Seed germination ecology of European mesic grasslands

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# Abstract

# Keywords

*Arrhenatheretalia*, *Arrhenatherion*, plant regeneration, seed germination, seed morphology, species-rich meadows, hay meadows, mesic pastures

# Introduction

European mesic grasslands are semi-natural open habitats that occupy moderately fertile and well-drained soils (Mucina et al. [2016](#ref-RN4698)). These plant communities developed in clearings of a formerly temperate woodland landscape as a consequence of anthropogenic intervention (Poschlod et al. [2009](#ref-RN5033)). At a time, they were novel plant communities with a composite flora made up of species from different origins: grasslands plants that existed in non-forested “islands” of the landscape, plus plants that evolved in parallel to human intervention by hybridisation or polyploidization (Poschlod et al. [2009](#ref-RN5033)). During historical times the botanical composition of mesic grasslands evolved following changes in human activities and management practices (Chytry). They are thus the result of a process of habitat domestication that is characteristic of Europe’s natural history (Flannery [2018](#ref-RN5034)). Nowadays, mesic grasslands maintained by agricultural practices are a definitory aspect of European landscapes, both real (Finck et al. [2002](#ref-RN4996)) and imagined (Judd & Judd [2017](#ref-RN5029)).

Traditional phytosociological classification of mesic grasslands puts emphasis on the separation between mesic meadows used for hay making versus mesic pastures maintained by grazing, but a recent revision at the European level showed that the main driver of variation in species composition is the intensity rather than the type of management (Rodríguez-Rojo et al. [2017](#ref-RN4893)). Indeed, changes in the intensity of management, including land abandonment and agricultural intensification, are threatening the maintenance of mesic grasslands in large parts of Europe (Carboni et al. [2015](#ref-RN4997)). For this reason the European Habitats Directive (92/43/EEC) has included in its lists of habitats of conservation interest those species-rich meadows that are traditionally managed by one or two annual cuts and light grazing.

One of the reasons for the high conservation interest of traditional mesic grasslands is their high species richness, and for this same reason they have been highlighted as a valuable source of natural seed materials to be used in ecological restoration, rewilding and urban greening (Haslgrübler et al. [2014](#ref-RN4894); Golińska et al. [2017](#ref-RN5030); Krautzer et al.). However, a lack of knowledge about species germination traits has already been identified as a bottleneck (Ladouceur et al. [2018](#ref-RN2241)) hampering the development of a competitive native seed industry (De Vitis et al. [2017](#ref-RN4665)) in Europe. Understanding seed germination is part of the practical scientific framework needed to tackle large-scale ecological restoration challenges (Merritt & Dixon [2011](#ref-RN3507)) and to maintain *ex situ* collections of plant genetic resources (Li & Pritchard [2009](#ref-RN3763)). When such a knowledge framework is available, ambitious regional schemes of seed-based landscape restoration can be designed (Jiménez-Alfaro et al. [2020](#ref-RN4666)).

Mesic grasslands are dominated by mesophilous grasses of the Poaceae family, which make up most of the biomass and define the structure of the vegetation. The dispersal unit in the grasses is generally the floret (**Fig. 1a**), a composite structure made up of the caryopsis or grain (a dry, indehiscent and monospermic fruit) and its surrounding bracts (i.e. modified leaves): the lemma and the palea. Removal of the lemma and palea, as well as puncturing the pericarp, can aid in seed germination (Probert et al. [1985](#ref-RN1248)). This structure is often accompanied by awns or hairs (**Fig. 1b**) that are assumed to aid dispersal, although it is not always the case that they do (Schonfeld [1983](#ref-RN1290)). Grass seeds have been reported as having physiological seed dormancy (Sprague [1940](#ref-RN5018); Dixon [1995](#ref-RN5024); Baskin & Baskin [2014](#ref-RN3214)) in various degrees, but germinability is usually high even without treating the seeds with cold stratification (Grime et al. [1981](#ref-RN3273); Schonfeld [1983](#ref-RN1290); Williams [1983c](#ref-RN1281); Bean [1984](#ref-RN1260); Froud-Williams et al. [1984](#ref-RN1271); Froud-Williams et al. [1986](#ref-RN1229); Froud-Williams [1987](#ref-RN1205); Dixon [1995](#ref-RN5024); Perez-Fernandez & Rodriguez-Echeverria [2003](#ref-RN789); Perez-Fernandez et al. [2006](#ref-RN680); Stanisavljevic et al. [2011](#ref-RN398); Oliveira et al. [2012](#ref-RN372); Wille et al. [2013](#ref-RN334); Stanisavljevic et al. [2015](#ref-RN223)). Freshly harvested seeds are comparatively more dormant, but dormancy tends to disappear quickly in dry storage (Sprague [1940](#ref-RN5018); Dixon [1995](#ref-RN5024)). Germination has been reported to occur at temperatures ranging from 5 to 30 ºC (Grime et al. [1981](#ref-RN3273); Williams [1983b](#ref-RN1292); Pannangpetch & Bean [1984](#ref-RN1261); Froud-Williams et al. [1986](#ref-RN1229); Probert et al. [1986](#ref-RN1230); Dixon [1995](#ref-RN5024)). Most Poaceae species have also been reported to germinate better in light than in darkness (Williams [1983b](#ref-RN1292); Williams [1983a](#ref-RN1280); Froud-Williams et al. [1984](#ref-RN1271); Probert et al. [1985](#ref-RN1248); Probert et al. [1986](#ref-RN1230); Probert [1986](#ref-RN1221); Thompson [1989](#ref-RN1172); Dixon [1995](#ref-RN5024)) and even to be inhibited to germinate by darkness (Froud-Williams et al. [1986](#ref-RN1229)). Nonetheless, species of *Bromus* have been reported as germinating better in darkness (Thompson [1989](#ref-RN1172)) and *Cynosurus cristatus* as being indifferent to light/darkness (Williams [1983b](#ref-RN1292); Williams [1983a](#ref-RN1280)). In *Poa trivialis*, germination was promoted by light but not by alternating temperatures (Froud-Williams [1987](#ref-RN1205)), although alternating temperatures did encourage some germination in darkness (Froud-Williams et al. [1986](#ref-RN1229)). Wild grass ecotypes usually have a germination response to alternating temperatures (Schonfeld [1983](#ref-RN1290); Williams [1983b](#ref-RN1292); Williams [1983a](#ref-RN1280); Pannangpetch & Bean [1984](#ref-RN1261); Probert et al. [1985](#ref-RN1248); Probert et al. [1986](#ref-RN1230); Probert [1986](#ref-RN1221); Thompson [1989](#ref-RN1172)), although this response is missing in some wild ecotypes and in the domesticated cultivars (Pannangpetch & Bean [1984](#ref-RN1261)), and some species such as *Lolium perenne* have been reported as insensitive to temperature alternation (Thompson [1977](#ref-RN1380); Williams [1983a](#ref-RN1280)).

Next in abundance to the grasses are the legumes of the Fabaceae family. Legumes contribute to the nutritional value of grassland fodder, as thanks to their N-fixating capabilities they have high N contents (Reiné et al. [2020](#ref-RN5028); Álvarez et al. [2021](#ref-RN5040)). The dispersal unit of most legumes is the seed itself (**Fig. 1c**) but in some species dispersal units are more complex, including indehiscent monospermic fruits (e.g. *Onobrychis*, **Fig. 1d**) or indehiscent monospermic fruit fragments, i.e. loments (e.g. *Ornithopus*, **Fig. 1e**). Legumes are generally hard-seeded, i.e. they have a water-impermeable seed coat which needs to become permeable before germination can happen (i.e. physical seed dormancy) (Grime et al. [1981](#ref-RN3273); Jones [1986](#ref-RN1215); Ehrman & Cocks [1996](#ref-RN5016); Kupferschmid et al. [2000](#ref-RN3073); Baskin & Baskin [2014](#ref-RN3214)). In *Medicago*, seeds that have not reached full maturity can germinate and are not yet impermeable, but the completion of maturation imposed coat impermeability, and thereafter the seed coat must be scarified to allow water imbibition and germination (Gresta et al. [2007](#ref-RN652)). Buried legume seeds are able to track the seasons, and in some species germination seems to be promoted by cold stratification and alternating temperatures (Van Assche et al. [2003](#ref-RN780)), some of these have been described as having combinational dormancy (i.e. physical + physiological) (Van Assche & Vandelook [2010](#ref-RN5037)). However, seeds have also been reported to germinate without any previous treatment (Marchiol et al. [2000](#ref-RN881); Nikolic et al. [2007](#ref-RN632); Kabouw et al. [2010](#ref-RN460); Oliveira et al. [2012](#ref-RN372)), and to lose dormancy during storage (Van Assche & Vandelook [2010](#ref-RN5037)). As in the grasses, legume seeds have been reported to germinate in high numbers across a range of temperatures from 5 to 25 ºC (Grime et al. [1981](#ref-RN3273); Gresta et al. [2007](#ref-RN652)). Legume seeds have been described as not responsive to light and capable of germinating in darkness (Silvertown [1980](#ref-RN1339); Grime et al. [1981](#ref-RN3273)).

Grasses and legumes are accompanied by a diversity of other families which, even if present in lower abundances, contribute to the aesthetic value of the meadows (Southon et al. [2017](#ref-RN5039); Chollet et al. [2018](#ref-RN5038)). They also add nutritional quality, being richer than grasses and legumes in specific elements (Reiné et al. [2020](#ref-RN5028); Álvarez et al. [2021](#ref-RN5040)). In many of the most frequent minor families (e.g. in the Asteraceae, Ranunculaceae, Polygonaceae, Lamiaceae, Dipsacaceae, Cyperaceae) the dispersal unit is the achene: dry, indehiscent and monospermic fruits. The morphology of these achenes is varied: cypselas with a hairy pappus in *Centaurea* (Asteraceae, **Fig. 1f**); beaked in *Ranunculus* (Ranunculaceae, **Fig. 1g**); hardened nutlets in *Prunella* (Lamiaceae, **Fig. 1h**); hairy in *Knautia* (Dipsacaceae, **Fig. 1i**); surrounded by a perigynium which aids in dispersal by water in *Carex* (Cyperaceae, **Fig. 1j**). In the genus *Sanguisorba* (Rosaceae), the dispersal unit is the urn-shaped receptacle containing one to three achenes (**Fig. 1k**). In the Apiaceae, it is the mericarp (**Fig. 1l**), an indehiscent monospermic fragment of the fruit. In some other minor families the dispersal unit is the seed itself, such as in the Caryophyllaceae (**Fig. 1m**), the Plantaginaceae (**Fig. 1n**), the Juncaceae or the hemiparasitic species of *Rhinanthus* (Orobanchaceae) (**Fig. 1o**). Our understanding of the germination ecology of these seeds is as varied as their morphology. In the Asteraceae, high germination without pretreatments has been reported in *Taraxacum officinale* (Washitani [1984](#ref-RN1276); Benvenuti & Pardossi [2016](#ref-RN194); RN120; Noronha [1997](#ref-RN963); Mezynski [1974](#ref-RN1428)) and *Hypochaeris radicata* (Oomes [1976](#ref-RN1403); Benvenuti & Pardossi [2016](#ref-RN194)) and *Achillea millefolium* (Oomes [1976](#ref-RN1403)). *Taraxacum officinale* germinated between 5 and 30 ºC (Masin et al. [2017](#ref-RN120); RN1428; Washitani [1984](#ref-RN1276)), and had higher germination in light (Thompson [1989](#ref-RN1172); Letchamo [1996](#ref-RN1015); Noronha [1997](#ref-RN963)) and in alternating temperatures (Mezynski [1974](#ref-RN1428)). In *Stachys officinalis* (Lamiaceae), seeds need either cold stratification, light or alternating temperatures to germinate (Wagner [2011](#ref-RN416); Kolodziejek et al. [2017](#ref-RN128)). Underdevelopped embryos that need to grow inside the seed before germination (i.e. morphological dormancy) are widespread in the Ranunculaceae and the Apiaceae (Jauzein & Mansour [1992](#ref-RN1112); Baskin & Baskin [2014](#ref-RN3214)). *Ranunculus repens* (Ranunculaceae) germinated between 10 and 25 ºC, but the germination percentages were low (Harris [1998](#ref-RN931)); the same species has been reported to respond to alternating temperatures, which can promote its germination even in darkness (Thompson & Grime [1983](#ref-RN3180)). In the Polygonaceae, *Rumex acetosa* can germinate inmediately after dispersal and between 7 and 27 ºC, while the congeneric *Rumex acetosella* does not, this differences is due to the former being able to germinate at constant temperatures in the darkness (Grime et al. [1981](#ref-RN3273); Van Assche et al. [2002](#ref-RN814)), while the latter has an absolute requirement for light (Van Assche et al. [2002](#ref-RN814)). In *Heracleum sphondylium* (Apiaceae), growth of the embryo only occurs below 10 ºC, in moist conditions (Jauzein & Mansour [1992](#ref-RN1112)). *Sanguisorba minor* (Rosaceae) increased its germination after abrasion of the seeds with bleach (Tavşanoğlu et al. [2015](#ref-RN226); Benvenuti & Pardossi [2016](#ref-RN194)), although germination without pre-treatment has also been reported (Ludewig et al. [2014](#ref-RN298); Tavşanoğlu et al. [2015](#ref-RN226)). Seeds of the hemiparasitic species *Rhinanthus angustifolius* and *Rhinanthus minor* (Orobanchaceae) required relatively long periods of cold stratification to germinate (Ter Borg [2005](#ref-RN4014)), and were able to germinate in the dark (Marin et al. [2019](#ref-RN2236)).

Although a wealth of studies has accumulated, a synthesis of the seed germination ecology of mesic grasslands is missing, despite their conservation, restoration and cultural significance. In this article, we present for the first time a synthesis of germination ecology in European mesic grasslands. To do so, we combined a newly-generated dataset with germination records from ENSCOBASE (Carta et al. [2021](#ref-RN5036)), the seed germination database of the European Native Seed Conservation Network (<http://enscobase.maich.gr/index.tml>); and the *SylvanSeeds* database of seed germination records for nemoral biome species (Fernández-Pascual [2021](#ref-RN4968)). To the resulting dataset containing 1,940 germination records from 32 European countries we applied Bayesian meta-analysis (Pappalardo et al. [2020](#ref-RN5000)) to test the seed germination response to environmental cues including scarification, stratification, temperature, alternating temperature and light. In addition, we also analyzed seed morphology (mass and shape) and the differences between the main functional groups of mesic grasslands: grasses (Poaceae), legumes (Fabaceae) and the other families.

# Materials and methods

## Selection of study species

To create a species list of representative mesic grassland species for inclusion in our analysis, we used a dataset of 118 vegetation relevés from three Western European regions with well-maintained mesic grasslands: 43 from Northern Portugal (Trás-os-Montes), 25 from the Cantabrian Mountains of Spain (Parque Nacional Picos de Europa) and 50 from the Pyrenees. This dataset represents a sample of mesic grassland diversity, as the plots were situated along a major stress gradient related to drought (Rodríguez-Rojo et al. [2014](#ref-RN2980)): two regions with a Temperate macroclimate, and Northern Portugal which is transitional between the Temperate and Mediterranean macroclimates (Rodríguez-Rojo et al. [2017](#ref-RN4893)). Furthermore, grasslands from the Pyrenees are closest to the central European optimum of mesic grassland vegetation (*Arrhenatheretalia elatioris* Tx. 1931) while Portuguese grasslands are in suboptimal areas at the limit of the distribution (Rodríguez-Rojo et al. [2017](#ref-RN4893)). Finally, traditional management of mesic grasslands is relatively well preserved in this area within the European context (Prince et al. [2012](#ref-RN4998); Guadilla-Sáez et al. [2019](#ref-RN4999)).

Each relevé had been conducted in a square area (25-100 m2 area) situated in the central part of a meadow avoiding the margins. The chosen meadows are maintained by traditional agricultural practices (mowing for hay-making once or twice per year and light grazing). Vegetation in the plots had been sampled in 2016-2017, at the peak of plant development, just before mowing. All vascular plant species in the plots had been recorded and assigned an abundance value using the transformation of the Braun-Blanquet scale to coverage (+ = 0.1%, 1 = 5%, 2 = 17.5%, 3 = 37.5%, 4 = 62.5% and 5 = 87.5%). All plant names in this manuscript follow the nomenclature of the Euro+Med plant database (<https://www.emplantbase.org/home.html>). Using all the relevés, we calculated the cumulative abundance of each species in the entire area. We removed 208 species with cumulative abundance values below 2%, considering them transient species that might have been recorded by chance and do not represent the core mesic grasslands flora. We used the remaining 117 species as the core list to retrieve seed germination data for this article.

## Seed collection and morphology

From the vegetation plots described above, we collected dispersal units (hereafter called seeds) during the dispersal seasons of 2016, 2017 and 2018. To describe seed morphology, we acquired images of 100-seed samples of each species using a flatbed scanner (Brother LC985) with a resolution of 200 dpi and a scanning area of 1024 x 1024 pixels (Bacchetta et al. [2008](#ref-RN2678)). We distributed the seeds on the scanner transparent glass, in a 10 x 10 grid. For each sample, and without moving the seeds, we repeated the scans with black and white backgrounds. In the case of the black background, we covered samples with a black box to avoid interference from environmental light. For the white background, we used the scanner cover. We digitized the obtained images and stored them in JPEG format (Joint Photographic Experts Group). We processed the scanned images using *ImageJ*, an open-source image processing program designed for scientific multidimensional images (Schneider et al. [2012](#ref-RN4708)). The program calculates several biometric parameters for each seed on the sample, among these, seed length and width. Using these values, we calculated seed shape as seed length divided by seed width. Additionally, we obtained seed mass for the study species from Kew’s Seed Information Database (Royal Botanic Gardens, Kew [2017](#ref-RN2987)).

## Seed germination data

We germinated seeds of the different species using three germination treatments to determine the effect of temperatures representative for the study area: 14/4 ºC representing the capacity of freshly-dispersed seeds to germinate at cool temperatures of spring and autumn, 22/12 ºC as the optimal summer temperature, and 30/20 ºC as sun-heated soil, e.g. soil exposed to sun after hay cutting. Additionally, we compared, at each of these temperature regimes, the germination of fresh seeds versus seeds subjected to a dormancy-breaking treatment. In the case of the Fabaceae, the treatment consisted in scarification by chipping the seed coat with a scalpel, to remove physical dormancy (Baskin & Baskin [2014](#ref-RN3214)). For the rest of the families, we used gibberellic acid GA3 (0,0645 mM) in darkness during 24h, as a treatment to remove potential physiological seed dormancy (Blandino et al. [2019](#ref-RN4976)). For each species and treatment, we sowed four Petri dishes with 25 seeds each. The germination substrate was 1% distilled water - agar. We sealed dishes with Parafilm to prevent desiccation. Trials took place in a germination chamber (KBW 400, Binder GmbH, Tuttlingen, Germany) with a 12/12 hour photoperiod (the light period corresponding to the higher temperature). Experiments lasted for four weeks, with germination scoring once per week. The germination criterion was 2 mm radicle emergence. After four weeks, we cut the seeds that failed to germinate and examined them under a magnifying glass. We classified them as normal when the embryo was visible and firm, empty when they lacked an embryo, and contaminated when they were moldy. We only considered normal seeds to calculate germination proportions and conduct subsequent analyses.

In addition to this experimental data, we retrieved seed germination records from ENSCOBASE (Carta et al. [2021](#ref-RN5036)), the seed germination database of the European Native Seed Conservation Network (<http://enscobase.maich.gr/index.tml>); and the *SylvanSeeds* database of seed germination records for nemoral biome species (Fernández-Pascual [2021](#ref-RN4968)). The combined dataset, including our own experimental data and the records from ENSCOBASE and *SylvanSeeds*, contained 1,940 germination records (i.e. germination proportions for a given seed lot of a species, recorded in a set of laboratory experimental conditions) from 32 European countries. There were 104 species in the dataset. Overall, 131,747 seeds had been used in the experiments. The range of experimental germination temperatures (weighted average of the daily thermoperiod) which had been used in the experiments spans from 2 to 31 ºC, with 1,146 records of constant temperatures (i.e. experiments that used the same temperature during all their duration) and 794 of alternating temperatures (i.e. experiments where different temperatures were applied during the day and the night, in diurnal cycles). Seeds had been exposed to light during some part of the diurnal cycle in 1,861 records or kept in total darkness in 79 records. Experiments had been performed with unstratified seeds (i.e. not subjected to a previous dormancy-breaking incubation) in 1,765 records and with stratified seeds (i.e. subjected to previous incubation in dormancy-breaking conditions, including treatments of wet incubation under cold, warm and combinations of cold and warm conditions) in 175 records. Finally, there were 212 records where GA3 had been applied, and 460 records where seeds had been scarified.

## Statistical analysis

To test the effect of the germination treatments on seed germination proportions, we performed a meta-analysis of the germination dataset by fitting binomial generalized mixed models with Bayesian estimation (Markov Chain Monte Carlo generalized linear mixed models, MCMCglmms) (Carta et al. [2021](#ref-RN5036); Fernández-Pascual et al. [2021](#ref-RN4965)) using the R package *MCMCglmm* (Hadfield [2010](#ref-RN4755)). We fitted the analysis separately for the three major groups of mesic grassland plants: Poaceae, Fabaceae, and the minor families. To account for the effect of a shared phylogeny in species traits, models included as a random effect a reconstructed phylogenetic tree for the study species. To create the phylogeny we used the R package *V.PhyloMaker* (Jin & Qian [2019](#ref-RN4753)) which contains an updated mega-tree of the seed plants based on Smith & Brown ([2018](#ref-RN4754)). We placed taxa absent from the mega-tree at the genus-level basal node. The phylogenetic tree is available in the data folder of the GitHub repository (see Data Availability Statement). Germination proportion was the response variable. Models had, as fixed effects, the experimental conditions (scarification, stratification, GA3, temperature, alternating temperature and light). Random effects included the phylogenetic tree, species identity, seed lot and source of the data. In all models, response variables were scaled so their contribution to the effect sizes could be compared. We used weakly informative priors in all models, with parameter-expanded priors for the random effects. Each model was run for 500,000 MCMC steps, with an initial burn-in phase of 50,000 and a thinning interval of 50 (De Villemereuil & Nakagawa [2014](#ref-RN4756)), resulting, on average, in 9,000 posterior distributions. From the resulting posterior distributions, we calculated mean parameter estimates and 95% Highest Posterior Density (HPD) and Credible Intervals (CI). We estimated the significance of model parameters by examining CIs, considering parameters with CIs overlapping with zero as non-significant. To estimate the phylogenetic signal of seed germination over all variables, we used Pagels’s lambda (λ) (Pagel [1999](#ref-RN4757)), estimated simultaneously with the models by calculating the mean of the posterior distribution and the 95% CI of λ as indicated by De Villemereuil et al. ([2014](#ref-RN4756)). When λ = 0, related taxa are no more similar than expected by chance, while when λ = 1, the trait is evolving following a constant variance random walk or Brownian motion model; intermediate values of λ indicate a phylogenetic correlation in trait evolution that does not fully follow a Brownian motion model (Pagel [1999](#ref-RN4757)). Detailed results of the MCMCglmms are available in the results folder of the GitHub repository (see Data Availability Statement).

To visualize the main patterns in the seed germination spectrum of mesic grassland plants, we did a Principal Component Analysis (PCA) as implemented in the package *FactoMineR* (Le et al. [2008](#ref-RN3166)). We performed the PCA ordination at the species level, i.e. calculating a series of continuous traits for each species. We transformed the final germination proportions to create a continuous variable for the germination cues (i.e. stratification, scarification, temperature, alternating temperatures and light). To do so, for each cue and species, we calculated a weighted average of the cue levels (in the case of temperature, cue levels were the temperature treatments; for the other cues the levels were 0 = absence and 1 = presence), weighting by the germination proportion at each level. This approach underrepresents the importance of the levels that were not tested for a given species, but can serve as a proxy of the response to the germination cues when visualized across the whole dataset; it must be stressed that this stage of the analysis serves only for visualization and not for hypothesis testing. We also included seed mass in the ordination. We left GA3 out of the PCA because its ecological interpretation is subordinated to stratification (as both cues break physiological seed dormancy). We also left seed length, width and shape out because these values were not available for all species.

# Results

## Seed morphology

The mass of these dispersal units in grassland grasses ranges from 0.1 to 4.1 mg, and they tend to be longer (5 mm on average) than they are wide (1.8 mm).

In mesic grasslands, the dispersal units of the Fabaceae are generally heavier than those of the Poaceae, ranging from 0.3 to 24.9 mg; and they are also rounder (2.5 mm long vs. 2.3 mm wide).

When comparing the morphology of all species (**Fig. 2**), the Poaceae appear as having relatively lighter and more elongated dispersal units, in contrast with the Fabaceae, which tend to have rounder and heavier seeds.

## Germination responses

When considering the full set of mesic grassland species, all of the six studied germination cues had a significant effect on final germination proportions (**Fig. 3**). The germination of grassland seeds was positively associated with scarification, stratification, GA3, alternating temperatures, and light. Average temperature had a negative effect, indicating a trend towards higher germination at lower temperatures. Averaging the whole dataset, the highest germination proportions were achieved at 20 ºC. Between 0 ºC and 20 ºC, germination proportions increased steadily with increasing temperatures. Above 20 ºC, germination declined more sharply, and grassland seeds rarely germinated at 30 ºC.

Some differences became apparent when dividing the dataset in the major floristic groups that compose mesic grassland vegetation (i.e. (i) the Poaceae as dominant and structural species, (ii) the Fabaceae as the second largest group, and (iii) the rest of the families). In the dominant group of the Poaceae (**Fig. 3**), no effect was found for stratification, GA3 or temperature. Stratification had a negative effect on germination. The major drivers of Poaceae germination appeared to be alternating temperatures and light, with both having a positive effect. In the group of the Fabaceae (**Fig. 3**), the largest positive effect on germination was produced by scarification, with no effect of stratification, GA3 or light. Temperature had a negative effect, and in fact Fabaceae species had higher germination proportion at temperatures under 20 ºC. Fabaceae seed germination also responded negatively to alternating temperatures. Finally, the remaining families (**Fig. 3**), the main cues having a positive effect on germination were stratification, GA3. alternating temperatures and light. These species did not respond to scarification nor to average temperature.

To describe the effects of the random factors, we will refer only to the full model that included all species in the dataset. The strongest effect was that of the phylogeny (mean = 6.48, CI = 3.7 - 9.79), followed by the source of the data (mean = 2.9, CI = 1.21 - 4.98) and the seed lot (mean = 1.28, CI = 0.94 - 1.61). The phylogenetic signal in the germination responses was relatively high (lambda = 0.75, CI = 0.65 - 0.84).

## Seed germination spectrum

The first PCA axis explained 27% of the variation (**Fig. 4**). The variables with the largest contribution to this first axis were the germination responses to scarification and alternating temperatures, and seed mass. This horizontal axis separated (i, left) species of the Poaceae and the minor families which responded to alternating temperatures and had smaller seeds from (ii, right) species of the Fabaceae which responded to scarification and had heavier seeds. Axis 2 explained 21% of the variability. The main contributing variables were the germination responses to light and stratification. This axis separated (iii, bottom) species from all groups that germinated better in light from (iv, top) species of the minor families which needed stratification to germinate and had higher germination in the dark.

# Discussion

Our meta-analysis of germination records showed that, overall, the environmental factors with the strongest effect to control seed germination in mesic grassland species were alternating temperatures, light and scarification. Alternating temperatures and light are usually considered to be micro-environmental cues that indicate, at a fine scale, the existence of safe sites for regeneration (Jumpponen et al. [1999](#ref-RN4719)). The diurnal alternation of temperatures decreases with burial depth in the soil, and the alternation is also reduced by vegetation cover (Thompson [1977](#ref-RN1380); Van Assche & Vanlerberghe [1989](#ref-RN4910); Saatkamp et al. [2011](#ref-RN4952)). Thus, a positive germination response to alternating temperatures could detect the depth of seed burial, and prevent the germination of seeds that are buried too deep for seedlings to survive before emerging from the ground (Bond et al. [1999](#ref-RN4898)). Perhaps more importantly in this habitat, alternating temperatures could also indicate that the vegetation cover has been diminished by either sowing or grazing, and therefore mark an appropriate time for seedling regeneration, when the competition by the established plants is lessened. Similar functions can be ascribed to the germination response to light, as physiologically-active light in significant amounts only travels the uppermost milliliters of soil (Tester & Morris [1987](#ref-RN4909)) and the quality of light will be affected by vegetation cover (Jankowska-Blaszczuk & Daws [2007](#ref-RN604)). Therefore, the germination response to alternating temperatures and light indicate conditions that are found immediately after mowing for haymaking, a predictable perturbation that occurs approximately at the same time every year, and which forces meadow plants to establish, grow and reproduce in the time frame determined by this perturbation (Grime [2006](#ref-RN4887); Klimešová et al. [2010](#ref-RN4888)). In addition, this traits would also promote the formation of a persistent soil seed bank (Williams [1983b](#ref-RN1292); Venn & Morgan [2010](#ref-RN4929); Jaganathan et al. [2015](#ref-RN4718)). On the other hand, it is worth mentioning that the Poaceae, which show the germination response to alternating temperatures and light, also have the more elongated seed shape, a trait that is thought to reduce the capacity of seeds to enter the soil seed bank in temperate grasslands (RN5014; Funes et al. [1999](#ref-RN5013)).

The strong effect of scarification was specifically related to the hard-seeded Fabaceae. In this family, physical dormancy as a result of an unpermeable seed coat works as a mechanism to detect seasonal cycles of temperature and humidity (Van Assche et al. [2003](#ref-RN780)). It has been also proposed that hardseedeness can avoid seed predation, by preventing the emission of olfactory cues that are elicited by seed imbibition and that can be perceived by seed predators (Paulsen et al. [2013](#ref-RN2568)). In the case of this study, Fabaceae seeds are the heaviest, and they clearly follow a separate regeneration strategy from that of the other families. While other groups rely on alternating temperatures and light as gap-detecting mechanisms, Fabaceae seeds do not respond to these factors, and instead they are mainly regulated by scarification, and they also respond to cooler temperatures than the rest of species. This would indicate that Fabaceae seeds tend to germinate when temperatures are cooler, and thus before or after the summer hay-making season. Their larger size, and the related larger reserves, could allow Fabaceae seedlings to emerge at times when the competition from the established vegetation would be higher.

The germination response to average temperatures and stratification is understood to detect cues related to seasonal cycling and macroclimatic variation (Finch-Savage & Leubner-Metzger [2006](#ref-RN3063) p. @RN5036). Apart from the aforementioned response to cool temperatures in the Fabaceae, the rest of the families did not show a response to average temperatures, further highlighting their reliance on micro-habitat cueing. Moreover, the dominant family of the Poaceae showed a negative response to cold stratification. The minor families, however, showed a divergence from the Poaceae strategy in their positive response to stratification. These subordinate species appear to require a period of cold stratification, indicating that overwintering has occured, before they can germinate (Baskin & Baskin [2014](#ref-RN3214)). These minor families are also the only group that shows a positive response to GA3, a phytohormone which can work as a substitute of cold stratification to overcome physiological dormancy (Bewley et al. [2013](#ref-RN3368)).

The positive phylogenetic signal found in the models highlights the phylogenetic clustering of regeneration strategies that we have described so far: (1) the dominant family of the Poaceae, showing a lack of responses to seasonal cues (average temperature and cold stratification), and relying instead on micro-niche cues (alternating temperatures, light) that can be associated to the yearly perturbation of mowing, (2) the second most-dominant family, the Fabaceae, which do not respond to the micro-niche cues, and regulate their germination timing through scarification and cooler germination temperatures, possibly because their larger size and reserves allow their seeds to decouple their emergence timing from the mowing perturbance, and finally (3) the minor families that respond to micro-cues in a similar way than the Poaceae, but which are differentieted from them by showing a positive response to cold stratification and G3, indicating that they rely on physiological seed dormancy.

* Less dormancy in grassland vs. arable populations (Froud-Williams et al. [1986](#ref-RN1229)). Herbaceous plants adapted to open habitats needed less cold stratification than congeneric species adapted to forest understories (Brink et al. [2013](#ref-RN3570)).
* Better germination without treatments (Haslgrübler et al. [2014](#ref-RN4894)).
* In Poland soil bank dominated by forbs of arable and weedy species, low representation of grasses and legumes, confirms the typical Arrhenatherion species do not form a seed bank (Janicka [2017](#ref-RN5032)). Soil bank is transient in hay meadows and related grasslands (Milberg [1992](#ref-RN5042); McDonald [1993](#ref-RN5041); Hutchings & Booth [1996](#ref-RN5026)). Regeneration limited by dispersal limitation (Kupferschmid et al. [2000](#ref-RN3073)).
* Mowing promotes germination and emergence (Kupferschmid et al. [2000](#ref-RN3073)). Mowing promotes autumn germination (Kahmen & Poschlod [2008](#ref-RN5025)).
* Question of clonal regeneration.
* Discuss second axis = geophytes vs hemicriptófitos?
* Conclusions/applications
* Discuss dormancy in freshly collected grasses, limitations of stored seed data (Sprague [1940](#ref-RN5018))

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# Author contributions

All authors contributed data. E.F.P. conceived the study and performed the analyses. E.F.P. wrote the manuscript with help from A.C. All authors revised the manuscript, and approved the final version.

# Data availability

The original data, R code for the analysis and creation of the manuscript can be accessed at the GitHub repository <https://github.com/efernandezpascual/meadows>. Upon publication, a version of record of the repository will be deposited in Zenodo.

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# Figures



Figure 1: Diversity of dispersal units in mesic grasslands: (a) Floret of *Cynosurus cristatus* (Poaceae); (b) floret of *Arrhenatherum elatius* (Poaceae); (c) seed of *Lathyrus pratensis* (Fabaceae); (d) legume of *Onobrychis viciifolia* (Fabaceae); (e) loment fragment of *Ornithopus perpusillus* (Fabaceae); (f) achene with pappus of *Centaurea scabiosa* (Asteraceae); (g) achene of *Ranunculus acris* subsp. *despectus* (Ranunculaceae); (h) nutlet of *Prunella grandiflora* (Lamiaceae); (i) achene of *Knautia nevadencis* (Dipsacaceae); (j) perigynium of *Carex binervis* (Cyperaceae); (k) receptacle of *Sanguisorba minor* (Rosaceae); (l) mericarp of *Carum verticillatum* (Apiaceae); (m) seed of *Cerastium fontanum* (Caryophyllaceae); (n) seed of *Plantago lanceolata* (Plantaginaceae); (o) seed of *Rhinanthus angustifolius* (Orobanchaceae).

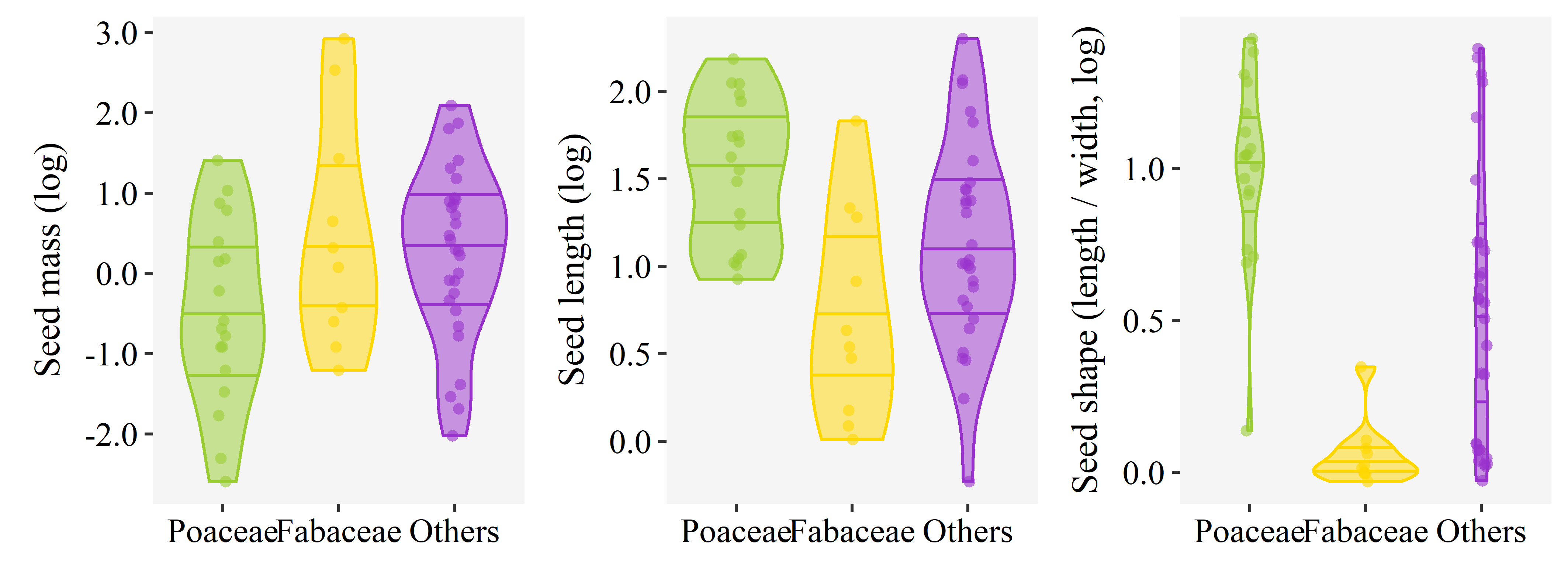


Figure 2: Seed size and shape in mesic grasslands. Kernel probability densities (violin plots) for seed mass, seed length and seed shape (length / width). The three horizontal lines within the probability densities represent the first quartile, the median and the third quartile of the data.

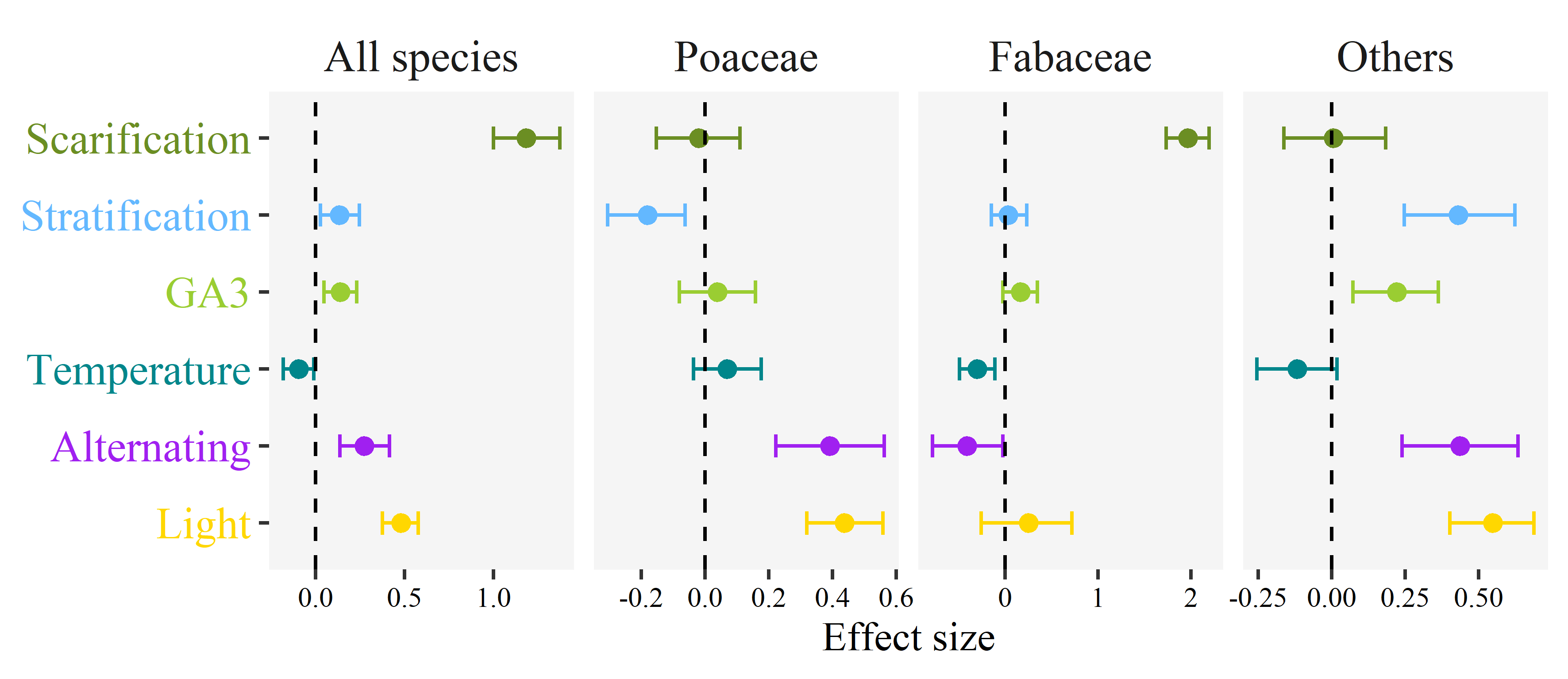


Figure 3: Effect of the germination environment on seed germination of mesic grassland species. Dots indicate the posterior mean of the effect size, and whiskers its 95 % credible interval. The line of zero effect is shown. When the credible intervals overlap with the zero-effect line, the effect is not significant. The figure shows the results of a model including all species, and of models including only the three main floristic groups of mesic grasslands (i.e. the Poacaea, the Fabaceae and the remaining families).

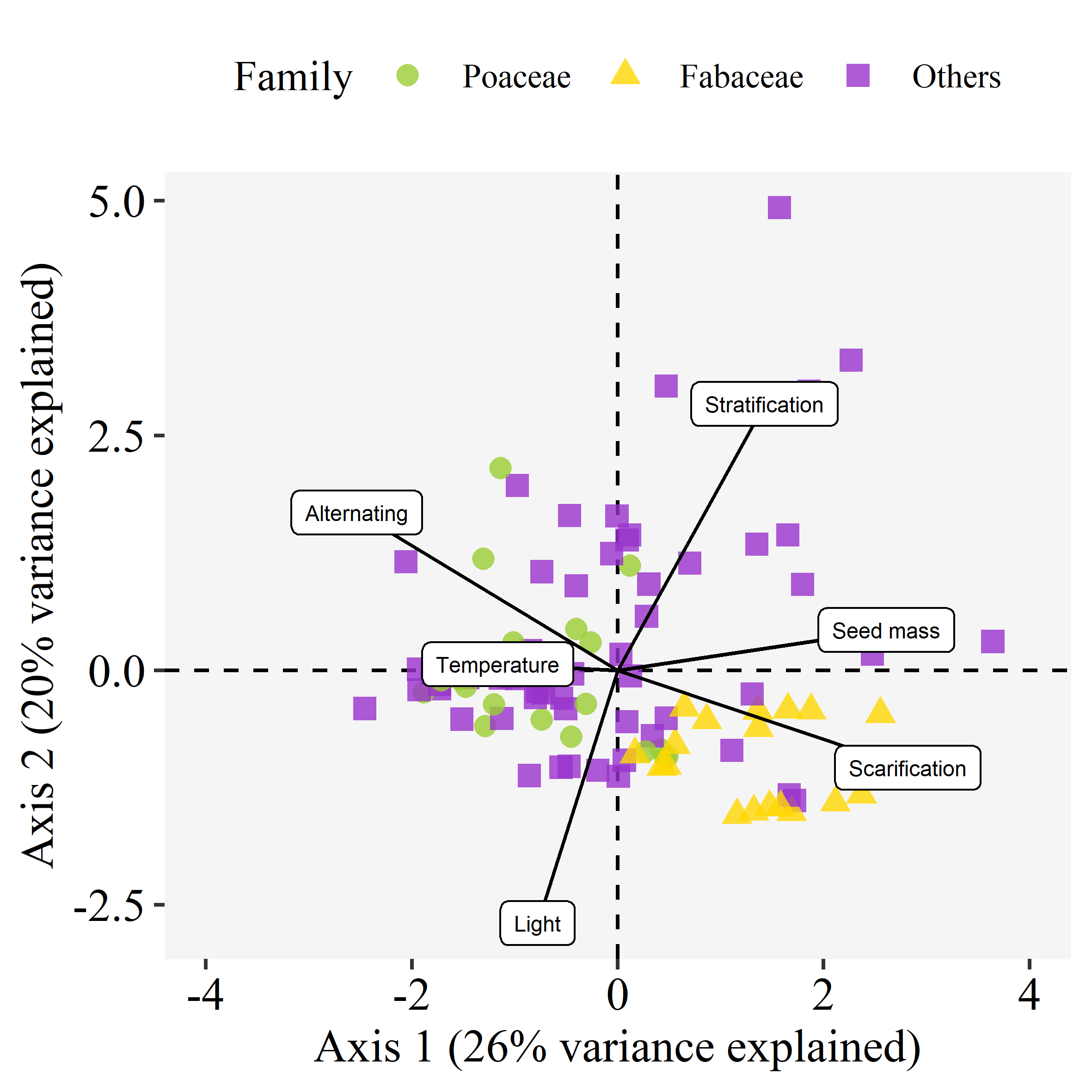


Figure 4: Principal Component Analysis ordination of the seed germination spectrum of mesic grassland species. Each symbol is a species, different symbols indicate the three major floristic groups of mesic grasslands Labels indicate the contribution of the variables to the axes.