alectorolophus density affects 1) plant diversity, 2) species composition reflected by community mean functional traits and environmental indicator values, and 3) grassland productivity and biomass quality.

Methods

Study system

To enhance representativeness and generality of results and conclusions, we conducted our study in 47 meadows in three distinct regions in Switzerland: Bernese lowland (N=30), the Bernese Oberland (N=13) and the Valais (N=4). Sites were selected by accurate point
coordinates on the occurrence of *Rhinanthus alectorolophus* provided by Info Flora (www.infoflora.ch), the national data and information center of the Swiss flora, as well as by own observations. All sites were non-intensively managed meadows which could be assigned to communities of the Arrhenatherion alliance in the Bernese lowland and mainly to communities of the Polygono-Trisetion alliance in the two regions in higher altitudes (sensu Delarze et al., 2015). We considered a site to be suitable if it was well accessible, not mown when the vegetation was assessed and when *Rhinanthus alectorolophus* was present in varying densities. In addition, the site had to be visually homogeneous, e.g. that it could be assigned to one vegetation type. Altitude ranged from 504 m.a.s.l. (Bernese lowland) to 1946 m.a.s.l. (Schynige Platte, Bernese Oberland). The maximum geographic distance between the regions was approximately 80 km. Mean annual precipitation varies among the regions with around 700 mm in the Valais, 1000 mm in the Bernese lowland and 2000 mm in the Bernese Oberland.

**Vegetation and biomass data**

Between mid-May and beginning of June 2015, we sampled the meadows in the Bernese lowland, in mid-June the ones in Valais and between mid and end of June the ones in the Bernese Oberland, to account for the different developmental stages of the vegetation along the altitudinal gradient. Within each meadow, we placed nine 20 cm × 20 cm plots along a density gradient of *Rhinanthus alectorolophus* (named *Rhinanthus* hereafter). For this, we first visually subdivided each grassland in zones with low, medium and high *Rhinanthus* density and randomly placed three plots in each zone to maximize the range from a minimum (mostly equivalent with the absence) to a maximum *Rhinanthus* density within each meadow (up to 95% *Rhinanthus* cover), ensuring that plots were distributed across the
whole meadow and that two plots of the same density class were not situated in the same
Rhinanthus patch. We chose small sample quadrats of 400 cm$^2$ because the parasitic effects
on host plants is limited to the close neighbourhood of the parasite (host plants are only
affected within 10 cm of the parasite; Gibson and Watkinson, 1992) and this plot size has
been already used in many previous studies (e.g., Pywell et al., 2004; Ameloot et al., 2006;
Mudrak et al., 2016). In each of the resulting 423 plots, we identified all vascular plant
species and estimated their percentage cover and the cover of bare ground.

We then harvested the aboveground biomass, separated Rhinanthus, other herb and grass
species, dried the samples for 48 h at 80 °C and weighed them separately with 0.01 g
precision. After this, we again pooled the three biomass samples of each of the nine plots
per meadow and ground them to pass a 0.5 mm sieve to test for variation of biomass quality
along the Rhinanthus-density gradient.

As indications for biomass quality, we analysed the ground biomass samples for nitrogen (N),
phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K), carbon (C), neutral detergent
fibre (NDF), acid detergent fibre (ADF), and lignin (ADL) by means of near infrared
reflectance (NIR) spectroscopy (Foley et al., 1998). We recorded the reflectance spectrum of
each sample between 1250 to 2350 nm at 1 nm intervals. Each sample scan consisted of 24
single measurements, which we averaged to one spectrum. Biomass nutrient concentrations
and fibre contents derived from calibration models previously established. For
methodological details on NIR spectral analyses, calibration models and the laboratory
reference measurements see Kleinebecker et al. (2011).

Community weighted mean traits and environmental indicator values
We requested data from the TRY – Plant Trait Database (Kattge et al., 2011) and the Seed Information Database (SID; Royal Botanic Gardens Kew, 2016) to compute community mean traits of seed mass, plant height, specific leaf area (SLA) and leaf dry matter content (LDMC).

Data was available for all herbaceous species (212 out of 231). We excluded woody species (19 out of 231) from community mean trait calculations because they only occurred as seedlings and play no significant role in mown grasslands but might strongly affect traits like average plant height and seed mass. For each species trait, we computed the median value of all data base records. We then used the abundance-weighted community mean of each trait per plot for further analyses.

Moreover, we calculated abundance weighted indicator values for moisture and fertility (values ranging from 1 to 5; see Landolt, 2010) of each plot. Indicator values give the position of the realized niche of plant species and by averaging them over all species per plot, these values yield information on the environmental conditions of the grasslands.

**Statistical analysis**

Data were analyzed using R (version 3.2.4, R Development Core Team, 2016). We first calculated the biomass of *Rhinanthus*, grasses and herbs relative to the total biomass per plot. As relative *Rhinanthus* biomass and *Rhinanthus* cover estimates were strongly correlated ($r = 0.78$, $p < 0.001$), we used the measured relative *Rhinanthus* biomass values for further analyses. Legumes are suggested to be well-suitable hosts for *Rhinanthus* (e.g., Cameron et al., 2006). However, as we did not separate the biomass of legumes from the one of other herbs, we calculated the cumulative legume cover to test whether legumes as a group are negatively affected by increasing *Rhinanthus* densities.
For each plot, we calculated species richness, effective diversity ($e^{H'}$, where $H'$ denotes Shannon diversity) and Pielou’s evenness ($J = H' / \log[S]$) using the vegan package (Oksanen et al., 2015).

We then computed linear mixed effect models to account for the study design using the nlme package (Pinheiro et al., 2014). Our response variables were the total plant diversity measures and those separately for the functional groups grasses and herbs, community mean traits and environmental indicator values for moisture and fertility, total biomass, grass and herb biomass, and biomass quality measures. *Rhinanthus* was omitted from calculations on diversity measures, community mean traits and environmental indicator values. The models for biomass were calculated with and without *Rhinanthus*. Models on biomass quality also included *Rhinanthus* as these measures were based on pooled total biomass samples. As results for species richness and effective diversity were qualitatively similar, we only show the ones for species richness. Fixed effects included region and the relative *Rhinanthus* biomass and its interaction with region. We also included relative *Rhinanthus* biomass as quadratic term because we suspected some non-linear relationships, but omitted the quadratic term if not significant. As one of the three observers did not sample the meadows in the Valais and to correct for individual differences of the observer in species knowledge, we included the observer identity as a co-factor. Meadow was fitted as random term to account for meadow-specific differences. Model assumptions were checked visually by plotting residuals vs. predicted values and with normal-quantile plots.

To model species response curves along the *Rhinanthus* gradient, we used the package eHOF (Jansen and Oksanen, 2013). Huisman-Olff-Fresco models (HOF models; Huisman et al., 1993; see also Oksanen and Minchin, 2002) are five hierarchical models with increasing complexity: I) no trend, II) monotone sigmoid with a maximum at one end of the gradient,
III) monotone sigmoid with a plateau, IV) unimodal symmetric and V) unimodal skewed. Jansen and Oksanen (2013) further developed the models by adding model VI) bimodal with equal maxima and VII) bimodal with different maxima. As species composition differed strongly across the three regions, we modelled species response curves along the Rhinanthus-density gradient separately for each region. For this, we used abundance data (percentage cover) and a Gaussian error family distribution for species with at least 10 occurrences in all plots of the particular region (following Peppler-Lisbach and Kleyer, 2009; Peper et al., 2011). Model selection was done by comparing Akaike information criterion (AIC) and 100 bootstrapping runs tested model robustness. This resulted in a list of HOF models for 36 species in the Bernese lowland, 30 species in the Bernese Oberland, and 16 species in the Valais (Tab. S3).

To obtain reliable species response curves, the whole extent of the distribution data of a species must be covered along a gradient (Lawesson and Oksanen, 2002; Normand et al., 2009). Therefore, we removed species described by model I) with no response to Rhinanthus density and model II) with a monotone sigmoid response with a maximum at one end of the gradient. For the remaining species, we extracted the optimum value from the HOF model, represented by the maximum predicted abundance. For model III) we selected the mid-point of the plateau, and for model VII) the higher of the two modes (Fig. S2).

**Results**

**Rhinanthus-density effects on plant diversity**

Although meadows in the Bernese lowland were less species rich (mean: 11.6 species per 400 cm²; min: 3; max: 27) compared with the ones at higher altitudes in the Bernese
Oberland (mean: 12.8; min: 5; max: 21) and the Valais (mean: 14.4; min: 6; max: 25), and the Bernese Oberland (mean: 0.26 rel Rhinanthus biomass; min: 0; max: 0.83) and the Valais (mean: 0.12; min: 0; max: 0.45) compared to the lowland (mean: 0.33; min: 0; max: 0.85), the effects of an increasing Rhinanthus density on all diversity measures were consistent among regions in most cases (indicated by the non-significant Rhinanthus density-by-region interactions, Table 1).

We found significant hump-shaped relationships of total, herb and grass species richness with relative Rhinanthus biomass (Fig. 1a, Tab. 1), with highest values at 31%, 27.4% or 33.6% relative Rhinanthus biomass, respectively. Compared with plots without Rhinanthus, total species richness increased by 12% (1.35 species per 400 cm²: 0.32 grass and 1.03 herb species on average) at this density level. At Rhinanthus densities >60%, species richness was even lower than in plots without Rhinanthus (Fig. 1a). In contrast, evenness (excluding Rhinanthus) increased linearly with increasing relative Rhinanthus biomass (Fig. 1b, Tab. 1).

Rhinanthus-density relationships with environmental indicator values and community mean traits

When excluding Rhinanthus from the community mean trait analysis, we found no significant effects of increasing Rhinanthus densities on abundance weighted indicator values for moisture and fertility and on seed mass and SLA (Tab. S1). This indicates that Rhinanthus did not affect the abiotic conditions to such an extent that it would translate in a shift in the community mean for these measures.
However, community mean plant height decreased with increasing relative *Rhinanthus* biomass (the quadratic relationship was also significant and plant height tends to increase again at the end of the *Rhinanthus* density gradient). In addition, the relationship of community mean LDMC and relative *Rhinanthus* biomass was U-shaped (Fig. S1, Tab. S1).

*Rhinanthus*-density effects on individual species responses

We found species to respond differently to varying *Rhinanthus* densities (Tab. S3). However, no consistent patterns to separate the effects of increasing *Rhinanthus* densities on functional groups were evident. As the optima of different grass species (e.g. *Bromus erectus*, *Cynosurus cristatus*, *Dactylis glomerata*, *Festuca* spp., *Phleum pratense*, *Poa* spp.), legume species (e.g. *Lathyrus pratensis*, *Lotus corniculatus*, *Medicago lupulina*, *Trifolium* spp.) or species with tap roots (e.g. *Leontodon hispidus*, *Taraxacum officinale*) were distributed along the whole gradient (Tab. S3; Figs. S2, S3), we were not able to identify consistent trends to point out functional groups which might be suitable hosts because they suffer from high *Rhinanthus* densities. However, these results underline the rather unspecific host selection of *Rhinanthus*.

*Rhinanthus*-density effects on functional group abundances and biomass quality

Excluding *Rhinanthus* led to a biomass decrease with increasing relative *Rhinanthus* biomass (Fig. 1d; Tab. 2). Biomass of herbs followed a similar U-shaped pattern with increasing relative *Rhinanthus* biomass (Fig. 1f, Tab. 2), whereas the biomass of grasses decreased linearly (Fig. 1e). Overall, the cumulative cover of legumes decreased linearly with increasing relative *Rhinanthus* biomass (Fig. 2, Tab. 2).
Including *Rhinanthus* led to a U-shaped relationship between the total biomass (including *Rhinanthus*) and the relative *Rhinanthus* biomass (Fig. 1c, Tab. 2). At the density level with the highest species richness (31% relative *Rhinanthus* biomass), yield was on average reduced by 26%.

In addition, neither nutrient concentrations (N, P, K, Ca, Mg) nor fibre content (neutral detergent fibre NDF, acid detergent fibre ADF or lignin) varied with *Rhinanthus* density (Tab. S2). This indicates that the presence of *Rhinanthus* has no effect on biomass quality of these meadows.

**Discussion**

*Rhinanthus* density effects on plant diversity and biomass

Using our new gradient approach showed that the species richness-*Rhinanthus*-density relationship followed an optimum curve with highest values at intermediate densities, based on corresponding increases in both grass and herb diversity. In contrast, studies using the 'all or nothing' approach, like Fibich et al. (2016) simply found a positive effect of the presence in 11 out of 16 hemiparasites on the plant diversity of grassland communities when analyzing more than 30,000 plots of the Czech National Phytosociological Database. Moreover, Joshi et al. (2000) experimentally showed that the presence of *Rhinanthus alectorolophus* in newly created grasslands in Switzerland increased species richness of unsown species. Similarly, the artificial introduction of *R. minor* also increased species richness of sown grasslands in England (Pywell et al., 2004; Westbury et al., 2006). However, results from studies using the 'all or nothing' approach may result in misleading
management recommendations as they do not account for changes along the *Rhinanthus*-density gradient.

The major mechanism promoting plant diversity seems to be the mediation of competitive balances in the community and the reduced productivity of grasslands (Davies et al., 1997; Smith et al., 2003; Bullock and Pywell, 2005; Mudrák et al., 2016). According to Ameloot et al. (2005; meta-analysis of experimental sowing studies) and Fisher et al. (2013; introduction experiment) the average reduction of vegetation biomass by *Rhinanthus* is 26%. Our findings partly confirm the positive *Rhinanthus* effects on plant diversity via reduced productivity as we found an increase of species richness (+12%) coinciding with a maximum biomass decrease (-26%). However, this changed along the *Rhinanthus*-density gradient with maximum species richness at 31% relative *Rhinanthus* biomass. At *Rhinanthus* densities >60%, we found species richness dropping below the average values of plots without *Rhinanthus*. This is in accordance to Gibson and Watkinson (1992; observational study in combination with a *Rhinanthus*-removal experiment), who found lower species richness in patches with high *R. minor* densities in diverse British meadows. Interestingly, beyond this threshold, *Rhinanthus* biomass even over-compensated the loss of total biomass. A reason might be the relation of these high densities to optimal environmental conditions, promoting nutritious hosts (e.g. Seel and Press, 1993; Cameron et al., 2006) and thereby maximizing *Rhinanthus* biomass.

As both grass and herb biomass decreased along the gradient and their diversity was enhanced at intermediate *Rhinanthus* densities, our results indicate that *Rhinanthus* parasitizes rather density-dependent than selectively. Given the rather unspecific behaviour of *Rhinanthus*, which is supported by our analysis on individual species responses along the *Rhinanthus* gradient where we found no consistent patterns, it might well be that the
belowground abundance of a host species, e.g. of species with a diffuse and wide spreading root system, at least partly influences the probability of being parasitized. Accordingly, Press and Phoenix (2005) stated that a parasite’s host specificity might simply be an artefact of host abundance, meaning that the probability of being encountered by a parasite is higher for abundant host species than for less abundant ones and that parasitism is often proportional to the belowground presence of a host species. However, our findings of the overall linear reduction of grass biomass and legume cover support the idea that some hosts with a diffuse and wide spreading root system and high nutrient content, and at the same time lacking adequate defence mechanisms are better suited (e.g., Cameron et al., 2006; Sandner and Matthies, 2016). This might in turn reduce the abundance of the whole functional group of suitable hosts, when *Rhinanthus* densities are increasing.

The positive response in evenness with increasing *Rhinanthus* densities also confirm the resource-availability theory and suggests that suitable host species are parasitized over-proportionally, given that these hosts have a diffuse and wide spreading root system and lack adequate defence mechanisms preventing penetration. Thereby competitive exclusion could be prevented and coexistence with subordinate species might be promoted (Gibson and Watkinson, 1992; Press, 1998; Phoenix and Press, 2005). Similar to our findings, Fibich et al. (2016) found hemiparasite presence to be positively related with the evenness of plant communities. In addition, Demey et al. (2015) reported a decrease in evenness after the removal of *Rhinanthus*. It seems that hemiparasitic plants can play key roles in mediating the competitive relationships and the stability in a plant community (Press, 1998; Pennings and Callaway, 2002). Chesson (2000) described this effect as an “equalizing mechanism”.

*Rhinanthus* densities varied among our study regions with highest mean and maximum densities in the Bernese lowland. It has been shown that *Rhinanthus* performance decreases
with altitude (Hargreaves et al., 2015), which might be a possible explanation for these regional differences. A possible explanation for the lowest density values in the meadows situated in the Valais might be the considerably lower mean annual precipitation in this region compared to the other two. Moreover, *Rhinanthus* densities are highly variable over time (Press and Phoenix, 2005; Ameloot et al., 2006; Cameron et al., 2009) and therefore one might expect a rather cyclic effect of *Rhinanthus* on plant diversity and the composition of the plant community. After reaching high densities and promoting subordinate plants, subsequent invasion by competitive species becomes more likely because *Rhinanthus* loses its advantage when suitable hosts are no longer available. The subsequent increase of rather competitive species with an extensive root system is then again beneficial for *Rhinanthus* as enough resources are again available. Thus, patches with high *Rhinanthus* densities appear to move through the meadow over time (Press and Phoenix, 2005; Cameron et al., 2009). However, spatially explicit monitoring over several years would be required to confirm whether *Rhinanthus* exhibits such cycles of temporal changes in patch density. Moreover, whether the distribution patterns of *Rhinanthus* populations vary along environmental or elevational gradients has not been tested, yet. In our study areas we had the impression that the distribution of *Rhinanthus* changes from aggregated to more random with increasing altitude. However, this needs to be tested in future studies. Until now, temporal dynamics in the community composition have not yet been monitored with permanent study plots. However, Cameron et al. (2009) calculated intransitive-competition networks between *Rhinanthus*, grasses and forbs based on pairwise competition experiments between *Rhinanthus minor* and three forbs as well as three grasses to simulate these changes over time. Their models suggested fluctuating abundances of the parasite and the other functional groups resulting in “shifting clouds” of *Rhinanthus* within a grassland (Cameron et
al., 2009). The lack of information on *Rhinanthus* densities in previous seasons is therefore a limitation of our study and all other previous ones investigating the effect of *Rhinanthus* on diversity and composition in only one year.

*Rhinanthus*-density effects on community composition

The effects of hemiparasites on vegetation structure and community mean traits were rarely considered in previous studies (but see Mudrák et al., 2016). We found a decrease in community mean plant height with increasing *Rhinanthus* biomass. Similarly, Mudrák et al. (2016), who compared plots with *Rhinanthus minor* to plots were they had experimentally removed *Rhinanthus*, also found a reduced community mean plant height in plots with *Rhinanthus* present. These shifts in the community composition towards smaller plant species might be explained by the stronger parasitization of rather large and competitive species with large root volumes or by exerting *Rhinanthus* density-dependent control of dominant plants facilitating coexistence and promoting smaller plants (Phoenix and Press, 2005). *Rhinanthus* performance, e.g. the individual biomass and seed production is better when suitable host groups are present (Sandner and Matthies, 2016) likely leading to a stronger suppression of the host in the next year. This might facilitate the establishment of less competitive, e.g. smaller plant species. In addition, hemiparasitic plants are in general considered to be rather weak light competitors (Matthies, 1995), especially sensitive to shading during the seedling stage (Těšítel et al., 2011). Together with the dominance and productivity reducing effects (Ameloot et al., 2005), smaller species and also the establishment of *Rhinanthus* itself are thus likely promoted under these *Rhinanthus*-engineered conditions. However, the significant quadratic relationship with plant height tending to increase again at the end of the *Rhinanthus* density gradient is biologically
doubtful because at these high Rhinanthus densities only few species have a large effect on the community mean plant height.

In contrast to Mudrák et al. (2016), who found a decrease of LDMC with Rhinanthus minor present, we found a U-shaped LDMC vs. Rhinanthus density relationship. Mudrák et al. (2016) assumed that this decrease might be due to the reduced grass abundance as grasses have distinct trait values compared to forbs. However, as they used the ‘all or nothing’ approach, comparing sites with and without hemiparasite the results might be not fully comparable to ours. Moreover, the other investigated community mean traits such as seed mass indicating colonization ability and SLA, related to fertilization and resource utilization of plants (e.g. Pfestorf et al., 2013), did not differ along the Rhinanthus-density gradient. This suggests that predominantly height as an indicator of light competition, and LDMC as an indicator for changed resource use, are directly affected by Rhinanthus. The minor effect of Rhinanthus on community composition is further substantiated by the absence of changes in the abundance-weighted indicator values. Although hemiparasites have high transpiration rates and low water use efficiencies (Phoenix and Press, 2005), this seems not to translate into higher abundance or number of species that prefer drier conditions.

Rhinanthus-density effects on biomass quality

Given the small impact of Rhinanthus on species composition and community mean traits, the absence of effects on fodder quality may not be surprising. As we are not aware of any other study addressing how the quality of biomass changes with an increasing abundance of (hemi-)parasitic plants, further studies are required to test whether our results hold for
other types of grasslands or other parasitic plants. Also testing for density effects on the 
quality of herb and grass biomass separately might be very interesting to investigate.

Methodological implications and perspectives for future studies

Our results clearly support the use of the gradient approach instead of the ‘all or nothing’ 
approach in order to account for the density-dependent role of hemiparasitic plants for the 
functioning of plant communities. Our findings further suggest that studies using the ‘all or 
nothing’ approach may result in misleading conclusions and management recommendations. 
However, the limitation of our study and all other previous ones investigating the effect of 
Rhinanthus on diversity and composition is that we captured only a snapshot of the dynamic 
Rhinanthus invasion. As we only investigated one year, we cannot distinguish between 
recently invaded plots and the ones where the parasite density had already declined 
because of the decreasing abundance of suitable hosts. We therefore suggest establishing 
permanent plots to monitor the dynamics of Rhinanthus populations over time. This would 
further allow analysing compositional and functional changes of the host community. 
Moreover, this might also help to find more consistent patterns of increasing Rhinanthus 
densities on particular plant species. Such an observational monitoring approach might also 
be combined with experimentally controlling Rhinanthus densities by seed addition in 
established plant communities in meadows. Furthermore, including varying environmental 
and elevational gradients in future studies would allow testing for differences in distribution 
patterns of Rhinanthus populations and their effects on plant community composition along 
these gradients in addition to changing Rhinanthus densities.
Recommendations for restoration and agriculture

Overall, our results highlight that the density rather than the pure presence of hemiparasites is particularly important to understand and control their functional role. In summary, our findings of increased plant diversity in line with a slightly lower yield but similar biomass quality clearly show that *Rhinanthus* is a suitable tool for grassland restoration if its density can be maintained at intermediate levels. We therefore recommend introducing *Rhinanthus* into meadows for which high hay production is not the only goal, e.g. in course of agri-environmental schemes aiming at a reduction of management intensity and the enhancement of biodiversity.

But, how can intermediate *Rhinanthus* densities be maintained after successful establishment? A suitable management action to control the abundance of *Rhinanthus* without eradicating the whole population is early mowing before fruit ripening during one year (more than one year of early mowing will likely eradicate the population; Magda et al., 2004). However, Blažek and Lepš (2015) and Mudrák et al. (2014) demonstrated that *Rhinanthus* abundance can be drastically reduced when a meadow is mown before seed ripening and that the population depends on annual seed production. We therefore suggest late mowing of strips or even leave uncut strips until the next year on 10–20% of a meadow, as it has been suggested to support invertebrate diversity without detrimental effects on plant diversity (van Klink et al., 2017). These uncut strips might act as refuge to preserve the seed source and thereby maintain the *Rhinanthus* population in a meadow.

**Funding**
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

We thank the editor Diethart Matthies as well as Renate A. Wesselingh and two further anonymous reviewers for their constructive comments and their efforts with our manuscript. The study has been supported by the TRY initiative on plant traits (http://www.try-db.org). The TRY initiative and database is hosted, developed and maintained by Jens Kattge and Gerhard Bönisch (Max Planck Institute for Biogeochemistry, Jena, Germany). TRY is currently supported by DIVERSITAS/Future Earth and the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig.

References


**Table 1**: Summary of linear mixed effect models testing the effects of relative *Rhinanthus* biomass (including quadratic term when significant) on plant diversity in 47 grasslands in Switzerland. $R^2$ denotes the squared correlation coefficient between predicted and observed values.

<table>
<thead>
<tr>
<th>Species richness</th>
<th>Evenness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>$df$</td>
</tr>
<tr>
<td>(Intercept)</td>
<td>1</td>
</tr>
<tr>
<td>Region</td>
<td>2</td>
</tr>
<tr>
<td>Observer</td>
<td>2</td>
</tr>
<tr>
<td>rel. <em>Rhinanthus</em> biomass linear</td>
<td>1</td>
</tr>
<tr>
<td>rel. <em>Rhinanthus</em> biomass quadratic</td>
<td>1</td>
</tr>
<tr>
<td>Region $\times$ rel. <em>Rhinanthus</em> biomass linear</td>
<td>2</td>
</tr>
<tr>
<td>Region $\times$ rel. <em>Rhinanthus</em> biomass quadratic</td>
<td>2</td>
</tr>
</tbody>
</table>

$R^2=0.47$  $R^2=0.43$  $R^2=0.39$  $R^2=0.25$

**Table 2**: Summary of linear mixed effect models testing the effects of relative *Rhinanthus* biomass (including quadratic term when significant) on biomass and cumulative percentage cover of legumes in 47 grasslands in Switzerland. $R^2$ denotes the squared correlation coefficient between predicted and observed values.

<table>
<thead>
<tr>
<th>Biomass</th>
<th>Cover</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>$df$</td>
</tr>
<tr>
<td>(Intercept)</td>
<td>1</td>
</tr>
<tr>
<td>Region</td>
<td>2</td>
</tr>
<tr>
<td>rel. <em>Rhinanthus</em> biomass linear</td>
<td>1</td>
</tr>
<tr>
<td>rel. <em>Rhinanthus</em> biomass quadratic</td>
<td>1</td>
</tr>
<tr>
<td>Region $\times$ rel. <em>Rhinanthus</em> biomass linear</td>
<td>2</td>
</tr>
<tr>
<td>Region $\times$ rel. <em>Rhinanthus</em> biomass quadratic</td>
<td>2</td>
</tr>
</tbody>
</table>

$R^2=0.42$  $R^2=0.52$  $R^2=0.35$  $R^2=0.40$  $R^2=0.33$
Figures

**Figure 1.** Relationships between A) species richness, B) evenness C) total biomass, D) biomass without *Rhinanthus*, E) biomass of grasses, and F) biomass of herbs and relative *Rhinanthus* biomass in nine plots in each of 47 meadows in three regions in Switzerland. Open circles indicate plots from Bernese lowland, triangles from Valais and plus signs from Bernese Oberland. Linear or curve-linear regressions are indicated for all data (bold black) and the three study regions Bernese lowland (thin black), Bernese Oberland (dark grey), Valais (light grey), separately. The regions were only fitted separately when they differed significantly from each other. In these cases, the lines/curves are limited to the maximum extent of *Rhinanthus* density in the particular regional data set.

**Figure 2.** Relationship between the cumulative percentage cover of legumes and the relative *Rhinanthus* biomass in nine plots in each of 47 meadows in three regions in Switzerland. Open circles indicate plots from Bernese lowland, triangles from Valais and plus signs from Bernese Oberland.
Figure 1.
Figure 2.
Appendix

Table S1. Summary of linear mixed effect models testing the effects of relative Rhinanthus biomass (including quadratic term when significant) on mean environmental indicator values and on the community mean traits specific leaf area (SLA) and leaf dry matter content (LDMC) in 47 grasslands in Switzerland. \( R^2 \) denotes the squared correlation coefficient between predicted and observed values.

<table>
<thead>
<tr>
<th>Community excluding Rhinanthus</th>
<th>df</th>
<th>Moisture</th>
<th>Nutrient</th>
<th>Seed mass</th>
<th>Plant height</th>
<th>SLA</th>
<th>LDMC</th>
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<td>0.0</td>
<td>0.986</td>
<td>1.5</td>
<td>0.234</td>
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<tr>
<td>rel. Rhinanthus biomass linear</td>
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<td>0.742</td>
<td>0.9</td>
<td>0.355</td>
<td>3.4</td>
<td>0.067</td>
</tr>
<tr>
<td>rel. Rhinanthus biomass quadratic</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Observer</td>
<td>2</td>
<td>0.4</td>
<td>0.646</td>
<td>0.3</td>
<td>0.710</td>
<td>1.2</td>
<td>0.288</td>
</tr>
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<td>Region × rel. Rhinanthus biomass linear</td>
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<td>1.1</td>
<td>0.319</td>
<td>0.2</td>
<td>0.795</td>
<td>0.3</td>
<td>0.732</td>
</tr>
<tr>
<td>Region × rel. Rhinanthus biomass quadratic</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\( R^2 < 0.36 \) \( R^2 = 0.38 \) \( R^2 = 0.21 \) \( R^2 = 0.43 \) \( R^2 = 0.42 \) \( R^2 = 0.34 \)
Table S2. Summary of linear mixed effect models testing the effects of relative *Rhinanthus* biomass on measures of biomass quality in 47 grasslands in Switzerland. C (carbon), nitrogen (N), phosphorus (P), Ca (calcium), K (potassium), Mg (magnesium), ADF (acid detergent fibre), ADL (lignin), and NDF (neutral detergent fibre). Quadratic terms were excluded because they were not significant. $R^2$ denotes the squared correlation coefficient between predicted and observed values.
<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>N</th>
<th>P</th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
<th>ADF</th>
<th>ADL</th>
<th>NDF</th>
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<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>(Intercept)</td>
<td>1</td>
<td>856087.9</td>
<td>&lt;0.001</td>
<td>2428.6</td>
<td>&lt;0.001</td>
<td>31043</td>
<td>&lt;0.001</td>
<td>2385.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Region</td>
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<td>8.3</td>
<td>0.001</td>
<td>6.6</td>
<td>0.003</td>
<td>12.1</td>
<td>&lt;0.001</td>
<td>5.9</td>
<td>0.006</td>
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<tr>
<td>rel. Rhinanthus biomass linear</td>
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<td>1.3</td>
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<td>1.1</td>
<td>0.293</td>
<td>0.1</td>
<td>0.776</td>
<td>0.3</td>
<td>0.566</td>
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<td>Region × rel. Rhinanthus biomass linear</td>
<td>2</td>
<td>1.0</td>
<td>0.376</td>
<td>2.1</td>
<td>0.111</td>
<td>1.4</td>
<td>0.245</td>
<td>1.3</td>
<td>0.270</td>
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</table>

R² = 0.61  R² = 0.66  R² = 0.42  R² = 0.19  R² = 0.52  R² = 0.74  R² = 0.45  R² = 0.51
Table S3. Parameters of the response curves (optimum, minimum, maximum, range and mean), the applied eHOF model (III monotone sigmoid with a plateau, IV unimodal symmetric, V unimodal skewed, VI bimodal with equal maxima, VII bimodal with different maxima) and its parameters (a-d), using percentage cover (abundance) data of the species with at least 10 occurrences in each of the three study regions.
Figure S1. The relationship between A) community mean plant height and B) community mean leaf dry matter content (LDMC) and relative *Rhinanthus* biomass in nine plots in each of 47 meadows in three regions in Switzerland. Open circles indicate plots from Bernese lowland, triangles from Valais and plus signs from Bernese Oberland.

Figure S2. Species ranges and optima along the *Rhinanthus*-density gradient for the species with at least 10 occurrences in each of the three study regions (A) Bernese lowland, (B) Valais and (C) Bernese Oberland, and a species response curve different from model I (no response) or model II (monotone sigmoid).

Figure S3. Response curves of species with at least 10 occurrences in each of the three study regions (A) Bernese lowland, (B) Valais and (C) Bernese Oberland along the *Rhinanthus*-density gradient. The parameters of the species response curves and the eHOF model are summarized in Table S3.
Figure S1.
Figure S2.
Figure S3.