

Opposite effects of litter and hemiparasites on a dominant grass under different water regimes and competition levels

Alejandro Loydi  · R. Lutz Eckstein · Tobias Gebauer · Kristin Ludewig · Annette Otte · Christoph Reisdorff · Kai Jensen · Tobias W. Donath

Received: 30 June 2017 / Accepted: 11 November 2017
© Springer Science+Business Media B.V., part of Springer Nature 2017

Abstract Direct and indirect biotic interactions may affect plant growth and development, but the magnitude of these effects may vary depending on environmental conditions. In grassland ecosystems, competition is a strong structuring force. Nonetheless, if hemiparasitic plant species are introduced the competition intensity caused by the dominant species may be affected. However, the outcome of these interactions may change between wet or dry periods. In order to study this, we performed a pot experiment

with different densities of the dominant species *Schedonorus arundinaceus* (1, 2 or 4 individuals) under constantly moist or intermittently dry conditions. The different *Schedonorus* densities were crossed with presence or absence of hemiparasites (either *Rhinanthus minor* or *R. alectorolophus*). Additionally, pots remained with bare ground or received a grass litter layer (400 g m⁻²). We expected that indirect litter effects on vegetation (here *Schedonorus* or *Rhinanthus*) vary depending on soil moisture. We measured *Schedonorus* and *Rhinanthus* above-ground biomass and C stable isotope signature ($\delta^{13}\text{C}$) as response variables. Overall, *Schedonorus* attained similar biomass under moist conditions with *Rhinanthus* as in pots under dry conditions without *Rhinanthus*. Presence of *Rhinanthus* also increased $\delta^{13}\text{C}$ in

Communicated by Marjan Jongen.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11258-017-0783-1>) contains supplementary material, which is available to authorised users.

A. Loydi (✉) · K. Ludewig · A. Otte
Institute of Landscape Ecology and Resource Management, Research Centre for BioSystems, Land Use and Nutrition (IFZ), Justus-Liebig University Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany
e-mail: aloydi@criba.edu.ar

A. Loydi
Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS), Departamento de biología, bioquímica y farmacia, Universidad Nacional del Sur (UNS) - CONICET, Camino La Carrindanga Km7, B8000FWB Bahía Blanca, Argentina

R. L. Eckstein
Department of Environmental and Life Sciences, Biology, Karlstad University, 651 88 Karlstad, Sweden

T. Gebauer · K. Ludewig · C. Reisdorff · K. Jensen
Applied Plant Ecology, Biocenter Klein Flottbek, University of Hamburg, Ohnhorststr. 18, 22609 Hamburg, Germany

T. Gebauer
Department of Geobotany, Faculty of Biology, University of Freiburg, Schaenzlestr. 1, 79104 Freiburg, Germany

T. W. Donath
Department of Landscape Ecology, Institute for Natural Resource Conservation, Christian-Albrechts-University Kiel, Olshausenstr. 75, 24118 Kiel, Germany

moist pots, indicating hemiparasite-induced water stress. Litter presence increased *Schedonorus* biomass and reduced $\delta^{13}\text{C}$, indicating improved water availability. Plants under dry conditions with litter showed similar biomass as under wet conditions without litter. Hemiparasites and litter had opposite effects: hemiparasites reduced *Schedonorus* biomass while litter presence facilitated grass growth. Contrary to our expectations, litter did not compensate *Schedonorus* biomass when *Rhinanthus* was present.

Keywords Drought · *Festuca arundinacea* Schreb. · Grassland · *Rhinanthus alectorolophus* · *Rhinanthus minor* · *Schedonorus arundinaceus* (Schreb.) Dumort. · Stable isotopes · Tall fescue

Introduction

At a local scale, plant community composition is the result of a balance between positive and negative interactions among their members and with other biota within the ecosystem (Lortie et al. 2004). These biotic interactions can be direct or indirect, both having important consequences on community composition and structure (Morin 2009).

Direct interactions among individuals, such as competition, predation or parasitism, affect growth and reproduction. In particular, parasitism reduces or delays host development, which may have an impact on direct interactions of the host with other non-parasitic species (Mudrák et al. 2014). Hemiparasitic plants are frequent in natural and semi-natural grasslands and they may play an important role in reducing competitive effects of the dominant grasses (Mudrák et al. 2014; Phoenix and Press 2005). This may increase grassland biodiversity by allowing subordinate species to establish (Ameloot et al. 2005). Due to this effect, hemiparasites such as *Rhinanthus* species are being actively used as a restoration tool in grassland restoration projects (Bullock and Pywell 2005; Mudrák et al. 2014; Pywell et al. 2004). However, the response of the dominant vegetation to *Rhinanthus* may change depending on the prevailing nutrient and stress level (Bullock and Pywell 2005). Apart from a direct, mostly negative, parasite-host interaction, the indirect effects on co-occurring species may shift from facilitative to competitive

under stressful environments such as drought (Bu et al. 2013; Napier et al. 2016) and alter the outcome of interactions among dominant and subordinate species (Press and Phoenix 2005). For example, when the parasitic species is attached to a host, high transpiration rates might be maintained even under drought conditions, water uptake by the host plants will increase (Jiang et al. 2010; Spasojevic and Suding 2011), soil water content will be reduced and, in turn, subordinate species will be weakened by lower water availability.

On the other hand, indirect interactions among plants are mediated through effects of living or dead plants on abiotic conditions such as soil moisture, light availability, pH or temperature that modulate growth conditions of the vegetation (Levine 1999; Resco de Dios et al. 2014). In the case of dead plant remains (i.e. litter), their indirect effects may accumulate over time and thus last much longer than direct effects of living individuals. Also, they have the potential to alter the outcome of direct interactions among plant populations in complex communities (Bergelson 1990; Ruprecht et al. 2010a). In this way, litter may exert different types of effects on plant communities. It can have negative *direct* effects on vegetation by creating a mechanical barrier for growth, especially during early stages of plant development (Jensen and Gutekunst 2003; Ruprecht and Szabo 2012). But litter may also have positive or negative *indirect* effects on vegetation, usually acting through changes in microenvironmental conditions (Deutsch et al. 2010). Litter reduces soil evaporation and temperature fluctuations (Eckstein and Donath 2005; Loydi et al. 2014), which has positive effects during dry periods or in areas of high insolation or with high temperatures (Loydi et al. 2013). Similarly, litter releases nutrients to the soil, increasing seedling growth (Berg 2000; Myers et al. 1997), but it may also release allelochemicals during decomposition, which will reduce or delay seed germination (e.g. Hovstad and Ohlson 2008; Inderjit et al. 2008; Loydi et al. 2015a; Ruprecht et al. 2010b). Litter can also lower the strength of competition in plant communities by delaying regrowth of established vegetation (Schmiede et al. 2013; Török et al. 2012). In a previous study, we found that the presence of a litter layer reduced seedling emergence but it also increased biomass of established fully-grown individuals of *Schedonorus arundinaceus* (Schreb.) Dumort. (syn. *Festuca arundinacea*

Schreb.), which in turn increased their competitive effect (Loydi et al. 2015b). Overall, litter seems to have stronger direct effects when vegetation grows from underneath the litter layer, as during germination or regrowth of individuals. Indirect effects on environmental conditions generated by litter are more important for already emerged or developed individuals (Hovstad and Ohlson 2009; Levine 1999; Violle et al. 2006).

Central European grasslands are systems where disturbance and competition are the main structuring forces (Pärtel et al. 2005) and litter represents an important agent for indirect interactions (Facelli and Pickett 1991). Additionally, among the wide array of plant functional groups in these grasslands, hemiparasitic plants, such as species of the genera *Rhinanthus*, *Euphrasia* and *Odontites*, are frequent (Westbury 2004; Wolfe et al. 2005). Thus, the direct and indirect effects that components of grassland systems, e.g. dominant grasses, hemiparasitic forbs, grass litter, exert upon each other may have profound consequences for community composition and structure. These effects seem to be stronger in communities with highly variable environmental conditions (Napier et al. 2016). To untangle these complex interactions will also help to increase the effectiveness of the application of *Rhinanthus* species to control dominant and unwanted grasses in restoration projects. However, to our knowledge, there have been no experimental studies addressing the potentially interactive effects of litter and hemiparasites on the performance of a dominant species under different levels of environmental stress. Therefore, we performed a pot experiment using different densities of the grass *S. arundinaceus* growing in the presence or absence of one of two congeneric hemiparasitic species (i.e. *Rhinanthus alectorolophus* and *R. minor*) with or without the presence of a grass litter layer. Pots were additionally subjected to constantly moist or intermittently dry conditions, the latter simulating drought. We evaluated *Schedonorus* and *Rhinanthus* performance measuring changes in plant aboveground biomass and stable carbon isotopes ratios ($\delta^{13}\text{C}$). Drought stress usually reduces aboveground biomass production. This is partly explained by a reduction in stomatal conductance. Under such conditions, the RubisCo enzyme is no longer able to discriminate among natural C isotopes, leading to an increasing ^{13}C presence in plant tissues. The resulting increase of the

carbon isotopic ratio ($\delta^{13}\text{C}$) can thus be used as an indicator of drought stress (Adams and Grierson 2001; Dawson et al. 2002). Therefore, carbon isotopic ratio is a sensitive indicator of drought stress in plants.

Multiple factors in our experiment may change moisture conditions: (i) constantly moist and intermittently dry conditions will have an impact on aboveground biomass and, as soon as stomatal closure increases, on ^{13}C isotopic signature, (ii) increasing levels of competition and the presence of hemiparasites will decrease water availability, and (iii) presence of a litter layer will improve water availability by reducing soil evaporation under drier conditions, thus counteracting water deficits due to the presence of hemiparasites. For this particular work, we wanted to test if the presence of litter and hemiparasitic species exert opposite effects on aboveground biomass depending on water availability. Based on current knowledge, we expect the following:

1. Hemiparasitic species have a negative effect on water availability and increase drought stress. Their presence will thus reduce *Schedonorus* biomass more strongly under intermittently dries than under moist conditions. They will indirectly reduce stomatal conductivity resulting in an increased carbon isotopic signature.
2. A litter layer will increase water availability under drought and thus weaken the negative effects of intermittently dry conditions on aboveground biomass (i.e. of *Schedonorus* and *Rhinanthus*). Plants will respond to higher water availability due to a litter layer with a higher stomatal conductivity resulting in a relatively low carbon isotopic signature.
3. Litter and hemiparasites presence exert opposite effects on *Schedonorus* growth. Thus, presence of a litter layer will compensate biomass reduction and isotope ratio changes due to presence of *Rhinanthus* spp.

Materials and methods

Study species

Schedonorus arundinaceus (tall fescue) is a cool-season perennial bunchgrass native to Europe. It is a common grass in natural and semi-natural grasslands

across Central Europe, occurring regularly together with *Rhinanthus* species (Gibson and Newman 2001). It is widely used as forage in its native range and many cultivars have been introduced as forage worldwide.

Parasitic species of the genus *Rhinanthus* (Orobanchaceae) are frequent in non-intensively managed grasslands in Europe and North-America (Ameloot et al. 2005; Jiang et al. 2010). The family contains appr. 45 species, all annual or perennial root hemiparasitic herbs (Wolfe et al. 2005) with grasses and legumes as their preferred host. Recently, *Rhinanthus* species have been used as a tool in several grassland restoration programmes, since introduction of *Rhinanthus* may be a low-cost method to reduce grass competition (Hejzman et al. 2011) and indirectly increase biodiversity. The selected *Rhinanthus* species differ in their habitat preference: *R. minor* is found in mesic habitats while *R. alectorolophus* is more frequent in slightly drier systems (Bullock and Pywell 2005).

Experimental design and data collection

We performed a completely randomised pot experiment to test the effect of competition (factor levels (k) = 3, low/medium/high levels of competition by *Schedonorus*), presence of plant hemiparasites (k = 3, absence/*R. minor*/*R. alectorolophus*), litter presence (k = 2, presence/absence) and moisture conditions (k = 2, intermittently dry/constantly humid) on biomass and carbon stable isotope ratios in leaf tissues of *Schedonorus* and *Rhinanthus*. For each combination ($2 \times 2 \times 3 \times 3 = 36$ combinations), we prepared 10 replicates ($N = 360$) of 4 L pots (18 cm diameter and 16 cm depth). Additionally, we prepared pots only with *R. minor* or *R. alectorolophus* (*moisture conditions* \times *litter* = 4 combinations per species) replicated 10 times each to evaluate the performance of hemiparasites without hosts. The experiment consisted of a total of 440 pots.

Schedonorus and *Rhinanthus* species were grown from seeds from a commercial seed supplier (Rieger-Hofmann® GmbH). *Schedonorus* seeds were germinated under greenhouse conditions and later transplanted to their respective pots. The transplants were made in November 2012 and plants were maintained outside for six months (autumn and winter) until the beginning of the experiment. Pots were filled with commercial potting soil (Fruhstorfer Erde®, Type P,

Industrie-Erdenwerke Archut GmbH, Lauterbach, Germany) composed of a mixture of peat, clay and humus (pH-CaCl₂ 5.7, 188 mg L⁻¹ nitrogen, 136 mg L⁻¹ P₂O₅ and 206 mg L⁻¹ K₂O). The competition treatment was established by planting one, two or four individuals of *S. arundinaceus* in each pot. Pots with the same potting soil were maintained under the same conditions and used for the treatments without competitors. *Rhinanthus* seeds were sown in germination trays and maintained wet for 6 months at 4 °C under dark conditions in a germination chamber for proper stratification. Afterwards, they were moved to greenhouse conditions for 2 weeks since attachment of *Rhinanthus* to the host starts 2 weeks after emergence (Davies et al. 1997). All *Rhinanthus* individuals had 2–4 fully developed leaves at the time of transplantation.

On 14th May 2013, all pots were placed in a greenhouse located close to Giessen, Germany (50°32'N, 8°41.3'E, 172 m a.s.l.). *Schedonorus* individuals were cut 2 cm above the soil surface and half of the pots received 400 g.m⁻² of air-dried grass litter (10 g per pot) without covering the grasses. The litter amount is within the range of 250 and 500 g m⁻² where positive effects on vegetation biomass in pot experiments can be expected (Loydi et al. 2013). Litter was collected from unfertilized grasslands. The other half of the pots was kept without litter (i.e. bare ground). Afterwards, one third of the pots with *Schedonorus* within each level of the competition treatment received five individuals of *R. minor* and the other third five individuals of *R. alectorolophus*. The rest was maintained without any hemiparasite species. Additionally, 80 pots without *Schedonorus* received five individuals of one *Rhinanthus* species in the presence or absence of a litter layer and under different moisture conditions (eight combinations, factors *hemiparasites* \times *litter* \times *moisture conditions*) and were maintained under the same conditions. These pots were used as controls to measure *Rhinanthus* growth without a host but under the effect of all the other treatments employed. However, they were not used in our statistical analyses (see below). After transplantation of all *Rhinanthus* individuals, all pots were maintained constantly moist for 11 days to avoid subsequent mortality. Nonetheless, 3% of the transplanted *Rhinanthus* (ca. 50 individuals) died during the first 4 days and were replaced by new individuals. We measured litter depth in a random subsample of 10

pots per *Schedonorus* treatment and the controls. Litter depth varied between 21.3 and 38.8 mm, with a mean of 29.6 mm, but there were no significant differences among treatments ($F_{3,36} = 1.43$, $p = 0.250$). Additionally, we measured the diameter of each *Schedonorus* tuft to test for random differences among treatments before the start of the experiment. As expected, the individual basal diameter of the *Schedonorus* plants differed significantly between the levels of competition, with 66.0 ± 0.5 , 51.1 ± 0.4 and 38.6 ± 0.3 mm plant⁻¹ (mean \pm SE) for pots with one, two and four individuals, respectively ($F_{2,324} = 1105.3$, $p < 0.001$, see Table S1 in supplementary information). There were no differences among the other treatments. At the same time, to ensure similar levels of nutrients in each pot, we added Osmocote exact standard (Everris GmbH, 16N:9P:12K + 2MgO + trace elements, with a 3–4 months release formulation) with watering at 4 g L⁻¹.

On 25th May, the experiment commenced by letting half of the pots become intermittently dry, while the other half was maintained constantly moist. To accomplish this, the pots in the intermittently dry treatment received 50 mL of water, while the constantly humid pots received 100 mL. After 20 days, the amount of water applied was doubled due to higher temperatures at the end of spring and beginning of summer. Watering events occurred when the treatment with the highest evapotranspiration but no *Rhinanthus* individuals turned dry (i.e. treatment with four *Schedonorus*, no litter, no *Rhinanthus* and moist conditions). We assumed that this treatment had the highest evapotranspiration since it had the highest *Schedonorus* green biomass, and was therefore used as an indicator for watering the rest of the treatments. This resulted in watering twice to three times a week. Volumetric soil water content was monitored using a ThetaProbe ML2x soil moisture sensor (Delta-T, Cambridge, UK) only in the pots without *Rhinanthus* ($n = 120$) to avoid damage of *Rhinanthus* seedlings or established host-parasite connections. This was done three times during the experiment: immediately, 1 and 2 days after watering at randomly chosen dates. At the end of the growing season, before the dry summer period (7th July), all aboveground biomass was collected from each pot, dried at 60 °C for 48 h and weighed. The experiment lasted for 43 days. For statistical analyses, we only used those pots with at

least two or more healthy and well-developed *Rhinanthus* individuals, i.e. those pots where *Rhinanthus* did actually parasitize on *Schedonorus*. This was assumed when individuals had five times more biomass than *Rhinanthus* individuals growing in the control pots without hosts (Jiang et al. 2007). This resulted in a slightly unbalanced design with a total of 335 pots (93% of the original design).

To analyse ¹³C stable isotope signatures of *Schedonorus* and *Rhinanthus* plants, we used four replicates per treatment using only those pots with four or five well-developed *Rhinanthus* individuals. A subsample of leaf biomass was used for analyses. Two grams of biomass were ground in a Retsch MM200 mill (Retsch GmbH, Hann, Germany) and subsequently stored dry in 2 mL Eppendorf tubes until further analysis. Samples of 3–4 mg were weighted into 4×6 mm tin cups (HEKAtech, Germany) for C isotope determination in a stable isotope ratio mass spectrometer (nu Horizon, Nu Instruments Limited, UK) linked to an elemental analyser (EURO-EA 3000, Euro Vector, Italy) in continuous flow configuration (set-up by HEKAtech, Germany).

Samples were measured against CO₂ reference gas that had been calibrated to L-glutamic acid reference materials USGS-40 ($\delta^{13}\text{C} = -26.39\text{‰}$ VPDB) and USGS-41 ($\delta^{13}\text{C} = -37.63\text{‰}$ VPDB) (International Atomic Energy Agency, Vienna, Austria). We used NIST-certified 2,5-bis(5-tert-butyl-2-benzoxazol-2-yl)thiophene (BBOT; 72.52% C; HEKAtech, Germany) as internal reference material for carbon isotope analysis. The $\delta^{13}\text{C}$ were expressed as

$$\text{isotope ratio } \delta(\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{stand}}} - 1 \right) \times 1000$$

where R represents the ratio of the fractional abundance of the heavy and light isotope (¹³C/¹²C) in the tissue sample (R_{sample}) or the reference substance used (R_{stand}).

Statistical analysis

All data were evaluated using factorial ANOVA to analyse the effects of competition, litter, moisture conditions and presence of hemiparasites. Hemiparasites were considered a factor only for *Schedonorus* data. Data were transformed using Box-Cox transformations to meet ANOVA assumptions (Legendre and

Legendre 1998). All factors were considered fixed. Due to the mortality of *Rhinanthus* individuals during the experiment, we included the number of *Rhinanthus* individuals that survived until the end of the experiment as a covariate in the ANOVA model for *Schedonorus* biomass to account for its potential effects on final biomass. Covariates were not necessary for *Schedonorus* stable isotopic composition since variation in *Rhinanthus* was minimal (4 or 5 individuals per pot, see above), nor for *Rhinanthus* biomass since there was no *Schedonorus* mortality during the experiment. In all cases, Tukey posthoc tests were performed. All statistical analyses were performed using Statistica (version 10.0, StatSoft Inc, Tulsa, OK, US).

Results

The number of live *Rhinanthus* individuals in each pot did not affect the outcome of the study since their presence as covariate in the ANOVA was non-significant ($p = 0.117$; Table S2). In general, the effects of *R. minor* and *R. alectorolophus* on *Schedonorus* did not differ significantly and both *Rhinanthus* species showed similar response to the analysed factors.

Soil moisture per pot

Soil moisture was higher in treatments with litter presence at all sampling dates (see Table 1 and Table S3). Already 1 and 2 days after watering

significantly higher moisture contents were found in the low competition compared to the other treatments (Table 1). Two days after watering (and right before the next watering event), litter had a positive effect on soil moisture only at medium competition levels but not at high or low competition level (Competition \times Litter interaction, $p = 0.043$, Table S3). At the same date, in well-watered pots low competition level showed a higher soil moisture than medium and high competition level but intermittently dry pots had similar soil moisture at all competition levels (Competition \times Watering interaction, $p = 0.0007$, Table S3).

Schedonorus biomass per plant

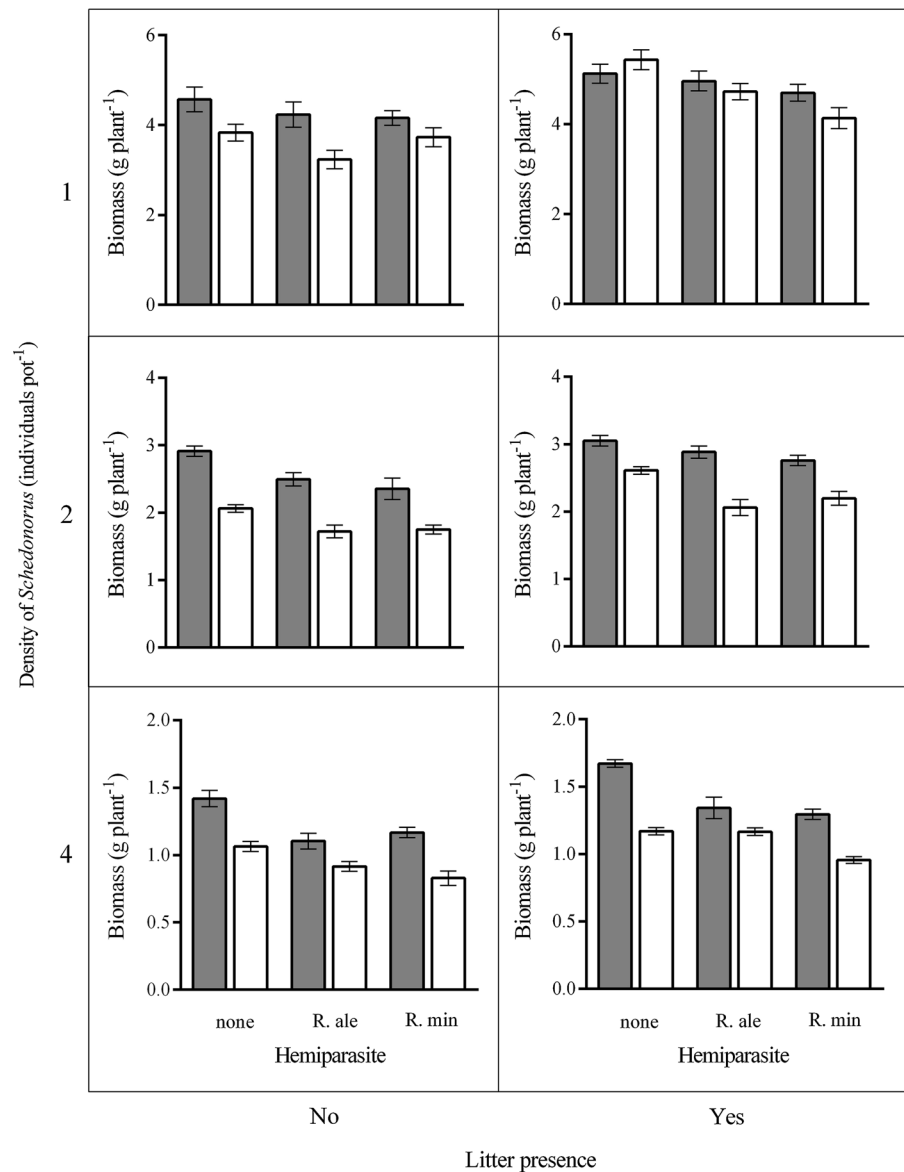
Overall, increased levels of competition reduced *Schedonorus* biomass per plant (Fig. 1). Also, within each competition treatment, intermittently dry conditions significantly reduced biomass (Fig. 1). Within each competition treatment, the presence of both hemiparasites significantly reduced biomass compared to pots without hemiparasites (Fig. 1), especially at medium and high competition intensity (Fig. S1). Overall, *Schedonorus* attained similar biomass under moist conditions with *Rhinanthus* as in pots under intermittently dry conditions without *Rhinanthus* (Fig. 1). The presence of litter increased *Schedonorus* biomass under both constantly wet and intermittently dry conditions and the combination of intermittently dry conditions with litter showed similar biomass as constantly wet conditions without litter (Fig. S2). There was no significant

Table 1 Mean soil moisture content (in % volumetric soil water content; \pm SE, $n = 10$) during the experiment under the different treatments

Factor	Levels	Sampling date		
		Immediately after watering	1-Day after watering	2-Days after watering
Competition	Low	34.93 \pm 1.24 ^a	29.35 \pm 0.91 ^b	13.70 \pm 1.21 ^b
	Medium	33.12 \pm 1.13 ^a	27.44 \pm 1.14 ^{ab}	9.98 \pm 0.63 ^a
	High	34.04 \pm 1.19 ^a	26.88 \pm 1.25 ^a	8.55 \pm 0.40 ^a
Watering	Wet	39.16 \pm 0.63 ^b	31.63 \pm 0.72 ^b	13.59 \pm 0.87 ^b
	Dry	28.79 \pm 0.71 ^a	24.15 \pm 0.78 ^a	7.99 \pm 0.24 ^a
Litter presence	Yes	36.95 \pm 0.89 ^b	32.12 \pm 0.68 ^b	12.12 \pm 0.85 ^b
	No	31.15 \pm 0.89 ^a	23.81 \pm 0.74 ^a	9.53 \pm 0.58 ^a

Different letters within each factor indicate significant differences among factor level means (Tukey test, $p < 0.05$)

Fig. 1 *Schedonorus* mean biomass per plant (in g per plant) in pots without hemiparasite species or with *Rhinanthus alectorolophus* (R. ale) or *Rhinanthus minor* (R. min) at different levels of competition and with presence or absence of a litter layer. Grey bars represent moist conditions and white bars represent dry conditions. Mean \pm 1 SE are shown



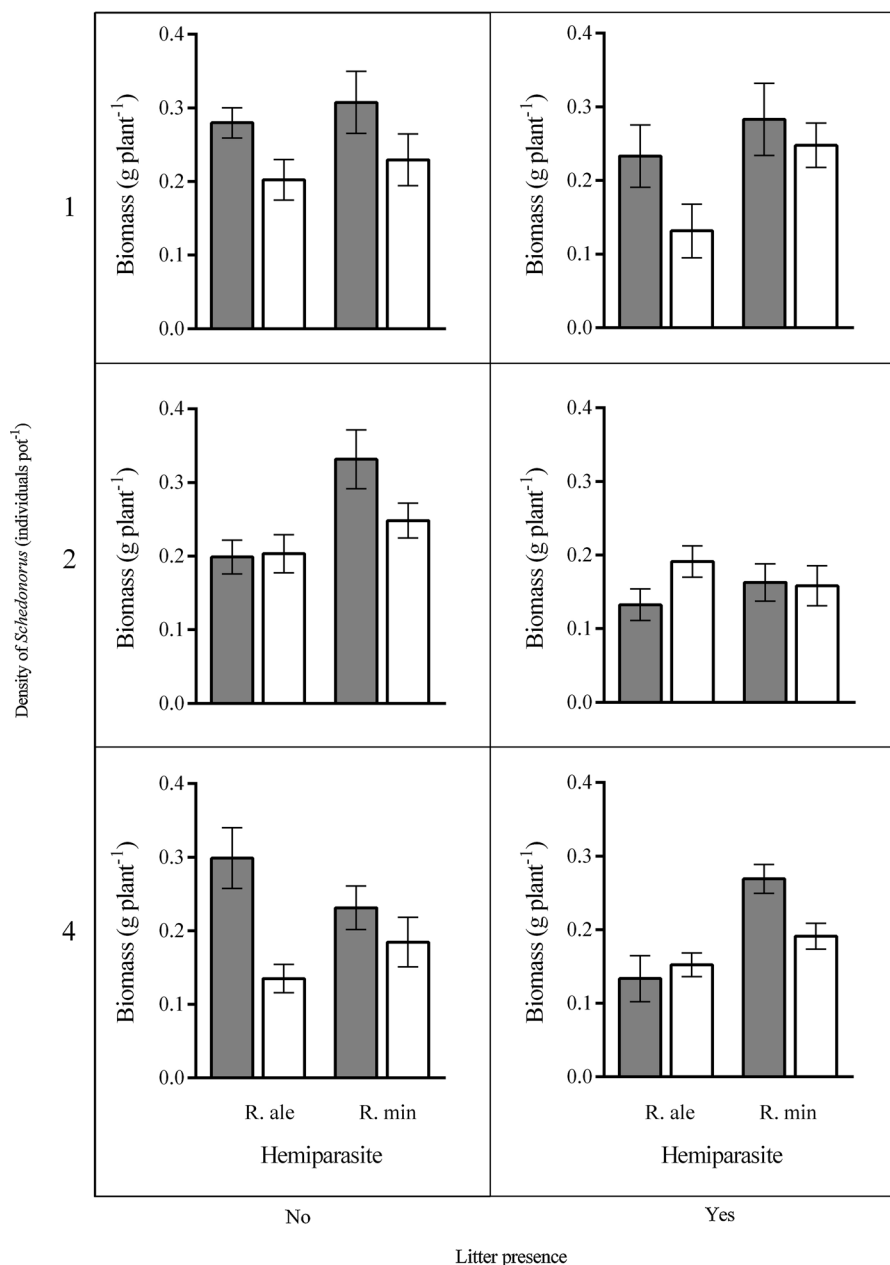
litter \times hemiparasite interaction ($p = 0.186$, Table S2), indicating that the presence of a litter layer did not compensate for negative effects of the hemiparasites on *Schedonorus* biomass.

Hemiparasite biomass

In comparison with *Schedonorus*, *Rhinanthus* biomass responded differently to various factors of the experiment (Table S4). The interaction among Competition \times Hemiparasite \times Litter was significant, showing a higher biomass in the absence of a litter

layer for all treatments, although the magnitude varied between species. In all cases, *R. minor* showed a higher biomass than *R. alectorolophus*. As in *Schedonorus*, dry conditions reduced *Rhinanthus* biomass, although this tended to happen at low competition levels ($p = 0.084$, Fig. 2). Unlike in *Schedonorus*, the presence of a litter layer reduced *Rhinanthus* biomass ($p = 0.0001$), although this was due to a higher biomass in constantly moist conditions without litter (Fig. 2).

Fig. 2 *Rhinanthus alectorolophus* (R. ale) and *Rhinanthus minor* (R. min) mean biomass per plant (in g per plant) at different levels of competition and with presence or absence of a litter layer. Grey bars represent moist conditions and white bars represent dry conditions. Mean \pm 1 SE are shown



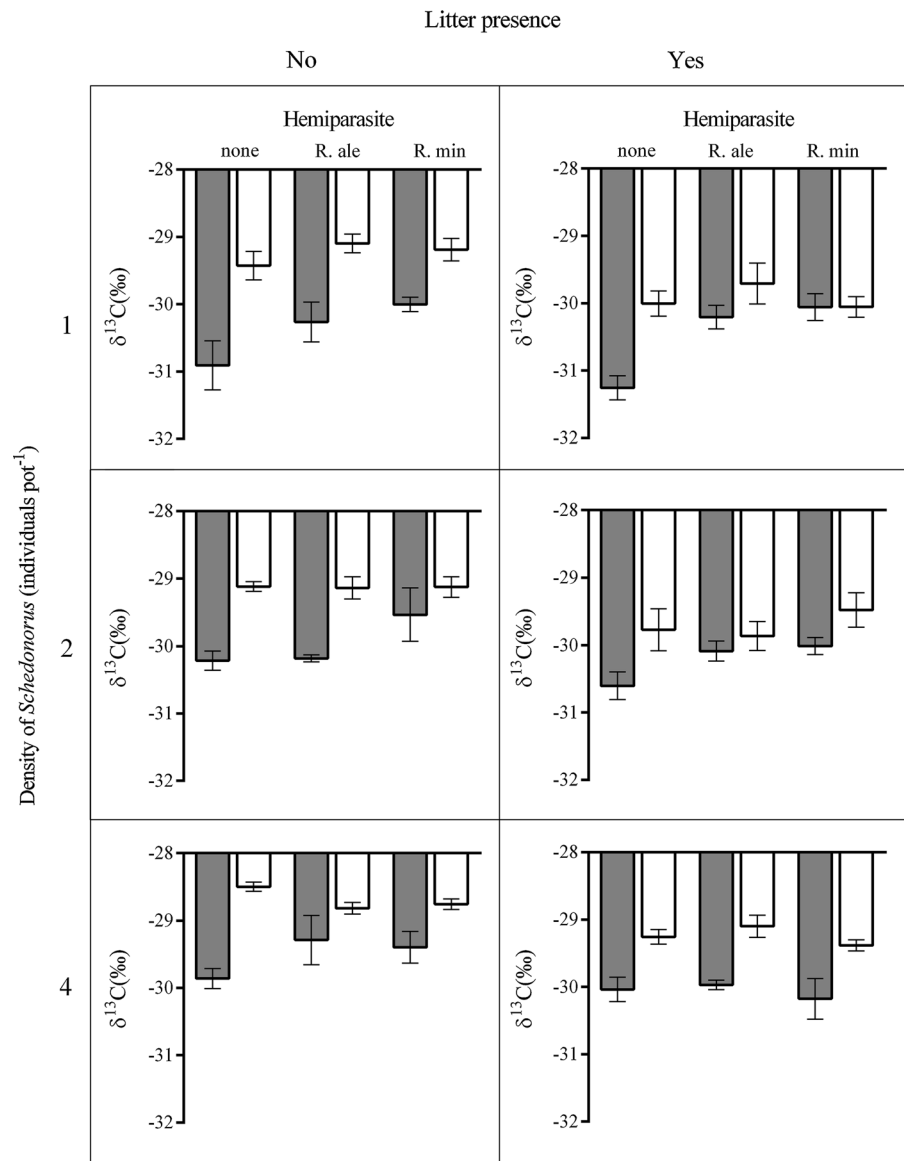
Schedonorus and *Rhinanthus*: ^{13}C isotope signatures

Intermittently dry conditions resulted in higher (less negative) $\delta^{13}\text{C}$ in *Schedonorus* leaves than constantly moist conditions, indicating relatively higher assimilation of ^{13}C (i.e. reduced isotope discrimination) in the former owing to stomata closure. The presence of hemiparasites did not affect $\delta^{13}\text{C}$ in dry pots. In

contrast, in constantly moist pots, the presence of any of the *Rhinanthus* species resulted in higher $\delta^{13}\text{C}$ indicating lower water availability due to hemiparasites (Fig. 3, Table S5).

When comparing different *Schedonorus* densities, the presence of *Rhinanthus* induced a water shortage at low competition levels (i.e. increased $\delta^{13}\text{C}$) but not at medium or high competition levels. In contrast, the presence of a litter layer improved water availability

Fig. 3 $\delta^{13}\text{C}$ values (in per mil) in *Schedonorus* leaf biomass in pots without hemiparasite species or with *Rhinanthus alectorolophus* (R. ale) or *Rhinanthus minor* (R. min) at different levels of competition and with presence or absence of a litter layer. Grey bars represent moist conditions and white bars represent dry conditions. Mean \pm 1 SE are shown



as shown by a reduction in $\delta^{13}\text{C}$ in both watering treatments (Fig. 3).

In the case of *Rhinanthus*, both species showed significantly higher (less negative) $\delta^{13}\text{C}$ under intermittently dry conditions ($\delta^{13}\text{C}$ under intermittently dry vs. moist conditions were -30.97‰ vs. -31.97‰ and -31.23‰ vs. -32.13‰ , for *R. alectorolophus* and *R. minor* respectively), indicating a water deficit. Across all other factors, there were also significant differences in $\delta^{13}\text{C}$ between low and high competition levels, but neither of these was different from medium competition levels.

Discussion

Our work showed that the presence of hemiparasite species had negative direct effects on *S. arundinaceus*, while presence of a litter layer had indirect positive effects. However, the strength of these effects changed with water availability and *Schedonorus* density.

Treatment effects on *Schedonorus arundinaceus*

Water stress reduces aboveground primary productivity and biomass (Knapp et al. 2002; Yahdjian and Sala

2006) and increases $\delta^{13}\text{C}$ in plant tissues (Moreno-Gutiérrez et al. 2012; Murphy and Bowman 2009). Both responses were observed in our experiment. Presence of hemiparasites reduced host biomass. We expected that *Rhinanthus* will also increase isotopic composition of host biomass, indicating higher water stress (Moreno-Gutiérrez et al. 2012). However, this was only found in well-watered pots. Intermittently dry pots had an overall higher $\delta^{13}\text{C}$ isotopic signature, but showed no differences in isotopic composition between pots with or without hemiparasites presence. Under dry conditions, *Rhinanthus* species had a direct effect reducing plant growth, but they were not an additional source of water stress. Thus, our data suggest that water stress through drought and through the presence of hemiparasites is not additive. Probably, the stress provoked in *Schedonorus* individuals due to the intermittently dry conditions was already above the threshold where additional water stress due to hemiparasites would change the $\delta^{13}\text{C}$ isotopic signature. Under natural conditions, during dry periods or in dry grasslands, the presence of hemiparasite species may probably not have any (or only weak) effects on vegetation, besides reducing available resources for growth (Ameloot et al. 2005; Jiang et al. 2010). Since even *Rhinanthus* growth is reduced under intermittently dry conditions, direct effects of hemiparasites on the host may be limited. Additionally, litter does not improve environmental conditions to a degree that compensates for *Rhinanthus* direct effects under dry conditions (but see below), or maybe *Rhinanthus* is not capable to reduce soil water availability for co-occurring species as we expected. Therefore, we hypothesised that in dry grasslands, the effectiveness of *Rhinanthus* species as a diversity-restoration tool is limited, but positive effects can be expected in mesic grasslands. This hypothesis should be empirically tested in future studies.

The presence of a litter layer may exert indirect positive effects on plants by slowing down the evaporation of water from the soil (Eckstein and Donath 2005). In our experiment, this effect lasted until the next watering event (see Table 1). Consequently, litter presence increased *Schedonorus* biomass by reducing drought stress. This observation is in line with the fact that *Schedonorus* biomass in intermittently dry pots covered with litter was similar to biomass in moist pots in the absence of a litter layer. However, isotopic composition between these two treatments was still different, with higher water stress

(i.e. higher $\delta^{13}\text{C}$ isotopic signature) in pots under intermittently dry conditions with a litter layer than in well-watered pots without litter (see Fig. 3). This indicates that *Schedonorus* in the intermittently dry pots was able to improve its water use efficiency slightly, which means a reduction of stomatal conductance in a range where carbon gain and, consequently, growth was not significantly slowed down. It is well known that litter increases soil moisture by acting as a barrier for water evaporation (Facelli and Pickett 1991), but this is the first time that this effect is demonstrated in terms of changes in carbon isotopic composition. Our results suggest that a litter layer cannot completely compensate intermittently dry conditions, but its presence may mitigate drought stress and thus counteract growth depression under reduced water availability. However, the role of litter in dry grasslands or during dry periods might be limited if dry conditions persist for too long.

The observed effect of competition in the experiment was already present at the beginning of the study, since the competition treatment was established 6 months before the start of the experiment. However, once the experiment started, our experimental approach did not produce a water deficit in the low competition treatment in the absence of *Rhinanthus* (Table 1), but there was an effect at medium or high levels of competition when water demand increased but water supply was kept constant. Also, the effect of *Rhinanthus* was different for the different competition treatments. In general, well-watered pots with *Rhinanthus* had the same biomass as intermittently dry pots without *Rhinanthus*, suggesting that *Rhinanthus* may have a strong effect on grass biomass (Hejerman et al. 2011) that will allow non-host species to compete with established grasses under natural conditions (Ameloot et al. 2005; Davies et al. 1997).

Treatment effects on *Rhinanthus* spp.

Rhinanthus biomass was similar for wet and dry conditions at medium and high densities of *Schedonorus*, where hemiparasite species may have acquired enough resources from the host (Gibson and Watkinson 1989; Westbury 2004). At low *Schedonorus* densities, one host individual was not enough to support optimal hemiparasite resource supply. Parasitism by several individuals on one host may limit available nutrients and constrain proper development

of the hemiparasite (Atsatt and Strong 1970). Contrary, higher density of *Schedonorus* individuals, and therefore higher biomass, did not translate into higher biomass of the hemiparasite species, probably because high host biomass incurs a competitive effect on the hemiparasite, most likely for light or space (see Loydi et al. 2015b). This is also reflected in the lower *Rhinanthus* biomass when a litter layer is present, since these pots were characterised by high *Schedonorus* biomass. Limited growth of the hemiparasites due to a reduced water supply was corroborated by higher $\delta^{13}\text{C}$ isotopic signature under intermittently dry conditions. However, there were no differences in carbon isotopic signature (or only minor differences) among levels of the other factors. This may suggest that the main effects of competitors or litter on *Rhinanthus* biomass were not mediated through changes in available water because carbon isotopic signature did not change. Instead, these changes suggest a competition effect between *Schedonorus* and *Rhinanthus* in pots with high *Schedonorus* densities. For example, Hejcman et al. (2011) reported that *R. minor* is affected by competition when aboveground productivity is high. On the other hand, litter may exert a negative mechanical effect on *Rhinanthus* at low *Schedonorus* densities, as reported by other authors (e.g. Mudrak et al. 2014). This direct negative effect of grass litter on *Rhinanthus* biomass may be partly the cause of higher *Schedonorus* biomass in these pots, being an indirect positive effect of litter on *Schedonorus* biomass.

Conclusions

In this work, we have shown some of the complex relations among biotic components of grassland habitats. Direct effects of hemiparasites and indirect effects of litter (acting through changes in environmental conditions) have opposite consequences for the growth of the widespread grass *S. arundinaceus*. Overall, litter seems to have a stronger effect on dominant grasses than presence of hemiparasites especially under dry conditions. Nonetheless, under well-watered conditions, hemiparasites may have a considerable effect on dominant vegetation without being affected by litter presence. Interactions between both factors (i.e. litter and hemiparasites) are complex: litter increases host biomass while reducing hemiparasite biomass, suggesting that litter accumulation in grassland may be a

benefit for the dominant vegetation, especially under dry conditions when the direct effect of hemiparasites is at the same time limited.

Acknowledgements This work was partly funded by the Alexander von Humboldt Foundation by means of a Georg-Forster-postdoctoral fellowship (A. L.). We thank Lena Kretz and Joseph Scholz-vom Hofe (both Giessen University) for invaluable help during the experiment.

References

- Adams M, Grierson P (2001) Stable isotopes at natural abundance in terrestrial plant ecology and ecophysiology: an update. *Plant Biol* 3:299–310
- Ameloot E, Verheyen K, Hermy M (2005) Meta-analysis of standing crop reduction by *Rhinanthus* spp. and its effect on vegetation structure. *Folia Geobot* 40:289–310
- Atsatt P, Strong DR (1970) The population biology of annual grassland hemiparasites. I. The host environment. *Evolution* 24:278–291
- Berg B (2000) Litter decomposition and organic matter turnover in northern forest soils. *For Ecol Manag* 133:13–22
- Bergelson J (1990) Life after death: site pre-emption by the remains of *Poa Annua*. *Ecology* 71:2157–2165
- Bu Z-J, Zheng X-X, Rydin H, Moore T, Ma J (2013) Facilitation vs. competition: does interspecific interaction affect drought responses in *Sphagnum*? *Basic Appl Ecol* 14:574–584
- Bullock JM, Pywell RF (2005) *Rhinanthus*: a tool for restoring diverse grassland? *Folia Geobot* 40:273–288
- Davies D, Graves J, Elias C, Williams P (1997) The impact of *Rhinanthus* spp. on sward productivity and composition: implications for the restoration of species-rich grasslands. *Biol Conserv* 82:87–93
- Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. *Annu Rev Ecol Syst* 33:507–559
- Deutsch E, Bork E, Willms W (2010) Soil moisture and plant growth responses to litter and defoliation impacts in Parkland grasslands. *Agric Ecosyst Environ* 135:1–9
- Eckstein RL, Donath TW (2005) Interactions between litter and water availability affect seedling emergence in four familial pairs of floodplain species. *J Ecol* 93:807–816
- Facelli JM, Pickett STA (1991) Plant litter: its dynamics and effects on plant community structure. *Bot Rev* 57:1–32
- Gibson D, Newman J (2001) *Festuca arundinacea* Schreber (F. elatior L. ssp. arundinacea (Schreber) Hackel). *J Ecol* 89:304–324
- Gibson C, Watkinson A (1989) The host range and selectivity of a parasitic plant: *Rhinanthus minor* L. *Oecologia* 78:401–406
- Hejcman M, Schellberg J, Pavlu V (2011) Competitive ability of *Rhinanthus minor* L. in relation to productivity in the Ren-gen Grassland Experiment. *Plant Soil Environ* 57:45–51
- Hovstad KA, Ohlson M (2008) Physical and chemical effects of litter on plant establishment in semi-natural grasslands. *Plant Ecol* 196:251–260

- Hovstad KA, Ohlson M (2009) Conspecific versus heterospecific litter effects on seedling establishment. *Plant Ecol* 204:33–42
- Inderjit, Seastedt TR, Callaway RM, Pollock JL, Kaur J (2008) Allelopathy and plant invasions: traditional, congeneric, and bio-geographical approaches. *Biol Invasions* 10:875–890
- Jensen K, Gutekunst K (2003) Effects of litter on establishment of grassland plant species: the role of seed size and successional status. *Basic Appl Ecol* 4:579–587
- Jiang F, Timergalina L, Kudoyarova G, Jeschke WD, Hartung W (2007) Growth and development of the facultative root hemiparasite *Rhinanthus minor* after removal of its host. *Funct Plant Biol* 34:237–245
- Jiang F, Jeschke WD, Hartung W, Cameron DD (2010) Interactions between *Rhinanthus minor* and its hosts: a review of water, mineral nutrient and hormone flows and exchanges in the hemiparasitic association. *Folia Geobot* 45:369–385
- Knapp AK, Fay PA, Blair JM, Collins SL, Smith MD, Carlisle JD, Harper CW, Danner BT, Lett MS, McCarron JK (2002) Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* 298:2202–2205
- Legendre P, Legendre L (1998) Numerical ecology. Elsevier Science BV, Amsterdam
- Levine JM (1999) Indirect facilitation: evidence and predictions from a riparian community. *Ecology* 80:1762–1769
- Lortie CJ, Brooker RW, Choler P, Kikvidze Z, Michalet R, Pugnaire FI, Callaway RM (2004) Rethinking plant community theory. *Oikos* 107:433–438
- Loydi A, Eckstein RL, Otte A, Donath TW (2013) Effects of litter on seedling establishment in natural and semi-natural grasslands: a meta-analysis. *J Ecol* 101:454–464
- Loydi A, Lohse K, Otte A, Donath TW, Eckstein RL (2014) Distribution and effects of tree leaf litter on vegetation composition and biomass in a forest-grassland ecotone. *J Plant Ecol* 7:264–275
- Loydi A, Donath TW, Eckstein RL, Otte A (2015a) Non-native species litter reduces germination and growth of resident forbs and grasses: allelopathic, osmotic or mechanical effects? *Biol Invasions* 17:581–595
- Loydi A, Donath TW, Otte A, Eckstein RL (2015b) Negative and positive interactions among plants: effect of competitors and litter on seedling emergence and growth of forest and grassland species. *Plant Biol* 17:667–675
- Moreno-Gutiérrez C, Dawson TE, Nicolás E, Querejeta JI (2012) Isotopes reveal contrasting water use strategies among coexisting plant species in a Mediterranean ecosystem. *New Phytol* 196:489–496
- Morin PJ (2009) Community ecology. Wiley, New York
- Mudrák O, Mládek J, Blázek P, Leps J, Dolezal J, Nekvapilová E, Tesitel J (2014) Establishment of hemiparasitic *Rhinanthus* spp. in grassland restoration: lessons learned from sowing experiments. *Appl Veg Sci* 17:274–287
- Murphy BP, Bowman DM (2009) The carbon and nitrogen isotope composition of Australian grasses in relation to climate. *Funct Ecol* 23:1040–1049
- Myers RJK, van Noordwijk M, Vityakon P (1997) Synchrony of nutrient release and plant demand: plant litter quality, soil environment and farmer management options. In: Cadisch G, Guiller K (eds) Driven by nature: plant litter quality and decomposition. CAB International, Wallingford, pp 215–229
- Napier JD, Mordecai EA, Heckman RW (2016) The role of drought-and disturbance-mediated competition in shaping community responses to varied environments. *Oecologia* 181:621–632
- Pärtel M, Bruun HH, Sammuli M (2005) Biodiversity in temperate European grasslands: origin and conservation. In: 13th international occasional symposium of the European Grassland Federation. Integrating efficient grassland farming and biodiversity: proceedings of the 13th international occasional symposium of the European Grassland Federation, pp 1–14
- Phoenix GK, Press MC (2005) Linking physiological traits to impacts on community structure and function: the role of root hemiparasitic Orobanchaceae (ex-Scrophulariaceae). *J Ecol* 93:67–78
- Press MC, Phoenix GK (2005) Impacts of parasitic plants on natural communities. *New Phytol* 166:737–751
- Pywell RF, Bullock JM, Walker KJ, Coulson SJ, Gregory SJ, Stevenson MJ (2004) Facilitating grassland diversification using the hemiparasitic plant *Rhinanthus minor*. *J Appl Ecol* 41:880–887
- Resco de Dios V, Weltzin JF, Sun W, Huxman TE, Williams DG (2014) Transitions from grassland to savanna under drought through passive facilitation by grasses. *J Veg Sci* 25:937–946
- Ruprecht E, Szabo A (2012) Grass litter is a natural seed trap in long-term undisturbed grassland. *J Veg Sci* 23:495–504
- Ruprecht E, Enyedi MZ, Eckstein RL, Donath TW (2010a) Restorative removal of plant litter and vegetation 40 years after abandonment enhances re-emergence of steppe grassland vegetation. *Biol Conserv* 143:449–456
- Ruprecht E, Józsa J, Ölvédi TB, Simon J (2010b) Differential effects of several “litter” types on the germination of dry grassland species. *J Veg Sci* 21:1069–1081
- Schmiede R, Ruprecht E, Eckstein RL, Otte A, Donath TW (2013) Establishment of rare flood meadow species by plant material transfer: experimental tests of threshold amounts and the effect of sowing position. *Biol Conserv* 159:222–229
- Spasojevic MJ, Suding KN (2011) Contrasting effects of hemiparasites on ecosystem processes: can positive litter effects offset the negative effects of parasitism? *Oecologia* 165:193–200
- Török P, Miglécz T, Valkó O, Kelemen A, Tóth K, Lengyel S, Tóthmérész B (2012) Fast restoration of grassland vegetation by a combination of seed mixture sowing and low-diversity hay transfer. *Ecol Eng* 44:133–138
- Violle C, Richarte J, Navas M-L (2006) Effects of litter and standing biomass on growth and reproduction of two annual species in a Mediterranean old-field. *J Ecol* 94:196–205
- Westbury DB (2004) *Rhinanthus minor* L. *J Ecol* 92:906–9027
- Wolfe A, Randle C, Liu L, Steiner K (2005) Phylogeny and biogeography of Orobanchaceae. *Folia Geobot* 40:115–134
- Yahdjian L, Sala OE (2006) Vegetation structure constrains primary production response to water availability in the Patagonian steppe. *Ecology* 87:952–962