

Soil seed bank formation during early revegetation after hydroseeding in reclaimed coal wastes

J. González-Alday^{a,b,*}, R.H. Marrs^b, C. Martínez-Ruiz^a

^a Área de Ecología, E.T.S. de Ingenierías Agrarias de Palencia, Universidad de Valladolid, Campus La Yutera, Avda. de Madrid 44, 34071, Palencia, Spain

^b Applied Vegetation Dynamics Laboratory, School of Biological Sciences, University of Liverpool, Liverpool, L69 7ZB, UK

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ABSTRACT

The soil seed bank is an important component of ecosystem resilience and represents a stock of regeneration potential in many plant assemblages; however, little is known about the initial development of seed bank during restoration. We characterized the size and composition of the soil seed bank in a reclaimed coal mine in Spain. For that, the initial seed bank of soil-forming material and cattle manure that was spread over it analyzed before hydroseeding. Later, the seed bank that developed in the two seasons (2.3 years) after hydroseeding was resampled, taking in consideration the distance to natural communities and topography. The seed bank increased from virtually nothing to 1813 seeds m⁻² over the study period, and was composed of mainly native species, which were more abundant near seed sources in the adjacent landscape. Topography only influenced the size of the hydroseeded species seed bank, with four species comprising approximately 45% of the seed bank. There were also variations in seed bank species number and composition in the different areas of the same mine. These results emphasize the necessity of taking care when including foreign species in a hydroseeding mixture, and of considering seed bank development within each area of a site in management planning. Otherwise differences may condition the future vegetation recovery from the desired target, creating very different communities in very close proximity.

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1. Introduction

The reconstruction of ecosystem function is one of the major challenges of restoration ecology (Aronson and van Andel, 2006), especially if the aim is to change degraded systems into ones that will be sustainable in the long term (Hobbs and Norton, 1996). The components that must be restored for long-term sustainability include some form of plant cover (Hobbs and Norton, 1996; Davy, 2002), and as an outcome, the formation of a soil seed bank. The soil seed bank represents a stock of regeneration potential in many plant assemblages (Dessaint et al., 1997; Richter and Stromberg, 2005) and is an important component of ecosystem resilience (Thompson, 2000). The soil seed bank is derived from a combination of stored seed that has been incorporated over many years plus seed input recently from the current species pool. The seed bank is, therefore, available to provide propagules for species turnover

(Pakeman and Small, 2005; Hui and Keqin, 2006) and to help recovery from perturbations (Swanton and Booth, 2004; Riemens et al., 2007). As a result, the seed bank, depending on environmental changes at the site (Leck et al., 1989), has the potential to produce changes in vegetation composition during ecological restoration in the longer term. However, if the seed bank contains a high proportion of alien species or generalist weedy species then there is an issue for medium-to-long-term sustainable restoration, especially when the aim is to restore semi-natural communities of high conservation value (Ghorbani et al., 2007). Clearly, where mineral wastes with a very limited starting seed bank are to be restored, the initial revegetation phase will be a crucial point in the initial development of the seed bank. Here, we investigated the development of the soil seed bank during the reclamation of coal wastes in Palencia, northern Spain.

The waste left after opencast coal extraction creates localized, highly degraded sites where there are statutory or planning requirements to ensure the restoration of vegetation cover (Cooke and Johnson, 2002; Moreno-de las Heras et al., 2008). In most cases, the wastes remaining following mineral extraction are highly degraded (Tordoff et al., 2000), and revegetation is limited through a lack of (a) a seed bank which has been lost as a result of mining activity (Mathis and Middleton, 1999), and (b) reduced

* Corresponding author at: Área de Ecología, E.T.S. de Ingenierías Agrarias de Palencia, Universidad de Valladolid, Campus La Yutera, Avda. de Madrid 44, 34071, Palencia, Spain. Tel.: +34 979 108321; fax: +34 979 108440.

E-mail addresses: josucham@agro.uva.es, josucham@yahoo.com (J. González-Alday).

opportunities for dispersal from nearby colonization sources (Andrés and Mateos, 2006); both contribute to slow natural succession (Ash et al., 1994; Bradshaw, 1997). In order to surmount these problems the traditional restoration approach has been to add seeds, usually using herbaceous species to provide rapid vegetation colonization, which will assist in soil protection (Vallejo et al., 2006). Usually, forage grass and legume species are sown (Martínez-Ruiz et al., 2007), thus minimizing expense (Pensa et al., 2004); however, these species may not be part of the planned final vegetation target at the site. The seed bank that develops on the site will be expected to contain seeds of these foreign species, as well as those that colonize from the wider species pool. Knowledge of seed colonization is therefore an important requirement for developing a full understanding of the restoration process.

The potential of the seed bank for assisting in restoring richness is well known (Davy, 2002). Given its importance and implications for ecosystem restoration, it is therefore surprising that there have been so few studies quantifying the change in seed banks during restoration (but see Pärtel et al., 1998; Wagner et al., 2006; Ghorbani et al., 2007). There is, however, considerable uncertainty about the role of introduced (i.e. foreign) species relative to native species during the vegetation restoration process, and the effects of site-specific, spatial variables on seed bank development. Understanding these questions is important, because differences in seed bank formation may change the future development of vegetation composition (Davy, 2002). Moreover, a large proportion of the sown species in the seed bank may slow the development of the target community, or it may even create a new community with a very different composition from the target (Muller et al., 1998).

The aim of this study, therefore, was to characterize the size and composition of the seed bank from the vegetation of a reclaimed coal mine in Spain, 2.3 years after hydroseeding. We hypothesized that during this period: (1) the seed density would increase caused by the addition of hydroseeded species and native seed colonization, (2) the species forming a seed bank would be composed mainly of hydroseeded (foreign) species, (3) the site-specific spatial variables (distance of different areas to natural communities and topography) would influence the size of the seed bank, and (4) the species composition of seed bank would be also affected by site-specific spatial variables.

2. Materials and methods

2.1. Site description and hydroseeding

The study site was located in 'Poza Sell', a 10 ha restored open-pit coal mine near Villanueva de la Peña, Palencia, northern Spain (1185 m a.s.l.; 42°50'N, 4°38'W). The climate is sub-humid Mediterranean (M.A.P.A., 1991). Annual mean temperature in the area is 9°C and average annual precipitation is 980 mm (mean values for 1932–2007, based on 'Cervera de Pisuerga Meteorological Station'; data provided by the Spanish Meteorological National Agency), with a dry season in summer. The vegetation surrounding the study area consisted of a complex matrix with grasslands (*Bromus mollis*, *Vulpia myuros*, *Plantago alpina*, *Arenaria montana*), crop fields (*Avena sativa*, *Secale cereale*), remnants of natural shrubland (*Rosa canina*, *Erica cinerea*) and *Quercus pyrenaica* woodland (González-Alday, 2005).

After coal mining ceased, the final open-pit was filled with coal waste from nearby mines and the surface was covered with 50–100 cm of finer-textured sediments, then cattle manure was spread over the soil (30 t ha⁻¹). Thereafter, the site was hydroseeded by the mining company 'UMINSA' (Unión Minera

del Norte S.A.) in October 2003. The hydroseeding slurry contained 150 kg ha⁻¹ of soluble chemical fertilizer (8N:15P:15K), and 210 kg ha⁻¹ of a seed mixture containing grasses (81 wt%) and herbaceous legumes (19 wt%). The seed mixture contained a mixture of seeds from commercial and crop sources (70% vs 30%, respectively). The commercial seed included *Festuca* spp., *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Trifolium pratense*, *Lotus corniculatus* and *Trifolium repens* in 9:2:2:2:1:1:1 proportions; and the crop species included *A. sativa*, *S. cereale*, *Medicago sativa* in 3:3:1 proportions.

2.2. Soil seed bank before hydroseeding

To assess the potential of the initial soil-forming material as a seed source for restoration, the composition and size of the soil seed bank was analyzed in October 2003, before hydroseeding. Eighty-four soil cores (10 cm diameter, 5 cm depth) were sampled randomly over the whole study site. The cores were grouped spatially into 14 groups and mixed thoroughly to produce 14 bulked samples; these samples were sieved through a 4 mm mesh to remove large stones. At the same time, and because the cattle manure was spread in a clearly differentiated layer on top of soil, it was sampled separately, but at the same sampling intensity and volume (84 samples of 393 cm³ each). These were also grouped spatially to 14 bulked samples. The soil and manure samples were spread (0.5 cm layer) in plastic trays (25 cm × 30 cm × 8 cm), along with two control trays filled with sterilized sand (2 cm layer) to detect contaminant seeds. All samples were cultivated under field conditions (temperature range 15–22°C and natural light conditions) from October 2003 until the point where no new seedlings had emerged for 4 weeks (12th June 2004). The number of emergent seeds was counted in each tray weekly, and every 2 months the soil in each tray was mixed to maximize germination (Kitajima and Tilman, 1996; Thompson et al., 1997). The emergent seedlings were identified using Muller (1978), Chancellor (1983) and Villarías (2006), counted and removed.

Finally, to assess the number of ungerminated seeds remaining in the soil, each of the samples was mixed for 30 min in a solution of sodium hexametaphosphate (50 g l⁻¹) (Decaëns et al., 2003; Makarian et al., 2007). This suspension was passed through two sieves (0.5 and 0.2 mm) and the material retained in each sieve was dried for 48 h and any intact seeds were counted and identify under a stereoscopic microscope (×10–40).

2.3. Soil seed bank after hydroseeding

In order to assess how the soil seed bank had developed during restoration, it was resampled at the end of February 2006, two growing seasons (2.3 years) after hydroseeding. At this time the sampling took place after the main germination period, but before new seed could disperse to assess the persistent part of the seed bank.

Sampling of the 10 ha mine was stratified at two levels to account for site topography (flat areas vs slopes) and distance to natural communities (close vs distant). Therefore four areas were selected: (1) a flat area in contact with natural vegetation (F_c), (2) an area also in contact with natural vegetation but on a 25° slope (S_c), (3) an area isolated from natural vegetation but on a flat area (F_i), and (4) an area also isolated from natural vegetation on a 25° slope (S_i).

Each of these four areas was divided in three strips running perpendicular to the maximum slope gradient. On the slopes these strips were located at different elevations (upper, middle, bottom), whereas on the flat sites they were located parallel to the regions divided in slope areas and represented geographical position on the site (south, middle and north).

Table 1
The number of seeds (number per 84 samples) and species composition of the soil-forming materials (soil and manure) applied before hydroseeding the 'Poza Sell' reclaimed open-pit coal mine, Villanueva de la Peña, Palencia, northern Spain. The probability (p) of detecting a seed of each species in a single sample is also given.

Soil			Manure		
Species	Number of seeds	p	Species	Number of seeds	p
<i>Briza media</i>	1	0.01	<i>Erodium cicutarium</i>	1	0.01
<i>Agrostis</i> spp.	1	0.01	<i>Vicea</i> spp.	1	0.01
<i>Sanguisorba minor</i>	1	0.01	<i>Dactylis glomerata</i>	1	0.01
<i>Arenaria</i> spp.	2	0.02	<i>Trifolium glomeratum</i>	2	0.02
<i>Cerastium</i> spp.	2	0.02	<i>Daucus carota</i>	2	0.02
<i>Capsella bursa-pastoris</i>	3	0.04	<i>Trifolium campestre</i>	4	0.05
Total	10	0.12		11	0.13

In each of these 12 strips, six 5 m × 5 m plots were located randomly. At each plot, eight soil cores were sampled at random locations using a soil corer with a diameter of 3 cm and 2 cm deep. The lower sampling depth was based on results of the study made before hydroseeding, which suggested that low seed numbers would be detected and it was thought unlikely that these would have been moved below 2 cm within 2.3 years. The eight soil cores per plot were mixed thoroughly to produce six samples per strip.

The method for assess the seed bank was replaced from emergence to the sieving/flotation technique (Barralis et al., 1986; Shaukat and Siddiqui, 2004), as a result of (a) the limited space in the greenhouse to cultivate all the samples at the same time, and (b) at view of the few seed found in the initial seed bank analysis, and because initial seed bank analysis was only planned to characterize the potential seed source of soil-forming material and manure, not to compare strictly with the new developed seed bank.

Soil from each plot was mixed in a solution of sodium hexameta-phosphate (50 g l⁻¹), and the seeds floating on the surface of the suspension collected. Thereafter, the suspension was passed through a four sieves with mesh widths of: 1.0, 0.8, 0.5 and 0.2 mm. All sieve fractions were dried for 48 h, and seeds were sorted with a stereoscopic microscope (×10–40). The germination ability of these seeds was assessed by placing them in Petri dishes with absorbent paper in a germination chamber at 25 °C, 14 h daylight (5–8 klx) and 15 °C, 10 h at night, for 3 months and watered three times a week (Reiné et al., 2004). The number of seeds germinating (criteria = appearance of the radicle) was counted weekly for 3 months (from 5th March to 5th June) (Bernhardt et al., 2008).

In all cases, seeds identification was made using Cappers et al. (2006) and Villarías (2006) and through comparison with reference specimens collected from the mine in spring and summer of 2006.

2.4. Data analyses

The pre-hydroseeding data were assessed using descriptive statistics and the following procedures were applied to the results obtained after 2.3 years. The seed bank data set was analyzed using both univariate and multivariate methods. Univariate analysis was performed with generalized linear mixed model (GLMM), where several response variables (total number of seeds, number of seeds of selected species groups (hydroseeded species, native species), the two most important species (*T. repens* and *L. perenne*), species richness, Shannon-Wiener and Simpson diversity indexes) were tested to evaluate if distance to natural community (close or isolated), topography for hydroseeded species (slope or flat), area (F_c , S_c , F_i , S_i) and slope elevation (upper, middle, bottom) or position in flat areas (south, middle, north) are assignable causes of variability in the seed bank formation (Pinheiro and Bates, 2000).

Within the GLMM, spatial variables nested from largest scale to smallest (distance to natural community or topography/

area/elevation-position) were included only as random effects. The model simplification guidelines for hierarchical data of Crawley (2007) were used. The minimal adequate models (MAMs) were derived through fitting of a model with all the spatial variables as random ones and the deleting the variables one at time, and then comparing the depleted model with the previous one using the ANOVA function and the chi-square statistic (χ^2) as the deletion test. The more general model is preferred to more simplified one if the p -value for the deletion test is lower than 0.05. After all, to assess if the random effects are required in the model comparisons vs null model were carried out. As well, the Akaike's Information Criterion (AIC; Akaike, 1973) was used to aid model selection (Pinheiro and Bates, 2000). For all MAMs the reduction of AIC (Δ AIC) was calculated as: Δ AIC = AIC_i – AIC_{min}, where AIC_i is the AIC of the initial model and AIC_{min} is of the minimal adequate model (Burnham and Anderson, 2002). Models were fitted using Laplace method, the log-link function and a Poisson error distribution for count data and the logit-link and a Binomial error distribution for the diversity data (Crawley, 2007).

Multivariate analysis was used to relate seed bank species composition to the spatial explanatory variables (distance to the natural community, area and elevation-position). The species dataset was reduced by removing all species which only occurred in one sample and a Hellinger transformation was then applied (Legendre and Gallagher, 2001). An initial detrended correspondence analysis produced eigenvalues of ($\lambda_1 = 0.39$, $\lambda_2 = 0.35$, $\lambda_3 = 0.30$, $\lambda_4 = 0.28$) and gradient lengths (GL) of (GL₁ = 3.06, GL₂ = 3.38, GL₃ = 2.55, GL₄ = 2.67) for the first four axes. The gradient lengths suggested that unimodal canonical correspondence analysis (CCA) was appropriate for subsequent analyses (Ter Braak and Šmilauer, 2002). Using CCA the number of constraining variables (distance to the natural community, area and elevation-position) was reduced using forward selection based in AIC as the selection criterion (Oksanen et al., 2007). Significance of the contrasts was assessed using permutation tests using the reduced model with 199 permutations. Standard deviational ellipses (95% confidence limits) were then used to illustrate each area in the biplot (Milligan et al., 2004).

All statistical analyses were implemented in the R software environment (version 2.7; R Development Core Team, 2008), using the LME4 package for GLMM (Bates and Sarkar, 2007) and the Vegan package for both multivariate analyses and the calculation of diversity indexes (Oksanen et al., 2007).

3. Results

3.1. The soil seed bank before hydroseeding (Table 1)

Few seeds were detected in either the soil or the manure before hydroseeding, and there were no species in common between the two substrates. In 84 samples, 10 seeds from six species were found in the soil and 11 seeds from six species in the manure. There was,

Table 2

Results of deletion test for random effects of generalized linear mixed models (GLMMs) for the different variables measured in the seed bank developing after hydroseeding the 'Pozo Sell' reclaimed open-pit coal mine, Villanueva de la Peña, Palencia, northern Spain.

Response variable	Model	AIC	Deletion test	χ^2	p-value	Δ AIC MAM
Number of seeds	Mod1 = 1 + (1 Site/Area/Ele.pos)	75.88				
	Mod2 = 1 + (1 Area/Ele.pos)	73.88	1 vs 2	0.001	0.999	
	Mod3 = 1 + (1 Ele.pos)	91.53	2 vs 3	19.645	<0.001	
	Mod4 = 1 + (1 Area)	71.88	2 vs 4	0.005	0.941	4.00
	Null model	108.37	4 vs null	36.482	<0.001	
Hydroseeded seeds	Mod1 = 1 + (1 Topo/Area/Ele.pos)	75.40				
	Mod2 = 1 + (1 Area/Ele.pos)	76.82	1 vs 2	4.427	0.042	
	Mod3 = 1 + (1 Topo/Ele.pos)	72.20	1 vs 3	0.001	0.999	3.20
	Mod4 = 1 + (1 Ele.pos)	73.48	3 vs 4	3.998	0.046	
	Mod5 = 1 + (1 Area)	76.78	3 vs 5	6.585	0.010	
	Null model	105.2	3 vs null	34.998	<0.001	
Native seeds	Mod1 = 1 + (1 Site/Area/Ele.pos)	110.12				
	Mod2 = 1 + (1 Area/Ele.pos)	110.31	1 vs 2	2.196	0.138	
	Mod3 = 1 + (1 Site/Ele.pos)	108.10	1 vs 3	0.001	0.999	2.02
	Mod4 = 1 + (1 Ele.pos)	138.70	3 vs 4	32.579	<0.001	
	Mod5 = 1 + (1 Area)	116.70	3 vs 5	10.564	<0.001	
	Null model	144.22	3 vs null	38.102	<0.001	
Richness	Mod1 = 1 + (1 Site/Area/Ele.pos)	43.66				
	Mod2 = 1 + (1 Area/Ele.pos)	41.75	1 vs 2	0.096	0.757	
	Mod3 = 1 + (1 Ele.pos)	46.34	2 vs 3	6.584	0.010	
	Mod4 = 1 + (1 Area)	39.83	2 vs 4	0.081	0.777	3.83
	Null model	49.17	4 vs null	9.335	<0.001	
<i>L. perenne</i>	Mod1 = 1 + (1 Topo/Area/Ele.pos)	112.30				
	Mod2 = 1 + (1 Area/Ele.pos)	110.27	1 vs 2	0.316	0.574	2.03
	Mod3 = 1 + (1 Ele.pos)	114.15	2 vs 3	5.880	0.015	
	Mod4 = 1 + (1 Area)	113.60	2 vs 4	5.327	0.021	
	Null model	146.53	2 vs null	38.260	<0.001	
<i>T. repens</i>	Mod1 = 1 + (1 Topo/Area/Ele.pos)	170.81				
	Mod2 = 1 + (1 Area/Ele.pos)	169.39	1 vs 2	0.585	0.444	
	Mod3 = 1 + (1 Ele.pos)	171.39	2 vs 3	3.998	0.046	
	Mod4 = 1 + (1 Area)	168.31	2 vs 4	0.918	0.338	2.50
	Null model	178.20	4 vs null	10.805	<0.001	

therefore, less than a 0.15 probability of detecting a seed in any given sample. This maximum seed density of 11 seeds per 84 samples of soil equates to 17 seeds m⁻², whereas manure, distributed homogeneously had a seed density of 10 seeds per 84 samples producing a maximum of 15 seeds m⁻². Neither species found in the soil nor manure was sown in the hydroseeding mix.

The most abundant species in the soil was *Capsella bursa-pastoris* (30%), and the most abundant family was Caryophyllaceae (40%) comprising two taxa (*Arenaria* spp. and *Cerastium* spp.). Those three taxa represent 70% of the seeds found in the soil. The species found

in the manure were dominated by the Fabaceae (64%) and *Trifolium campestre* was the main species (36%).

3.2. The soil seed bank after hydroseeding (Tables 2 and 3)

The seed bank size 2.3 years after mine restoration increased considerably reaching a total of 738 germinated seeds of 46 species corresponding to an overall seed density of 1813 seeds m⁻². The total seed number variability is originated mostly among areas (Table 2), with a greater number of seeds in S_c (14.78 ± 0.61; Table 3)

Table 3

Seed number mean values per unit area or elevation-position ± S.E. in brackets for the different variables measured in the seed bank developing after hydroseeding the 'Pozo Sell' reclaimed open-pit coal mine, Villanueva de la Peña, Palencia, northern Spain. Elevation-position mean values are calculated grouping the samples from different zones but from the same position in the slopes.

Random variable	Number of seeds	Hydroseeded seeds	Native seeds	Richness	<i>L. perenne</i>	<i>T. repens</i>
Area						
F _c	9.22 (0.66)	2.89 (0.25)	6.33 (0.68)	5.56 (0.42)	0.33 (0.16)	1.00 (0.29)
S _c	14.78 (0.61)	6.78 (0.60)	8.00 (0.97)	7.50 (0.40)	2.89 (0.47)	1.55 (0.40)
F _i	7.39 (0.63)	3.38 (0.36)	4.00 (0.49)	4.06 (0.26)	0.89 (0.29)	0.94 (0.29)
S _i	9.61 (0.70)	5.94 (0.62)	3.67 (0.42)	5.17 (0.28)	1.39 (0.32)	2.66 (0.65)
Elevation-position						
Ele-upper	12.33 (1.31)	4.75 (0.63)	7.58 (1.44)	6.50 (0.67)	0.83 (0.20)	2.83 (0.75)
Ele-mid	11.58 (0.88)	6.50 (0.65)	5.08 (0.80)	5.67 (0.40)	2.66 (0.59)	1.92 (0.53)
Ele-bott	12.67 (1.12)	7.83 (0.73)	4.83 (0.86)	6.83 (0.51)	2.92 (0.53)	1.58 (0.72)
Pos-south	7.33 (0.38)	3.17 (0.34)	4.17 (0.49)	4.17 (0.27)	0.58 (0.36)	0.83 (0.30)
Pos-mid	7.58 (0.99)	2.83 (0.42)	4.75 (0.85)	4.25 (0.43)	0.50 (0.29)	1.00 (0.41)
Pos-north	10.00 (0.81)	3.42 (0.40)	6.58 (0.87)	6.00 (0.52)	0.75 (0.25)	1.08 (0.36)

compared to the rest. The number of seeds from the hydroseeded species represented only 46% of the seed bank, and was influenced by topography (Table 2), with a greater density on the slopes (6.36 ± 0.43) than in flat areas (3.14 ± 0.22). At the same time, hydroseeded seeds density varied significantly among the elevations on the slopes (Table 2), with a greater number of seeds at the bottom (7.83 ± 0.73) and middle parts (6.5 ± 0.65) than in upper ones (4.75 ± 0.63 ; Table 3).

The native species represented 54% of the total seeds found, and showed that there was a significant effect attributable to site and elevation-position (Table 2), with greater density when close to natural communities (7.17 ± 0.60) than in isolated areas (3.83 ± 0.32) and with greater density in upper elevation of the slopes (7.58 ± 1.44), and in north position of the flat areas (6.58 ± 0.87 ; Table 3).

The total species richness at each plot ranged from 2 to 10 species. The mean species richness showed a significant variation among areas (Table 2), with greater values than the rest in S_c (7.5 ± 0.40) and the lowest in F_i (4.06 ± 0.26 ; Table 3). Results for the two diversity indexes showed the same statistical results as richness so are not reported here in detail but the Shannon-Wiener diversity ranged from 1.82 ± 0.09 in F_i to 2.56 ± 0.09 in S_c , whereas Simpson diversity ranged from 0.68 ± 0.02 in F_i to 0.79 ± 0.02 in S_c .

3.3. Individual species in the soil seed bank after hydroseeding (Tables 2 and 3)

Seeds from only 6 out of the 10 hydroseeded species were detected, the most abundant being *T. repens* (15%), *L. perenne* (13%), *P. pratensis* (9%) and *Festuca* spp. (8%), the remaining two hydroseeded species (*T. pratense*, *P. pratense*) were present at <1%. *T. repens* seeds were more abundant in S_i than in the rest (S_i : 2.67 ± 0.65 ; Tables 2 and 3), whereas *L. perenne* had a greater density than the rest in S_c (S_c : 2.89 ± 0.47) and the lowest in F_c (F_c : 0.33 ± 0.16). *L. perenne* also showed significant variation between the elevation-positions on the slopes (Table 2), with a greater number of seeds at the bottom (2.92 ± 0.53) and middle parts (2.66 ± 0.59) than in upper ones (0.83 ± 0.20 ; Table 3). *P. pratensis* and *Festuca* spp. did not show significant variation between topography, areas or elevation-positions, whereas *T. pratense* and *P. pratense* were not present in sufficient quantity to fit models.

Forty native species were found, the most abundant being *T. campestris* (11%) and *T. glomeratum* (9%). Both of these species were detected in low numbers in the manure applied over the mine; but after 2.3 years neither species showed significant variation between sites, areas or elevation-positions. As all of the other 38 native species were present in low numbers; models could not be fitted.

3.4. Overall species composition of the soil seed bank after hydroseeding (Fig. 1 and Table 4)

Area was the only explanatory variable included in the model after forward selection in CCA reducing the AIC of the null model from 184 to 182, and the significance of the model was $p < 0.005$. The constrained inertia within this CCA was 0.55 and $\lambda_1 = 0.25$ and $\lambda_2 = 0.20$. The species plot (Fig. 1a) showed three of the four main hydroseeded species (*T. repens*, *Festuca* spp. and *P. pratensis*) near the centre of the ordination and the fourth (*L. perenne*) nearby. The overall distribution of species reflected the sites of soil collection (Fig. 1b). All areas showed some degree of overlap around the origin, because the soil composition in each area contains the seeded species. However, all four areas occupied a different region of the ordination space. The slope isolate area (S_i) was located on the right hand side and contained *T. tomentosum* and *Poligonum per-*

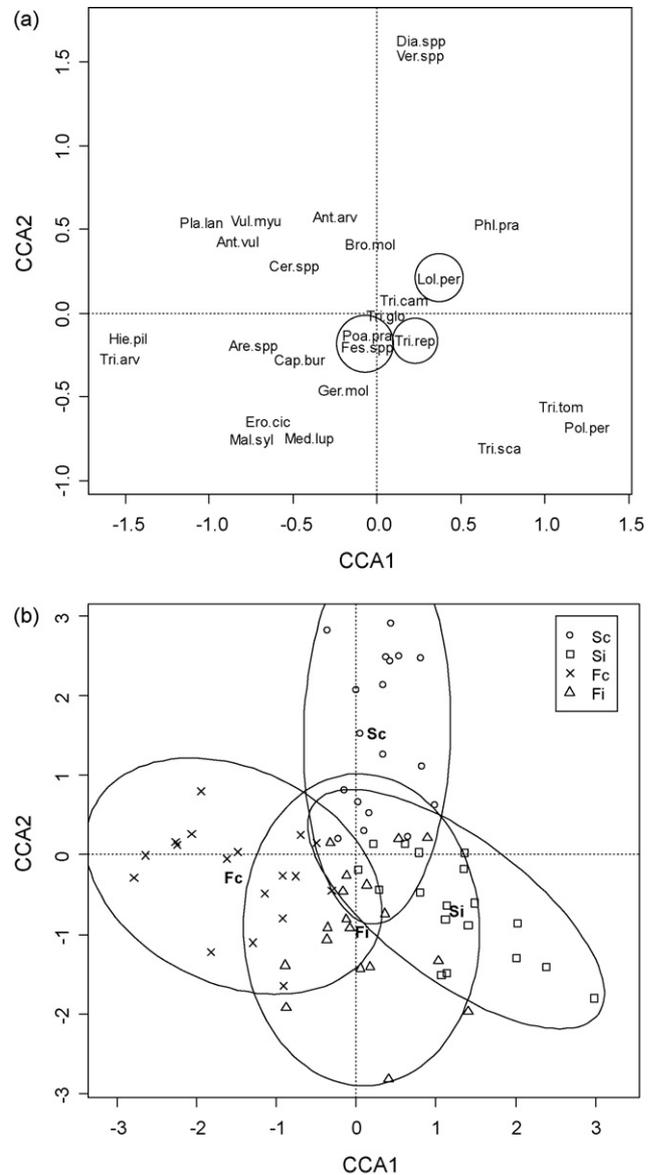


Fig. 1. Constrained CCA ordination plots of areas of the species composition of the seed bank 2.3 years after hydroseeding at the 'Poza Sell' reclaimed open-pit coal mine, Villanueva de la Peña, Palencia, northern Spain; the ordination was constrained on Area (S_c , F_c , F_i , S_i). (a) Species; hydroseeded species are circled, (b) plot positions within each area along with their SD ellipses (95% confidence limits). Species codes: *Anthemis arvensis*=Ant.arv; *Anthyllis vulneraria*=Ant.vul; *Arenaria* spp.=Are.spp; *Bromus mollis*=Bro.mol; *Capsella bursa-pastoris*=Cap.bur; *Cerastium* spp.=Cer.spp; *Dianthus* spp.=Dia.spp; *Erodium cicutarium*=Ero.cic; *Festuca* spp.=Fes.spp; *Geranium molle*=Ger.mol; *Hieracium pilosella*=Hie.pil; *Lolium perenne*=Lol.per; *Malva sylvestris*=Mal.syl; *Medicago lupulina*=Med.lup; *Phleum pratense*=Phl.pra; *Plantago lanceolata*=Pla.lan; *Poa pratensis*=Poa.pra; *Polygonum persicaria*=Pol.per; *Trifolium arvense*=Tri.arv; *T. campestris*=Tri.cam; *T. glomeratum*=Tri.glo; *T. repens*=Tri.rep; *T. scabrum*=Tri.sca; *T. tomentosum*=Tri.tom; *Veronica* spp.=Ver.spp.; *Vulpia myuros*=Vul.myu.

sicaria; the flat area in contact with the reference community (F_c) was located on the left hand side with *T. arvense* and *Hieracium pilosella* as the characteristic species, although it shared certain native species (*V. myuros*, *Plantago lanceolata*, *Anthyllis vulneraria*, *Cerastium* spp.) with the slope contact area (S_c) (Fig. 1b). Axis 2 separated the slope in contact with the natural community (S_c) on the upper part, with *Veronica* spp. as characteristic species, from the flat isolate area (F_i) that had showed less directional change (Fig. 1b).

Table 4

Number of seeds per square meter for the different species found in each area in the seed bank developing after hydroseeding the 'Poza Sell' reclaimed open-pit coal mine, Villanueva de la Peña, Palencia, northern Spain. Some seeds were not identified at the end of the experiment are recorded as Indet., but their family in brackets were included.

Species	F_c	S_c	F_i	S_i
<i>Trifolium repens</i>	177	275	167	472
<i>Trifolium pratense</i>	–	20	–	–
<i>Festuca</i> spp.	118	167	108	187
<i>Poa pratensis</i>	157	196	167	128
<i>Phleum pratense</i>	–	29	–	20
<i>Lolium perenne</i>	59	511	157	246
<i>Bromus mollis</i>	79	128	–	49
<i>Vulpia myuros</i>	59	59	–	–
<i>Trifolium campestre</i>	138	344	128	187
<i>Avena sulcata</i>	–	10	–	–
<i>Anthemis arvensis</i>	29	79	20	–
<i>Verbena officinalis</i>	–	29	–	–
<i>Plantago lanceolata</i>	10	10	–	–
<i>Dianthus</i> spp.	–	108	–	–
<i>Ranunculus sardous</i>	–	20	–	–
<i>Anthyllis vulneraria</i>	20	10	–	–
<i>Geranium molle</i>	20	10	20	10
<i>Capsella bursa-pastoris</i>	88	29	88	10
<i>Trifolium glomeratum</i>	118	265	147	118
<i>Minuartia mediterranea</i>	49	59	29	–
<i>Veronica</i> spp.	–	88	–	–
<i>Cerastium</i> spp.	88	69	–	29
<i>Arenaria</i> spp.	69	29	39	–
<i>Herniaria hirsuta</i>	–	29	–	–
<i>Erophyla verna</i>	–	10	–	–
<i>Erodium cicutarium</i>	39	–	39	–
<i>Malva sylvestris</i>	10	–	10	–
<i>Medicago polymorpha</i>	20	–	20	–
<i>Medicago lupulina</i>	20	–	39	–
<i>Trifolium scabrum</i>	–	–	108	98
<i>Cirsium</i> spp.	10	–	20	–
<i>Trifolium tomentosum</i>	–	–	–	39
<i>Rumex crispus</i>	–	–	–	20
<i>Polygonum persicaria</i>	–	–	–	29
<i>Lactuca serriola</i>	–	–	–	20
<i>Myosotis ramossissima</i>	–	–	–	10
<i>Centaurea cyanus</i>	–	–	–	20
<i>Trifolium arvense</i>	108	–	–	–
<i>Satureja acinos</i>	29	–	–	–
<i>Hieracium pilosella</i>	49	–	–	–
<i>Sanguisorba minor</i>	29	–	–	–
<i>Silene</i> spp.	39	–	–	–
Indet. (Caryophyllaceae)	–	10	–	–
Indet. (Poaceae)	–	10	–	–
Indet. (Dicotyledons)	–	–	–	10
Indet. (Fabaceae)	–	10	–	–

4. Discussion

The development of the seed bank during the early stages of the restoration of coal wastes by hydroseeding has been shown to be affected by the distance from natural communities, and by topography in the case of the hydroseeded species. The results also illustrate that seed bank development varied spatially across the site with differences found between four different areas; these results were in agreement with Ghorbani et al. (2007), who suggest that the seed bank formation appears to occur at different speeds and directions even in close locations.

4.1. The pre-treatment seed bank

Very low seed numbers were detected in the soil before hydroseeding was applied; moreover, the species composition was relatively limited and was formed mainly of species with very small seeds, i.e. ruderals (*Arenaria* spp., *Cerastium* spp. and *C. bursa-pastoris*; Pakeman and Marshall, 1997). The low seed numbers were

expected because the topsoil used had been stockpiled for 2 years and it is well known that such storage reduced seed numbers and viability by hypoxia and high concentrations of carbon monoxide (Davy, 2002). At the same time, the topsoil handling on this site mixed the topsoil with sediments from deeper parts of the open-cast pit, which will inevitably dilute the amount of viable seeds available for restoration.

Seeds could also be derived from the manure that was also added during the initial soil preparation (Malo and Suárez, 1995; Pakeman et al., 2002). However, in this study the number of seeds found in the manure was very low compared to those obtained from cattle manure elsewhere (18.5 seeds per dung corer with a diameter of 5 cm and 5 cm deep (Dai, 2000); 10.3 seeds per gram of dung (Traba et al., 2003); or 10–100 seeds m^{-2} (Cosyns et al., 2005)).

4.2. Seed bank development 2.3 years after hydroseeding

4.2.1. Size of the seed bank

An important result was that the soil seed bank increased from virtually nothing to 1813 seed m^{-2} over the study period, thus the first hypothesis is accepted. Nevertheless, it must take in consideration that the different seed bank methods used (core sizes and seed identification methods) may partially influence this increase. But at the same time, the size of the seed bank here is similar to those from other areas with low vegetation cover in Spain (e.g. in the badlands of the Upper Llobregat basin in Pyrenees; Guàrdia et al., 2000), and in alpine ski trials in France (Isselin-Nondedeu and Bedécarrats, 2007). However, the seed numbers are much lower than would be expected after conversion from conventional to organic farming systems, with an increase from 4000 to 17,000 seeds m^{-2} in a 3-year period (Albrecht and Sommer, 1998).

Surprisingly, the seed bank was not composed mainly of hydroseeded species as we had originally hypothesized; native species dominated the seed bank but there was a substantial component of hydroseeded species. Thus, the second hypothesis is partially accepted. This dominance by native species may be due to either (a) an increase in seed inputs from plants germinating from the initial inoculums present in the manure and soil, or (b) dispersal of seeds from the surrounding vegetation. The evidence we collected supported the second explanation because we notice that the seed density of native species in the soil increased when the distance between the sampling point and the nearest natural community was shorter. Distance from seed source is an important constraint for colonization (Lichter, 2000); seed colonization potential depends on its dispersal mechanisms and the distance that have to travel (Traba et al., 2003; Wagner et al., 2006).

Moreover, only two species that were present in the manure (*T. campestre* and *T. glomeratum*) were found in the vegetation after restoration (González-Alday and Martínez-Ruiz, 2007) and here were the most important native species found in the seed bank, reaching similar densities to species added during hydroseeding. In view of the very low numbers of these species found in the manure at the start, it is likely that the increase in the seed bank comes from (a) seed production from plants that germinated from the manure, and (b) seed from the surrounding species pool. Unfortunately, the exact pathway remains unknown.

4.2.2. Effect of location on seed bank development

The size of the seed bank was influenced by distance between the sampled and the adjacent natural communities which acted as seed sources, thus hypothesis three is accepted. However, the species richness and diversity of the soil seed bank appeared less sensitive measures and differed only between the different treated areas of the mine.

The lowest richness and diversity values were found on the flat isolated area (F_i), and the greatest values were found in slope area that was in contact with the natural community (S_c), suggesting that isolation decreases the probability of colonization (Quintana-Ascencio and Menges, 1996; Pärtel et al., 1998; Geertsema and Sprangers, 2002). This pattern was not so clear in F_c and S_i , because in spite of sharing the same species richness; the slope isolate area (S_i) richness was mainly composed by hydroseeded species (2.7 hydroseeded species out of 5.2), whereas the flat in contact with the natural community (F_c) is mainly composed by native species (4 native species out of 5.6).

The influence of topography only affected the size of the seed bank of the hydroseeded species. The greater seed bank size of hydroseeded species on slopes compared to the flat areas may be caused by a combination of factors: (a) differential application of seed, the hydroseeding was applied from the bottom of the slope, and it is possible that more spray/seeds were applied near the sprayer; (b) secondary movement caused of seed in runoff (Chambers and MacMahon, 1994), which was considerable in the early stages of restoration when vegetation was sparse and seed incorporation into the soil should have been relatively easy (Ghorbani et al., 2007). The relative importance of each of these possible explanations required further investigation. In contrast on the flat areas, where secondary movement of seeds is not so important (Chambers and MacMahon, 1994), the plants might act as seed traps (Bullock and Moy, 2004) reducing the number of seeds that reach the soil surface and therefore form the soil seed bank.

Previous studies of seed bank size in the badlands of the Upper Llobregat basin in Pyrenees (Spain) have reported no differences between topographic positions on slopes (Guàrdia et al., 2000). In contrast, our results showed that there was an increase in size of the hydroseeded species seed bank from the top to the bottom of the slope. The slope is, at least on this site, a factor that affects the seed dispersal; essentially because of gravity seeds cannot move upslope (Mack, 1995), and any movement will be down-slope. Downward movement could be enhanced by post-dispersal movement of the seeds by surface runoff (Chambers and MacMahon, 1994), especially for round seeds such as *Trifolium* spp. (Isselin-Nondedeu and Bedécarrats, 2007) or elongated seeds like *L. perenne*. Indeed, *L. perenne* seeds showed the same distribution pattern in the seed bank as the hydroseeded species, being affected by topography and increasing down-slope.

4.2.3. Effect of location on seed bank species composition

The seed bank species composition varied between the different areas in the same mine, thus the fourth hypothesis is accepted. These results support the conclusions of Ghorbani et al. (2007), who found differences in propagule bank formation between sites and at locations within a site.

It is known that during the first stages the sown and ruderals species dominate (Muller et al., 1998); in our case the same pattern was observed with the seed bank developed mainly by hydroseeded species, and ruderals (*Arenaria* spp., *Cerastium* spp. and *C. bursapastoris*) from the previous soil and manure applied. However, some native recruited species (*P. lanceolata*, *B. mollis*, *Dianthus* spp. and *V. myuros*) characteristics of the surrounding vegetation and important for restoration, as well as early colonizers wind dispersal species (*H. pilosella* and *T. tomentosum*) also appeared in an important size. Especially, on close to natural community areas (S_c and F_c), suggesting the importance of short distances to natural communities to improve the colonization potential (Wagner et al., 2006).

Particularly noteworthy is that four of sown species (*L. corniculatus*, *M. sativa*, *A. sativa* and *S. cereale*) did not appear in the seed bank and another two (*P. pratense* and *T. pratense*) had a very low seed

number. The reason for this might be that these species had a very low cover during the establishment phase on this mine (González-Alday et al., 2008). Their lower plant cover would have reduced seed production (Scursoni et al., 2007), and subsequently the amount of seeds that might be incorporated into the seed bank. These results have important implications for mine restoration, since the inclusion of those foreign species did not generate a seed bank, and therefore through time the foreign species are likely to be replaced by native species that will develop a seed bank (Muller et al., 1998). The other four hydroseeded species (*T. repens*, *L. perenne*, *P. pratensis* and *Festuca* spp.) comprised approximately 45% of the seed bank, and this will undoubtedly help these species persist (D'Antonio and Meyerson, 2002). As a result, they could present problems for vegetation composition in the medium-to-long-term restoration of target semi-natural communities (D'Antonio and Meyerson, 2002; Ghorbani et al., 2007).

5. Conclusions

Finally, our results emphasize that the development of a seed bank is an important part of the restoration process. At the same time, it seems to be essential that management plans take in consideration the differences in seed bank development of each site. Otherwise those differences may condition the future vegetation recovery, creating very different communities in very close areas.

When the restoration area is close to natural communities the native species component of the seed bank density increased, making easier the development of community towards finally vegetation target. On the other hand, where commercial foreign species are added through hydroseeding, they must be chosen with care, because some species become an important part of the seed bank, which may cause long-term problems. However, further investigation is needed to assess the long-term dynamics of hydroseeded and native recruited species and the factors that may limit seed establishment, once seeds arrive at the mine.

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