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Establishment of rare flood meadow species by plant material transfer: Experimental tests of threshold amounts and the effect of sowing position

Ralf Schmiede[a,*], Eszter Ruprecht[b], R. Lutz Eckstein[a], Annette Otte[a], Tobias W. Donath[a]

[a] Department of Landscape Ecology and Resource Management, Interdisciplinary Research Centre, Justus-Liebig-University Gießen, Heinrich-Buff-Ring 26-32, DE-35392 Gießen, Germany

[b] Hungarian Department of Biology and Ecology, Babeş-Bolyai University, Republicii Street 42, RO-400015 Cluj-Napoca, Romania

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ABSTRACT

The transfer of freshly cut seed-containing plant material is a widely applied method to re-establish grassland of high biodiversity. Still, the amount of plant material applied varies greatly across restoration projects. Therefore, we set up a two-year common garden experiment where we assessed the effect of plant material amount (0, 400, 800, 1600, 3200 g m^{-2}) and relative seed position (on top and beneath a litter layer) on seedling establishment, seedling fate and seedling fitness of eight target species for restoration of alluvial meadows.

Most seedlings (85.6%) emerged within the first year. Cumulative seedling emergence and final seedling establishment across all species were highest on control plots and low litter plots but were very low or failed completely, at 1600 and 3200 g m^{-2}, respectively. In general, large-seeded species were significantly more successful than smaller seeded species. Relative seed position had only a small impact on seedling emergence and establishment but was decisive for seedling survival at high litter quantities. Across all species, seedlings that died had a significantly lower relative height than surviving seedlings. Interestingly, co-occurring resident grassland vegetation had a neutral rather than negative impact on the response variables.

Our results suggest an upper threshold of 1000 g m^{-2} for the amount of plant material applied in grassland restoration, since higher amounts will inhibit seedling establishment. The prompt emergence of most seedlings during the first vegetation period highlights the importance of creating optimal conditions for seedling establishment already in the early phase of vegetation development on restoration sites.

1. Introduction

Species-rich floodplain meadows are among the most threatened plant communities in Central Europe (Joyce and Wade, 1998) and are therefore protected by the Fauna Flora Habitat Directive of the European Union (92/43/ECC). Along the northern Upper Rhine, Germany, restoring species-rich meadows on arable fields via self-greening started in the early 1980s. As found before (cf. Bakker and Berendse, 1999), seed limitation caused by depleted soil seed banks and the rarity of long-distance dispersal events from remnant stands restricted the re-colonization through plants, except for common grassland species (Bissels et al., 2005; Donath et al., 2003). This motivated several large-scale restoration projects in the region that aimed at the re-establishment of species-rich *Arrhenatherion*-, *Cnidion*- and *Molinion* meadows. In these projects seeds of typical flood-meadow species were introduced via the transfer of freshly cut propagule-containing plant material from remnant stands to former arable fields and meadows (Donath et al., 2007; Hölzel and Otte, 2003) and to previously sward-disturbed species-poor grasslands (Schmiede et al., 2012). The subsequent establishment of a large number of rare flood-meadow species showed that the transfer of seed-containing plant material is an adequate technique to overcome seed limitation (for recent reviews, see Kiehl et al., 2010 and Török et al., 2011).

However, Kiehl et al. (2010) point out that the amounts of plant material considered appropriate for restoration differ substantially between projects aiming to restore different vegetation types. The dry weight of applied plant material ranges from ca. 300 g m^{-2} in

* Corresponding author. Present address: Anhalt University of Applied Sciences, Department of Agriculture, Ecotrophology and Landscape Development (Department 1), Strenzfelder Allee 28, DE-06406 Bernburg, Germany. Tel.: +49 3471 355 1233; fax: +49 3471 355 1235.

E-mail addresses: R.Schmiede@loel.hs-anhalt.de (R. Schmiede), eszter.ruprecht@ubbcluj.ro (E. Ruprecht), Lutz.Eckstein@umwelt.uni-giessen.de (R. Lutz Eckstein), Annette.Otte@umwelt.uni-giessen.de (A. Otte), Tobias.W.Donath@umwelt.uni-giessen.de (T.W. Donath).

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relatively low-productive communities such as dry calcareous grasslands or mesic *Arrhenatherion* grasslands (Kiehl et al., 2006) to 1500 g m^{-2} in high-productive grasslands like floodplain meadows (Donath et al., 2007).

Beyond acting as a mere carrier for seeds of the target species, the plant material itself (i.e. litter) very likely influences germination and establishment of transferred plant species and will very likely also affect resident species. Litter can influence nutrient availability due to decomposition and may therefore affect productivity, composition and biodiversity of plant communities (Facelli, 1994; Facelli and Facelli, 1993; Foster and Gross, 1998). On a finer scale, litter may have an impact on germination and establishment of plants by changing chemical and physical conditions (Facelli and Pickett, 1991b).

A number of studies have shown that chemical effects of litter may have a negative impact on seedlings (e.g. Ruprecht et al., 2008), but these seem to be less important than physical effects (Hovstad and Ohlson, 2008; Xiong et al., 2001). Dead plant material decreases light availability for seedlings and acts as a mechanical barrier that retards growth above and below-ground (Eckstein and Donath, 2005; Jensen and Meyer, 2001; Donath and Eckstein, 2010; Rotundo and Aguiar, 2005). On the other hand, a moderate litter layer may also promote seedling emergence since, especially under dry conditions, seeds beneath the litter cover experience less water and temperature stress (Loydi et al., 2013; Facelli and Pickett, 1991a; Rotundo and Aguiar, 2005). In addition to these environmental factors, seed size is closely related to germination and seedling fate. Various studies have shown that large-seeded species show higher growth rates and survival under harsh conditions, such as drought, shade or competition, than small-seeded species (e.g. Burmeier et al., 2010; Bruun and Brink, 2008; Donath and Eckstein, 2010; Gross, 1984; Leishman et al., 2000; Turnbull et al., 1999). Under these circumstances, three mechanisms related to seed size may have a strong influence on the performance of seeds during early seedling establishment (Leishman et al., 2000; Quero et al., 2007): the reserve effect, i.e. larger seeds retain a larger proportion of reserves after germinating, the metabolic effect, which results in low relative growth rates of seedlings from large seeds, and the seedling-size effect, i.e. seedling's size increases with seed size. In the early cotyledon-stage, the reserve and seedling size effect mostly determine survival of seedlings. Later, in the leaf-stage, growth and survival depend on seedling morphology and physiology, while the importance of seed-size related effects decrease (Leishman et al., 2000; Moles and Westoby, 2004).

A number of studies have analyzed the effect of different amounts of litter on germination and establishment of seedlings (Eckstein and Donath, 2005; Jensen and Gutekunst, 2003; Xiong et al., 2001) but fewer have assessed the effects of seed position (Donath and Eckstein, 2012; Rotundo and Aguiar, 2005; Ruprecht and Szabó, 2012). These studies showed that the effect of seed position depends on seed size (Donath and Eckstein, 2010): the smaller the seeds, the more easily do they percolate deeper into the litter resulting in similar starting conditions for seeds from on top and beneath the litter cover when germination starts.

The above mentioned processes will be especially important in seed-containing plant material applied for restoration purposes, a measure widely used in ecological restoration (cf. Kiehl et al., 2010; Török et al., 2011), because seeds will be distributed within the plant material cover. However, despite its high practical relevance the effect of seed position on seedling establishment, fate and fitness has – to our knowledge – not been studied under natural conditions. Therefore, we analyzed the effects of different amounts of litter and sowing position on early seedling establishment, seedling fate (survival) and seedling fitness (height, dry weight) of target species under field conditions. We addressed the following questions:

(i) Do the effects of litter on seedling establishment of target species vary with seed size and how does this response change with litter quantity and seed position?
(ii) How does litter amount and seed position affect fate and fitness of established seedlings?

2. Methods

2.1. Experimental design

Eight meadow species, targets for restoration and nature conservation, which are typical constituents of species-rich flood-meadows (phytosociological alliances *Arrhenatherion*, *Cnidion* and *Molinion*) along the northern Upper Rhine (cf. Donath et al., 2003), were selected for a field seed sowing experiment. The species belonged to six different plant families and had different seed masses: *Arabis nemorensis* (*Brassicaceae*; 0.09 mg), *Inula salicina* (*Asteraceae*; 0.13 mg), *Galium boreale* (*Rubiaceae*; 0.65 mg), *Selinum carvifolia* (*Apiaceae*; 0.67 mg), *Viola pumila* (*Violaceae*; 1.20 mg), *Serratula tinctoria* (*Asteraceae*; 2.06 mg), *Peucedanum officinale* (*Apiaceae*; 4.77 mg) and *Iris spuria* (*Iridaceae*; 18.53 mg). Nomenclature follows Wisskirchen and Haeupler (1998).

Data for seed masses are averages of measurements on 10 subsamples of 100 seeds each. The selected species cover almost the complete seed size spectrum of alluvial meadows (Hölzel and Otte, 2004). Our test species were separated into two groups based on a seed mass threshold of 1 mg, which corresponds to mean seed size in different grassland types (Leishman et al., 1995; Moles and Westoby, 2006): small-seeded species (seed mass <1 mg) and large-seeded species (seed mass $\geqslant$1 mg). Seeds were collected in bulk from autochthonous populations on flood-meadows at the northern Upper Rhine in July–August 2006. Seed collection included at least 50 different plant individuals from three sites each. Seeds cleaned from appendages were subsequently dry-stored in darkness at room temperature (ca. 20 °C) until sowing.

The experiment was conducted in mesic grassland within a common garden near Giessen, Germany (50°32′N, 8°41.3′E, 172 m a.s.l.) which was ploughed and afterwards levelled with a harrow in February 2007 before the setup of the experiment. The experimental design was a blocked split-plot (Gomez and Gomez, 1984), with five blocks. Within each block, we established five mainplots (3 m × 3 m), each mainplot randomly receiving one of the five litter treatments (400, 800, 1600, 3200 g m^{-2}, and 0 g as a control). Each mainplot (*litter quantity*) contained 16 quadratic subplots (each 0.1 m^2) consisting of all combinations of the factors *species identity* (eight levels) and *seed sowing position* (two levels, i.e. sown on top and beneath the litter cover). Each subplot was surrounded by a buffer of ca. 0.3 m to avoid side effects from neighboring plots. Randomisation of the treatment levels was performed on the mainplot and subplot scale. One hundred seeds of a single species were sown in each subplot in February 2007. Each *species* × *sowing position* × *litter quantity* was replicated five times.

Litter quantities correspond to annual litter production of low (400 and 800 g m^{-2}), medium (1600 g m^{-2}) and high productive (3200 g m^{-2}) floodplain meadows (Donath et al., 2004) and represent, except for 3200 g m^{-2}, quantities of plant material regularly applied in restoration projects (Kiehl et al., 2010; Török et al., 2011). The grass litter originated from a mesic unfertilized grassland site dominated by *Poa pratensis*, *Agrostis stolonifera*, *Arrhenatherum elatius* and *Dactylis glomerata* that harboured none of the study species.

Seedling establishment was followed for two years on five census occasions: end of July, beginning of October 2007, end of April, end of July, and mid October 2008. At each counting, newly established seedlings were marked and their height measured (except for *Arabis nemorensis*, which was omitted from the analysis