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Short communication

# Fungal-mediated mortality explains the different effects of dung leachates on the germination response of grazing increaser and decreaser species☆

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ABSTRACT

Depending on their response to grazing, grassland species can be categorized as grazing increasers or decreasers. Grazing by livestock includes several different activities that can impact species differently. Recent evidence suggest that one of these actions, dung deposition, can reduce the germinative performance of decreaser species, thus favouring increasers. The present study tested the hypothesis that decreased germinative success of decreaser species is caused by a greater activity of fungal pathogens under the influence of dung leachates. We performed a phytotron experiment analysing the germination and fungal infections of fourteen species from Mediterranean grasslands. Species were grouped into phylogenetically-related pairs, composed of an increaser and a decreaser species. Seeds of each species were germinated under four different treatments (control, dung leachate addition, fungicide addition and dung leachate and fungicide addition), and the differences in germination percentage, germination speed and infection rate between each increaser species and its decreaser counterpart were analysed. Decreaser species were more affected by mortality than increaser ones, and these differences were higher under the presence of dung leachates. The differences in germinative performance after excluding the effect of seed mortality did not differ between treatments, showing that the main mechanism by which dung leachates favour increaser species is through increased mortality of the seeds of decreaser species. Drastic reductions in the number of dead seeds in the treatments including fungicide addition further revealed that fungal pathogens are responsible for these differences between species with different grazing response. The different vulnerabilities of increaser and decreaser species to the increased activity of fungal pathogens under the presence of dung leachates seems the main reason behind the differential effect of these leachates on species with different grazing response.


## 1. Introduction

Grazing by domestic livestock has substantial effects on the structure and composition of herbaceous plant communities (Noy-Meir et al., 1989; Peco et al., 2005). Depending on their response to grazing in terms of presence or abundance, several grassland species are categorized as grazing increasers or decreasers (Vesk and Westoby, 2002). Although grazing activity is generally perceived as a single action, it is composed of different activities, such as defoliation, trampling and faeces and urine deposition (Dobarro et al., 2013). Each component of livestock activity can potentially have a specific impact on different plant species, ultimately leading to differences in the relative abundances of many species between grazed and non-grazed areas (Del-Val and Crawley, 2005; Díaz et al., 2007; Kohler et al., 2006, 2004). A deeper understanding of the mechanisms that lead to the different responses of plant species under the different components of grazing is an essential step to understand and predict the consequences that changes in grazing regimes have on grassland diversity.

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Among the components of livestock activity, defoliation and trampling seem to be the ones with the greatest impacts (Dobarro et al., 2013; Kohler et al., 2006, 2004). In accordance, a majority of the research aiming at characterizing the mechanisms leading to the different grazing responses of increaser and decreaser species has focused on the effects of defoliation. Experimental evidence suggests that increaser species have a greater tolerance to defoliation (Del-Val and Crawley, 2004, 2005) and higher relative growth rates (Leoni et al., 2009) than decreaser species. However, although the reproductive stage is often neglected when studying differences in grazing response between species, germination and seedling establishment are fundamental determinants of the grassland specific composition with a great abundance of annual plants, such as Mediterranean ones (Espigares and Peco, 1995; Marañón, 1998; Peco et al., 2009). Therefore, studying the effects of grazing on the germination and establishment stages may provide clues about the influence of livestock in Mediterranean grasslands.

Recent studies have shown that the seeds of grazing increaser and decreaser species respond differently under conditions associated to grazing activities. For example, the germination rates of increaser species are reduced when the spectral composition of light indicates the presence of potential competitors (Dobarro et al., 2010). In addition, Carmona et al. (2013) showed that the proportion of decreaser species seeds that germinate, as well as their germination speed, declined under the presence of dung leachates. By contrast, the seeds of increaser species were not affected by dung leachates (Carmona et al., 2013). Most importantly, changes in germination were not proportional to leachate concentrations, but rather displayed marked thresholds, suggesting that the effect of dung leachates on germination did not depend on its concentration but on its mere presence or absence (Carmona et al., 2013). Such a response was apparently consistent with greater pathogen activity in the leachates environment, which would affect the two kinds of species in different ways.

Fungi are considered as the most important pathogens for seeds (Baskin and Baskin, 2001; Kirkpatrick and Bazzaz, 1979; Kremer, 1993), reducing their survival and germination rates (Blaney and Kotanen, 2001; Crist and Friese, 1993; Schafer and Kotanen, 2004). The abundance and composition of fungal communities is context-dependent; several factors affect soil fungi, including soil temperature, moisture or litter characteristics (Dalling et al., 2011; Mordecai, 2012; Ruprecht et al., 2008; Schafer and Kotanen, 2003). Among these factors, grazing by large herbivores increases the biomass and affects the composition of soil fungal communities (Bardgett and Leemans, 1997; Jirout et al., 2011). Further, not all plant species are equally sensitive to fungal attacks (Leishman et al., 2000; Orrock and Damschen, 2005), a feature that can eventually translate into important differences in their abundances and distributions (Gallery et al., 2010). In this context, the combination of grazing-mediated changes in fungal communities and species-specific seed susceptibilities to fungal attack may be one of the determinants of the differences in composition of grazed and ungrazed sites.

In this paper, we present an experiment aimed at discerning whether the different effects of dung leachates on the germination response (germination percentage and speed) of increaser and decreaser species described in Carmona et al. (2013) are caused by different susceptibilities to fungal pathogens. Because phylogeny can influence the grazing response and susceptibility to fungal attack, we select pairs of confamilial species with contrasting responses to grazing. Specifically, we hypothesize that the rate of fungal infections on the seeds of grazing decreaser species will be higher under a treatment of dung leachates, whereas increaser species will be less susceptible to these conditions. If our hypothesis is correct, differences in seed fungal infections between species with different grazing responses will increase in favour of increasers under the presence of cattle dung leachates, but this increase will be largely reduced or eliminated with the addition of fungicide. Finally, if fungal attacks merely affect seed viability, but not the germination response of the surviving seeds, the differences observed in Carmona et al. (2013) should disappear once that seed mortality due to fungal attacks is taken into account.

## 2. Material and methods

In July 2010, we collected seeds of 14 herbaceous species belonging to five different families in Mediterranean grasslands situated 35 km North of Madrid, Spain (40°43′N, 3°43′W, zone description in Peco et al., 2006). We selected the same species used in a previous study that found differences in the effects of cattle dung leachates on the germination of seeds of increaser and decreaser species (Carmona et al., 2013). To control for the effect of phylogeny, species were organized into 4 confamilial and 3 congeneric pairs, each one containing a grazing increaser and a grazing decreaser species (Carmona et al., 2013): *Brassica barrelieri* (L.) Janka paired with *Alyssum granatense* Boiss. and Reuter (*Brassicaceae*); *Spergularia purpurea* (Pers.) D. Don with *Silene scabriflora* Brot. (*Caryophyllaceae*); *Astragalus pelecinus* (L.) Barneby with *Vicia lathyroides* L. (*Fabaceae*); *Poa annua* L. with *Micropyrum tenellum* (L.) Link (*Poaceae*); *Trifolium glomeratum* L. with *Trifolium strictum* L (*Fabaceae*); *Plantago coronopus* L. with *Plantago lanceolata* L. (*Plantaginaceae*); and *Vulpia muralis* with *Vulpia ciliata* Dumort (*Poaceae*). The classification of species to each grazing response group was made according to abundance data from grazed and ungrazed plots in the same area (Carmona et al., 2015, 2012; Peco et al., 2006, 2005). Only species with significant differences between grazed and ungrazed plots and present in more that 10% of the plots were used (see Appendix 1 for a more detailed description of the methods used for this classification).

In January 2011, we collected 3 kg of fresh cattle dung from 30 different dung pats from the area where seeds were collected. After two weeks of drying in a greenhouse, the dung samples were cut-up in small pieces, and thoroughly mixed together. Then, we crumbled the dry dung and recorded its weight. After that, we added 3 L of distilled water per kilogram of dung and placed the resulting mixture in a 9 cm diameter plunger coffee-maker and applied a 0.2 kg/cm$^2$ pressure for 30 s, obtaining a highly concentrated dung leachate. The proportion of water added is similar to that of fresh dung (Dickinson et al., 1981) and therefore, we expect this leachate concentration to be equivalent to the maximal concentration of dung leachates under field conditions. Finally, we diluted this leachate adding 2.5 parts of distilled water for each part of dung leachate in order to get a leachate concentration similar to the 10% concentration of Carmona et al. (2013). This leachate concentration maximizes differences in germination and seedling development between species with differing responses to grazing (Carmona et al., 2013), and is therefore optimal to analyse the effect of dung leachates on germination.

To test for the differential effects of fungi on increaser and decreaser species, we produced four different treatments: (1) Excrement treatment (E) consisting in the dung leachate described above; (2) Excrement and Fungicide treatment (EF) consisting in the addition of the 1.5 g of the fungicide BELPRON C-50 to each 100 g of the dung leachate; (3) a Control (C) consisting in distilled water; finally, (4) a water and Fungicide treatment (CF), consisting in a 1.5% w/w dilution of fungicide in distilled water, was used in order to detect any possible deleterious effect of fungicide on the measured variables. Any significant difference between the C and CF treatment would indicate such an effect, compromising the