The impact of dung on inter- and intraspecific competition of temperate grassland seeds

Tanja Milotić\textsuperscript{a,b,*} and Maurice Hoffmann\textsuperscript{a,b}

\textsuperscript{a} Terrestrial Ecology Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium (corresponding author)

\textsuperscript{b} Research Institute for Nature and Forest, Kliniekstraat 25, 1070 Brussels, Belgium

*Corresponding author: tanja.milotic@inbo.be

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Abstract

Questions: In temperate grasslands, seeds of numerous dry-fruited plant species are dispersed via ingestion and subsequent defecation by grazing animals. Depending on the herbivore species and season, dung pats may contain a large assemblage of conspecific or heterospecific seeds competing for space, light and nutrients in the space-limited environment of an individual dung pat. In an environment rich in nutrients, such as herbivore dung, the outcome of inter- and intraspecific competition might differ from situations where nutrients are limiting. Additionally, dung pats being small and spatially isolated habitats with very specific conditions may impact competitive interactions as well. Besides the plant-soil interactions on competition known from literature, the specific quality and structure of dung pats might provoke more complex interactions between different seed densities and species combinations.

Methods: We conducted a greenhouse competition experiment using three common perennial grassland species. *Agrostis stolonifera, Trifolium pratense* and *Trifolium repens* were used in two-species combinations with different proportions of each species and in monocultures. Seeds were sown in three seed densities (50, 150 and 250 seeds) and the effects of cattle and horse dung on establishment, growth and flowering were tested.

Results: Interactions, most probably attributable to interspecific competition, differed between species mixtures. Seeds sown in polycultures generally emerged sooner, but the resulting seedlings had lower relative growth rates compared with seeds sown in monocultures. Increased biomass was measured for each species when growing in polycultures while evidence for intraspecific competition was found in monocultures. *T. pratense* developed relatively more flowers when plants were growing in polycultures compared with monocultures. Few effects of seed densities were found, although higher seed densities led to lower establishment success in both monocultures and polycultures. Adding dung generally increased the time needed
emerge, relative growth rates and flowering, but decreased establishment success in monocultures.

Conclusions: Both seed density and the presence of dung shape the post-dispersal fate of seeds. While high seed densities imply a cost due to lower germinability, the nutritive environment of dung acts as a compensation resulting in faster growth and an increased investment in reproductive tissues.

Keywords: dispersal, dispersal success, dung, endozoochory, grassland species, interspecific competition, intraspecific competition, plant competition, post-dispersal seed fate

Running head: Seedling competition in dung
Introduction

Seed dispersal in general, and specifically endozoochory, has the potential to be advantageous to plants (1) by reducing mortality near the mother plant caused by density-dependent factors ('escape hypothesis') (Connell 1971, Janzen 1970); (2) by facilitating the establishment of new populations ('colonization hypothesis') (Howe & Smallwood 1982, Cain et al. 2000); and (3) by transporting seeds towards micro sites characterized by the specific narrow range of environmental conditions which favour establishment ('directed dispersal hypothesis') (D'hondt et al. 2012, Cavallero et al. 2012, Wenny 2001). In the past decades, numerous studies measured the endozoochorous dispersal potential of a wide array of dry-fruited grassland species (e.g., Malo & Suarez 1995b, Pakeman et al. 2002, Pakeman et al. 1999, Bakker & Olff 2003, Cosyns et al. 2005a & D'hondt & Hoffmann 2011), but few studies examine the next steps in plant establishment. The decisive step in seed dispersal depends on the post-dispersal success of seeds and includes the processes of secondary seed dispersal, seed predation, germination, establishment, growth and the outcome of competitive processes (Wang & Smith 2002, Nathan & Muller-Landau 2000, Traveset et al. 2001).

Seeds dispersed through endozoochory end up being deposited in a dung environment which can alter germination success and the subsequent establishment, growth and flowering (Milotić & Hoffmann 2016a, Ramos-Font et al. 2015). Fresh dung contains high concentrations of growth promoting macronutrients and essential trace minerals (Traveset et al. 2001, Haynes & Williams 1993, Lupwayi et al. 2000). However, it must be noted that only a fraction of this nutrient load is inorganic and readily available for plant uptake (Jørgensen & Jensen 1997). Furthermore, both the dung C: N: P stoichiometry and the rates of dung decomposition and nutrient release are closely related with herbivore diet and digestive physiology (Sitters et al. 2014). Factors such as humidity (Dickinson & Craig 1990, Dickinson et al. 1981), presence of dung fauna (Lovell & Jarvis 1996, Sitters et al. 2014), chemical composition of dung (Ouédraogo et al. 2004) and vegetation type at the deposition site (Shepherd et al. 2000) define the rate at which dung mineralizes in natural...
ecosystems. Besides the high nutrient content of dung, low concentrations of phytotoxic compounds have been measured in fresh dung which may alter the activity of enzymes regulating germination rate and seedling growth (Berendse & Möller 2009, Farnsworth 2008, Luna & Moreno 2009, Marambe et al. 1993). The specific moist conditions within dung pats may have a considerable impact on the early life stages of plants. The moisture content of dung pats is closely related to the decomposition stage and weather conditions (Dickinson et al. 1981) and may even greatly differ between various parts of the dung pat (Underhay & Dickinson 1978). As the concentration of inorganic nutrients increases and the phytotoxic compounds gradually disappear from decomposing dung, the nutritive environment of the dung pat is most useful in the growth phase of plants.

Furthermore, as most seeds contain a reserve of mineral and organic nutrients to nourish the embryo in its initial stages of establishment (Fenner and Thompson, 2005), the poor nutrient availability of fresh dung is expected to be of minor importance in the first life stages of plants. In addition to the effects of dung, precipitation may play a significant role in germination and seedling growth (Tjelele et al. 2015).

Dung pats often contain a high concentration of germinable seeds belonging to a wide range of species. In temperate dune grasslands, Cosyns et al. (2005a) for example found an average 463 seedlings per litre of cattle or horse dung (summer sampling) belonging to a total of 31 species. Seed density clearly varies with seed availability in the environment, herbivore type and size, and season (Cosyns et al. 2005a, Malo & Suarez 1995b) and a high variability of seed concentrations and species combinations have been found in dung. Seed dispersers may therefore not only affect plant fitness by the act of dispersal itself, but by the different combinations and densities they deposit seeds in dung (Loiselle 1990).

Despite the presumption that post-dispersal competition for resources modifies the establishment success of seedlings, post-dispersal competition is rarely quantified in endozoochory studies. As competing plants deprive each other of resources such as space, light, water and nutrients,
germination in an environment rich in nutrients, such as herbivore dung, might alter the outcome of the competition process compared to a situation where nutrients are limiting. Furthermore, complex interactions might exist between different seed densities, species combinations and the quality and structure of dung. Previous research measured increasing interspecific competition with artificial fertilisation levels (Gu et al. 2012), and faster germination times for seeds sown in highly competitive environments (Orrock & Christopher 2010). The specific environmental conditions in dung pats might therefore trigger a shift in species co-existing patterns which may locally result in an increase of small-scale species richness (Cosyns et al. 2006).

To contribute to improved understanding of at least some aspects of zoochorous dispersal, we conducted a competition experiment using three perennial grassland species with a comparable seed size (see Bruun & Poschlod 2006 and D’hondt & Hoffmann 2011). In an attempt to disentangle the effects of different species combinations and seed densities in the presence of dung with the effects of digestive processes in the gut, undigested seeds were used in an experiment in a controlled greenhouse environment. Two clover species (Trifolium pratense and Trifolium repens) with short germination times (with mean germination times of respectively 1.9±0.2 and 1.5±0.0 days measured for seeds of the same seed batch in Milotić & Hoffmann 2016c) and one grass species (Agrostis stolonifera) with moderate germination times (10.1±1.8 days in Milotić & Hoffmann 2016c) were used in two-species combinations with different proportions of each species and in monocultures.

Three seed densities were used to assess the effects of different competition levels on the establishment, growth, flowering and biomass of the experimental species. We studied the effects of adding dung of cattle and horses; two herbivore species with a different digestion strategy (respectively ruminants and hindgut fermenters) and which are commonly used in temperate European nature management.

In this study we seek an answer to the following questions:
1. How are inter- and intraspecific competition processes shaping the post-dispersal seed fate of different combinations of two early germinating legumes and one slower germinating grass species?

2. To what extent are these competitive processes affected by seed density over a gradient of low, medium and high seed density?

3. How does the dung of two herbivores with distinct digestive strategies affect seedling establishment and growth?
Materials and methods

Selected species and seed quality test

Three perennial species were used as model species in the experiments: *Agrostis stolonifera* L., *Trifolium pratense* L. and *Trifolium repens* L. All selected species are common in temperate European grasslands and have been found emerging from dung samples (e.g., Cosyns et al. 2005a) and were able to germinate in controlled feeding trials (e.g., Cosyns et al. 2005b, D'hondt & Hoffmann 2011). Seeds have been purchased in a webshop specialised in seeds of wild plants grown in a temperate climate (Berkshire, UK, www.herbiseed.com) as it was practically unfeasible to sample such large quantities of wild seeds in their natural habitat. As such, seeds of a high and homogeneous quality were used in the experiments, although eventual maternal effects were not completely ruled out using this approach. Seed quality was tested in a standardised lab environment in a germination trial during 60 days. For each species, 5 replicates were used and per replicate 50 seeds were sown in Petri dishes filled with 1% water agar substrate. Newly germinated seeds were counted daily during the first 30 days and once every two days in the last part of the trial. Mean time to germination (MTG) (Ranal & Garcia De Santana 2006) and the proportion of germinated seeds (germinability) were derived from the count data.

Experimental design

The establishment, biomass, growth and flowering of the selected species were assessed in three dung treatments: cattle and horse dung and a dung-free control treatment. Furthermore, different densities were used: 50 (low density), 150 (medium density) and 250 seeds (high density) and in different combinations of monocultures and polycultures in order to assess different levels of inter and intraspecific competition. Experimental seed densities were derived from the densities of seeds found germinating in cattle and horse dung in previous research conducted in temperate dune grasslands (Cosyns 2004). Polycultures consisted of 2 species which were sown in either equal (50%-50%) or unequal (20%-80% or 80%-20%) proportions of the total seed density of 50, 150 or 250 seeds
per replicate (Table 1). All possible combinations of species mixtures, dung treatments and seed
densities were made. Round plastic plant pots (diameter: 15 cm, height: 16 cm) with drainage holes
were filled with a 1:1 mixture of sand and compost. The compost was homogenised and mixed
before usage and contained nutrients for the first 6-8 weeks of plant growth (1.25 kg/m³ with N-P-K
concentrations of 14-16-18) according to the manufacturer (www.structural.be). Planting pots were
put in an unheated greenhouse. Temperature and relative humidity ranged between 15.2 °C and 24.7
°C, and 79.6 % and 96.7 % during the experiment. In order to reduce the influence of the specific
micro-climate at the edges, plastic rings (diameter: 9 cm, height: 5 cm) were inserted in the centre of
the plant pots. In each ring, intact seeds were sown either mixed with cattle or horse dung or on top
of the substrate in the control treatment. Each combination of dung treatments, seed densities and
species mixtures was replicated five times for each of the three species with a total of 540 pots in the
entire experiment (Table 1). Fresh dung was collected from 10-15 stabled cattle and horses fed on a
similar diet of pellet feed and hay in order to keep contamination with wild seeds minimal. The
sampled dung was mixed prior to application in order to obtain homogeneous cattle and horse dung
samples. In the dung treatments, rings were filled with 2 cm of either cattle or horse dung after
which the seeds were evenly mixed with the dung layer, while in the control treatment seeds were
put on top of the substrate. In addition to these control pots which served as a measure for the
effects of dung on plant establishment and growth and contained seeds, blank pots were installed as
a quality control measure. These blank pots had either a top layer of dung or bare substrate and the
emergence of any seedlings was monitored as a control for seed contamination originating from the
sand-compost mix, dung or surroundings. Experimental and blank pots were put in a complete
randomised design in order to minimise the impact of location-based environmental fluctuations.
Plant pots were watered manually on a daily basis and no artificial light was used. The experiment
started on May 30th 2012 when all species were sown and ended after 100 days. Emergence timing
was monitored by counting emerged seedlings every two days during the first two weeks of the
experiment and weekly thereafter. Height and flowering (if applicable) were measured after 40, 70
and 100 days. The height of the tallest individual of each species per pot was measured by lifting up
the hanging parts or tillers. Flowering was expressed as the sum of the number of flower buds,
flowers and fruits of all plants of each species present in each pot. After 100 days, plant
establishment was measured by counting the number of plants present at that moment in each pot
with a distinction between juvenile, flowering and non-flowering adult plants. Plants were harvested
and separated into below- and above-ground parts and dried to a constant weight at 65 °C after
which biomass was measured.

Data analysis

Emergence timing was analysed using a Cox proportional hazard model with dung type, seed density,
competing species and a competing species: proportion interaction term as covariates.

In each census interval, relative growth rates (RGR) were derived from the height of the tallest
individual of each species per pot:

\[ RGR = \frac{\ln(H_t) - \ln(H_{t-\Delta t})}{\Delta t} \]

where \( H_t \) is the maximum height at time \( t \), \( H_{t-\Delta t} \) is the height in the previous interval and \( \Delta t \) is the
length of the time interval (Hunt 1982). The effects of dung types and seed densities on RGR were
assessed using repeated measures ANOVAs with a proportion: competing species interaction term.
RGR was log\(_{10}\) transformed and Tukey posthoc tests were applied.

Establishment success in monoculture pots was expressed as the percentage of the sown seeds that
established to juvenile plants and flowering or non-flowering adults at the end of the 100-day
experiment. Two-way ANOVAs were used to analyse the effects of dung addition and seed densities
for each tested plant species. If needed to meet the assumptions for parametric tests, establishment
success logit transformed. Significantly differing dung types and seed densities were identified using
Tukey posthoc tests.
Competition between species in polycultures was expressed as relative neighbour effect (RNE) (Markham & Chanway 1996). Two sets of RNE values were calculated using relative establishment success or individual biomass in polycultures and monocultures as follows:

$$RNE = \frac{(M_{\text{mono}} - M_{\text{poly}})}{x}$$

with $M_{\text{mono}}$: mean establishment success or harvested biomass in monocultures, $M_{\text{poly}}$: mean establishment success or harvested biomass in polycultures and $x=M_{\text{mono}}$ if $M_{\text{mono}} > P_{\text{poly}}$; $x=M_{\text{poly}}$ if $M_{\text{poly}} > M_{\text{mono}}$

RNE values range between -1 and 1, with positive values indicating interspecific competition and negative values indicating facilitation between species (Weigelt & Jolliffe 2003). Differences in RNE between dung types, seed densities and competing species were assessed with ANOVAs with a proportion: competing species interaction term. t-tests were used to identify whether RNE differed significantly from 0.

The bivariate relationships between root and shoot biomass and between the average biomass of individual plants and flower counts were analysed using standardised major axis regressions (SMA) with plant species, dung types, competing species, seed densities and proportions as fixed factors. SMA is an alternative for classical regression techniques and is useful in situations where both the $x$ and $y$ dimensions are subjected to measurement errors (Warton et al. 2006). First, the data were tested for common slopes between species, dung types or seed densities. If the slopes did not differ, we tested for common elevations (or $y$-intercepts) between dung types as the lines fitted to the dung types may both represent a shift along their common slope and/or a shift in elevation (Warton et al. 2012). Models were tested for normality and residual distribution and if needed, data were log$_{10}$ transformed.
All analyses were performed using R version 3.3.1. (R Core Team 2016). The 'survival' package version 2.38-3 (Therneau, 2015) and the 'smatr' package version 3.4-3 (Warton et al. 2012) were used for respectively survival analyses and SMA regressions.
Results

In the seed quality test interspecific differences in germination timing were found ($F_{2,12} = 95.624$, $p<0.001$) as *Agrostis stolonifera* (MTG (se): 10.1 (1.8) d) needed more time compared to *Trifolium pratense* and *Trifolium repens* (MTG (se): respectively 1.9 (0.2) and 1.5 (0.0) d). Also, the proportion of germinated seeds after 60 days differed between species ($F_{2,12} = 191.001$, $p<0.001$) with the lowest germinability for *A. stolonifera* (22.0 (3.6) %) while nearly all *T. repens* seeds germinated (97.2 (0.4) %) and 88.8 (2.6) % of the seeds of *T. pratense*.

In the greenhouse experiments, all tested species emerged later when sown in dung ($z = -5.087$, $p<0.001$) (Fig. 1). Seed densities generally did not affect emergence timing ($z = -0.370$, $p = 0.711$), although *T. pratense* emerged slightly faster in high densities compared to low seed density ($z = 2.403$, $p = 0.016$). All species emerged later when sown in polycultures ($z = -2.329$, $p = 0.020$) and in most cases, emergence was faster in case seeds were dominant in a mixture ($z = 6.158$, $p<0.001$). No seedlings emerged in the blank pots which served as a proxy for the measurement of seed contamination.

For all tested species relative growth rate (RGR) was higher when seeds were sown in cattle or horse dung compared to the control treatment. *Trifolium pratense* and *T. repens* grew respectively 3 and 1.5 times faster when sown in cattle dung compared to horse dung ($F_{2,256} = 26.631$, $p<0.001$ and $F_{2,238} = 15.148$, $p<0.001$ respectively). RGR of *A. stolonifera* and *T. repens* decreased significantly over time. While seed densities and different species combinations did not differ in RGR, monocultures consistently had a higher RGR compared to polycultures irrespective of the species that were competing (Table 2).

In monocultures, the presence of dung resulted in lower establishment success, especially when seeds were sown in cattle dung (both *Trifolium* species) and overall establishment success decreased with increasing seed densities (Fig. 2, Appendix 1, Appendix S1). Significantly more seeds of the *Trifolium* species emerged compared to *A. stolonifera* ($F_{2,132} = 40.624; p<0.001$). Establishment success
of *A. stolonifera* and *T. pratense* seedlings in polycultures was altered by interspecific competition effects while the establishment of *T. repens* was not affected by the presence of competing species when sown in 50%-50% combinations with *A. stolonifera* (Fig. 3). Relative neighbour effects (RNE) were affected by the presence of dung, seed densities and competing species (Appendix, Appendix S2). More specifically, individual plant biomass was relatively higher in polycultures for all tested species. Furthermore, in most cases, differences between species combinations were found (Fig. 4, Appendix, Appendix S2). In *A. stolonifera* and *T. pratense* intraspecific competition was stronger when seeds were sown in horse dung compared to the control and cattle dung treatments while dung addition did not have any effect on *T. repens* RNE (Appendix, Appendix S2).

Species generally differed in root: shoot biomass allocation and *T. repens* invested more in aboveground biomass (Fig. 5, Appendix, Appendix S3 and A 4). The addition of cattle dung resulted in a lower root: shoot ratio in both *Trifolium* species compared to the control treatment. Furthermore, the root: shoot ratio was higher in monocultures compared to polycultures for *T. pratense* and *T. repens*. Proportionally more flowers developed on *A. stolonifera* in monocultures than in combination with *T. pratense* while the opposite effect was found for *T. pratense* as significantly more flowers appeared in polycultures compared to monocultures. Plants grown in cattle (both *Trifolium* species) and horse (*T. repens*) dung developed more flowers compared to the control treatment and relatively fewer flowers appeared in high densities of *T. pratense* (Fig. 5, Appendix, Appendix S5).
Adding dung generally increased the time to emergence, which is in line with the findings of previous research (Miller 1995, Meyer & Witmer 1998, Ramos-Font et al. 2015) and has been ascribed to the rather unfavourable environment of fresh dung. Animal excrements may contain toxic compounds with adverse effects on seed survival and germination (Marambe et al. 1993, Welch 1985). In some cases, dung facilitates fungal and bacterial growth with a mortal effect on seeds (Meyer & Witmer 1998, Traveset et al. 2007) while in other cases dung was found to protect seeds from being attacked by parasites and predators (Fragoso et al. 2003). On the other hand, once germinated, the growth of young seedlings is in some cases promoted in the dung environment, most probably due to the fertilising effect of this micro-environment (Traveset et al. 2001, Milotic & Hoffmann 2016a).

Furthermore, both the structural and chemical composition of dung is known to vary between herbivore species (e.g., between ruminants and non-ruminants (Holter 2016)) or with diet (e.g., brown bear dung composed of animal vs. vegetal material (Traveset et al. 2001)). According to Holter (2016), ruminant dung generally consists of smaller particles and has a higher C: N ratio while the water content is in the same order of magnitude. In our experiment, a hard crust developed soon after application of cattle dung while the appearance of the used horse dung remained structurally unchanged during the whole experimental period. The slower emergence rate and lower establishment success of seedlings in cattle dung might be related with this dry and hard top layer (Grellier et al. 2012). The structural composition and water potential of soils are further determinants of plant health and are known to differ greatly between soil types (MacDonald 1994). Plant growth is closely related to the availability of macro and micro nutrients and toxic elements in the soil environment (Mengel & Kirkby 1978). Therefore, the differing nutrient concentrations in ruminant and non-ruminant dung could result in a species-specific response in plant development (Dai 2000, Jorgensen & Jensen 1997). As fresh dung contains relatively high levels of toxic compounds and most nutrients are bound in organic compounds that need to be mineralized before
becoming available for plant uptake, dung might have adverse effects on the early life stages of plants (Cosyns et al. 2005, Ramos-Font et al., 2015). Furthermore, dung may also promote the growth of fungi and bacteria which may damage seeds and inhibit germination and seedling survival (Clark & Wilson 2003, Traveset et al. 2007).

As high seed densities generally have a negative impact on plant establishment, a selection pressure can be expected for tactics that overcome this competition, e.g., early germination or induced dormancy (Loiselle 1990, Murray 1998). Orrock and Christopher (2010) measured shorter germination times when intraspecific competition was greater in a bird-dispersed shrub and early germinated plants grew larger and had higher growth rates as they had more time to grow without competitors. Likewise examples of the advantages of early germination can be found in both intraspecific (e.g., Black & Wilkinson 1963) and interspecific (e.g., Bergelson & Perry 1989) seed mixtures while other species might postpone germination until the subsequent season in highly competitive environments (Turkington et al. 2005). In a lab experiment, Linhart (1976) measured an increased germination rate in *Trifolium* and *Agrostis* species when sown in high densities. Although we should note that we used different methods and species, we did not measure any effect of seed density on germination timing. Overall, high seed densities led to a reduced establishment success in monocultures which may be attributed to intraspecific competition or to the mechanism of autotoxicity that results in an inverse correlation between seed abundance and germination success (Barnea et al. 1992, Loiselle 1990, Murray 1998). Plants could be able to escape sibling competition in high densities through an adaptation of the proportion of dormant seeds and seeds that germinate instantaneously (Cheplick 1992, Hyatt & Evans 1998).

The functional equilibrium theory (Brouwer 1962) postulates that the allocation of biomass shifts towards roots when belowground resources (e.g., nutrient level and water) are low whereas shoot biomass increases in case aboveground resources (e.g., CO₂ and light) are limiting. Doing so, plants optimise their ability to compete for limiting resources (Poorter & Nagel 2000). Our results partly
support this theory, as both *Trifolium* species indeed had a higher root biomass in the control
treatment. Nevertheless, few interactions between dung treatments and seed densities were found
which is in contrast with the differing root: shoot biomass allocation in *Plantago lanceolata* with
varying seed densities and nutrient levels found by Berendse and Möller (2009) or the increased
belowground competition Aerts *et al.* (1991) found in fertilised heathland plants.

In all tested species individual plant biomass was relatively higher in polycultures which suggests that
intraspecific competition has stronger effects than interspecific competition. Higher biomass of
species in polycultures has been previously found in natural ecosystems and increases with
increasing nutrient levels (Gu *et al.* 2012, Li & Watkinson 2000). On the other hand, lower
establishment of *A. stolonifera* and *T. pratense* has been found in polycultures, so rather than
producing more biomass spread over a large number of individuals, the average biomass per
individual was greater in polyculture stands. In monocultures, both *Trifolium* species invested more
in belowground biomass which suggests that intraspecific competition is harsher than interspecific
competition in our species mixtures. Furthermore, through a symbiotic relationship with soil bacteria
of the genus *Rhizobia* in their root nodules both *Trifolium* species are able to bind atmospheric
nitrogen into ammonium and gain a competitive advantage. In grass-clover mixtures, Nesheim and
Boller (1991) noted an increased nitrogen fixation when clovers were growing in combination with
grasses whereas Ledgard and Steele (1992) measured reduced nitrogen fixation when N fertiliser was
used. In our experiments, we indeed measured a higher allocation to *Trifolium* root biomass in the
control treatments. The presence of dung following endozoochorous dispersal might, therefore,
change the competitive interactions of species mixtures, affect the development of root mutualists
or act as a potential source of inoculum. Furthermore, the reproductive effort decreases with
increasing seed density in *T. pratense* which could be related to the fact that individual plants are
smaller in high seed densities and can, therefore, allocate a smaller proportion of their biomass in
reproductive tissues (Weiner 1988). Our results suggest that the presence of dung and competition
affects endozoochorous dispersal success. However, we should keep in mind that we conducted our
experiment in a standardised greenhouse environment which differs from the natural situation. As we directly put dung on the substrate we did not account for competition by already established vegetation. Large herbivores deposit a rather large quantity of dung per defecation and their dung often smothers and kills the existing vegetation at the deposition site and consequently creates a gap for seedling establishment (Traveset 1998, Williams & Haynes 1995). In a dung deposition experiment in temperate dune grasslands, Cosyns et al. (2006) measured an increase in small-scale species richness as species co-existing patterns shifted from a dominance by monocotylous species towards a more diverse assemblage of dicotylous and monocotylous species. Therefore, dung pats not only seem to trigger inter- and intra-specific competition processes between their seed load, but also create safe sites in the existing vegetation enabling the germination and establishment of endozoochorously dispersed seeds.

Throughout the experiment we used a 2 cm thick layer of dung, which should enable the germination of the rather large seeded test species (see e.g., Grundy et al. 2003), but in natural situations cattle dung pats are frequently thicker than 2 cm (Malo & Suarez 1995a) and horses often exhibit latrine behaviour by which both dung and dispersed seeds becomes aggregated (Edwards & Hollis 1982). Furthermore, due to practical considerations, intact seeds were used while ingested seeds undergo a sequence of mechanical, thermal and chemical processes (Milotić & Hoffmann 2016b). A decreased germination success has been observed after gut passage in many species (e.g., Cosyns et al. 2005b). In a gut passage experiment using cattle, D'hondt and Hoffmann (2011) found an increased germination success of T. pratense while A. stolonifera seeds were less likely to germinate after gut passage. On the other hand, both of our tested Trifolium species had a lower germinability after being fed to horses and sheep (Cosyns et al. 2005b). Therefore, our results should rather be interpreted as the outcome of one particular step in the complex process of endozoochory, and we can assume that an even lower germination success would have been found when using ingested seeds.
We conclude that the variable concentrations and species combinations in dung pats may trigger processes of inter- and intraspecific competition and hence define the post-dispersal fate of endozoochorously dispersed seeds. While high seed densities imply a cost due to lower germinability and establishment, the nutritive environment of dung acts as a compensation in the later life stages of plants resulting in faster growth and an increased investment in reproductive tissues in competitive superior species.

Author’s contribution

Tanja Milotić and Maurice Hoffmann conceived and designed the experiment and wrote the paper, Tanja Milotić did all practical and analytical work.

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**Supporting information**

**Appendix S1** - Two-way ANOVA results with establishment success in monocultures as measured variable and dung type and seed density as nominal variables. Establishment success is expressed as the percentage of sown seeds that established to visible plants at the end of the experiment and was analysed for each size class. Significant results are written in bold.

**Appendix S2** - ANOVA results with RNE based on establishment success and biomass as measured variable and dung type, seed density and competing species as nominal variables and a nested proportion: competing species term.

**Appendix S3** - Likelihood ratios and p-values for the SMA regressions between root and shoot biomass and between flower count and total biomass by species with dung types, seed densities and competing species as factors.

**Appendix S4** - Standardized major axis (SMA) regression coefficients between root and shoot biomass by species and factor. Only factors which differ significantly after a likelihood test are listed (see **Appendix S3**). Factor levels marked with different letters differ significantly (p<0.05) after multiple comparisons with Šidák correction.

**Appendix S5** - Standardized major axis (SMA) regression coefficients between flower counts and total biomass by species and factor. Only factors which differ significantly after a likelihood test are listed (see **Appendix S3**). Factor levels marked with different letters differ significantly (p<0.05) after multiple comparisons with Šidák correction.
Table 1 - Experimental scheme with all treatment combinations of dung types, seed densities and species combinations. Numbers indicate the number of seeds of the focal species (in bold) and competing species (between brackets). Each combination was replicated 5 times.

<table>
<thead>
<tr>
<th>Dung type</th>
<th>Seed density</th>
<th>Focal species</th>
<th>Agrostis stolonifera</th>
<th>Trifolium pratense</th>
<th>Trifolium repens</th>
</tr>
</thead>
<tbody>
<tr>
<td>control, cattle or horse</td>
<td>low</td>
<td>Agrostis stolonifera</td>
<td>50 (0)</td>
<td>10 (40), 25(25) or 40(10)</td>
<td>10 (40), 25(25) or 40(10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trifolium pratense</td>
<td>10 (40), 25(25) or 40(10)</td>
<td>50 (0)</td>
<td>10 (40), 25(25) or 40(10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trifolium repens</td>
<td>10 (40), 25(25) or 40(10)</td>
<td>10 (40), 25(25) or 40(10)</td>
<td>50 (0)</td>
</tr>
<tr>
<td>medium</td>
<td></td>
<td>Agrostis stolonifera</td>
<td>150 (0)</td>
<td>30 (120), 75(75) or 120(30)</td>
<td>30 (120), 75(75) or 120(30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trifolium pratense</td>
<td>30 (120), 75(75) or 120(30)</td>
<td>150 (0)</td>
<td>30 (120), 75(75) or 120(30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trifolium repens</td>
<td>30 (120), 75(75) or 120(30)</td>
<td>30 (120), 75(75) or 120(30)</td>
<td>150 (0)</td>
</tr>
<tr>
<td>high</td>
<td></td>
<td>Agrostis stolonifera</td>
<td>250 (0)</td>
<td>50 (200), 125(125) or 200(50)</td>
<td>50 (200), 125(125) or 200(50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trifolium pratense</td>
<td>50 (200), 125(125) or 200(50)</td>
<td>250 (0)</td>
<td>50 (200), 125(125) or 200(50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trifolium repens</td>
<td>50 (200), 125(125) or 200(50)</td>
<td>50 (200), 125(125) or 200(50)</td>
<td>250 (0)</td>
</tr>
</tbody>
</table>
Table 2 - Repeated measures ANOVA results in order to evaluate the effect of dung, seed density, competing species and species proportion on relative growth rate (RGR).

<table>
<thead>
<tr>
<th>species</th>
<th>factor</th>
<th>df</th>
<th>F-value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agrostis stolonifera</em></td>
<td>dung</td>
<td>2</td>
<td>11.704</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>seed density</td>
<td>2</td>
<td>0.488</td>
<td>0.616</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>1</td>
<td>4.591</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>competing species</td>
<td>2</td>
<td>19.785</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>proportion</td>
<td>4</td>
<td>0.803</td>
<td>0.600</td>
</tr>
<tr>
<td></td>
<td>dung: seed density</td>
<td>4</td>
<td>0.697</td>
<td>0.600</td>
</tr>
<tr>
<td></td>
<td>dung: time</td>
<td>2</td>
<td>3.374</td>
<td>0.345</td>
</tr>
<tr>
<td></td>
<td>density: time</td>
<td>2</td>
<td>1.081</td>
<td>0.933</td>
</tr>
<tr>
<td><em>Trifolium pratense</em></td>
<td>dung</td>
<td>2</td>
<td>26.631</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>seed density</td>
<td>2</td>
<td>1.115</td>
<td>0.330</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>1</td>
<td>0.007</td>
<td>0.933</td>
</tr>
<tr>
<td></td>
<td>competing species</td>
<td>2</td>
<td>193.693</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>proportion</td>
<td>4</td>
<td>1.084</td>
<td>0.365</td>
</tr>
<tr>
<td></td>
<td>dung: seed density</td>
<td>4</td>
<td>2.566</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>dung: time</td>
<td>2</td>
<td>11.952</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>density: time</td>
<td>1</td>
<td>10.061</td>
<td>0.002</td>
</tr>
<tr>
<td><em>Trifolium repens</em></td>
<td>dung</td>
<td>2</td>
<td>15.148</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>seed density</td>
<td>2</td>
<td>0.162</td>
<td>0.851</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>1</td>
<td>9.910</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>competing species</td>
<td>2</td>
<td>24.325</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>proportion</td>
<td>4</td>
<td>0.489</td>
<td>0.744</td>
</tr>
<tr>
<td></td>
<td>dung: seed density</td>
<td>4</td>
<td>0.679</td>
<td>0.607</td>
</tr>
<tr>
<td></td>
<td>dung: time</td>
<td>1</td>
<td>8.058</td>
<td>0.005</td>
</tr>
</tbody>
</table>
Focal species proportion (%)

Agrostis stolonifera

Trifolium pratense

Trifolium repens

Competing species

- Agrostis stolonifera
- Trifolium pratense
- Trifolium repens

RNE establishment
all species  
- Agrostis stolonifera  
- Trifolium pratense  
- Trifolium repens

Trifolium repens dung types  
- control  
- cattle  
- horse

Trifolium pratense dung types  
- control  
- cattle  
- horse

Trifolium pratense competing species  
- Agrostis stolonifera  
- monoculture  
- Trifolium pratense  
- Trifolium repens

Trifolium pratense seed density  
- low  
- medium  
- high

Trifolium repens dung types  
- control  
- cattle  
- horse

Trifolium repens competing species  
- Agrostis stolonifera  
- monoculture  
- Trifolium pratense  
- Trifolium repens

Trifolium repens  
- flower count  
- individual biomass

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We conducted a competition experiment using three common perennial grassland species in different combinations and densities. By adding dung, we examined plant establishment, growth and flowering following endozoochory. High seed densities implied a cost due to a reduced establishment while the nutritive environment of dung acted as a compensation resulting in faster growth and an increased investment in reproductive tissues.
The impact of dung on inter- and intraspecific competition of temperate grassland seeds

Tanja Milotić and Maurice Hoffmann

Supporting information

Contents

Appendix S1 - Two-way ANOVA results with establishment success in monocultures as measured variable and dung type and seed density as nominal variables. Establishment success is expressed as the percentage of sown seeds that established to visible plants at the end of the experiment and was analysed for each size class. Significant results are written in bold.

Appendix S2 - ANOVA results with RNE based on establishment success and biomass as measured variable and dung type, seed density and competing species as nominal variables and a nested proportion: competing species term.

Appendix S3 - Likelihood ratios and p-values for the SMA regressions between root and shoot biomass and between flower count and total biomass by species with dung types, seed densities and competing species as factors.

Appendix S4 - Standardized major axis (SMA) regression coefficients between root and shoot biomass by species and factor. Only factors which differ significantly after a likelihood test are listed (see Appendix S3). Factor levels marked with different letters differ significantly (p<0.05) after multiple comparisons with Šidák correction.

Appendix S5 - Standardized major axis (SMA) regression coefficients between flower counts and total biomass by species and factor. Only factors which differ significantly after a likelihood test are listed (see Appendix S3). Factor levels marked with different letters differ significantly (p<0.05) after multiple comparisons with Šidák correction.
Appendix S1 - Two-way ANOVA results with establishment success in monocultures as measured variable and dung type and seed density as nominal variables. Establishment success is expressed as the percentage of sown seeds that established to visible plants at the end of the experiment and was analysed for each size class. Significant results are written in bold.

<table>
<thead>
<tr>
<th>Species</th>
<th>Factor</th>
<th>Df</th>
<th>All</th>
<th>Non-flowering</th>
<th>Flowering</th>
<th>Juvenile</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agrostis stolonifera</em></td>
<td>dung</td>
<td>2</td>
<td>F: 6.649; p=0.003</td>
<td>F: 2.726; p=0.079</td>
<td>F: 7.605; p=0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>seed density</td>
<td>2</td>
<td>F: 4.604; p=0.017</td>
<td>F: 1.515; p=0.233</td>
<td>F: 3.891; p=0.030</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dung: density</td>
<td>4</td>
<td>F: 1.872; p=0.137</td>
<td>F: 0.537; p=0.709</td>
<td>F: 1.905; p=0.131</td>
<td></td>
</tr>
<tr>
<td><em>Trifolium pratense</em></td>
<td>dung</td>
<td>2</td>
<td>F: 67.459; p&lt;0.001</td>
<td>F: 26.554; p&lt;0.001</td>
<td>F: 37.908; p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>seed density</td>
<td>2</td>
<td>F: 4.714; p=0.015</td>
<td>F: 5.799; p=0.007</td>
<td>F: 34.252; p&lt;0.001</td>
<td>F: 9.342; p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>dung: density</td>
<td>4</td>
<td>F: 1.542; p=0.211</td>
<td>F: 3.071; p=0.028</td>
<td>F: 14.701; p&lt;0.001</td>
<td>F: 1.189; p=0.332</td>
</tr>
<tr>
<td><em>Trifolium repens</em></td>
<td>dung</td>
<td>2</td>
<td>F: 33.246; p&lt;0.001</td>
<td>F: 0.006; p=0.994</td>
<td>F: 1.726; p=0.192</td>
<td></td>
</tr>
<tr>
<td></td>
<td>seed density</td>
<td>2</td>
<td>F: 4.063; p=0.026</td>
<td>F: 1.484; p=0.240</td>
<td>F: 2.020; p=0.147</td>
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</tr>
<tr>
<td></td>
<td>dung: density</td>
<td>4</td>
<td>F: 0.990; p=0.425</td>
<td>F: 0.349; p=0.843</td>
<td>F: 0.584; p=0.677</td>
<td></td>
</tr>
</tbody>
</table>
Appendix S2 - ANOVA results with RNE based on establishment success and biomass as measured variable and dung type, seed density and competing species as nominal variables and a nested proportion: competing species term.

<table>
<thead>
<tr>
<th>Focal species</th>
<th>Factor</th>
<th>df RNE&lt;sub&gt;establishment&lt;/sub&gt;</th>
<th>F</th>
<th>p</th>
<th>df RNE&lt;sub&gt;biomass&lt;/sub&gt;</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agrostis stolonifera</em></td>
<td>dung type</td>
<td>2</td>
<td>3.444</td>
<td>0.034</td>
<td>2</td>
<td>3.750</td>
<td>0.034</td>
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<tr>
<td></td>
<td>seed density</td>
<td>2</td>
<td>115.2</td>
<td>&lt;0.001</td>
<td>2</td>
<td>7.078</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>competing species</td>
<td>1</td>
<td>3.251</td>
<td>0.073</td>
<td>1</td>
<td>4.420</td>
<td>0.043</td>
</tr>
<tr>
<td></td>
<td>proportion</td>
<td>4</td>
<td>8.661</td>
<td>&lt;0.001</td>
<td>3</td>
<td>3.586</td>
<td>0.024</td>
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<tr>
<td></td>
<td>dung : density</td>
<td>4</td>
<td>110.9</td>
<td>&lt;0.001</td>
<td>4</td>
<td>1.610</td>
<td>0.194</td>
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<tr>
<td><em>Trifolium pratense</em></td>
<td>dung type</td>
<td>2</td>
<td>8.727</td>
<td>&lt;0.001</td>
<td>2</td>
<td>27.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>seed density</td>
<td>2</td>
<td>5.536</td>
<td>0.004</td>
<td>2</td>
<td>20.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>competing species</td>
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<td>9.740</td>
<td>0.002</td>
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<td>6.360</td>
<td>0.013</td>
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<tr>
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<td>proportion</td>
<td>4</td>
<td>4.689</td>
<td>0.001</td>
<td>4</td>
<td>1.423</td>
<td>0.230</td>
</tr>
<tr>
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<td>dung : density</td>
<td>4</td>
<td>2.845</td>
<td>0.025</td>
<td>4</td>
<td>15.3</td>
<td>&lt;0.001</td>
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<tr>
<td><em>Trifolium repens</em></td>
<td>dung type</td>
<td>2</td>
<td>55.5</td>
<td>&lt;0.001</td>
<td>2</td>
<td>0.240</td>
<td>0.787</td>
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<tr>
<td></td>
<td>seed density</td>
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<td>27.62</td>
<td>&lt;0.001</td>
<td>2</td>
<td>3.309</td>
<td>0.039</td>
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<tr>
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<td>competing species</td>
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<td>7.591</td>
<td>0.006</td>
<td>1</td>
<td>0.264</td>
<td>0.608</td>
</tr>
<tr>
<td></td>
<td>proportion</td>
<td>4</td>
<td>1.258</td>
<td>0.287</td>
<td>4</td>
<td>2.801</td>
<td>0.028</td>
</tr>
<tr>
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<td>dung : density</td>
<td>4</td>
<td>11.836</td>
<td>&lt;0.001</td>
<td>4</td>
<td>5.114</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Appendix S3 - Likelihood ratios and p-values for the SMA regressions between root and shoot biomass and between flower count and total biomass by species with dung types, seed densities and competing species as factors.

<table>
<thead>
<tr>
<th>species</th>
<th>factor</th>
<th>likelihood ratio (df)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>root: shoot ratio</td>
<td>all</td>
<td>species 72.66 (2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Agrostis stolonifera</td>
<td>dung types 0.16 (2)</td>
<td>0.923</td>
</tr>
<tr>
<td></td>
<td></td>
<td>seed density 1.44 (2)</td>
<td>0.486</td>
</tr>
<tr>
<td></td>
<td></td>
<td>competing species 4.10 (2)</td>
<td>0.129</td>
</tr>
<tr>
<td></td>
<td>Trifolium pratense</td>
<td>dung types 6.86 (2)</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td></td>
<td>seed density 1.47 (2)</td>
<td>0.480</td>
</tr>
<tr>
<td></td>
<td></td>
<td>competing species 100 (2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Trifolium repens</td>
<td>dung types 11.82 (2)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>seed density 2.28 (2)</td>
<td>0.319</td>
</tr>
<tr>
<td></td>
<td></td>
<td>competing species 7.74 (2)</td>
<td>0.021</td>
</tr>
<tr>
<td>flower count: biomass</td>
<td>Agrostis stolonifera</td>
<td>dung types 0.14 (2)</td>
<td>0.932</td>
</tr>
<tr>
<td></td>
<td></td>
<td>seed density 3.05 (2)</td>
<td>0.217</td>
</tr>
<tr>
<td></td>
<td></td>
<td>competing species 7.44 (2)</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>Trifolium pratense</td>
<td>dung types 12.76 (2)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>seed density 8.88 (2)</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>competing species 26.49 (2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Trifolium repens</td>
<td>dung types 16.66 (2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>seed density 1.12 (2)</td>
<td>0.572</td>
</tr>
<tr>
<td></td>
<td></td>
<td>competing species 1.79 (2)</td>
<td>0.409</td>
</tr>
</tbody>
</table>
Appendix S4 - Standardized major axis (SMA) regression coefficients between root and shoot biomass by species and factor. Only factors which differ significantly after a likelihood test are listed (see Appendix S3). Factor levels marked with different letters differ significantly (p<0.05) after multiple comparisons with Šidák correction.

<table>
<thead>
<tr>
<th>Species, factor</th>
<th>Factor level</th>
<th>n</th>
<th>$\alpha_{SMA}$ (95% CI)</th>
<th>$\beta_{SMA}$ (95% CI)</th>
<th>$r^2$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>all</td>
<td></td>
<td>453</td>
<td>-0.47 (-0.55; -0.38)</td>
<td>0.81 (0.77; 0.85)</td>
<td>0.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$^a$ Agrostis stolonifera</td>
<td></td>
<td>70</td>
<td>-0.55 (-0.80; -0.32)</td>
<td>1.02 (0.89; 1.17)</td>
<td>0.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$^a$ Trifolium pratense</td>
<td></td>
<td>187</td>
<td>-0.73 (-0.93; -0.54)</td>
<td>0.91 (0.84; 0.99)</td>
<td>0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$^b$ Trifolium repens</td>
<td></td>
<td>196</td>
<td>-0.21 (-0.29; -0.13)</td>
<td>0.59 (0.55; 0.64)</td>
<td>0.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$^a$ Trifolium pratense dung types</td>
<td>control$^a$</td>
<td>42</td>
<td>-0.82 (-1.10; -0.53)</td>
<td>1.04 (0.93; 1.16)</td>
<td>0.80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$^b$ cattle$^b$</td>
<td></td>
<td>16</td>
<td>-0.64 (-0.96; -0.32)</td>
<td>0.83 (0.73; 0.95)</td>
<td>0.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$^b$ horse$^b$</td>
<td></td>
<td>12</td>
<td>-1.12 (-1.54; -0.70)</td>
<td>1.02 (0.88; 1.18)</td>
<td>0.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>competing species</td>
<td>monoculture$^a$</td>
<td>45</td>
<td>-0.05 (-0.24; 0.15)</td>
<td>0.80 (0.74; 0.86)</td>
<td>0.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$^b$ Agrostis stolonifera</td>
<td></td>
<td>79</td>
<td>-0.62 (-0.79; -0.45)</td>
<td>0.80 (0.74; 0.86)</td>
<td>0.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$^c$ Trifolium repens</td>
<td></td>
<td>63</td>
<td>-0.47 (-0.62; -0.32)</td>
<td>0.80 (0.74; 0.86)</td>
<td>0.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$^a$ Trifolium repens dung types</td>
<td>control$^a$</td>
<td>70</td>
<td>-0.14 (-0.23; -0.05)</td>
<td>0.61 (0.56; 0.66)</td>
<td>0.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$^b$ cattle$^b$</td>
<td></td>
<td>59</td>
<td>-0.31 (-0.43; -0.20)</td>
<td>0.61 (0.56; 0.66)</td>
<td>0.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$^b$ horse$^b$</td>
<td></td>
<td>67</td>
<td>-0.26 (-0.36; -0.15)</td>
<td>0.61 (0.56; 0.66)</td>
<td>0.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>competing species</td>
<td>monoculture$^a$</td>
<td>25</td>
<td>-0.32 (-0.61; -0.03)</td>
<td>0.74 (0.60; 0.91)</td>
<td>0.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$^{ab}$ Agrostis stolonifera</td>
<td></td>
<td>88</td>
<td>-0.25 (-0.39; -0.11)</td>
<td>0.59 (0.53; 0.68)</td>
<td>0.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$^b$ Trifolium pratense</td>
<td></td>
<td>83</td>
<td>-0.12 (-0.21; -0.02)</td>
<td>0.53 (0.48; 0.59)</td>
<td>0.76</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
**Appendix S5** - Standardized major axis (SMA) regression coefficients between flower counts and total biomass by species and factor. Only factors which differ significantly after a likelihood test are listed (see **Appendix S3**). Factor levels marked with different letters differ significantly (p<0.05) after multiple comparisons with Šidák correction.

<table>
<thead>
<tr>
<th>Species, factor</th>
<th>Factor level</th>
<th>n</th>
<th>$\alpha_{\text{SMA}}$ (95% CI)</th>
<th>$\beta_{\text{SMA}}$ (95% CI)</th>
<th>$r^2$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>all</td>
<td></td>
<td>453</td>
<td>-1.49 (-1.74; -1.24)</td>
<td>1.37 (1.27; 1.47)</td>
<td>0.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Agrostis stolonifera</em> competing species</td>
<td>monoculture$^a$</td>
<td>19</td>
<td>-2.24 (-3.69; -0.79)</td>
<td>1.88 (1.30; 2.72)</td>
<td>0.45</td>
<td>0.002</td>
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<tr>
<td></td>
<td><em>Trifolium pratense</em>$^a$</td>
<td>22</td>
<td>-0.54 (-1.10; 0.02)</td>
<td>0.98 (0.72; 1.34)</td>
<td>0.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td><em>Trifolium repens</em>$^{ab}$</td>
<td>29</td>
<td>-1.81 (-2.71; -0.91)</td>
<td>1.45 (1.12; 1.88)</td>
<td>0.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Trifolium pratense</em> dung types</td>
<td>control$^a$</td>
<td>42</td>
<td>-8.04 (-11.25; -4.82)</td>
<td>0.76 (0.63; 0.93)</td>
<td>0.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>cattle$^b$</td>
<td>16</td>
<td>-10.93 (-18.71; -3.15)</td>
<td>1.30 (1.05; 1.61)</td>
<td>0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>horse$^{ab}$</td>
<td>12</td>
<td>-9.16 (-17.18; -1.13)</td>
<td>1.02 (0.79; 1.32)</td>
<td>0.09</td>
<td>0.026</td>
</tr>
<tr>
<td>seed density</td>
<td>low$^a$</td>
<td>61</td>
<td>-9.68 (-15.98; -3.38)</td>
<td>1.12 (0.90; 1.39)</td>
<td>0.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>medium$^a$</td>
<td>63</td>
<td>-15.82 (-24.13; -7.51)</td>
<td>1.40 (1.12; 1.74)</td>
<td>0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>high$^b$</td>
<td>63</td>
<td>-7.91 (-13.25; -2.56)</td>
<td>0.86 (0.69; 1.09)</td>
<td>0.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>competing species</td>
<td>monoculture$^a$</td>
<td>45</td>
<td>-12.92 (-19.04; -6.79)</td>
<td>0.58 (0.43; 0.78)</td>
<td>0.04</td>
<td>0.186</td>
</tr>
<tr>
<td></td>
<td><em>Agrostis stolonifera</em>$^b$</td>
<td>79</td>
<td>-6.85 (-10.93; -2.78)</td>
<td>1.27 (1.11; 1.45)</td>
<td>0.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td><em>Trifolium repens</em>$^{ab}$</td>
<td>63</td>
<td>-10.65 (-15.30; -6.01)</td>
<td>1.42 (1.21; 1.66)</td>
<td>0.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Trifolium repens</em> dung types</td>
<td>control$^a$</td>
<td>70</td>
<td>-3.51 (-5.91; -1.11)</td>
<td>1.56 (1.25; 1.95)</td>
<td>0.15</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>cattle$^b$</td>
<td>59</td>
<td>-9.54 (-15.03; -4.04)</td>
<td>2.40 (2.02; 2.86)</td>
<td>0.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>horse$^b$</td>
<td>67</td>
<td>-10.33 (-14.78; -5.89)</td>
<td>2.77 (2.35; 3.27)</td>
<td>0.55</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>