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The fate of herbaceous seeds during topsoil stockpiling: Restoration potential of seed banks

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ABSTRACT

Topsoil removed during linear infrastructure construction is one of the most valuable resources for the ecological restoration of roadslopes, as it contains high concentrations of micro-organisms, nutrients and seeds. During construction work, topsoil is stockpiled in a way that can harm seed germination and survival capacity. In order to assess the effects of topsoil storage time and seed burial depth on seed survival, germination and mortality, an experiment with three replicates was conducted using two factors: time (1–6 months) and burial depth (0, 5, 30 and 50 cm). At each stockpile depth we buried 25 seeds from 10 natural grassland species (5 families) in permeable nylon sachets. Seed mass and seed functional responses to light (LI) and diurnal temperature fluctuations (DTF) were also measured for each species. Seed survival, germination and mortality during stockpiling were analyzed using binomial GLMs. Explanatory variables were family, depth of burial, time, seed mass, LI and DTF responses. Seed survival decreased with storage time but increases with depth. Seed losses were due to seed germination and mortality in the stockpile. Germination percentage increased with time but decreased with burial depth. This parameter was negatively related to LI and DTF. Mortality increased significantly with time and depth and was negatively related to seed mass.

Results show that there may be a loss of viable seeds in topsoil stockpiles, particularly in the case of large seeds. Our results also underline the functional role of light requirements for germination and the detection of diurnal temperature fluctuations as mechanisms to achieve higher soil seed persistence. The correct management of topsoil is decisive for the restoration of roadslopes. Seed germination potential decrease with time of storage and burial depth, consequently a serious loss of seeds conducts an impoverishing of topsoil, which is the main natural resource for the restoration of these disturbed ecosystems. Viable seed loss is lower in deeper layers of the stockpile, so short-period topsoil storage and bigger stockpiles could reduce seed loss and increase thus the restoration potential of topsoil.

1. Introduction

Recently built roadslopes are hostile environments for the recovery of vegetation cover due to a lack of nutrients, the coarse texture of the embankment material (Sheldon and Bradshaw, 1977; Jim, 2001; Elmarsdottir et al., 2003), the lack of organic matter (Muzzi et al., 1997; Albaladejo et al., 2000) and the initial lack of viable seeds in the substrate (Balaguer, 2002). Additionally, there is significant erosion in these systems due to their steep slopes and scarce initial plant cover (Grace, 2002; Bochet and García-Fayos, 2004), with high environmental, security and economic costs (Arnáez and Larrea, 1995; Nicolau, 2002). Roadslopes thus have particular characteristics in which the restoration of the vegetation cover is of great importance and complexity for the infrastructure management companies (García-Fayos et al., 2000). Its recovery begins once the road infrastructure is completed through techniques such as hydroseeding (Enríquez et al., 2004; Matesanz et al., 2006), plantations (Jochimsen, 2001; Holl, 2002), and/or geotextile mesh (Rickson, 2006; Benik et al., 2003; Mitchell et al., 2003). Topsoil, the top 30 cm of soil removed during the initial construction stage of linear infrastructure projects, is usually spread on the roadslopes prior to the use of any of these techniques (Bote et al., 2005; Tormo et al., 2007; Mola et al., 2011). This topsoil has a high content in organic matter and is important on account of its high content in native seeds (Rokich et al., 2000; Holmes, 2001; Tormo et al., 2007), nutrients and microorganisms, all of which contribute to the establishment

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and stabilization of the plant cover (Zhang et al., 2001; Bote et al., 2005; Tormo et al., 2007). Topsoil is normally stored for varying periods of time and later spread when the slopes are completed. It is described in environmental impact abatement regulations as one of the major ecosystem restoration measures for ensuring a diverse, self-sustaining ecosystem that can withstand disturbance (Newman and Redente, 2001). However, little research has been done on the most suitable method for topsoil stockpiling and management with a view to optimizing the viable and germinable seed content once it has been spread across the slope.

Current practices include topsoil storing in the form of stockpiles or trapezoidal ridges no more than 2 m high to prevent compaction of the underlying layers (Balaguer, 2002). The reasons for these stockpile dimensions have not been tested, and temperature, light, soil moisture and other factors may influence the viability of the buried seeds (Baskin and Baskin, 1998; Scoles and DeFalco, 2003). Moreover, if the topsoil is to remain stockpiled for more than six months, nitrogen-fixing legumes are often sown or fertilizers added to preserve the soil properties (Balaguer, 2002). The effectiveness of these techniques is also questionable, since topsoil contains its own seed bank whose richness and diversity can prompt the establishment of plant cover, although the effect of this action is unknown.

The timing and depth of the topsoil stockpile process has considerable impact on the number of viable seeds. The relationship between burial depth and seed persistence is one aspect that has been studied in different environments (Leishman and Westoby, 1998; Benvenuti et al., 2001; Grundy et al., 2003), focusing on the relationship between seed burial and germination (Chen and Maun, 1999) and emergence ability (Ward et al., 1996; Bowen et al., 2005; Hall et al., 2010). Rokich et al. (2000) found that after 3 years of storage, over 50% of the seed germination capacity is lost, and Scoles and DeFalco (2003), detected a 79% loss of seeds during the topsoil stockpiling phase in a road construction experiment. We know that the seed burial depth in the topsoil spread following storage influences the seed germination and establishment capacity, with the optimum depth equal to or less than 2 cm for herbaceous species (Andrade et al., 1997; Torma and Hodi, 2000; Traba et al., 2004). Many seed bank studies have found that most seeds lie in the top 5 cm (Fenner and Thompson, 2005; Traba et al., 2006). We also know that in seasonal environments such as temperate and Mediterranean zones, the bank's viable seed content peaks in late summer (Thompson and Grime, 1983; Ortega et al., 1987). However, to date little research has been done on the functional traits of the seeds that make them more vulnerable to long periods of storage in soil (Saatkamp et al., 2010). The combination of seed burial experiments with studies of the morphological and physiological characteristics of seed germination, will facilitate the prediction of the germination and survival of buried seeds (Milberg and Anderson, 1998; Baskin and Baskin, 2006).

The overall aim of the present study is to experimentally analyze the effect of burial depth and topsoil storage time on the restoration potential of the seeds contained in stockpiles. The specific research questions are the following: (a) What are the effects of burial time and depth on the survival, germination and seed mortality percentages? (b) What are the effects of burial time and depth on the germination of seeds that survive stockpiling? (c) Are these parameters related to seed functional traits such as mass and germination response to light and temperature changes? and (d) How do environmental conditions in the stockpile (temperature, soil moisture, daily temperature fluctuation) vary in terms of time and depth?

2. Material and methods

2.1. Study site and species

The study area was northwest of Madrid (Spain, 40°32′39″N, 3°41′10″W), surrounded by grasslands of annual species resulted from the abandonment of agriculture about 40 years ago. *Bromus hordeaceus*, *Bromus tectorum* and *Avena barbata* are some of the dominant species. Some scattered individuals of broom, *Retama sphaerocarpa*, are also present in the site which is located on sandstone, conglomerates and acidic clays. Average annual rainfall was 400 mm, with average temperature minima and maxima of 7.6 °C and 21 °C respectively (AEMET. Historical average 1971–2000).

In June and July 2009, seeds were collected from 10 abundant grass species from five families in the study area. The selected species were: *Tolpis barbata* (L.) *Gaertn.* and *Crepis capillaris* (L.) Wallr. (Compositae); *Bromus hordeaceus* L. and *Vulpia muralis* (Kunth) Nees, (Poaceae); *Trifolium campestre* Schreb. in Sturm and *Trifolium dubium* Sibth. (Fabaceae); *Petrorhagia nanteuilii* (Burnat) P.W. Ball and Heywood and *Spergularia purpurea* (Pers.) D. Don (Caryophyllaceae) and *Plantago coronopus L.* and *Plantago lagopus* L. (Plantaginaceae). Seeds from at least 10 individuals from a large population were collected and mixed. Seed material was stored in dry, dark lab conditions prior to the experiments.

2.2. Seed trait measurement

Three parameters were measured to characterize the collected seeds: seed mass and the effect of light and daytime temperature fluctuations on germination. Average seed mass was calculated by weighing 10 samples of 50 seeds from each species, except for *Spergularia purpurea* for which we weighed 10 samples of 100 seeds due to their small size.

The effects of light and diurnally fluctuating temperatures on germination were measured in two controlled phytotron experiments (Ibercex V-450-D ASL, SA, Madrid), with OSRAM L 18 W/20 fluorescent lamps. The seeds were placed in 5 cm diameter Petri dishes on filter paper (73 g m^{-2}) above a 2.5 g vermiculite substrate. The number of germinated seeds per plate was noted at the end of the experiment after 21 days in the phytotron. Distilled water was added daily to maintain moisture. The specific experimental conditions of each experiment were:

(a) *Light response*: Three replicates containing 25 seed from each of the 10 species were incubated for 16 h (25 °C) light and 8 h (15 °C) darkness, and compared with three replicates of 25 seeds from the same ten species for 16 h (25 °C) and 8 h (15 °C) darkness. The plates in darkness were covered with aluminum foil, with water added in this case in camera obscure. A light response index (LI) was defined as the percentage of germinations under light conditions divided by the sum of the percentages of germination in light and darkness.
(b) *Diurnal temperature fluctuation response*: Three replicates containing 25 seeds from each of the 10 species were incubated for 16 h (25 °C) light and 8 h (15 °C) dark, with a further three replicates maintained for 16 h (20 °C) light and 8 h (20 °C) darkness. We calculated the diurnal temperature fluctuation response index (DTF) as the germination percentages under temperature fluctuations, divided by the sum of germination percentages under temperature fluctuations plus germination percentages under constant temperature conditions.