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Response of drought and fertilization in *Erica andevalensis* seed banks: Significance for conservation management

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ABSTRACT

The present study attempts to investigate the size, composition and seedling dynamics of the seed bank of a metalliferous and vulnerable species, *Erica andevalensis*. Samples were taken during spring and autumn from two different sites. We also studied the effects of nutrient solution, irrigation from the river Tinto and irrigation deficit on seeding establishment and survival. Only *E. andevalensis* and Poaceae species emerged from the seed banks, although the former was dominant (98%). Germination and seedling establishment was totally inhibited by the waters of the river Tinto. Seed density was high in the soils of both seed banks irrigated with water and nutrient solution. We found no seasonal differences in the seed bank and number of germinated seeds and mortality rate and density were similar. From the standpoint of restoration management, the results indicate that the seed bank is a very important factor for successful species establishment.

1. Introduction

Seed banks act as a reservoir of plant propagules (Johnson and Bradshaw, 1979; Clemente et al., 2007) and play an important role in ecological restoration (Van der Valk and Pederson, 1989; Mitchell et al., 1998) and conservation of rare species (Fischer and Matthies, 1998). Currently, studies on seed banks in natural vegetation refer to their use for conservation of rare species (Aparicio and Guisande, 1997; Fischer and Matthies, 1998) and provide criteria for choosing the most viable method for restoring degraded areas (Salemaa and Uotila, 2001). The absence of vegetation cover enhances the risk of soil erosion, reduces the water holding capacity of the soil and facilitates leaching of heavy metals into ground water (Freedman and Hutchinson, 1980; Derome and Nieminen, 1998). Vegetation may also improve nutrient conditions in the soil (Cobb et al., 2000) and provide the basis for establishment of self-sustaining vegetative cover (Chan et al., 2003). Mining usually causes extreme damage to soil and vegetation because the original ecosystems have been grossly disturbed or buried in the process (Bradshaw, 2000). Mine tailings are difficult to revegetate due to the lack of organic matter, severe nutrient limitations, and potential metal toxicity (Bradshaw and Johnson, 1992). Therefore, mine tailings may require addition of organic amendments (e.g. biosolids) and mineral fertilizer in order to promote plant establishment (Santibáñez et al., 2007; Conesa et al., 2007). Phytostabilization is a remediation technology based on the use of appropriate plant species and used to immobilize contaminants in soil through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants (Cunningham et al., 1995; Padmavathiamma and Li, 2007; Pilon-Smits and Freeman, 2006).

Revegetation of degraded soils is therefore essential, and *Erica andevalensis* Cabezudo & Rivera was proposed for use in revegetation plans intended to promote the development of self-sustaining vegetative cover on contaminated soils in pyretic mining areas (Rossini Oliva et al., 2011; Monaci et al., 2011). *E. andevalensis* is a vulnerable species clearly associated with mining activities in the Andévalo area (Huelva, SW of Spain) and in Southern Portugal (São Domingo). Studies on *E. andevalensis* have focused mostly on germination behavior and requirements (Aparicio, 1995; Rossini Oliva et al., 2009a), species distribution (Aparicio, 1999), metal accumulation in plant tissues (Turnau et al., 2007; Monaci et al., 2011) or composition of phenolics (Márquez-García et al., 2009). Soil seed banks beneath vegetation on soils polluted by heavy metals might conceivably comprise living seeds of species that have long been eradicated from the established phase by heavy-metal stress (Meerts and Grommesch, 2001). No studies examine seed bank dynamics or seedling survival in this species and, since the

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seed bank is a key component of vegetation dynamics, detailed knowledge of seed banks might be of great practical interest for managing restoration of the study area.

The main objectives of this study are: 1) to assess the seed bank dynamics of *E. andevalensis* in two different sites in the mining area of Riotinto 2) to study the size and composition of the seed bank 3) to determine the effects of soil fertilization and irrigation with water from the river Tinto on germination number and mortality rate 4) to assess the impact of water deficit on seedling mortality. This would enable us to determine whether the tailings can be recovered naturally without chemical treatments and to establish how species survival is affected by drought.

2. Materials and methods

2.1. Study area and seed bank collection

The Riotinto mining area (Huelva province) is located in the Southwest of the Iberian Peninsula and covers an area greater than 4000 ha affected by mining and extraction activities. This has constituted one of Spain's most important mining areas in the last few centuries and it has been altered over more than 2000 years of history. Many authors have confirmed metal contamination therein (Soldevilla et al., 1992; Rodríguez et al., 2007; Monaci et al., 2011). The area has a Mediterranean climate with a mean annual rainfall of 750 mm and a mean annual temperature of 17 °C. The natural vegetation is mainly based on plant species with xerophitic characteristics, and *Erica* spp. are dominant. This area, together with southern Portugal, contains most of the sites where *E. andevalensis* can be found. Soil samples were collected in March 2008 on the banks of the river Tinto (RT) and in October in Peña de Hierro (PH). These two dates were chosen in order to allow for possible seasonal variations in the density of germinable seeds. Thus, autumn samples were intended for detection of species with transient seed banks and in order to obtain an estimate of maximum seed density after dispersal. Spring samples were intended to assess only the persistent seed banks. In these sites we observed young seedlings of *E. andevalensis* in autumn and spring (Fig. 1). The two sites present a conspicuous difference in floristic composition. In PH, *E. andevalensis* and *Erica australis* are the dominant species throughout the whole site, which is an open heathland afforested with pines, whilst in RT, close to the Tinto river headwater, the samples were collected from the banks where many other species can be found growing close to *E. andevalensis* (*E. australis*, *Cistus ladanifer*, *Cistus monspeliensis*, *Cistus salvifolius*, *Cistus populifolius*, *Nerium oleander*, *Ulex eriocladus* and *Elychrisum stoechas*). The main soil properties, total metals and available nutrients (such as water extracted) for both sampling points are shown in Table 1. For the study of seed banks, collection of small and numerous samples of soil is usually recommended, as opposed to a small number of large samples (Roberts, 1981). In each site we chose a plot of 6 m × 6 m for seed bank sampling and sampled seed banks within the 2 m × 2 m plot; to this end we randomly sampled 16 soil samples at a depth of 5 cm.

The germination experiments were performed in the University of Seville's greenhouse; soil samples were air dried, stones and plant fragments were removed, samples were mixed and distributed in different trays (20 × 15 × 4 cm). Holes were pierced in the bottom of each tray to enable exchange of water and ions. Environmental conditions consisted of natural photoperiods and a relative humidity of 70%. Three replicates were established for each experiment. Three different experiments were performed in the RT seed bank: one seed bank soaked with distilled water (DW), a second one irrigated with water from the river Tinto (RTW − pH 2.1) and the last one was irrigated with nutrient solution (NS) containing essential macro- and micro-nutrients at pH 6. We used a nutrient solution containing (in mM): NO_3^-, 4; $H_2PO_4^-$, 1; SO_4^{2-}, 2.5; K, 4; Ca, 2; Mg, 1. The micronutrients, except for Cu and Zn (as the soils are enriched by these two elements), were supplied as prescribed in the Long Ashton nutrient formula (Hewitt, 1966) and Fe was provided as 4 mg/l Fe-EDDHA. This nutrient solution composition was previously used for hydroponic cultivation of *E. andevalensis* (Rossini Oliva et al., 2010).

We applied the watering solutions regularly to maintain the moisture of the soil and to promote germination. Before starting the different treatments, trays were kept moist with distilled water at 4 °C for 20 days to increase the germination of this species (Aparicio, 1995).

Fig. 1. Seedlings of *Erica andevalensis* in the field.