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TESIS DOCTORAL:

INFLUENCIA DE FACTORES ABIÓTICOS EN LA REVEGETACIÓN TEMPRANA TRAS HIDROSIEMBRA DE ESTÉRILES DE CARBÓN

INFLUENCE OF ABIOTIC FACTORS IN EARLY REVEGETATION AFTER HYDROSEEDING OF COAL MINES

Presentada por Josu González Alday para optar al grado de doctor por la Universidad de Valladolid

Dirigida por:
Dra. Carolina Martínez Ruiz
Dr. Robert H. Marrs

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Contents

Abstract

Open cast mining generates large environmental impacts where there are statutory or planning requirements to ensure restoration. In such damaged systems the aim of the ecological restoration should be to develop a long-term self-sustaining ecosystem, which resembles the natural ecosystems that were present before the mining operations took place. Nevertheless, the effective restoration of mines is a complex process, hampered mainly by the complete removal of vegetation and soil disturbance. As the restoration is implemented, there is an excellent opportunity to study the successional dynamics of vegetation development and its constraints in these newly-created ecosystems.

This thesis analyzed the vegetation succession processes after the restoration of open cast coal mines located in the Palencia province (Spain). The study was performed at two scales: short term (1-6 years after restoration) and medium term (1-40 years after restoration or abandonment), with the aim of providing recommendations for the effective restoration of these areas. In short term the aim was mainly to analyze the success of the restoration and the factors influencing the vegetation establishment, while the medium-term aim was to describe the species turnover and the potential successional constraints.

In short term, hydroseeded species were successful in slopes revegetation, mainly during the first two years. Native species, forced to colonize from the surroundings by the absence of a seed bank in the soil, increased in number and cover with age. Stochastic factors (climate and colonization) or abiotic (topography) played an important role in the composition and abundance of both native and hydroseeded species. In contrast, soil factors did not control species composition during succession, but some of them changed during the successional process (increase of organic matter, sand content), thus creating better conditions for subsequent native species establishment and development. The species composition and vegetation structure (richness, diversity and cover) was converging with the reference community in the six years after restoration.

The medium-term plant succession was conditioned by the restoration method used; whether topsoil was added or not. In non-topsoiled mines, substrate-based constraints arrested succession leading to the formation of a community characterized by early-successional species. In contrast, top-soiled mines showed the classical pattern of species turnover, with annual and perennial species richness peaks and an increasing complexity in vegetation structure during succession. From environmental and soil variables only age since restoration and pH conditioned the succession, although it moved in the desired direction approaching the composition of native vegetation, developing a native shrub community in 15 years and a young forest in 30 years.

Resumen

La minería a cielo abierto genera grandes impactos ambientales cuya restauración es una necesidad urgente. El principal objetivo de la restauración ecológica de estas zonas es la creación de ecosistemas auto-suficientes a largo plazo que se asemejen a la vegetación previa antes de la actividad minera. Sin embargo, la restauración de manera efectiva de las minas es un proceso complejo, dificultado principalmente por la total eliminación de la vegetación y la alteración del suelo. No obstante, durante los procesos de restauración se dispone de una excelente oportunidad para estudiar la dinámica sucesional de la vegetación y los factores ambientales que la condicionan.

En esta tesis se analizan los procesos sucesionales de la vegetación durante la restauración de minas de carbón a cielo abierto localizadas principalmente en la provincia de Palencia (España). El estudio se realiza a dos escalas: a corto plazo (1-6 años tras la restauración) y a medio plazo (1-40 años tras la restauración o abandono), con el objetivo de proporcionar recomendaciones para la restauración efectiva de estas áreas. A corto plazo se pretende analizar principalmente el éxito de la restauración y los factores que condicionan el establecimiento de la vegetación, mientras que a medio plazo se pretende describir tanto la sustitución de especies como los factores que limitan la sucesión vegetal.

A corto plazo, las especies hidrosembradas tuvieron éxito en la revegetación, principalmente durante los dos primeros años. Las especies autóctonas, obligadas a colonizar desde los alrededores por la ausencia de un banco de semillas en el suelo, aumentaron en número y cobertura con la edad. Los factores estocásticos (clima y colonización) o los factores abióticos (topografía) juegaron un papel importante en la composición y abundancia de especies tanto hidrosembradas como autóctonas. Por el contrario, los factores edáficos no condicionaron la composición de especies, aunque sufrieron cambios durante el proceso sucesional (incremento de materia orgánica) que favorecieron las condiciones para el establecimiento de nuevas especies. La composición de especies y la estructura de la vegetación se aproximaron a la vegetación de referencia en los seis años tras de la restauración.

La sucesión vegetal a medio plazo se vio condicionada por el método de restauración, es decir, si los estériles se recubrieron de suelo o no. En las minas no recubiertas de suelo las limitaciones debidas a las características del suelo ralentizaron la sucesión, dominando especies de etapas sucesionales jóvenes. Por el contrario, las minas recubiertas de suelo mostraron el patrón clásico de sustitución de especies, con picos en la riqueza de especies (primero especies anuales y después perennes) y un aumento en la complejidad de la estructura de la vegetación con la edad. Únicamente dos de las variables ambientales y edáficas estudias (la edad desde la restauración y el pH) condicionaron la sucesión vegetal, aunque ésta progresó con el tiempo en la dirección deseada acercándose a la composición de la vegetación autóctona, desarrollándose una comunidad arbustiva natural en 15 años y un bosque joven en 30 años.

Chapter 1

Open-cast coal mine closure

Introduction

Introduction

Mining is often accompanied by large-scale environmental disturbance that leaves a landscape with no vegetation and very poor soil-forming materials on which an ecosystem can develop (Herath et al. 2009). In such damaged systems the aim of the ecological restoration should be to develop a healthy, long-term, self-sustaining ecosystem (Hobbs & Northon 1996), which resembles the semi-natural or natural ecosystems that were present before the mining operations took place. As the restoration is implemented, there is an excellent opportunity to study the successional dynamics of vegetation development in these newly-created man-made ecosystems (Bradshaw 1983). The investigation of these man-induced successions in restored mines has both practical and theoretical importance (Grant 2009). Practically, because information on successional development might help guide future ecological restoration treatments, hence making the restoration process more effective (Walker & del Moral 2009), and helping to identify management intervention points (Grant 2009). Theoretically, because a scientific approach to restoration can provide insights into fundamental ecological theory, including understanding the initial phases of vegetation establishment (Robbins & Matthews 2009), and how the vegetation community structure, soil function and seed bank develop (del Moral & Walker 2007).

The coal mining operations in northern Spain (León and Palencia; Fig. 1) have changed markedly over the past 35 years, from the traditional underground mining to the modern surface mining (open-cast extraction). Approximately 5,000 ha of the land surface of this region is currently affected, generating localized impacts, where there are statutory or planning requirements to ensure high-quality restoration (Cooke & Johnson 2002; Moreno-de las Heras et al. 2008). The methods for this land restoration have also evolved over the last 35 years. At the start of this period no management intervention was carried out and vegetation developed through natural colonization. More recently, active restoration management has been implemented; initially through reshaping the landscape and improving the baseline soil-forming materials, but over the last 20 years through the use of a more technical restoration process with seed introduction. However these approaches have not always been successful in creating self-sustaining ecosystems. Therefore, a better understanding of mechanisms that govern successional processes after open-cast coal mining in this area is urgently needed to inform management decisions. These decisions should be based on both scientific knowledge (Prach et al. 2007) and economic considerations (Bradshaw 2002).

The initial re-vegetation phase of any restoration scheme provides the starting point for succession; once established, this vegetation will help determine the course of vegetation development at least in the short-term (Hobbs & Norton 1996; Hobbs & Harris 2001). However, little is known about the abiotic factors that control the composition and abundance of species during the early establishment phase, which are very important to improve restoration success.

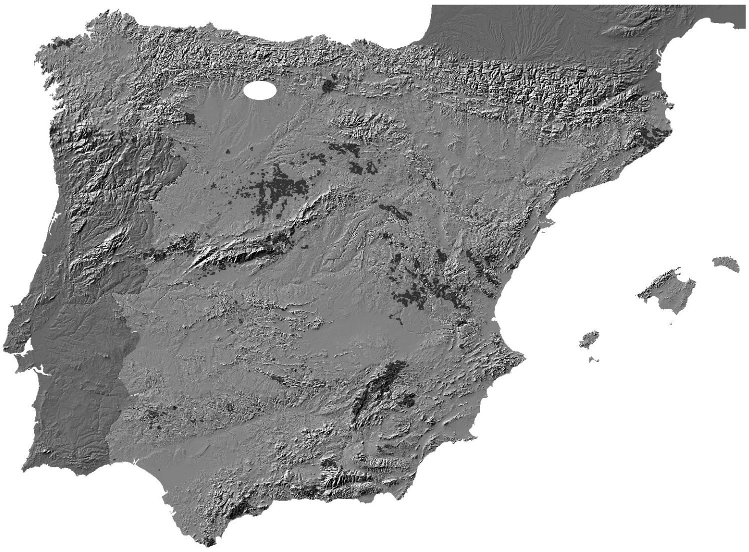

Figure 1. Geographic location of the study areas in Valderrueda-Guardo-Cervera coalfield in the León and Palencia regions of North Spain (white ellipse).

Overall, the main objective during the initial re-vegetation phase is the creation of a functioning ecosystem with some stable plant cover, and as an outcome, a soil seed bank. The soil seed bank is derived from a combination of stored seed and seed inputs from the current species pool. The seed bank is, therefore, available to provide propagules for species turnover (Pakeman & Small 2005; Hui & Keqin 2006) and to help recovery from perturbations (Swanton & 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 medium- to longer-term. Clearly, as mineral wastes have a very limited starting seed bank, the initial re-vegetation phase is a crucial point in the seed bank development.

Once re-vegetation is achieved, other processes come into play including species assembly rules, rates of species colonization and extinction, and both positive and negative species interactions; all of which interact producing species turnover and structural change (Bartha et al. 2003; Walker & del Moral 2008). At the same time, the changes in vegetation composition and structure can influence soil conditions directly (Isermann 2005). Vegetation development enhances the accumulation of carbon, nitrogen and nutrients (Marrs & Bradshaw 1993; Walker & Del Moral 2009), modifying the physical and chemical structure of the soil (Dölle & Schmidt 2009), and improving soil conditions for subsequent species colonization and development (Frouz et al. 2008). Therefore, by following the course of succession during the first years of restoration (short-term), those spatial and temporal changes in vegetation structure, soil parameters and species patterns that determine the initial outcomes of succession can be identified and measured; thus providing insights into fundamental community dynamics and restoration success (Picket et al. 2008; Walker & del Moral 2009).

In order to get a deeper insight into the mechanisms that govern successional processes, this study also focused on the characterization of medium-term vegetation succession in coal mines. Although, short-term successional studies (here 1-6 years) provides information on restoration success at the establishment phase, studies over longer term are needed to assess how the ecosystem responds once established. Thus, medium-term studies (here 1-40 years) were used to identify vegetation change and species turnover in the longer-term. They also provide evidence on whether the restoration objectives were being met and the time scales needed, or about the abiotic and biotic factors that may arrest medium-term successional process (Walker et al. 2006). Our knowledge of post-restoration successions could be improved if we elucidated these points, which are essential for solving practical restoration problems through succession.

As a consequence of the large impact of mining activities and their statutory requirements for restoration, it is of great importance to establish adequate restoration practices for these new-created ecosystems. Moreover, similar degraded ecosystems, that face the necessity to be restored, exist all over the world, and their importance is increasing due to accelerating land degradation in many countries. Studying succession process in coal mined areas may help in the development of worldwide insights in the restoration of large-scale man-made disturbances.

Main questions:

Short-term

1. Does aspect affect the early growth dynamics of hydroseeded species? Chapter 2.
2. How is the soil seed bank formed during the early revegetation process? Chapter 3.
3. What is the importance of topography and climate during the early revegetation process? Chapter 4.
4. Does the course of succession on a revegetated mine converge with a reference community in terms of species composition and vegetation structure? Chapter 5.
5. Which are the interactions of physicochemical soil development and vegetation succession (composition and structure) during the early revegetation process? Chapter 6.

Medium-term

6. How are plant medium-term successional processes influenced by local landscape and soil factors? Chapter 7.
7. How do functional groups (life-forms) and plant dispersal strategies richness and cover change during succession? Chapter 8.

In this research, extensive descriptive studies were carried out at two levels: short-term (1-6 years after restoration) and medium-term (1-40 years after restoration or abandonment). Short-term changes in vegetation succession and related constraints were studied following permanent plots (diachronic approach) at large reclaimed mine in 2003 (Chapters 2, 4, 5 and 6). At this mine, soil seed bank formation was analyzed as well (Chapter 3). Finally, for characterizing the medium-term vegetation succession at 31 mine sites and relate this to potential successional constraints, the space-for-time substitution (synchronic approach; Pickett, 1989) was used (Chapters 7 and 8).

Appendix included two related works done simultaneously with this research, and applying the methodologies learned. In this case, different forest management practices were analyzed in natural Mediterranean maritime pine (*Pinus pinaster*) forest.

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Chapter 2

Sampling quadrat of 0.25 m^2

The influence of aspect on the early growth dynamics of some frequently hydroseeded species in coal reclamation

Josu González-Alday, Rob H. Marrs & Carolina Martínez-Ruiz

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Abstract

Question: Does aspect affect hydroseeding success and the development of vegetation during early vegetation establishment on the steep slopes of coal wastes during the restoration process?

Location: Open-pit coal mine near Villanueva de la Peña, northern Spain.

Methods: In the first year after hydroseeding, we monitored the dynamics of hydroseeded species in three permanent plots of 20 m^2 on north- and south-facing slopes every two months. Soil properties and weather conditions were also monitored.

Results: Aspect was related to total plant cover during early revegetation, and south-facing slopes had the lowest cover. Aspect also influenced the early dynamics of hydroseeded grasses and legumes establishing on these slopes. Grass cover was greater on the north slope throughout the study, but differences in plant cover between north and south slopes appeared later for the legumes. Aspect also affected the relative contribution of both of grasses and legumes to the total plant cover, with grasses dominant on both northern and southern slopes, except during the summer on the southern slope. The species with the greatest difference in cover between the north- and south-facing slopes were *Festuca* spp., *Lolium perenne* and *Trifolium repens*.

Conclusion: In coal mine restoration areas of Mediterranean climates, differences in the development of hydroseeded species depended on the slope of the coal mine restoration areas, and this information is of importance to managers in selecting species for use in restoration.

Key words: Amended wastes; coal mining; revegetation; solar radiation; topography.

Abbreviations: HSI = Hydroseeding Success Index; ISSS = International Society of Soil Science; LMM = Linear Mixed Model; Ri = Radiation index.

Nomenclature: Tutin et al. (1964-1980)

Introduction

The aspect of a site influences the vegetation dynamics of natural communities (Cantlon 1953; Churchill 1982; Kutiel 1992; Bennie et al. 2006; Martínez-Ruiz & Marrs 2007) but little is known about the role of aspect in the development of vegetation on reclaimed coal mines, particularly in the Mediterranean region. The effect of aspect is primarily caused by the angle of the sun in relation to the soil surface (Dubayah 1994; Torri 1996; Evans & Winterhalder 2000; Oberhuber & Kofler 2000), which in turn affects soil surface temperature, evaporation rate and soil moisture (Kirkby et al. 1990). In the northern hemisphere, the solar radiation received at the soil surface is less on north-facing slopes than on south-facing ones (Mayland 1972; Dubayah 1994), and accordingly vegetation develops more readily on north-facing slopes (Andrés et al. 1996; Sternberg & Shoshany 2001; Bochet & García-Fayos 2004), partly because of increased water availability (Tormo et al. 2006).

The large-scale destruction of natural areas due to road construction and mining has created a large amount of bare slopes with differing aspects in Spain. In these situations, vegetation re-establishment has an important role in controlling soil loss and runoff (Andrés & Jorba 2000; Tordoff et al. 2000; Singh et al. 2002), and hence, the stability of slopes (Andrés & Jorba 2000). Usually restoration practitioners use sowing techniques to enhance vegetation establishment (Davy 2002), because there is often a lack of a seed bank, as in coal restoration areas (Middleton 1995; Mathis & Middleton 1999), and there may also be dispersal limitations from adjacent seed sources in patches of natural vegetation (Ash et al. 1994; Bradshaw 2000; Donath et al. 2003; Novak & Prach 2003; Foster & Tilman 2003). To overcome this lack of seed availability, hydroseeding is one seed application method that is used routinely to revegetate coal mine areas in Spain; this technique involves the application of a homogeneous suspension of water and seeds over the land surface (Roberts & Bradshaw 1985; Albadalejo et al. 2000; Brofas & Varelides 2000).

Aspect is one site factor that has been implicated as critical in determining the success of hydroseeding in land restoration (Andrés et al. 1996; Cano et al. 2002; Bochet & García-Fayos 2004; Martínez-Ruiz et al. 2007). The objective of this study was, therefore, to investigate the effect of aspect on the establishment of various species of legumes versus grasses, which were hydroseeded in a coal restoration area in Spain. We hypothesized that the: (1) total plant cover and hydroseeding success would differ on north versus south slopes, and (2) development of individual species and different functional groups of species would differ over time on north versus south slopes.

Methods

Site description and hydroseeding

This study was conducted between 2004 and 2005 in ‘Pozo Sell’, a 10 ha restored open-pit coal mine near Villanueva de la Peña, Palencia, northern Spain (1185 m a.s.l.; 42º50’N, 4º38’W). The climate is sub-humid Mediterranean (M.A.P.A. 1991), with a rainy season in autumn and spring and dry season in July through August (mean annual precipitation = 995 mm yr^{-1}; Fig. 1). 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*), and remnants of natural shrubland (*Rosa canina*, *Erica cinerea*, *Cistus laurifolius*, *Juniperus communis*) and *Quercus pyrenaica* woodland (González-Alday 2005).

After coal mining ceased, the final open pit was filled with coal wastes from nearby mines. The site was graded to a slope of 25º, and the surface was covered with 50-100 cm of finer textured sediments and cattle manure (30 t ha^{-1}).

The study area was hydroseeded by the mining company U.M.I.N.S.A. (Unión Minera del Norte S.A.) in October 2003 following the covering of coal wastes. 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% in weight) and herbaceous legumes (19% in weight). The seed mixture contained a mixture of seeds from commercial and crop sources (70 versus 30%, respectively). The commercial seed included *Festuca* spp., *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Trifolium pratense*, *Lotus corniculatus* and *T. repens* in 9:2:2:2:1:1:1 proportions; and the crop species included *Avena sativa, Secale cereale, Medicago sativa* in 3:3:1 proportions.

Vegetation sampling

The vegetation development following hydroseeding was examined in three permanent plots of 20 m^2 on both north- and south-facing slopes (aspects). Within each plot, eight 0.25 m^2 quadrats were positioned randomly and marked permanently on the first sampling date. The percentage (%) covers of all species present in each quadrat were estimated visually by the same observer, every two months from January 2004 to January 2005.

Soil sampling and analyses

Soil was sampled in January 2004 to a depth of 10 cm with a soil auger (diameter 8 cm) at two random points near each of the established quadrats within each plot. Eight soil samples were collected near each quadrat within a plot, and bulked and composited by plot; hence there were six soil replicates per slope type (north and south) to evaluate potential differences in initial soil characteristics.

Soil samples were air-dried, sieved (≤ 2 mm) and analysed for texture using the Bouyoucos-method (Day 1965) and ISSS classification (FAO-UNESCO-ISRIC 1988). Soil organic matter was determined using the k-dichromate oxidation method (Walkley 1947). Soil pH and electrical conductivity were measured with a conductivity meter in deionized water (ratio 1: 2.5) (Allen 1974). Soil total nitrogen was measured using the Kjeldahl-method (Bremner & Mulvaney 1982). Available P was measured using the Olsen method (Olsen & Sommers 1982). Exchangeable K^+ was extracted with 1N ammonium acetate at pH 7 by shaking for 30 min and determined using atomic absorption spectroscopy (Allen 1974).

Radiation index calculation

For each aspect, the radiation index (*Ri*) pattern was characterized during 2004. *Ri* estimates the proportion of potential direct irradiance intercepted by the slope for a given solar zenith and azimuth (Oke 1987), and is calculated as $Ri = \cos\beta \cos Z + \sin\beta \sin Z \cos(\psi - \gamma)$, where β is the angle of slope, Z is the solar zenith (angle of the sun from vertical), ψ is the solar azimuth (angle of the sun from due north), and γ is the slope aspect measured as azimuth angle. Values of ψ and Z were used from daily data at 13.00 hours, to represent maximum conditions of sward temperature and evaporative demand. A maximum *Ri* value of 1 is achieved when a slope is perpendicular to the direct radiation beam, and a value of 0 when the slope is parallel to the beam (Bennie et al. 2006).

Data analyses

The hydroseeding success index (HSI, ranging from 0 to 1) of Matesanz et al. (2006) was used to determine the relative contribution of hydroseeding to the community, as: HSI = HydC/TC, where HydC is the absolute cover of hydroseeded species and TC is the total vegetation cover.

The possible effects of aspect (north or south), month (7 levels) and interaction between aspect and month (fixed factors) on total plant cover, hydroseeded species cover, grass and legume cover, and HSI index were analysed using Linear Mixed Models (LMM) with the Restricted Maximum Likelihood method (REML). In all cases permanent plots were treated as random factors. Finally, working over the model matrix, contrasts were carried out to test differences between fixed factor levels (Pinheiro & Bates 2000). Consequently, the Bonferroni correction was used to adjust for the significance level for each *t*-test (Sokal & Rohlf 1995). Thus, the critical probability levels of $\alpha = 0.007$, 0.025 and 0.0125 were used to assess the significance of aspect, month and seasons (groups of months) respectively. Differences between aspects in soil properties were determined using *t*-tests. Vegetation cover data were arcsine square-root transformed before statistical analyses (Zar 1996). All statistical computations were implemented in the R software environment (version 2.6; R Development Core Team 2007), using the NLME package for LMM (Pinheiro et al. 2007).

Results

Climate and radiation index

During the sampling period, annual precipitation and temperature were atypical for the study area (Fig. 1). The weather in 2004 was warmer and drier than the 32-year mean (mean annual temperature: 10.4 vs. 9 ºC, respectively; total precipitation: 701 vs. 294 mm, respectively).

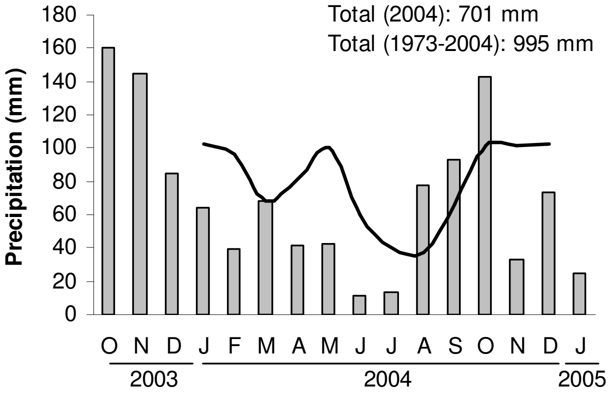

Figure 1. Seasonal precipitation pattern for ‘Pozo Sell’ open-pit coal mine from 2003 through 2004, based on the nearest Meteorological Station at Guardo, Spain (42º47’N, 4º50’W; 1110 m a.s.l.; data provided by the Spanish Institute of Meteorology), 16 km west of Villanueva de la Peña. The solid line is the 32-year monthly mean rainfall (period 1973-2004) for this station.

The autumn climate, during and just after hydroseeding application, was also atypical; October and November 2003 experienced a greater than average precipitation (59 and 44 mm above average, respectively, Fig. 1). In the sampling year of 2004, the fall season (August through October) also had a greater than average rainfall (41, 26 and 42 mm above average, respectively). During summer (June through July, 2004) the rainfall was below average resulting in a summer drought.

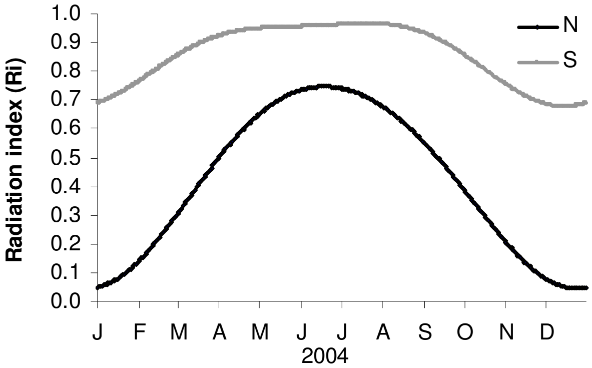

Figure 2. Daily radiation index at 13.00 hours on the north- and south-facing slopes from January through December 2004 for the ‘Pozo Sell’ open-pit coal mine, Villanueva de la Peña, Spain.

Ri was greater on south- than on north-facing slopes during 2004, with maximum differences in winter (Fig. 2). The duration of the *Ri* peak lasted from April to August on the southern slope, whereas the peak was confined to June on the northern one.

Soil properties

Soil properties did not differ between the north- and south-facing slopes (Table 1). Soil texture was clay loam, with a pH value that was moderately alkaline and organic matter content below 2% for both aspects. Total nitrogen, available phosphorus and exchangeable potassium were greater on northern than southern slopes (Table 1), whereas C/N was very similar in both aspects.

Total plant cover

The LMM analysis of total plant cover showed a significant aspect by month interaction ($F_{[6,24]} = 4.52$, $p < 0.01$). Total plant cover was always greater on the northern slope than on the southern one, except for May ($t = 0.96$, $p = 0.394$), and increased by 340% from January 2004 to January 2005 on the northern slope, and 287% on the southern slope (Fig. 3a). Nevertheless, the aspect x month interaction (Fig. 3a), between March and May suggested a greater increase in cover on the southern slope than on the northern one ($t = 3.86$, $p = 0.018$), and between July and September, a sharper drop on the southern than on the northern slope ($t = 3.70$, $p = 0.020$).

Table 1. Mean values ± SE of soil physico-chemical properties on north- and south-facing slopes in the 'Pozo Sell' open-pit mine, Villanueva de la Peña, Spain. *P-values* derived from a *t*-test are presented (n = 6).

Soil properties	North	South	*t*-value	*p*-value
Sand (%)	48.54 ± 0.88	44.64 ± 1.90	1.63	0.14
Silt (%)	24.23 ± 0.33	25.23 ± 1.33	0.69	0.51
Clay (%)	27.23 ± 0.67	30.13 ± 1.54	1.53	0.16
pH	8.19 ± 0.04	8.26 ± 0.04	1.08	0.31
Electrical conductivity (mmhos.cm^{-1})	0.13 ± 0.01	0.11 ± 0.01	1.43	0.18
Organic matter (% weight)	1.49 ± 0.19	1.15 ± 0.29	0.90	0.39
Total Nitrogen (mg.kg^{-1})	1100 ± 100	900 ±100	1.39	0.19
C/N	7.78 ± 0.70	7.21 ±1.45	0.04	0.74
Available phosphorus (mg.kg^{-1})	50.43 ± 2.50	28.20 ± 10.20	1.80	0.10
Exchangeable potassium (mg.kg^{-1})	204.65 ± 46.20	147.63 ± 10.50	1.00	0.34

Hydroseeding success

The ten hydroseeded species were recorded in both aspects at every sampling date, although the two annual species (*Avena sativa*, *Secale cereale*) disappeared in September (see below, and Figs. 5a-5b). In addition, the relative contribution of hydroseeded species to the total plant cover was high on both aspects (HSI >0.8: Fig. 3b), noting that HSI values ranged from 0 to 1. However, only significant monthly differences were found ($F_{[6,24]} = 4.70$, $p < 0.01$).

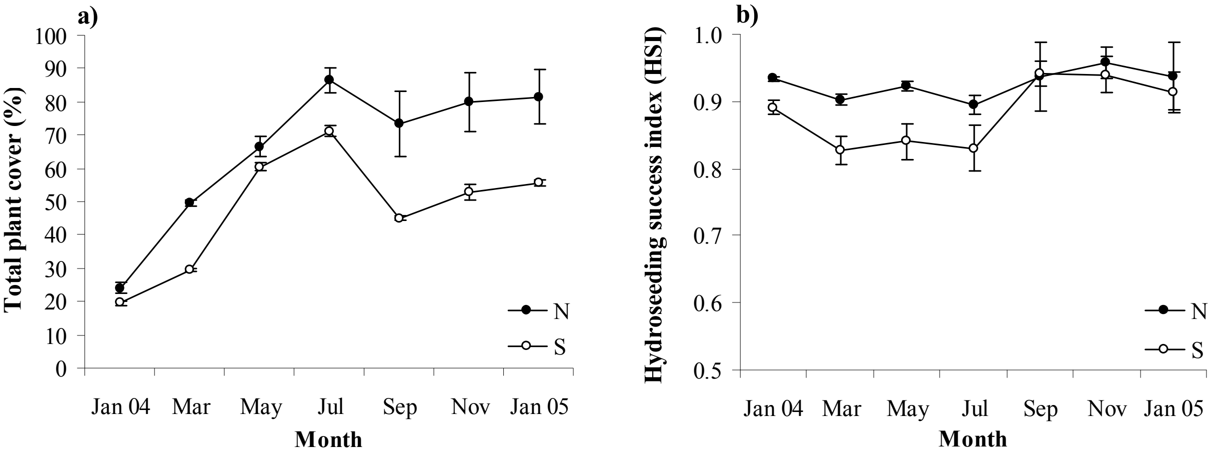

Figure 3. Total percent (%) plant cover and hydroseeding success index (HSI) from January 2004 through January 2005 on the north- and south-facing slopes (mean ± SE) in the 'Pozo Sell' open-pit coal mine, Villanueva de la Peña, Spain.

Cover of hydroseeded grasses and legumes

The hydroseeding mixture included seeds from six grasses and four legumes. The LMM of the cover of the hydroseeded grasses and legumes had a significant aspect by month interaction (grasses: $F_{[6,24]} = 2.64$, $p < 0.05$; legumes: $F_{[6,24]} = 3.52$, $p < 0.05$).

Grass cover was greater on the northern slope than on the southern one throughout the study (Fig. 4a). Nevertheless, legume cover only differed between aspects from September to November 2004 (aspect x month interaction) (Fig. 4b). During this period, legume cover was lower on the southern than on the northern slope (t = 3.56, p = 0.023), after a decline of legume cover between July and September on the southern slope (t = 4.04, p < 0.015). On the northern slope, however, legume cover did not change from July 2004 to January 2005.

Comparing grasses versus legumes within each aspect, the north slope had greater grass than legume cover ($F_{[1,4]}$ = 8.20, p < 0.05; see Figs. 4a–4b). On the south slope, grass and legume cover did not differ ($F_{[1,4]}$ = 2.71, p = 0.175), whereas results showed a significant aspect by month interaction effect ($F_{[6,24]}$ = 5.23, p < 0.001), suggesting higher grass than legume cover during winter (t = 10.72, p < 0.001).

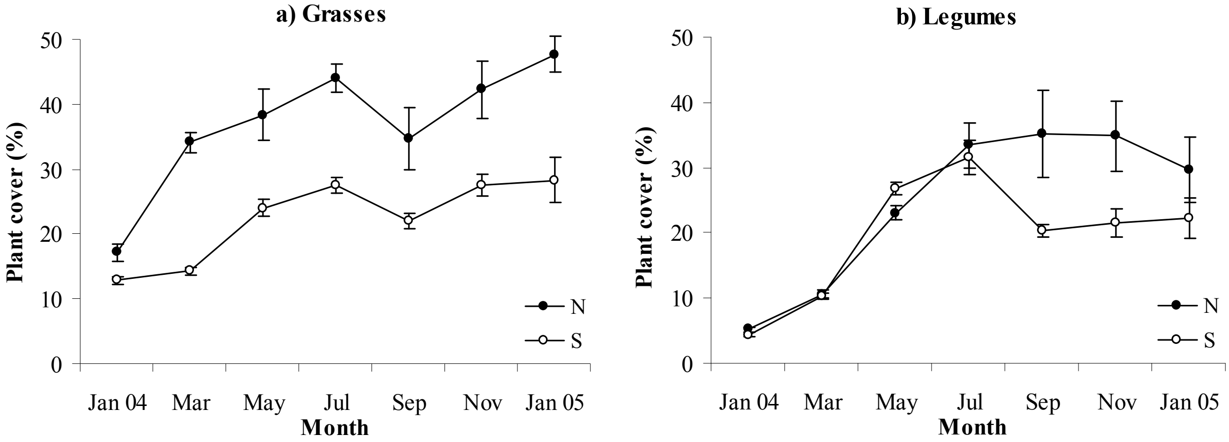

Figure 4. Mean percent (%) plant cover ± SE of hydroseeded grasses and legumes from January 2004 through January 2005 on the north- and south-facing slopes (mean) in the 'Pozo Sell' open-pit mine, Villanueva de la Peña, Spain.

Cover trends of ten hydroseeded species

Two species (*Lotus corniculatus*, *Trifolium pratense*) showed no significant aspect effect or aspect x month interaction (Table 2). Five species showed only a significant response to aspect, and three species showed a significant aspect x month interaction (Table 2; Fig. 5). Among species influenced by aspect, five types of responses through time were observed: (1) an increase in cover from January to May or July 2004 (*Avena sativa* and *Secale cereale*, Figs. 5a–5b), decreasing later in the season, disappearing by September, increasing at the end of the study period (annuals with greater cover on north-facing slopes); (2) a steady increase in cover through time, with cover always greater on the north-facing slope (*Lolium perenne*, Fig. 5c); (3) a large increase in cover until July or September, but decreasing more markedly later on the southern slope (*Trifolium repens*, Fig. 5d); (4) fairly constant cover starting in January 2004 (*Festuca* spp., Fig. 5e), with greater cover on the north-facing slope; and (5) little increase in cover until May, and with fluctuation, but overall decrease in cover until January 2005 (*Phleum pratense* and *Poa pratensis*, Fig. 5g and 5i).

Table 2. The summary results of linear mixed models testing the effects of aspect, month and their interaction on hydroseeded species cover. The *F* values of the fixed factors and their significance (*$p<0.05$; **$p<0.01$; ***$p<0.001$) are presented.

	Aspect	Month	Aspect x Month
df	1,4	6,24	6,24
Grasses			
Festuca spp.	23.50**	04.23**	2.45
Lolium perenne	07.24*	26.84***	5.05**
Phleum pratense	09.14*	18.65***	4.28**
Poa pratensis	03.35	16.45***	8.50***
Avena sativa	33.72**	58.37***	6.64***
Secale cereale	17.80*	49.88***	8.50***
Legumes			
Lotus corniculatus	00.86	15.60***	0.50
Medicago sativa	00.11	27.24***	2.54*
Trifolium pratense	04.44	09.40***	1.74
Trifolium repens	02.10	19.91***	6.03***

Discussion

The development of herbaceous vegetation after hydroseeding coal wastes in this Mediterranean region is affected by aspect. Grass cover was always greater on north- compared to south-facing slopes, and this result was in agreement with other studies (Martínez-Ruiz et al. 2001, 2007; Martínez-Ruiz & Fernández-Santos 2005; Martínez-Ruiz & Marrs 2007). For legumes, differences in cover related to aspect took longer to develop, e.g., legume cover was lower on south facing slopes by autumn one year after hydroseeding, probably because of the strong summer drought in 2004.

Given that the soils on both north- and south- facing slopes were treated in the same way and had similar macroclimatic conditions in this study, the influence of aspect was probably mediated through its effects on microclimate (Bennie et al. 2006), and the responses on these mine wastes are similar to those from motorway slopes in Spain (Cano et al. 2002; Bochet & García-Fayos 2004; Tormo et al. 2006). Our results indicate that aspect influences vegetation dynamics after hydroseeding through a combination of direct effects of differences in microclimate through the differing amounts of solar radiation received (Kutiel 1992), and the relationship of solar radiation to water availability (Kutiel 1992; Sternberg & Shoshany 2001; Bochet & García-Fayos 2004). Together these factors reduce vegetation cover on southern slopes compared to northern ones.

Vegetation cover on the south-facing slopes also responded negatively to precipitation fluctuations during 2004. In particular, a greater increase in plant cover was found on southern compared to northern slopes between March and May as result of early spring precipitation, and a greater reduction in cover between July and September as a consequence of summer drought. These differential responses during the season emphasise the importance of sampling over the entire growing season for assessing the effects of aspect on vegetation change (Kennedy & Addison 1987).

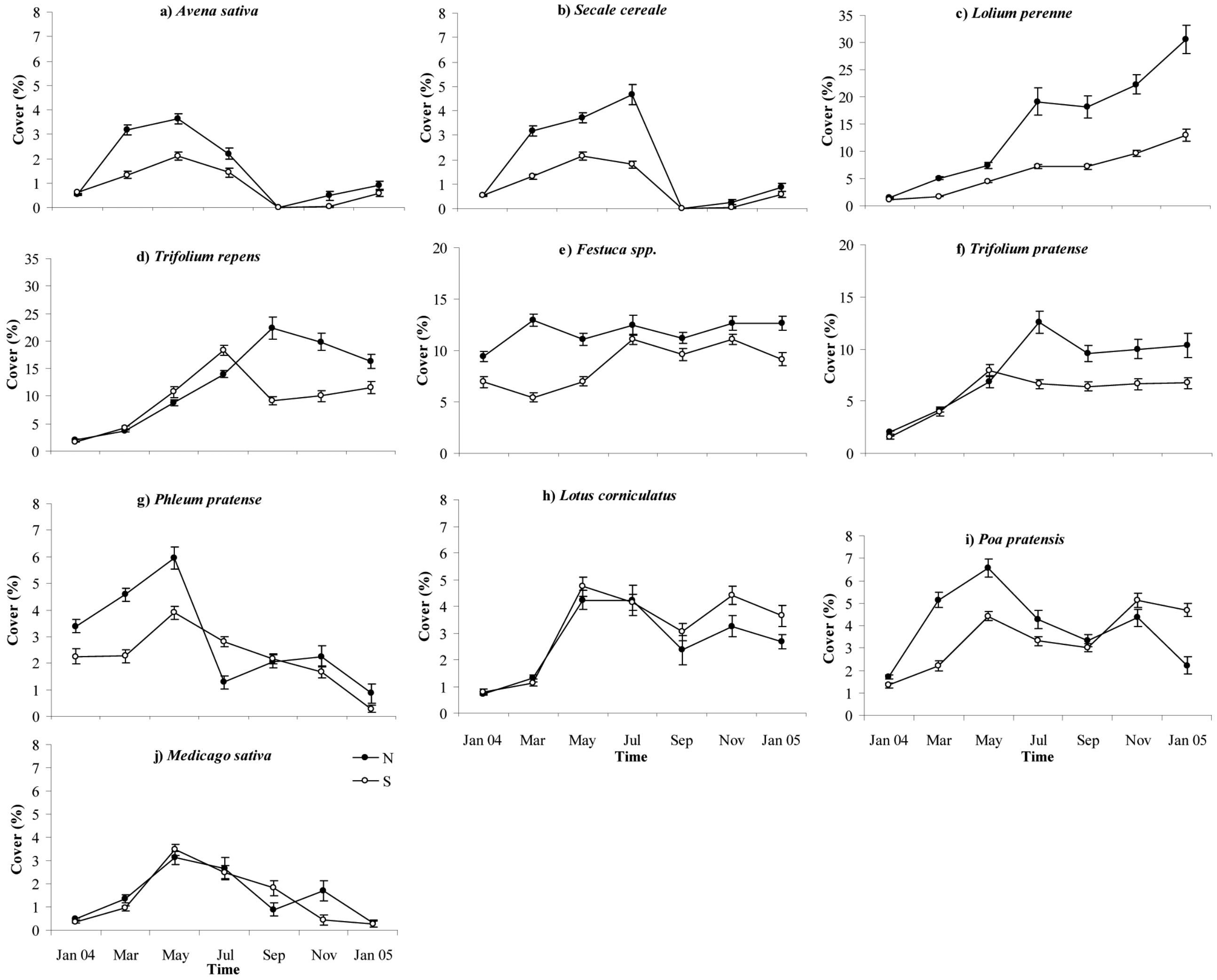

Figure 5. Mean percent (%) cover ± SE of ten hydroseeded species from January 2004 through January 2005 on north- and south-facing slopes in the 'Pozo Sell' open-pit mine, Villanueva de la Peña, Spain. See Table 2 for details of interactions.

Previous studies in Spain have reported a low success rate for hydroseeding in soil restoration, especially where commercial seeds of non-native species were used (Andrés & Jorbá 2000; Matesanz et al. 2006; Martínez-Ruiz et al. 2007). In contrast, this study showed that hydroseeding was successful in revegetating these coal mine areas; the hydroseeded species established and provided soil protection (HSI > 0.8) on both aspects through time. We can speculate that revegetation was successful in this study because of: (1) the time of hydroseeding (autumn; Roberts & Bradshaw 1985); (2) weather conditions (Andrés et al. 1996; Cano et al. 2002) with greater than average precipitation than usual during, and just after, sowing; and (3) site conditions matching the species selected for sowing. In spite of this, aspect had a short-term influence on hydroseeding success, with southern slopes having a relatively low cover. However, native species, especially annuals, became an important component of the total cover on the southern slopes on year after hydroseeding (González-Alday 2005).

Aspect also influenced the relative contribution of total plant cover of hydroseeded grasses versus legumes. On northern slope, there was generally a greater grass compared to legume cover, but on the southern slope, we found a seasonal variation in the relative contribution of grass versus legumes to total plant cover (as reported by Andrés et al. 1996), with grasses dominating during winter and autumn and legumes in early summer. It is possible that the cold autumn and winter during this study reduced legume cover, as they are more susceptible to low temperatures than grasses (Badaruddin & Meyer 2001; Márquez et al. 2006).

At the level of individual species, the effect of aspect was especially important for *Festuca* spp. and *Lolium perenne*, which contributed most to the grass cover, especially on the northern slope. Indeed, *L. perenne* provided 38% of northern-slope plant cover 15 months after hydroseeding. As the optimal germination of *Lolium* and *Festuca* occurs under relatively moist conditions (Sheldon & Bradshaw 1977), this might explain their greater cover on the hydroseeded north-facing slope through time. *Lolium* spp. has been also reported as a highly competitive species (González-Ponce 1998; Hoffman & Isselstein 2004) that might prevent the mid- and long-term establishment of autochthonous species (Matesanz et al. 2006).

Aspect clearly influenced *Phleum pratense*, which had a greater cover on the northern slope until May, after which its cover was reduced on both slopes. The sensitivity of *P. pratense* to water deficit (Sheaffer et al. 1992) together with the high temperatures reached in 2004 might have reduced its competitive ability relative to *L. perenne* (Höglind et al. 2001). The influence of aspect on annual species (*Avena sativa*, *Secale cereale*) was only significant during the active time of their life cycle, with both species having greatest cover on the northern slope.

In contrast, the effect of aspect on the cover of individual legumes in general was lower than on individual grasses. It is likely that the large variability of *T. pratense* cover on the northern slope was responsible for the lack of significance of aspect for this species. Nevertheless, *Trifolium pratense* and *T. repens* had greater cover on northern compared to southern slopes, but only from July or September, respectively, showing a delay in their response to aspect in accordance with that previously argued for the legume functional group as a whole. These two legume species contributed most of the legume cover on both aspects. The establishment of *Medicago sativa* was lower than expected probably because it was sown in the autumn; the optimal sowing time for this species is early spring in this region (González-Alday 2005).

Finally, our results emphasize the necessity of taking into account the aspect of slope in the selection of species used for revegetation, particularly by hydroseeding. Otherwise short-term vegetation establishment will be substantially reduced under the more severe conditions on south-facing slopes. Further investigations of the effect of aspect are needed to assess its effects in the longer term, specifically to monitor long-term successional dynamics of both hydroseeded and naturally recruited species.

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Chapter 3

Different seeds of colonizer species

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

Josu González-Alday, Rob H. Marrs & Carolina Martínez-Ruiz

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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 spread over it was 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 1,813 seeds m^{-2} over the study period, and was composed mainly by 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.

Key words: Distance to natural community, topography, foreign species, seed colonization, seed species composition, native species, minimal adequate models.

Introduction

The reconstruction of ecosystem function is one of the major challenges of restoration ecology (Aronson & van Andel 2006), especially if the aim is to change degraded systems into ones that will be sustainable in the long-term (Hobbs & Norton 1996). The components that must be restored for long-term sustainability include some form of plant cover (Hobbs & 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 & 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 & Small 2005; Hui & Keqin 2006) and to help recovery from perturbations (Swanton & 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 re-vegetation 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 restoration of coal wastes in Palencia, northern Spain.

The wastes left after open-cast coal extraction creates localized, highly-degraded sites where there are statutory or planning requirements to ensure the restoration of vegetation cover (Cooke & 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 & Middleton 1999), and (b) reduced opportunities for dispersal from nearby colonization sources (Andrés & 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.

Methods

Site description and hydroseeding

The study site was located in ‘Pozo Sell’, a 10 ha restored open-pit coal mine near Villanueva de la Peña, Palencia, northern Spain (1185 m a.s.l.; 42º50’N, 4º38’W). The climate is sub-humid Mediterranean (M.A.P.A. 1991). Annual mean temperature in the area is 9 ºC and average annual precipitation is 980 mm (mean values for 1932–2007, based on ‘Cervera de Pisuerga Meteorological Station’, 12 km east of ‘Pozo Sell’; data provided by the Spanish Institute of Meteorology). Rainfall is not distributed regularly throughout the year, and most rain falls during spring and autumn, 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*, *Cistus laurifolius*, *Juniperus communis*) 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 U.M.I.N.S.A. (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% in weight) and herbaceous legumes (19% in weight). The seed mixture included 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 *T. repens* in 9:2:2:2:1:1:1 proportions; and the crop species included *Avena sativa, Secale cereale, Medicago sativa* in 3:3:1 proportions.

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 cm^3 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 × 30 × 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 ºC 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 two months the soil in each tray was mixed to maximize germination (Kitajima & Tilman 1996; Thompson et al. 1997). The emergent seedlings were identified using Muller (1978), Chancellor (1983) and Villarías (2006), counted and removed; unidentifiable seedlings were transplanted to individual pots and grown on for later identification.

Finally, to assess the number of ungerminated seeds remaining in the soil, each of the samples was mixed for 30 minutes 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).

Soil seed bank after hydroseeding

In order to assess how the soil seed bank had developed during restoration, it was re-sampled 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 25º 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 25º 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).

In each of these 12 strips, six 5 × 5 m^2 plots were located randomly. At each plot, 8 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 8 soil cores per plot were mixed thoroughly to produce 6 samples per strip. These composite samples were sieved to remove large plant fragments and stones.

The method for assess the seed bank was replaced from emergence to the sieving/flotation technique (Barralis et al. 1986; Shaukat & 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 hexametaphosphate (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 ºC, 14 hr daylight (5–8 klux) and 15 ºC, 10 hr at night, for 3 months and watered 3 times a week (Reiné et al. 2004). The number of seeds germinating (criteria = appearance of the radicle) was counted weekly for three months (from 5th March to 5th June) (Bernhardt et al. 2008).

In all cases, seeds identification were 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.

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 analysed 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-Wienner 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 & 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 versus null model were carried out. As well, the Akaike's Information Criterion (AIC, Akaike 1973) was used to aid model selection (Pinheiro & Bates 2000). For all MAMs the reduction of AIC (ΔAIC) was calculated as: $\Delta AIC = AIC_i - AIC_{min}$, where AIC_i is the AIC of the initial model and AIC_{min} is of the minimal adequate model (Burnham & 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 & Gallagher 2001). An initial Detrended Correspondence Analysis produced eigenvalues of (λ_1= 0.39, λ_2= 0.35, λ_3= 0.30, λ_4= 0.28) and gradient lengths (GL) of (GL_1= 3.06, GL_2= 3.38, GL_3= 2.55, GL_4= 2.67) for the first four axes (ter Braak & Šmilauer 2002). The gradient lengths suggested that unimodal Canonical Correspondence Analysis (CCA) was appropriate for subsequent analyses. 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 & Sakar 2007) and the VEGAN package for both multivariate analyses and the calculation of diversity indexes (Oksanen et al. 2007).

Results

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 6 species were found in the soil and 11 seeds from 6 species in the manure. There was, 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}. No species found in the soil nor manure was sown in the hydroseeding mix.

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 probability (p) of detecting a seed of each species in a single sample is also given.

Soil			**Manure**		
Species	No. of seeds	p	Species	No. of seeds	p
Briza media	1	0.01	*Erodium cicutarium*	1	0.01
Agrostis spp.	1	0.01	*Vicia* spp.	1	0.01
Sanguisorba minor	1	0.01	*Dactilis glomerata*	1	0.01
Arenaria spp.	2	0.02	*Trifolium glomeratum*	2	0.02
Cerastium spp.	2	0.02	*Daucus carota*	2	0.02
Capsella bursa-pastoris	3	0.04	*Trifolium campestre*	4	0.05
Total	10	0.12		11	0.13

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 3 taxa represent 70% of the seeds found in the soil. The species found in the manure were dominated by the *Fabaceae* (64%) and *T. campestre* was the main species (36%).

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	Chisq	*p*-value	ΔAIC 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	
L. perenne						
	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	
T. repens						
	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	

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 geminated 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) 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).

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.

Random variable	Number of seeds	Hydroseeded seeds	Native seeds	Richness	*L. perenne*	*T. repens*
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)

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.

Overall species composition of the soil seed bank after hydroseeding (Fig. 1)

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 persicaria*; 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 (*Vulpia 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. and *Dianthus* spp. as characteristic species, from the flat isolate area (F_i) that had showed less directional change (Fig. 1b).

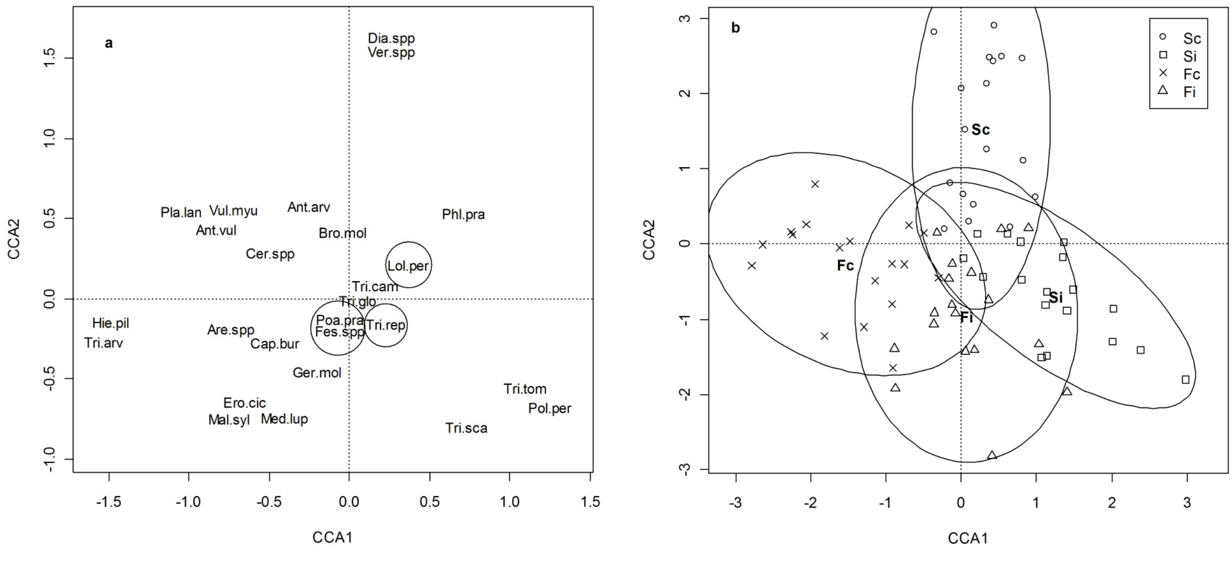

Figure 1. Constrained CCA ordination plots of areas of the species composition of the seed bank 2.3 years after hydroseeding at the 'Pozo Sell' reclaimed open-pit coal mine, Villanueva de la Peña, Palencia, northern Spain; the ordination was constrained on Area (S_c, F_c, F_i, S_i). (a) Species; hydroseeded 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. campestre* = 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.

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.

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 *Capsella bursa-pastoris*; Pakeman & 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 opencast 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 & 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)).

Seed bank development 2.3 years after hydroseeding

Size of the seedbank

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; Guardia et al. 2000), and in alpine ski trials in France (Isselin-Nondedeu & Bedecarrats 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 & 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 (Gonzalez-Alday & Martinez-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.

Effect of location on seedbank 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 & Menges 1996; Partel et al. 1998; Geertsema & 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 & 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 &

MacMahon 1994), the plants might act as seed traps (Bullock & 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 (Guardia 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 & MacMahon 1994), especially for round seeds such as *Trifolium* spp. (Isselin-Nondedeu & Bedecarrats 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.

Effect of location on seedbank 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 (Gonzalez-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 & 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 & Meyerson 2002; Ghorbani et al. 2007).

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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Chapter 4

Summer day and winter day in Pozo Sell

The importance of topography and climate on short-term revegetation of coal wastes in Spain

Josu G. Alday, Rob H. Marrs & Carolina Martínez-Ruiz

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Abstract

The initial colonization phase is the crucial start point for succession and, therefore, for restoration. However, little is known about abiotic factors that influence the early stages of revegetation dynamics on restored coal mines, particularly in a Mediterranean climate. This information is crucial for improving our ability to restore land despoiled by mining. Here, we characterized the short-term plant community development in the first 3 years after hydroseeding on a topographically diverse reclaimed open-pit coal mine in Spain. Topography influenced both community composition and diversity producing different trajectories between the three different aspects (north-facing, south-facing, flat). Hydroseeded species provided most of the initial vegetation cover, which brought about most of the compositional differences between these aspects. We also found that the changing climate through the growing season also influenced floristic composition and diversity. Summer drought reduced the cover of hydroseeded perennial species and allowed native species to colonize. These results emphasize the need to take topography into account when developing restoration management plans in Mediterranean ecosystems, and an acknowledgement that climate might drive the succession in the desired direction, hence increasing success in restoring mining impacts.

Key words: Hydroseeding; aspect; growing season; principal response curves; temperature and precipitation; diversity; dry periods.

Introduction

A central issue in community ecology is to understand the factors that control the composition and abundance of species in ecological communities (Menninger & Palmer 2006), and this must include interactions between abiotic and biotic factors (Larkin et al. 2006). Knowledge of how these processes operate is particularly important in restoration ecology, where new ecological communities need to be created on highly degraded sites (Hobbs & Norton 1996). In such situations there are also excellent new opportunities to examine and test ecological theory (Menninger & Palmer 2006). Here, we test the effect of a range of abiotic variables on vegetation development on restored open-cast, coal mine in northern Spain.

The initial re-vegetation phase of any restoration scheme provides the starting point for succession; once established this vegetation will help determine the course of vegetation development at least in the short- and medium-term (Hobbs & Norton 1996; Hobbs & Harris 2001). However, very little is known about early-vegetation establishment during ecological restoration on coal mines in Mediterranean climates (Martínez-Ruiz et al. 2007).

Aspect and slope are two important environmental variables already identified as important in structuring community during mine restoration in Mediterranean climates (Martínez-Ruiz et al. 2007; Martínez-Ruiz & Marrs 2007), mainly because of its effects on microclimate and solar radiation (Bennie et al. 2006). In Mediterranean ecosystems, aspect interacts with summer drought to influence species composition, diversity and community persistence (Chiarucci & Maccherini 2007; Valladares & Gianoli 2007).

The coal mining operations in the northern Spain (León and Palencia) have changed markedly over the past 35 years from the traditional underground coal mines to open-cast extraction. Aproximately 5,000 ha of the land surface of this region is currently affected, generating localized impacts. There are, however, statutory requirements to ensure high-quality restoration (Cooke & Johnson 2002; Moreno-de las Heras et al. 2008). The traditional way of tackling this restoration work was to use an engineering solution, where the landscape was re-modelled, the soil condition improved, and as limited recolonization processes the site was then sown with herbaceous species (Gonzalez-Alday et al. 2008). However, more recently, seeds have sown using hydroseeding, although this approach has not always been successful and there was often failure in early establishment phase (Vandvik et al. 2005). There is therefore a need for an improved knowledge of the factors that control the composition and abundance of species during the early establishment phase, which is important to improve restoration success in these situations.

Here, we assess the interaction of topography (aspect and slope) with micro-climate, both previously identified as important constraints in restoration schemes in Mediterranean climates (Gretarsdottir et al. 2004; Martínez-Ruiz et al. 2007; Martínez-Ruiz & Marrs 2007) on initial vegetation development. In order to, we used a structured monitoring survey fitted into an ongoing practical land restoration scheme in Spain. The study was carried out immediately after hydroseeding a topographically diverse open-pit coal mine where the entire area of bare coal mine wastes was restored. We tested four hypotheses: (1) topography would influence revegetation dynamics, (2) growing season would also influence vegetation dynamics; this would be caused mainly by climatic variability (rainfall and temperature), (3) the species compositional differences caused by topography would occur mainly through differential colonization of native species from the surrounding area, and (4) overall species diversity would also be affected by topography and would change through time in a similar manner to community change. The ultimate aim was to provide an improved understanding of the factors controlling vegetation establishment immediately after sowing these degraded soils (Walker & del Moral 2003), and help to create improved restoration procedures (Menninger & Palmer 2006).

Methods

Site description and hydroseeding

The study site was located in ‘Pozo Sell’, a 10 ha reclaimed open-pit coal mine near Villanueva de la Peña, Palencia, northern Spain (1,185 m a.s.l.; lat 42º50’N, long 4º38’W). The climate is sub-humid Mediterranean (M.A.P.A. 1991), with an annual mean temperature of 9 ºC and average annual precipitation of 980 mm (1932–2007 means from Cervera de Pisuerga meteorological station). Rainfall is not distributed regularly throughout the year; most rain falls during spring and autumn and there is a dry season in summer. The vegetation surrounding the site consisted of a complex matrix of grasslands (*Bromus mollis*, *Vulpia myuros*, *Plantago alpina*, *Arenaria montana*), crop fields (*Avena sativa, Secale cereale*), remnants of natural shrubland (*Rosa canina*, *Erica cinerea*, *Cistus laurifolius*, *Juniperus communis*) and *Quercus pyrenaica* woodland (González-Alday et al. 2009).

After coal mining stopped, the open pit was filled with coal wastes from nearby mines and the surface was covered with 50-100 cm of fine-textured sediments and a layer of cattle manure (30 t ha^{-1}). Thereafter, in October 2003 the entire site was hydroseeded by the mining company (Unión Minera del Norte S.A.). The hydroseeding slurry contained soluble chemical fertilizer (150 kg ha^{-1}; 8N:15P:15K), and a seed mixture (210 kg ha^{-1}) containing grasses and herbaceous legumes (81:19 by weight). The seed mixture included *Festuca* spp., *Avena sativa*, *Secale cereale*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Trifolium pratense*, *Lotus corniculatus*, *Medicago sativa* and *T. repens* in 9:3:3:2:2:2:1:1:1:1 proportions.

Vegetation sampling

Vegetation development following hydroseeding was examined in three different areas (north- and south-facing slopes (25º) and a flat area), placing in each area three permanent plots of 20 m^2 (n=9). Within each plot, eight 0.25 m^2 quadrats were positioned randomly and marked permanently on the first sampling date (January 2004). The cover (%) of all vascular plant species present in each quadrat was estimated visually by the same observer every two months from January 2004 to September 2006 (n=17). The use of plant cover and richness for characterize the short-term revegetation dynamics was based in previous work by Prach & Pyšek (2001) and Martinez-Ruiz et al. (2007); we accept this measure confounds density and growth. The quadrats were monitored over three growing seasons (considering growing season the period from October to September): Season 1 = 2003/2004; Season 2 = 2004/2005; Season 3 = 2005/2006. The seedlings started to establish in January 2004, so this date was used as the baseline.

Climate

The climatic data were provided by Spanish Meteorological National Agency from the Cervera de Pisuerga meteorological station (lat 42º51' N, long 4º30' W; 1,113 m a.s.l, 12 km East of ‘Pozo Sell’). Average precipitation (mm) and temperature (ºC) were summarized monthly over the study period, and for each of the three growing seasons.

Data analysis

The climate data were analyzed using descriptive statistics, whereas the vegetation dataset was analysed using both multivariate and univariate methods. Multivariate methods were used to test the first three hypotheses on community composition. Before analysis the species dataset was reduced by removing all species which only occurred in fewer than five quadrats. An initial Detrended Correspondence Analysis (DCA) produced eigenvalues of λ_1=0.27, λ_2=0.12, λ_3=0.09, λ_4=0.08 and gradient lengths (GL) of GL_1=2.36, GL_2=1.97, GL_3=1.67, GL_4=1.48 for the first four axes. The gradient lengths suggested that linear based ordination methods were appropriate (ter Braak & Šmilauer 2002), and these were used in all subsequent analyses. First, a Principal Components Analysis (PCA) was carried out to describe community composition. Partial Redundancy Analysis (pRDA) was then used to test Hypotheses 1 and 2. For Hypothesis 1, whether topography influenced revegetation dynamics was tested by analyzing the site × time interaction; here the overall effect of time and the variation between the experimental plots was partialled out. Hypothesis 2, whether growing season and climatic variability (rainfall and temperature) influenced vegetation dynamics, was tested by assessing the interactions between sites, growing seasons and climatic variables (precipitation and temperature); here the effects of the successional trends of the different sites were partialled out. In these pRDA analyses, significance was assessed with randomization tests using the reduced model and 999 permutations.

Thereafter, Hypothesis 3 was tested using Principal Response Curves (PRC, van den Brink & ter Braak 1999). PRC was used to test whether vegetation development would be influenced differentially by topography because of differential colonization from native species adjacent each area. In this analysis, the change in plant community composition through time in north and south sites were contrasted against the flat reference site. Month was included as a covariate to control the overall temporal trend. Both axes of the PRC analyses were inspected and significance was tested with randomization tests using the reduced model and 199 permutations stratified within month. In addition, randomization tests (999 permutations) were performed at each sampling date to evaluate the significance between sites.

Hypothesis 4, that overall species diversity paralleled community change, was tested using generalized linear mixed models (GLMM). GLMMs were used to derive responses between the diversity variables (species richness and Shannon-Weiner diversity index) and interactions between site, growing season and time. Site (north, south, flat), growing seasons (1, 2, 3) were treated as categorical fixed factors, time as a continuous factor, and time nested within plot were included as random factors to account for temporal and spatial autocorrelation (Pinheiro & Bates 2000). Model simplification for hierarchical data followed Crawley (2007) using the Bayesian Information Criterion (BIC, Venables & Ripley 2002; Pinheiro & Bates 2000). Models were fitted using the 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). As the conclusions from both species richness and Shannon-Wiener index were similar, only the species richness results are presented here.

All statistical analyses were implemented in the R software environment (version 2.7.2; R Development Core Team 2008), using the LME4 package for GLMM (Bates et al. 2008) and the VEGAN package for both multivariate analyses and the calculation of diversity indexes (Oksanen et al. 2008).

Results

Climate

The climate data observed during the three growing seasons was extremely variable and atypical for the study area. In all three growing seasons the site was warmer and drier than the 76-year growing seasons mean (Table 1). These differences were especially important during early summer (June, July) with higher temperatures and lower rainfalls than average, producing summer droughts over the study period.

Table 1. Temperature and precipitation during (a) the summer, and (b) the three growing seasons studied compared to the 76-year growing seasonal mean.

	Temperature (ºC)				**Rainfall (mm)**			
	Growing season			76-Years mean	Growing season			76-Years mean
	1	2	3		1	2	3	
(a) June-July	17	18	19	16	26	8	21	43
(b) Annual	9.3	9.4	9.3	8.9	880	543	626	988

Community description

One hundred and five vascular plant species were recorded over the three growing seasons. All the hydroseeded species were common most of them with cover values greater than 5%, especially during the first and second growing seasons. The cover of 16 species characteristic of the reference community, including *Bromus mollis*, *Vulpia myuros*, *Arenaria montana* and *Minuartia mediterranea* all increased over the study period. At the same time, 22 species, including *Inula montana*, *Agrostis castellana* and *Thymus vulgaris* were found only once, and this usually occurred in the third growing season.

In terms of community composition, the first two axes of the PCA explained 64% of the variation of the data (Fig. 1). The primary floristic gradients reflected the effects of both time and environment (sites). The starting positions are all located in the lower left quadrant of the plot; whereas the first and second growing season spring plots are located at the central and right area and third spring plots are near the top. This indicates temporal trajectories diagonal to the PCA axis 1 (50% variance). The second PCA axis (14% variance) mainly reflects differences in sites (environments), with north-facing plots having lower scores, and south- and flat-plots with progressively greater scores, indicating the effect of topography on revegetation.

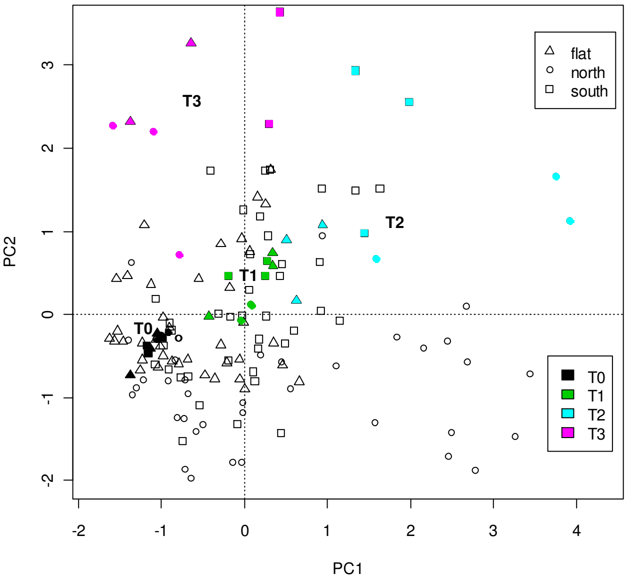

Figure 1. PCA ordination diagrams of the first two axes of plots, from the three growing seasons experiment at the 'Pozo Sell' reclaimed open-pit coal mine, Villanueva de la Peña, Palencia, northern Spain. Compositional change for the spring samplings over the course of three growing seasons is shown, where T0 indicate the first sampling date (January of 2004) and T1, T2 and T3 the spring samplings (May) for the years 2004, 2005 and 2006. The diagrams below represent the plots of each site in each growing season, whereas the arrows are the trajectories of compositional change of site plots in each season.

Hypothesis 1 and 2; Does topography and growing season influence revegetation dynamics?

Both hypotheses were tested using multivariate pRDA (Table 2). Hypothesis 1 tested the effect of topography (north, south and flat aspects) on its own. The effect of topography on compositional trends through time accounted for 5% of the variance in the data and was statistically significant (*p*<0.02), so this hypothesis was accepted.

Table 2 Summary of the partial RDA analyses of species cover during the first three growing seasons after restoration of the 'Pozo Sell' open-pit coal mine, Villanueva de la Peña, Palencia, northern Spain. The effects of the model for each analysis are specified in Variables, and the Covariables indicate the conditional variables; the effects of the covariables are removed before constraining the other variables. The significance (999 permutations) and the percentage of the total floristic variation in the data accounted for by each model are reported. P = 9 plots; T = time; S = the three different sites (north, south, flat); G = the three growing season (1, 2, 3); Pr = precipitation; Te = temperature; M = months.

Testing for effect of	Variables	Covariables	Variance	Pseudo-*F*	*p*(999)
Plot	P		17%	3.56	<0.001
Time	T	P	8%	15.23	<0.001
Different trend among sites (Hyp1)	S × T	P + T	5%	2.80	0.013
Site specific growing season effects (Hyp2)	G × S	S × T + P + T	17%	7.22	<0.001
Site specific precipitation effects (Hyp2)	Pr × S × T	S × T + P + T	6%	1.90	0.010
Site specific temperature effects (Hyp2)	Te × S × T	S × T + P + T	12%	4.55	<0.001
Total explained by Plot and Time	P × T		30%	3.33	<0.001

Hypothesis 2 tested whether revegetation dynamics varied with growing season and climate variability (precipitation and temperature). When the effects of growing season were added to the model the effect was significant ($p<0.001$) and the explained variance increased to 17%. When the two climate variables related to growing season (precipitation and temperature) were tested they were also significant ($p\leq0.010$), explaining 6% and 12% of the variance in the data, respectively. Thus, Hypothesis 2 was also accepted, although there are potentially important interactions between sites and growing seasons. As 30% of the total variance in the data is explained by the differences among plots and sampling dates (model P×T; Table 2), clearly other unmeasured environmental factors are important in influencing revegetation.

Hypothesis 3; Are compositional differences among topographies caused mainly by native species?

This hypothesis was tested using PRC; the PRC model accounted for 28% of the variation in species composition and was significant (pseudo-F=3.96, p=0.005). PRC graphs were plotted for axes 1 and 2 in order to visualize and interpret the effects of site (environment) and growing seasons on species composition through time; in both the successional trends of north and south sites were compared to the flat site as the reference x-axis (Fig. 2). The first and second PRC axes were statistically significant (199 permutations, $p<0.05$).

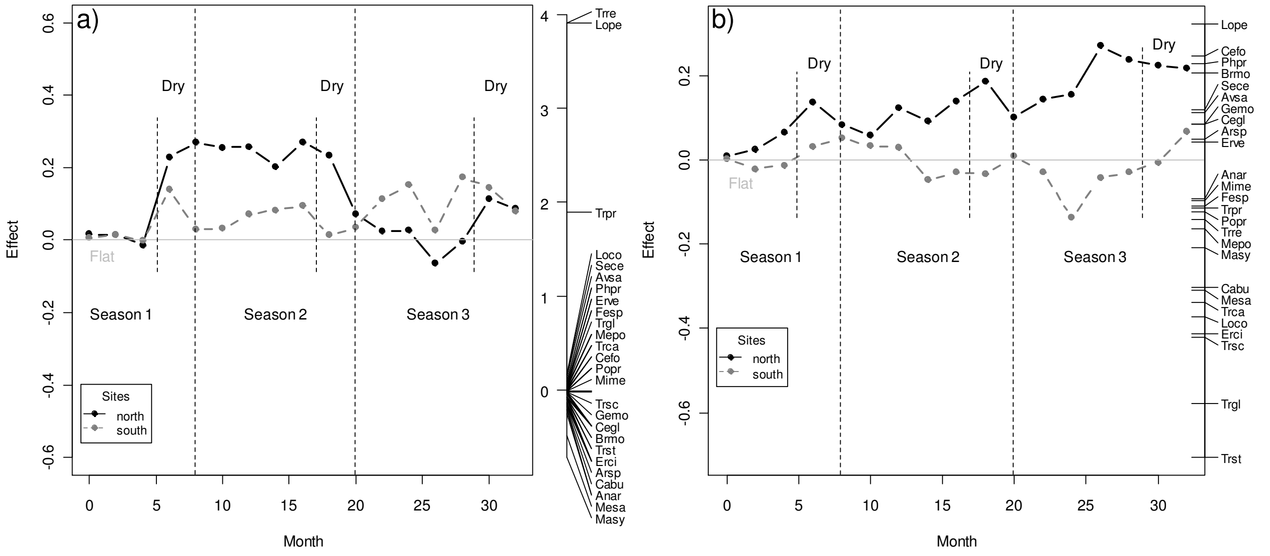

Figure 2. Principal Response Curve diagrams, showing the effect of sites (environments) on species composition through three growing seasons: (a) PRC axis 1, (b) PRC axis 2. The responses of the most common species are shown on the right. Values deviating from the reference values of 0 (flat reference site) indicate site effects. Species codes: *Anthemis arvensis* = Anar; *Arenaria* spp. = Arsp; *Avena sativa* = Avsa; *Bromus mollis* = Brmo; *Capsella bursa-pastoris* = Cabu; *Cerastium glomeratum* = Cegl; *Cerastium fontanum* = Cefo; *Erodium cicutarium* = Erci; *Erophyla verna* = Erve; *Festuca* spp. = Fesp; *Geranium molle* = Gemo; *Lactuca serriola* = Lase; *Leontodon taraxacoides* = Leta; *Lolium perenne* = Lope; *Lotus corniculatus* = Loco; *Malva sylvestris* = Masy; *Medicago polymorpha* = Mepo; *Medicago sativa* = Mesa; *Minuartia mediterranea* = Mime; *Phleum pratense* = Phpr; *Poa pratensis* = Popr; *T. campestre* = Trca; *T. glomeratum* = Trgl; *T. pratense* = Trpr; *T. repens* = Trre; *T. scabrum* = Trsc; *T. striatum* = Trst; *Secale cereale* = Sece.

The PRC diagram from axis 1 (Fig. 2a) illustrated that during the first months the north and south curves stayed close to the zero line control of the flat site, and showed no significant difference from it (p>0.05). At the end of first growing season during the dry period (July-September 2004) the north and south curves start to deviate from the flat site and the north site deviated the most (p<0.05). These differences were maintained throughout the second growing season, until the next dry period when all sites converged to a similar species composition (September 2005, p>0.05). During the third growing period the north and south sites reversed, with the north site becoming more similar in composition to the flat site than the south one. This reversal reflects the influence of climatic variability in the revegetation trajectories of each site and in the subsequent growing seasons (Hypothesis 2). In this analysis the site responses were associated positively with *T. repens* and *Lolium perenne,* which had the highest positive weights, followed by *T. pratense* (Fig. 2a) and five hydroseeded species (*Lotus corniculatus*, *Secale cereale*, *Avena sativa*, *Phleum pratense* and *Festuca* spp.) which were also positive values, but were much lower. The cover of these species was favoured in the north site, especially during second growing season. Species with negative weights were mainly native species (*Anthemis arvensis*, *Arenaria* spp.) and some ruderals as *Malva sylvestris* or *Capsella bursa-pastoris* (Fig. 2a).

On axis 2 (Fig. 2b) the curve for south site stayed close to the flat site indicating similar community composition, whereas the north site started deviating from the flat site at the second sampling date, and this deviation in species composition increased through the second and third growing seasons. In this analysis (Fig. 2b) the main weights were negative and composed mainly of native species (e.g. *T. striatum*, *T. glomeratum*, *T. scabrum*, *Erodium cicutarium*, *T. campestre*) and two hydroseeded species (*Lotus corniculatus*, *Medicago sativa*). These species were important in differentiating the north site from the reference site (flat) and especially from the south site, because south site had the least native species cover. The broad pattern that emerges is that the first PRC axis explained 16% of the variance in the data, and demonstrated compositional differences caused mainly by hydroseeded species, whereas the second PRC axis explained 5% of the variance in the data and showed the compositional differences were caused by native species. Hypothesis 3 is, therefore, not supported as the main differences were produced by hydroseeded species and not by native ones.

Hypothesis 4; Are changes in overall diversity affected by topography and do they mirror community composition?

Species richness ranged from 10 to 37 species per plot. The GLMM analysis showed that the trend in species diversity was not influenced by the interactions between sites, growing seasons and time (Table 3, Fig. 3). In each season, species richness showed the same trends, richness increased to a peak in early spring (March-May), and decreased thereafter in the dry season

(June-September). There were important differences between sites and growing seasons (Main effects; Table 3), with greater values on the flat site in each growing season followed in order by the south and north sites. Species richness increased in each growing season; season 3 had greater values than the other two (Table 3, Fig. 3).

Table 3 The relationship between species richness at 'Pozo Sell' reclaimed open-pit coal mine, Villanueva de la Peña, Palencia, northern Spain in relation to environmental variables derived using Generalized Linear Mixed Modelling (GLMM). The Minimum Adequate Model is presented (ΔBIC = 122.03): the intercept refers to the flat site in Season 1, and the magnitude of the effects is calculated as the estimated difference from the intercept.

Parameters	Estimate (±SE)	z value	*p*-value
Intercept	2.81 (0.07)	38.98	<0.001
Season 2	-0.14 (0.09)	-1.42	0.154
Season 3	0.30 (0.14)	2.20	0.028
North	-0.25 (0.06)	-4.03	<0.001
South	-0.19 (0.06)	-3.13	0.002
Time	0.05 (0.01)	3.52	<0.001
Time^2	-0.01 (0.001)	-4.08	<0.001

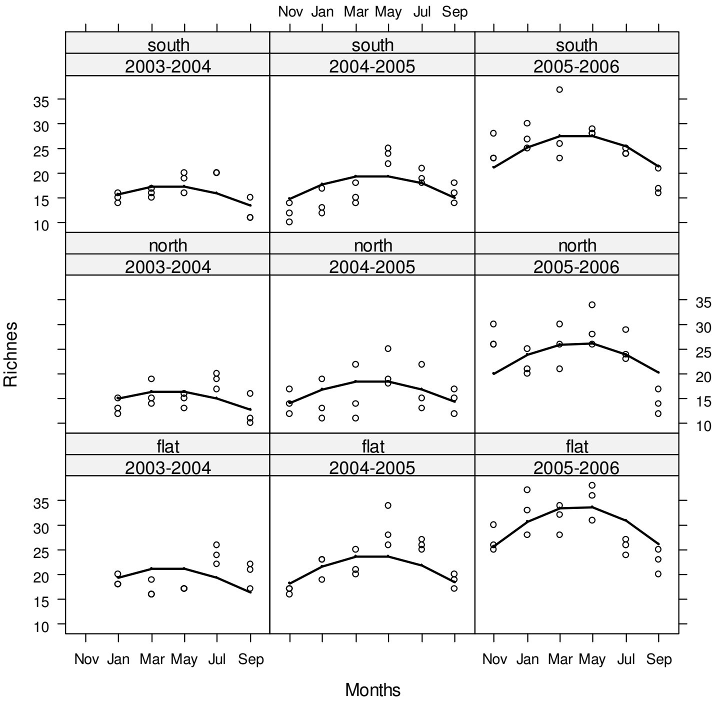

Figure 3. Species richness per plot through the three growing seasons (03/04, 04/05, 05/06) in each of the three sites (north, south and flat). The fitted line represents the minimal adequate GLMM (Table 3), some of the variance in the data originates from random variables included in the model.

Discussion

Hypothesis 1 and 2; Does vegetation success vary with topography and season?

Plant community composition was affected by topography; different successional trajectories were found on the three aspects through the three growing seasons. The flat and south sites maintained a similar species composition, whereas the north site diverged from them in two different ways: (a) there was a greater development of hydroseeded species during the second growing season, and (b) the north site maintained a greater cover of these hydroseeded species. These results may be caused by a combination of two factors. First, by the differential success of hydroseeding species in relation to aspect; in Mediterranean areas moister north facing slopes tend to have a greater proportion of perennial species than drier aspects (Madon & Médail 1997; González-Alday et al. 2008), and in our study many of these perennials were hydroseeded species. Second species colonization from the surrounding species pool, which was more prevalent in the south and flat sites, might have been a biotic feedback effect with seedling establishment of autochthonous species reduced through competition from the greater abundance of hydroseeded species (Matesanz et al. 2006; González-Alday & Martínez-Ruiz 2007). Colonizing seedlings and juveniles are very sensitive to such biotic competition (Harper 1977).

Growing season also affected community composition on all three aspects; the most likely cause is the variability of precipitation and temperature. Since, the greatest changes in composition were found during dry periods of each growing season supports this hypothesis. During the dry season growth and development are very limited, the probability of survival is reduced, and there is therefore, a strong selection pressure allowing the survival of well adapted species (Caccianiga et al. 2006). Annuals escape the dry summer as seed, whereas perennials must use resources to survive (Clary 2008). The drier conditions on the south and flat aspects clearly reduced the cover of hydroseeded perennial species (Joffre & Rambal 1993), and this favoured colonization of the newly created gaps by autochthonous species (Holl 2002; Martínez-Ruiz et al. 2007).

This detailed study over the 3 years has indicated that extreme events, such as the prolonged drought in the second growing season impacted on the north site, making it more similar to the flat site, rather than south sites by reducing the cover of hydroseeded species. Thus micro-climatic effects during the growing season are likely to be important environmental drivers of change in the early phases of restoration of herbaceous communities under Mediterranean conditions (Clary 2008).

The large amount of unexplained variance detected in these analysis suggests that there are other important unmeasured environmental factors that influence the community compositional change at the more local scale; for example, physico-chemical substrate characteristics (Whisenant 2002; Marrs 2002), or the role of soil micro-organisms (Allen et al. 2002). The community dynamics of these developing ecosystems clearly involves interactions between many drivers, both biotic and abiotic (Davy 2000), and further investigations of these additional processes and their interactions are needed.

Hypothesis 3; Are compositional differences during the early phases of revegetation caused mainly by native species?

Surprisingly, the compositional differences between sites were not caused mainly by native species as we had originally hypothesized. The reason is that hydroseeding species provided most of the initial vegetation cover especially at the north site (González-Alday et al. 2008). This study confirmed that the cover of hydroseeded species was greater during the first three growing seasons.

Nevertheless, the cover of native species, increased continually through the three growing seasons on all aspects. This increase may be due to either (a) an increase in colonization rates from the surrounding vegetation, i.e. there is a greater probability of success with time (Holl, 2002), (b) an increase of plant growth of the established species (Jochimsen 2001), or (c) a superior ability of native species to establish in disturbed sites because populations are adapted to these local environments (Urbanska 1997). Here, we found an increasing number of species characteristic of the surrounding vegetation through time (e.g. *A. arvensis*, *Cerastium fontanum*, *B. mollis*, *V. myuros*, *M. mediterranea*, *T. campestre* or *T. striatum*), and importantly for restoration, that the ongoing succession the vegetation is moving in the desired direction, especially on the south and flat sites which had the lowest cover of hydroseeded species.

The spontaneous processes of colonization have been used as an important restoration practice in central Europe (Prach & Pyšek 2001; Kirmer & Mahn 2001). Here, spontaneous colonization, being not massive, helps to redirect successional processes towards natural community. Therefore, could be interesting to consider spontaneous revegetation potential in restoration programs.

Hypothesis 4; Are changes in overall diversity affected by topography and do they mirror community composition?

The lack of interaction between sites, growing seasons and time in species richness produced stable patterns in every growing season, which reflects the annual cycle of species composition change in Mediterranean ecosystems (Madon & Médail 1997; Blondel & Aronson 1999). Richness increased in autumn and spring, the periods when most seedling emergence occurs (Lloret et al. 2004), but reduced in summer probably because of limited water availability (Piñol et al. 1995). This result is consistent with the annuals acting as re-selected ruderals at the local scale in areas of annual seasonality (Clary 2008), and Grime's (2001) proposal that much of primary succession should be characterized by stress tolerators.

Particularly noteworthy is the consistent increase in species richness through the three growing seasons. This might be expected, because as time since restoration increases there is a greater chance of the arrival of new species (Begon et al. 2006). At the same time, however, there was an effect of aspect aspect related plant community variation also affected species diversity (Hutchinson et al. 1999); the lowest richness values were found at the north site, suggesting a reduction through biotic competition between hydroseeded and native species (Begon et al. 2006).

Conclusions

The results of this study demonstrate that the short-term vegetation establishment can influence the successional development of restoration schemes. For Mediterranean ecosystems, it is essential when planning restoration projects to accept that differences by topography will affect the outcome of succession. In particular, it will influence the persistence of hydroseeded species, and this in turn will influence the colonization of native species from the surrounding vegetation.

In a Mediterranean climate the variability of precipitation and temperature through the growing season is also important. The disturbance produced by summer drought can have a considerable effect on the floristic composition and diversity during restoration. It produced conditions that allowed other species to colonise and redress the balance with the introduced perennial hydroseeded species. Clearly this impact may change if drought increases under new climate scenarios (Rambal & Debussche 1995).

At the same time, this study showed that the combination of compositional responses to sites, growing seasons and climate variability create complex compositional patterns, which limits the predictability of succession even in adjacent locations. Further investigation is needed to assess the importance of other environmental abiotic factors at more local (plot level) or general scales (site level), during the short- and long-term time scales, as well as the factors that may limit native species colonization processes and recruitment.

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Chapter 5

View of the reference community

Vegetation convergence during early succession on coal wastes: a six-year permanent plot study

Josu G. Alday, Rob H. Marrs & Carolina Martínez-Ruiz

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Abstract

Question: Does the course of succession on a coal mine restored by hydroseeding converge with a reference community in terms of species composition and vegetation structure? What is the rate of succession on hydroseeded plots and the balance between local colonization and extinction rates? Which native or hydroseeded species determine the successional process?

Location: A large reclaimed coal mine in the north of Palencia province, northern Spain (lat 42º50'N, long 4º38'W).

Methods: We monitored annually, between 2004 and 2009, vascular plant species cover in 9 permanent plots (20 m^2 each one) at a restored mine; these plots were set up in a structured way to account for site aspect (north, south and flat). Three identical permanent plots were set up in the surrounding reference community and monitored in 2004 and 2009. Detrended Correspondence Analysis was used to assess successional trends and rates of succession, generalized linear mixed models to derive patterns of vegetation structural changes and turnover, and Huisman-Olff-Fresco modeling to illustrate the response of individual species through time.

Results: The three restored mine areas exhibited a successional trend towards the reference community trough time, although the speed of convergence differed. Moreover, an increasing divergence through time was found among them, higher than the dissimilarity reduction with respect to the reference community. Richness, diversity and native species cover increased linearly trough time, whereas hydroseeded species cover decreased. Hydroseeding success was different in the three areas in 2004, being negatively related with native species colonization rates. The response patterns trough time of 10 hydroseeded species and 20 most common native species were described.

Conclusions: The results indicate that vegetation structural parameters converge with reference community really fast, whereas compositional convergence needs much longer time frames. At the same time, successional compositional trajectories and rates are area-dependent, and hence are a function of initial species composition.

Key words: Floristic composition; vegetation structure; species turnover; HOF modelling; species responses.

Abbreviations: AIC = Akaike Information Criterion; DCA = Detrended Correspondence Analysis; GLMM = Generalized Linear Mixed Models; HOF = Huisman-Olff-Fresco Models; PMAV = Permutational Multivariate Analysis of Variance; REML = Restricted Maximum Likelihood.

Nomenclature: Tutin et al. (1964-1993).

Introduction

Mining is a major environmental disturbance which often leaves a landscape with no vegetation and very poor soil-forming materials on which an ecosystem can develop (Herath et al. 2009). In such damaged systems the aim of the ecological restoration should be to develop a healthy, self-sustaining ecosystem, which is similar to the semi-natural or natural ecosystems that were present before the mining operations. As restoration proceeds, there is an excellent opportunity to study the successional dynamics of vegetation development in these newly-created man-made ecosystems (Bradshaw 1983; Alday et al. 2010). The investigation of these early successions in restored mines has both practical and theoretical importance (Grant 2009). Practically, because information on successional development might help guide future ecological restoration treatments, hence making the restoration process more effective (Walker & del Moral 2009), and identifying management intervention points (Grant 2009). Theoretically, because scientific approaches to restoration can provide insights into fundamental ecological theory, including understanding the initial phases of vegetation establishment (Robbins & Matthews 2009), and how the vegetation community structure develops (del Moral & Walker 2007).

When a mine is restored, the success of the initial revegetation treatments provides a starting species pool for the subsequent succession (Alday et al. 2010). Once this pool is established, other processes come into play including species assembly rules, rates of species colonization and extinction, and both positive and negative species interactions; all of which interact producing species turnover and structural change (Bartha et al. 2003; Walker & del Moral 2008). Therefore, by following the course of succession for the first years of the restoration process, those spatial and temporal changes in vegetation structure and species patterns that determine the initial outcome of succession can be identified and measured; thus providing insights into fundamental community dynamics and restoration success (Picket et al. 2008; Walker & del Moral 2009).

A fundamental part of any ecological restoration projects must be the identification of a target reference community to serve as a model against which to evaluate the success of the restoration (S.E.R.I. 2004). The most usual way to achieve this is to select an undisturbed reference community from the area surrounding the restored site (e.g. Martínez-Ruiz et al. 2007), or one typical of the local region (Aronson et al. 1995; Holl 2002). Thereafter, restoration success can be determined in two ways: first, through a comparison of the structure and function of the restored site with the reference community; and second, by calculation of the direction and speed of the trajectory between the restored site and the reference one (Mitchell et al. 2000; Fagan et al. 2008). One common approach to evaluate restoration success is to monitor vegetation change in permanent plots set up in both restored and reference sites (Kirmer & Mhan 2001; Prach & Pyšek 2001); here change through time provides a test of whether the sites are similar through time, i.e. convergence (del Moral 2007). In this paper, we investigated whether the community composition that developed on a restored open-cast mine in Spain was converging towards reference sites and the progress towards the desired target during the six years after the initial restoration works.

Open cast coal-mining affects around 5,000 ha of land in the León and Palencia regions of northern Spain, where there are mandatory requirements to ensure vegetation restoration (Moreno-de las Heras et al. 2008). During the last 10 years, one of the most common mine land restoration schemes in this regions has been to reshape the landform, improve the baseline soil-forming materials, and then introduce by hydroseeding selected herbaceous species that are adapted to disturbed and early-successional conditions. This approach has been called ‘passive relay floristic approach’; where practitioners assumed that the initial species will facilitate the colonization of medium- and late-successional native species through time, and the resulting restored community will be similar to surrounding native plant communities (McClain et al. 2010). However, the efficacy of this approach in achieving both the species composition and vegetation structure of reference sites has rarely been tested. There is also little information on the response of species introduced within the initial starting vegetation or the native colonizers through time.

In this paper, we attempt to address these issues. We followed the vegetation dynamics for the first 6 years after restoration in permanent plots on a restored coal mine in northern Spain, and compared the results with the vegetation of a surrounding reference plant community. As this site experiences a Mediterranean climate, and it is known that site aspect is a major factor controlling vegetation development in such situations (Martínez-Ruiz & Marrs 2007; González-Alday et al. 2008; Alday et al. 2010), the permanent plots were set up in a structured way to account for site aspect. This case study, therefore, embeds scientific monitoring of vegetation change within an ongoing practical mined land restoration scheme, and provides information of direct relevance to guide restoration practice (Bradshaw 1997; Picket et al. 2001). Specifically, we aimed to answer the following questions: (1) Does the course of succession on a coal mine restored using the standard restoration approach noted above (landform reshaping plus hydroseeding) converge with a reference community in terms of species composition and vegetation structure (richness, diversity and cover)? (2) What is the rate of succession on the restored mine? (3) What is the balance between local colonization and extinction rates on the restored mine? and (4) Which native or hydroseeded species determine the successional process? It was hoped that this information would lead to an improved restoration methodology, thus increasing effectiveness, and to generate knowledge to provide a more predictive understanding of successional dynamics during restoration.

Methods

Site description and restoration treatment

The study site was located in ‘Pozo Sell’, a 10 ha reclaimed open-pit coal mine near Villanueva de la Peña, Palencia, northern Spain (1.185 m a.s.l.; lat 42º50’N, long 4º38’W). The climate is sub-humid Mediterranean with a mean annual temperature of 9 ºC and average annual precipitation of 980 mm. Rainfall is distributed seasonally with most rainfall in spring and autumn, and with a pronounced dry season in summer. The vegetation surrounding the site comprises a complex matrix of grasslands (*Arenaria montana*, *Bromus mollis*, *Plantago alpina*, *Vulpia myuros*), crop fields (*Avena sativa*, *Secale cereale*), remnants of natural shrubland (*Cistus laurifolius*, *Erica cinerea*, *Juniperus communis*, *Rosa canina*) and *Quercus pyrenaica* woodland (Alday et al. 2010).

After mining stopped, the open pit was filled with coal wastes from nearby mines and the surface was covered with 50-100 cm of fine-textured sediments and a layer of cattle manure (30 t ha^{-1}). Thereafter, in October 2003 the entire site was hydroseeded using a slurry containing soluble chemical fertilizer (150 kg ha^{-1}; 8N:15P:15K), and a seed mixture (210 kg ha^{-1}) of grasses and herbaceous legumes (81:19 by weight). The seed mixture included *Festuca* spp., *Avena sativa*, *Secale cereale*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Trifolium pratense*, *Lotus corniculatus*, *Medicago sativa* and *T. repens* in 9:3:3:2:2:2:1:1:1:1 proportions.

Vegetation sampling

The change in plant species composition following restoration was examined in three separate areas differing in aspect: (1) a north-facing slope, (2) a south-facing slope, and (3) a flat area; both sloped areas were approximately 25º. In each of these areas three permanent plots of 20 m^2 were randomly located (restored mine; n=9), as well as in the shrub-land community surrounding the restored mine (reference community; n=3). Within each of these 12 plots, eight 0.25 m^2 quadrats were positioned randomly in January 2004 and marked permanently (Alday et al. 2010).

The cover (%) of all vascular plant species present in each quadrat was visually estimated by the same observer every spring (May-June) for six years (2004-2009) at the restored mine. The vegetation of the reference community was monitored twice; in the spring of 2004 and repeated in 2009. The mean cover of each species in the eight quadrats per plot was used to obtain an estimate of the cover of vascular plant species in each plot at each sampling occasion.

Data analysis

All statistical analyses were implemented in the R software environment (version 2.10; R Development Core Team 2009), using the VEGAN package for multivariate analyses (Oksanen et al. 2010), the LME4 package for GLMM (Bates & Maechler 2009) and the GRAVY package for HOF modeling (Oksanen 2003).

Change in floristic composition through time

Detrended Correspondence Analysis (DCA) was used to compare the change is species composition of the restored mine to the reference community. In this analysis the species dataset was down-weighted to reduce the influence of rare species. The VEGAN function ENVFIT (Oksanen et al. 2010) was then used to aid the interpretation of the output. ENVFIT was used to overlay (1) the centroids for each area (north-, south-, flat, reference) in each year, and (2) time as vector onto the species ordination plot. Successional trends for each area were displayed as lines connecting the time series of centroids. As the first DCA axis was highly correlated with time (τ=0.67, p<0.001), it represented a gradient of temporal turnover in species composition. Therefore, the rate of succession was estimated for reclaimed areas as the rate of change in site ordination scores along first axis (DCA-1 site scores; Matthews & Endress 2010).

Species heterogeneity and turnover

The Bray-Curtis dissimilarity index was calculated to compare species composition of the restored mine and the reference community in both 2004 and 2009. This index is a sensitive measure of heterogeneity (Robbins & Matthews 2009) and ranges from zero (complete similarity) to one (no species in common). The significance of the differences in Bray-Curtis average distances between restored mine and reference community were computed using a permutational multivariate analysis of variance (PMAV) with 1000 permutations (Oksanen et al. 2010).

In order to understand the processes of community change, the species turnover for each plot was calculated. Using species presence-absence data from the restored mine (n=9), the proportional species gained (G_p), loss (L_p) and turnover rates (T_p) were calculated following Matthews & Endress (2010). The slopes of these variables (G_p, L_p, T_p) for each plot were calculated to determine if the rates increased (positive slope) or decreased (negative slope) over time.

Change in vegetation structure

Generalized linear mixed models (GLMMs) were then used to determine the relationship between area and time on community composition variables (species richness, Shannon–Wiener diversity index, cover of hydroseeded and native species and species turnover). In these analysis, area (north-, south-facing, flat) were treated as categorical fixed factors, time was treated as a continuous fixed factor, and time nested within plot were included as random factors to account for temporal and spatial autocorrelation (Pinheiro & Bates 2000). Model simplification data followed Crawley (2007) using the Akaike Information Criterion (AIC, Pinheiro & Bates 2000). Models were fitted using the GLMER function, the REML method and data were arcsine-transformed (arcsin ($\sqrt{x}/100$)) for percentage data (%), whereas the Laplace method, the log-link function and a Poisson error distribution was used for count data (Crawley 2007). All values are reported as the mean ± standard error of the fixed factors, and the magnitude of the effects is calculated as the estimated difference from the flat area. Fitting mixed effects models allow inferences to be made about the fixed effects, which represent average characteristics of the population presented by areas, and the variability between plots.

Response of individual species through time

In order to illustrate the response pattern of individual species through time, the cover of (a) the 10 hydroseeded species, and (b) the 20 most common native species were fitted using Huisman-Olff-

Fresco (HOF) models (Huisman et al. 1993). HOF models allow testing of alternative responses to ecological gradients using a hierarchical set of five response models, ranked by their increasing complexity (Model I, no species trend; Model II, increasing or decreasing trend to a maximum; Model III, increasing or decreasing trend below the maximum attainable response; Model IV, symmetrical response curve; Model V, skewed response curve; Huisman et al. 1993). The species cover data were transformed (arcsin (√x/100)) and a Gaussian error structure used (Huisman et al. 1993). The AIC statistic was used to select the most appropriate model for each species; smaller values of AIC indicate better (more parsimonious) models.

Results

Eighty-seven vascular plant species were recorded in the restored mine during the six-year period. All of the ten hydroseeded species were found, especially during the first 3 years. The remaining 77 species included some uncommon species (*Clinopodium vulgare*, *Crocus nudiflorus*, *Crucianella angustifolia*, *Medicago arabica*), early colonizers and ruderals (*Brassica nigra*, *Capsella bursa-pastoris*, *Lactuca* spp., *Malva sylvestris*) and species characteristic of the reference community (*Avenula sulcata*, *Helianthemun hirtum*, *Hieracium pilosella*, *Trifolium campestre*). In contrast, sixty-eight vascular plant species were recorded in the reference community over the two sampling dates. The most abundant herbaceous species were: *Aira caryophyllea*, *Astragalus sempervirens*, *Bromus mollis*, *Ononis repens* and *Plantago alpina* and most abundant shrubs were: *Crataegus monogyna*, *Erica vagans*, *Rosa canina*, *Thymus mastigophorus* and *Thymus zygis*.

Change in floristic composition through time

The DCA of species composition produced eigenvalues (λ) of 0.49, 0.12, 0.09 and 0.07, and gradient lengths (GL) of 3.90, 1.62, 1.30 and 1.01 for the first four axes respectively. The species-time biplot showed that the passive fit of time was significant (*p*=0.006; Fig. 1a), and increased towards the positive end of the first axis. Hydroseeded species, such as *Avena sativa*, *Secale cereale*, *Festuca* spp., *Lotus corniculatus*, *Trifolium pratense* and *T. repens* were found at the negative end of axis 1, being negatively correlated with time. Early-successional species and ruderals such as *Capsella bursa-pastoris*, *Erodium cicutarium*, *Anthemis arvensis* and *Lactuca* spp. occupied an intermediate position on the ordination, and species that tended to be more common on the reference community, including *Arenaria montana*, *Avenula sulcata*, *Bromus mollis* and *Helianthemun hirtum* were found at the positive end of axis 1 and correlated positively with time.

The positions of the centroids of the four areas (north, south, flat, reference) for each year revealed several interesting patterns of species compositional dynamics (Fig. 1b). Two different results are apparent. The first is the move of centroids on axis 1, where all three restored mine

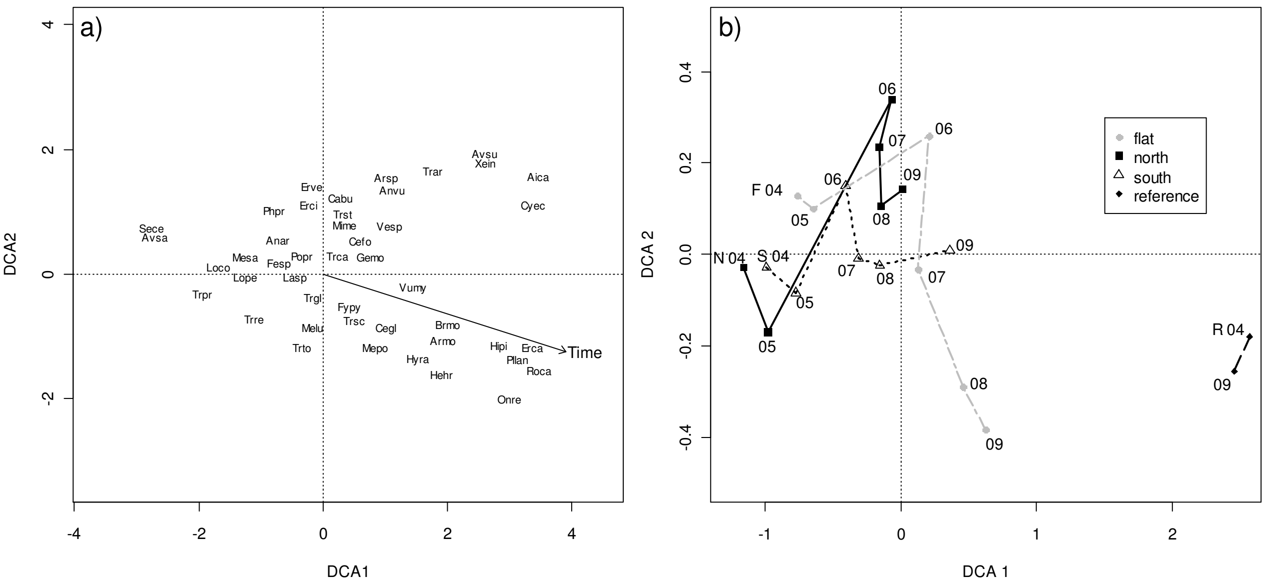

Figure 1. DCA ordination for the first two axes of floristic composition data from 9 permanent plots between 2004 and 2009 at the Pozo Sell reclaimed open-pit coal mine, and 3 permanent plots of reference community (only monitored in 2004 and 2009) at Palencia, northern Spain. (a) Species ordination with Time fitted as passive variable over ordination. Species codes: Aica = *Aira caryophyllea*; Anar = *Anthemis arvensis*; Anvu = *Anthyllis vulneraria*; Armo = *Arenaria montana*; Arsp = *Arenaria* spp.; Avsa = *Avena sativa*; Avsu = *Avenula sulcata*; Brmo = *Bromus mollis*; Cabu = *Capsella bursa-pastoris*; Cefo = *Cerastium fontanum*; Cegl = *C. glomeratum*; Cyec = *Cynosurus echinatus*; Erca = *Eryngium campestre*; Erci = *Erodium cicutarium*; Erve = *Erophila verna*; Fesp = *Festuca* spp.; Fypy = *Filago pyramidata*; Gemo = *Geranium molle*; Hehr = *Helianthemun hirtum*; Hipi = *Hieracium pilosella*; Hyra = *Hypochoeris radicata*; Lasp = *Lactuca* spp.; Lope = *Lolium perenne*; Loco = *Lotus corniculatus*; Melu = *Medicago lupulina*; Mepo = *M. polymorpha*; Mesa = *M. sativa*; Mime = *Minuartia mediterranea*; Onre = *Ononis repens*; Phpr = *Phleum pratense*; Pllan = *Plantago lanceolata*; Popr = *Poa pratensis*; Roca = *Rosa canina*; Sece = *Secale cereale*; Trar = *Trifolium arvense*; Trca = *T. campestre*; Trgl = *T. glomeratum*; Trpr = *T. pratense*; Trre = *T. repens*; Trsc = *T. scabrum*; Trst = *T. striatum*; Trto = *T. tomentosum*; Vesp = *Veronica* spp.; Vumy = *Vulpia myuros*; Xein = *Xeranthemum inapertum*. (b) Ordination of the centroids, representing replicate averages of each site per year are used for enhance the interpretability (04-09 indicate the last two digits of monitoring year).

areas are moving through time towards the reference community (moving from left to right on axis 1). The speed of convergence of the three restored mine areas towards the reference community differed; i.e. between 2004-2009 the flat area moved 1.39 units along axis 1 (0.28 units yr^{-1}) compared to 1.35 (0.27 yr^{-1}) and 1.17 (0.23 yr^{-1}), on the south- and north- facing areas respectively. However, in the tree restored mine areas the units moved per year decreased with time. The second is the move of centroids on axis 2, although in 2004 at the start of restoration all mine areas were relatively similar each other (especially the two sloped areas) there was an increasing divergence through time. The flat area is close to the reference community on this axis, but the other areas lag behind.

Species heterogeneity and turnover

The mean Bray-Curtis dissimilarity index between the three restored mine areas was 0.26±0.01 in 2004, and the mean distance between reclaimed areas and reference community was three times greater (0.95±0.01; PMAV with 1000 permutations; $p<0.001$, $r^2=0.77$). However, by 2009, the dissimilarity between the restored mine areas and the reference community were reduced to

0.82±0.01 (13% of reduction), although they were still significant (PMAV with 1000 permutations; $p<0.001$, $r^2=0.44$). Moreover, the dissimilarity among restored mine areas increased through time, reaching 0.53±0.02 in 2009 (27% of increase).

The GLMMs showed that on the restored mine both the inter-annual species gains (G_p) and losses (L_p) declined between 2004-2009, independently of the area considered (Fig. 2). However the decrease is sharper for gains (G_p slope=-0.10±0.01; t-value=6.45, $p<0.01$ vs. L_p slope=-0.05±0.01; t-value=-4.76, $p<0.01$). This indicated that the number of colonizers per year was reducing faster than species losses. Overall species turnover (T_p) declined with time from 0.46±0.03 between 2004 and 2005 to 0.25±0.02 five years later (slope=-0.07±0.01; t-value=-7.37, $p<0.01$), as a consequence of these decreasing rates of species gains and losses.

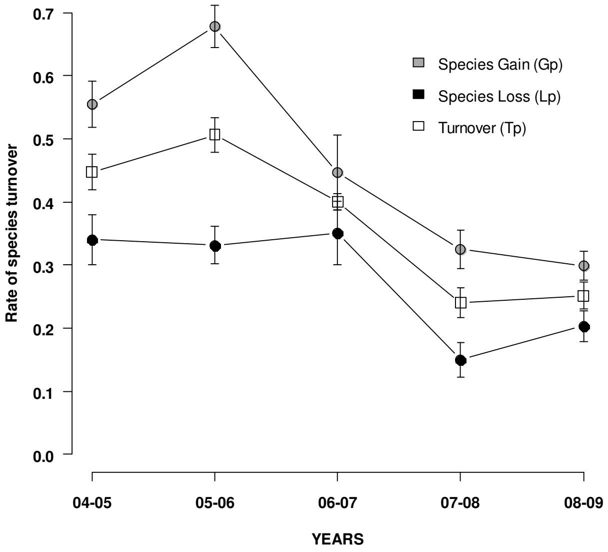

Figure 2. Turnover of herbaceous species between each pairs of years in the restored Pozo Sell mine. Plotted values are 9 permanent plots mean ± SE.

Change in vegetation structure

Richness at the restored mine increased from 21±0.9 to 36±0.8 species per plot between 2004 and 2009, almost reaching the value of the reference community (41±0.6 in 2009). GLMMs showed that species richness increased linearly over time, with a common positive slope for all areas (slope=0.10±0.015; z-value=6.66, $p<0.01$), but with different intercepts for each area in 2004 (north-facing=-0.16±0.06, z-value=-2.68, $p<0.05$; south-facing =-0.10±0.06, z-value=-1.63, $p=0.10$; flat=3.10±0.01, z-value=44.35, $p<0.001$). This result suggests different colonization rates for areas and plots in 2004 (Fig. 3a). A similar result was found for diversity which also increased linearly over time with the same slope for all areas (slope=0.012±0.01, t-value=8.01, $p<0.01$; Fig. 3b) and different intercepts. Here, in 2009, the mean diversity index per plot was 3.00±0.07 compared to the reference community's value of 3.26±0.04.

The cover of hydroseeded species per plot decreased progressively over the six years from 60%±5.30 to 24%±4.23, with a corresponding increase in colonization by native species from

13%±1.4 to 55%±2.9 (Figs. 3c, d). The response was linear in both cases although the slope is steeper for the increase in native species cover (slope=0.10±0.01; t-value=0.88, $p<0.001$) than for the reduction in hydroseeded species cover (slope=-0.08±0.006; t-value=-11.67, $p<0.001$). The different intercepts found for both areas in 2004 with respect to the hydroseeded species cover reflected differential hydroseeding success in the different areas of the restored mine (north-facing=0.32±0.08, t-value=4.17, $p<0.01$; south-facing=0.18±0.08, t-value=2.33, $p<0.05$; flat=0.78±0.06, t-value=13.61, $p<0.001$). Particularly noteworthy is that there is a negative correlation between hydroseeded species cover and native species richness during 2004-2005 (r= -0.82, $p<0.001$).

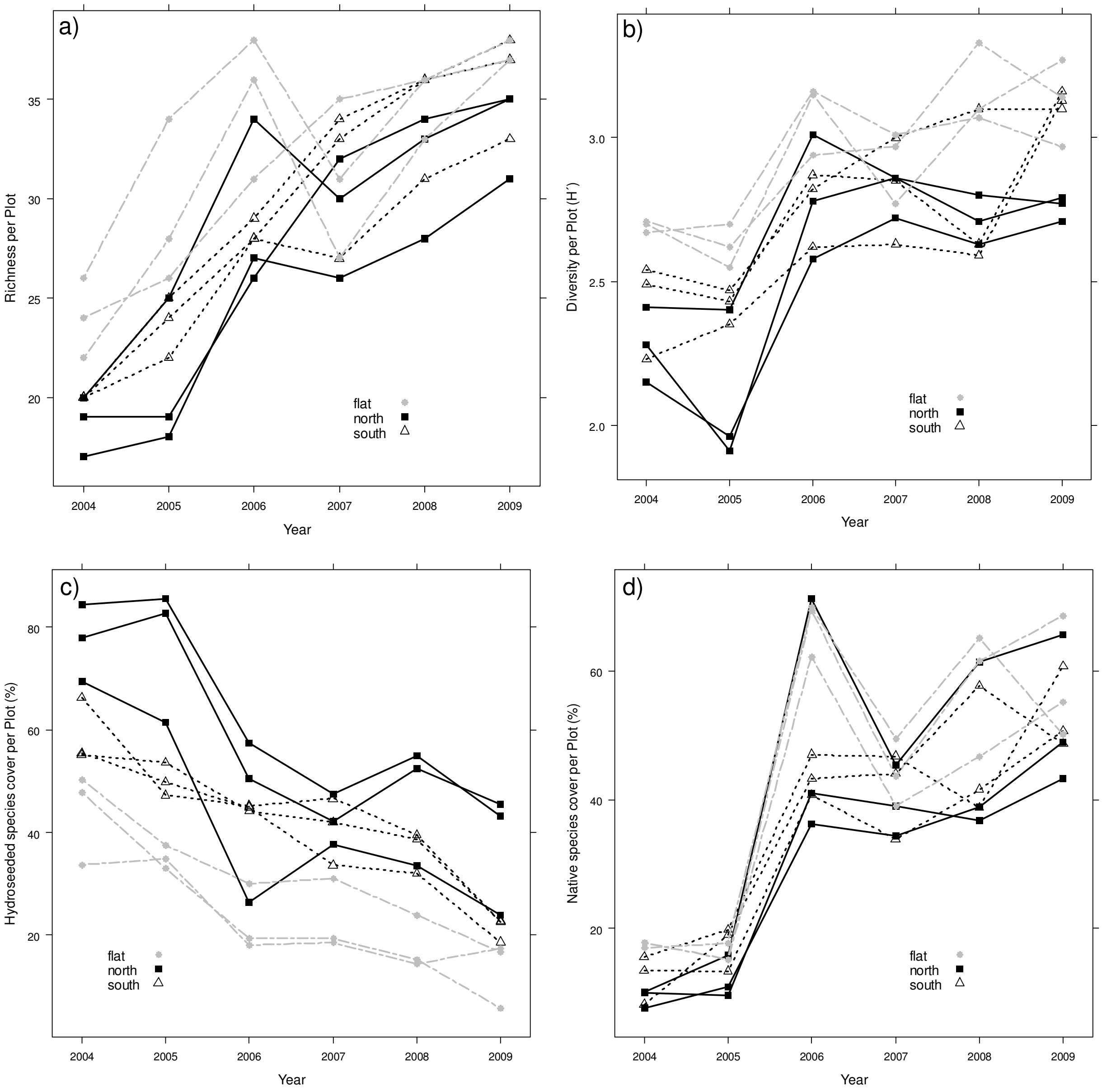

Figure 3. Changes over time in structure measures of 9 permanent plots distributed in three areas (flat, north- and south-facing slopes) at the restored Pozo Sell mine. (a) Species richness, (b) Diversity, (c) Hydroseeded species cover (%), (d) Native species cover (%).

Response of individual species through time

The cover of 8 of the 10 hydroseeded species decreased with time since restoration, whereas most of the native species showed an opposing, increasing response (Fig. 4, 5). For the hydroseeded species three groups were identified based on the type of significant response. Group 1 contained 6 species (*A. sativa*, *L. perenne*, *L. corniculatus*, *T. repens*, *T. pratense*, *S. cereale*) which cover declined through time (HOF models II, IV); Group 2 comprised 3 species (*Festuca* spp., *M. sativa*, *P. pratensis*) which either maintained a stable cover for 4-5 years and declined steeply (HOF model III), or showed a skewed response with an optimum at 4 years and subsequent decline (HOF Model V). Finally, Group 3 contained just *P. pratense;* this species showed not response to time and had a stable cover of 8% (HOF model I).

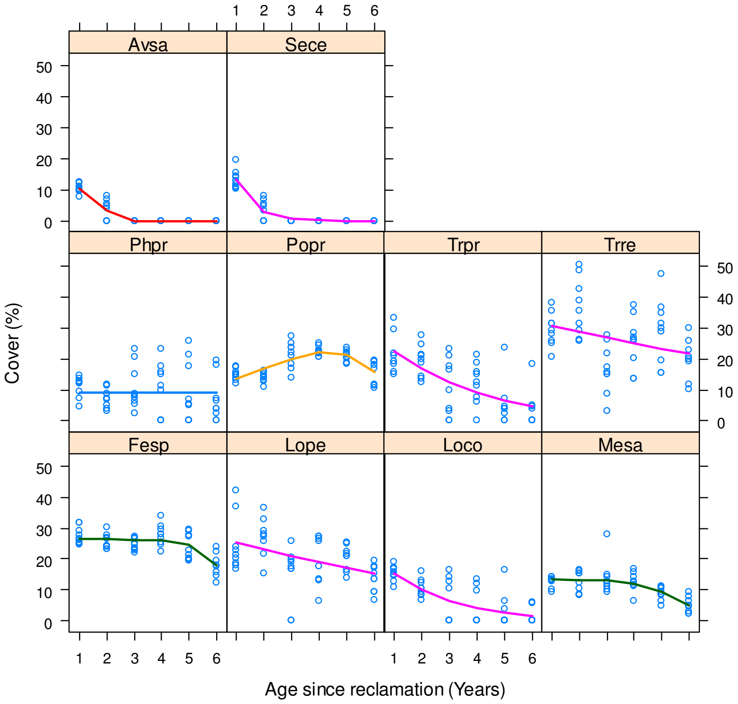

Figure 4. Significant response curves of the 10 hydroseeded species with respect to age since restoration, at the Pozo Sell reclaimed open-pit coal mine. Species codes: Avsa = *A. sativa*; Fesp = *Festuca* spp.; Loco = *L. corniculatus*; Lope = *L. perenne*; Mesa = *M. sativa*; Phpr = *P. pratense*; Popr = *P. pratensis*; Trpr = *T. pratense*; Trre = *T. repens*; Sece = *S. cereale*.

Although the cover of the most common native species increased through time, they could also be separated into three response groups (Fig. 5). Group 1, with species that showed a continually increase through time (HOF model II), and included two herbs (*Capsella bursa-pastoris*, *Medicago polymorpha*), and one woody species (*Helianthemun hirtum*). Group 2 comprised 10 species (*Arenaria montana*, *Arenaria* spp., *Bromus mollis*, *Cerastium fontanum*, *C. glomeratum*, *Geranium molle*, *Hypochoeris radicata*, *Trifolium scabrum*, *T. striatum*, *Vulpia myuros*); these species cover increased and reached a maximum between 3 and 4 years after restoration (HOF model III). Finally, Group 3 contained seven species with unimodal/skewed response (HOF models IV, V). This group showed a turnover of species: *Erodium cicutarium*, *Erophila verna* and *Minuartia* *mediterranea* peaked at 3 years; *Anthemis arvensis*, and *T. campestre* peaked at 4 years; and both *Filago pyramidata* and *Veronica* spp. which peaked at 5 years after restoration.

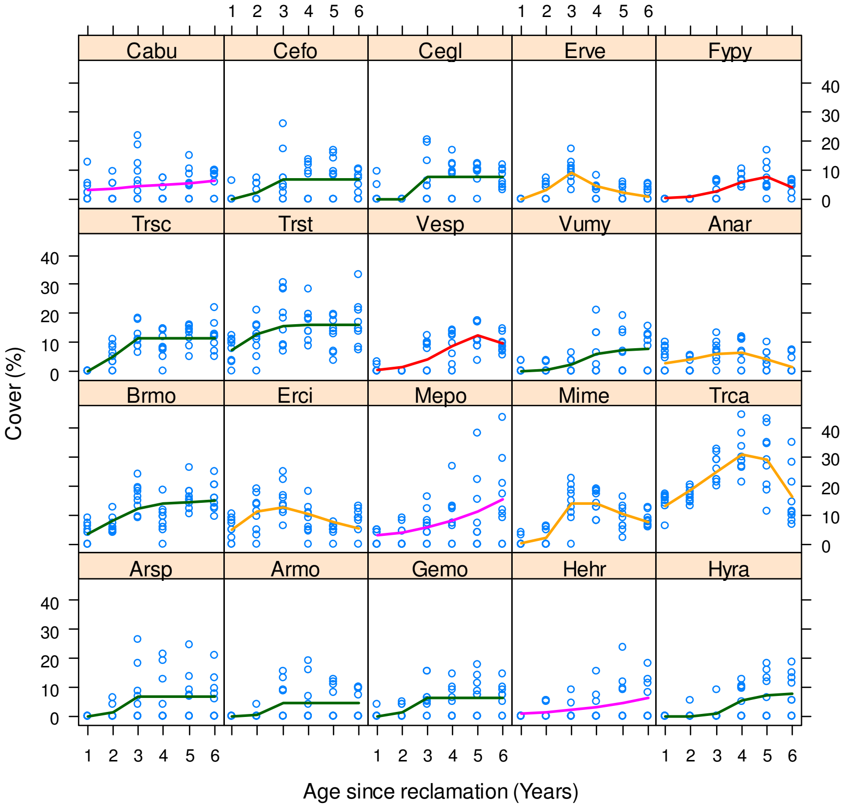

Figure 5. Significant response curves of the most abundant native species with respect to age since restoration, at the Pozo Sell reclaimed open-pit coal mine. Species codes: Anar = *A. arvensis*; Armo = *A. montana*; Arsp = *Arenaria* spp.; Brmo = *B. mollis*; Cabu = *C. bursa-pastoris*; Cefo = *C. fontanum*; Cegl = *C. glomeratum*; Erci = *E. cicutarium*; Erve = *E. verna*; Fypy = *F. pyramidata*; Gemo = *G. molle*; Hehr = *H. hirtum*; Hyra = *H. radicata*; Mepo = *M. polymorpha*; Mime = *M. mediterranea*; Trca = *T. campestre*; Trsc = *T. scabrum*; Trst = *T. striatum*; Vesp = *Veronica* spp.; Vumy = *V. myuros*.

Discussion

Vegetation convergence (composition and structure)

An important result was that vegetation structure (richness, diversity and cover) and the species compositional trajectories in the three restored mine areas (north, south and flat) are becoming increasingly similar (converging) with the reference community over time, albeit at different speeds. These successional trends are driven by a reduction in hydroseeded species cover and an increase of native richness and cover, which is consistent with the relay floristic approach. However, most of the native species that have colonized within the 6-year period were classified as widespread species with long distance dispersal mechanism (anemochorus and zoochorus species cf. González-Alday & Martínez-Ruiz 2007). As a consequence, reliance on the relay floristic restoration approach may be adequate for species with good dispersal mechanisms, but may inappropriate for less common species or with limited dispersal abilities. These conclusions mirror those from other studies of vegetation restoration using relay floristic approach in mined sites (Parrotta & Knowles 1999; Parrotta & Knowles 2001; Holl 2002).

The second noteworthy result was that in 2004 the species composition in the three restored mine areas was more similar than it was in 2009, essentially showing some divergence along the trajectory towards the reference community, and this divergence was greatest in the flat area. These divergent trajectories may be caused by a combination of (1) differential success of establishment of the hydroseeded species (greatest in north followed by south and flat areas, effect of topography; Alday et al. 2010) and (2) by the greater establishment of autochthonous species. It is well known that a successful early establishment of a founder population, here hydroseeded species, can have important implications for successional trajectories (del Moral & Larcher 2005), producing diverse trajectories even in close locations. These results are in agreement with other studies that demonstrate the frequency of multiple trajectories in early successions (del Moral 2007; Prach & Hobbs 2008; del Moral et al. 2010).

In three restored areas, the reduction in cover of hydroseeded species was accompanied by a significant increase in species richness, diversity and native species cover through time. These general patterns of richness, diversity and native cover changes over the first six years after restoration are consistent with other successional studies on different surfaces, including old-fields (Gibson et al. 2005), glacier forelands (Robbins & Matthews 2009), mine sites (Martínez-Ruiz et al. 2007) and lava flows (Cutler 2010). The richness and diversity (H´) increase was produced by the arrival of early successional species, including annuals, ruderals and some species common of the reference community. These new arrivals increased in cover and collectively had a greater cover than the hydroseeded species after 6 years. The cover of native species appeared to increase sharply between 2005 and 2006, which was almost certainly produced by the impact of an exceptionally dry year (Alday et al. 2010). It is likely that these new colonizers were better adapted to disturbance produced by the drought prevalent in Mediterranean region than hydroseeded species (Lawesson & Oksanen 2002; Alday et al. 2010).

Of course the reason for including some hydroseeded species during restoration was because of the very poor soil seed bank before treatment (González-Alday et al. 2009). The rapid cover produced by hydroseeded species reduced soil erosion (Albadalejo et al. 2000) and the extreme habitat conditions as both organic matter and nutrients accumulate (Bossuyt et al. 2003), which might facilitate the establishment of new individuals. However, it is well known that species already present at site have a competitive advantage over invaders as long as no further disturbance occur (Wiegleb & Felinks 2001). Therefore, the competitive interactions between hydroseeded and native species might limit further site colonization (Matesanz et al. 2006). Our results support the second explanation, because during the first 3 years some plots yet to experience dense hydroseeded species cover (north and south areas), retarded the colonization of native species, evidenced by the inverse relationship between hydroseeded species cover and richness (colonization rate), and resulting in a less diverse assemblages.

In six years, despite that richness and diversity on the restored mine reached similar levels to those at the reference community, native cover and especially species composition still differed considerably from reference area, with a Bray-Curtis dissimilarity index of 0.82. Therefore, a much longer time frame is needed for the restored sites to achieve a similar community composition and structure to the reference community. Similar conclusions were reached by McClain et al. (2010) for a riparian forest restored using passive relay floristic approach.

The rate of succession and local colonization and extinction rates on the restored mine

The rate of succession towards the reference community was different across areas. The DCA scores of flat area moved closer to the reference community than either the north- or south-facing slopes. This is due to differences in the establishment of hydroseeded and native species, discussed above. However, the three mined areas showed a reduction in the rate of change with time. Variability in early successional compositional rates among areas or plots has been demonstrated in other studies of ecological restoration (Prach et al. 1993), and in successional studies of lava flows (del Moral 2007) and glacial plains (Marteinsdóttir et al. 2010), where a mosaic patterns of vegetation results. The existence of differences in the early successional rates based on species composition suggests that within-area constraints and stochastic factors (e.g. extreme drought) appear to play an important role influencing species assembly and succession (Pickett et al. 2001; Marteinsdóttir et al. 2010).

The reclaimed areas studied here followed the general temporal patterns in rates of plant species changes during early succession defined by Anderson (2007). First, colonization rates (species gains) started high and declined trough time. Here this was complicated by an initial increase (2005-2006), which probably occurred because species began to colonize from surrounding landscape, and there was an initial increase in species richness. Second, when richness approached the number of species in the available reference community (here at 2007), the probability that a colonizing species is a new one declines (Foster & Tilman 2000, Bartha et al. 2003). At the same time, any new propagules face a reduced number of available microsites for germination, and an increased competitive pressure from stabilised vegetation composed of both hydroseeded and native species (Matthews & Endress 2010), hence the rate of species colonization declines. Third, extinction rates (loss rate) were always lower than colonization rates and showed a shallowed slope. Fourth, species turnover declines as it is the sum of the species gain and losses, suggesting that it is driven by the same mechanisms that drive them (colonization, reduction of available microsites and competition; Anderson 2007). Therefore, if the restoration was based on a relay floristic approach it would require medium turnover rates until most of the target species establish. Our results are not consistent with this, since during the first four years important changes in species composition occur mainly by the incorporation of wide spread native species, and turnover decelerates over time increasing the community stability (eg.

Odum 1969). This suggests that much longer time frame will be needed to achieve our ecological restoration objective.

Native or hydroseeded species determined successional process

The success of the initial restoration treatments provides the starting species pool for successinal process (Lepš et al. 2007; Alday et al. 2010). As a consequence, during the two first years after restoration (2004-2005) succession was mostly determined by the performance of hydroseeded species behavior, either by competitive interactions or by adaptation to environmental conditions (Alday et al. 2010). The hydroseeded species provided most of the initial vegetation cover in three mine areas, mainly through the high cover of *T. repens*, *T. pratense*, *Festuca* spp. and *Lolium perenne*, which formed dense communities with little chance for establishment of native species (Ninot et al. 2001; Alday et al. 2010). In later years the cover of hydroseeded species decreased; although only three species disappeared (*Avena sativa*, *Secale cereale* and *Lotus corniculatus*) three species maintained a cover greater than 10% (*T. repens*, *Festuca* spp. and *Lolium perenne*). However, the effect of hydroseeded species although decreased in importance persisted for the whole experimental period. These results were not in agreement with the hypothesis that commercial seed mixtures act as facilitating species, growing fast the first years after hydroseeding and gradually vanishing as more competitive species colonize (Mitchley et al. 1996; Merlin et al. 1999).

The lack of a seed bank on this mine (González-Alday et al. 2009) meant that native seeds had to arrive via dispersal from neighboring vegetation. As a consequence, most of the native species were animal- or wind-dispersed; e.g. *A. montana*, *B. mollis, Cerastium fontanum, M. mediterranea, T. campestre, T. striatum or V. myuros*. These species were the first colonizers with a cover that peaked at 3 years after restoration (2006) and they maintained stable cover values thorough. Therefore, from 2006 these native species started to be important in cover and were the responsible of the vegetation composition was moved towards reference community, conditioning the successional process (eg. Lanta & Lepš 2009). On the other hand, some native species turnover on plant cover peaks was produced, mainly by ruderal species (*Erodium cicutarium*, *Erophyla verna*, *Anthemis arvensis*, *Veronica* spp.), which showed a fast growing until their optima being later out-competed by hydroseeded and native species.

Conclusions

After 6 years of mine restoration vegetation structure (richness, diversity and plant cover) is approaching this of the reference community. In contrast, species composition although is becoming similar, six years latter still differed considerably from the reference community, suggesting that much longer time-frame is needed to achieve our ecological restoration objective. At the same time, the increasing differences in species composition between areas and the different successional compositional rates indicate that within-area constraints appear to play an important part in species assembly and succession, producing diverse trajectories even in close locations.

The present work showed that the reliance on the relay floristic approach may not be adequate for less common species or dispersal limited, therefore much longer time-frames are needed with this method to achieve that restored sites had a similar species composition than reference community. Finally, these results identified the main species involved in the short-term succession of coal mines in the region and helped guide future restoration strategies.

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Chapter 6

Old soil materials

Soil and vegetation development during early succession on coal wastes: a six-year permanent plot study

Josu G. Alday, Rob H. Marrs & Carolina Martínez-Ruiz

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Abstract

One of the objectives of ecological restoration is the development of a fully functioning soil. However, little is known about how new-formed soil parameters change during early stages of revegetation dynamics on restored coal mines, particularly in a Mediterranean climate. This information is crucial for improving our ability to restore land despoiled by mining. Here, we investigated the short-term soil changes and their relation with vegetation development during early succession on coal wastes located in Palencia (Spain). Soil and vegetation changes were monitored during 6 years after restoration at 9 permanent plots differing in aspects (north-, south- and flat areas). Soil variables showed a general trend over time of increasing soil organic matter, total carbon and nitrogen, sand content and exchangeable calcium, but a reduction in soil pH, clay and lime contents, whereas EC, P, Mg^{2+} and K^{+} showed no change through time. More importantly, these changes in soil properties were independent of the three areas with differing aspects, suggesting that age since restoration alone is the agent driving change in these soil variables at least in short-term. In addition, vegetation structural/functional changes were related with the accumulation of organic matter and sand content, it seems that they are involved in the mechanism that induce these soil changes, favoring the increase of plant community complexity in such mined areas. Surprisingly, floristic compositional changes were not related with soil factors, maybe because the soil-forming material was sufficiently good to prevent soil factors controlling species development. In any case, these results supported the idea that floristic compositional differences in short-term are mainly driven by a combination of stochastic and abiotic factors.

Key words: Soil disturbance; physicochemical soil properties; floristic composition; vegetation structure; soil organic carbon; restoration works.

Introduction

Mining is a major environmental disturbance which often leaves a landscape with no vegetation and very poor soil-forming materials for ecosystem development (Herath et al. 2009). In such damaged systems, the objective of ecological restoration should be the restoration of healthy, long-term, self-sustaining ecosystems (Hobbs & Northon 1996). One of the key components for ecosystem long-term sustainability is the development of a fully functioning soil (Moreno de las Heras 2009; Dölle & Schmidt 2009); with appropriate soil biota and microbial process (Frouz et al. 2008), and vegetative cover (Dazy et al. 2008). Indeed a self-sustaining restored ecosystem should have at least a semblance of the original soil dynamics (Walker & Del Moral 2009). Therefore, the knowledge of how soil develops during restoration is of particular importance to guide future ecological restoration works (Abreu et al 2010).

When a mine is restored, the initial restoration treatment provides a starting soil material, which can be very poor, and a species pool for the subsequent succession (Alday et al. 2010a). During succession, there are changes in vegetation composition and structure which can influence soil conditions directly (Isermann 2005). Vegetation development enhances the accumulation of carbon, nitrogen and nutrients in the soil (Marrs & Bradshaw 1993; De Kovel et al. 2000; Walker & Del Moral 2009), modelling the physical and chemical structure of habitats (Dölle & Schmidt 2009). As a result, these processes improve the soil conditions for subsequent species colonization and development (Frouz et al. 2008). Therefore, in order to predict medium-term changes in vegetation composition and structure, knowledge of changes (rates of change) in nitrogen, organic matter and proportion of nutrients in the soil during the initial restoration period is needed.

The changes of soil parameters and vegetation development over succession have been studied in various types of landscapes such as china clay wastes (Marrs et al. 1980a,b; Roberts et al. 1980), abandoned fields (Knops & Tilman 2000), urban sites (Schadek et al. 2009), glaciers (Hodkinson et al. 2003) and forests (Matlack 2009). However, there have been few attempts to carry out studies on vegetation succession and soil changes in restoration projects of coal mined sites in Mediterranean climates (e.g. Moreno-de las Heras 2009), and specially following permanent plots.

In the northern Spain, particularly in the provinces of León and Palencia, open cast coal mining has caused extensive damages in vegetation and soil characteristics (affecting ca. 5,000 ha). During the last 10 years, one of the most common mine land restoration schemes in this regions has been to reshape the landform, improve the baseline soil-forming materials, and then introducing herbaceous seeds by hydroseeding. Although, some experimentations have already been undertaken to validate this method and specifically to elucidate the importance of abiotic factors limiting vegetation establishment and development (González-Alday 2008; Alday et al. 2010a), there is little information about soil processes or changes in physicochemical soil properties during coal mines post-restoration successions.

In this paper, we attempt to address these issues. We followed the changes in vegetation and soil in permanent plots for 6 years after restoration was implemented on a coal mine in northern Spain. Permanent plot studies offer valuable insights into soil, nutrients and vegetation changes during succession (Dölle & Schmidt 2009). The aim here was to explore the interactions of physicochemical soil development and vegetation succession (composition and structure) and to discuss their potential functional relationships. Specifically, we aimed to answer the following questions: (1) How do soil parameters in a restored mine change over time? (2) Are soil parameters affecting the early floristic composition dynamics? and (3) What is the relationship between the soil factors and the vegetation structure variables (richness, diversity and cover)? It was hoped that this information would lead to improve ecological restoration methods, increasing their effectiveness and help to gain more predictive understanding of mine restoration dynamics.

Methods

Site description and restoration treatment

The study was carried out in 'Pozo Sell', a 10 ha reclaimed open-pit coal mine near Villanueva de la Peña, Palencia, northern Spain (1.185 m a.s.l.; lat 42º50'N, long 4º38'W). The climate is sub-humid Mediterranean with mean annual temperature of 9 ºC and average annual precipitation of 980 mm seasonally distributed, i.e. with a rainy season in autumn and spring and a pronounced dry season in summer. The vegetation surrounding the site comprises a complex matrix of grasslands (*Arenaria montana*, *Bromus mollis*, *Plantago alpina*, *Vulpia myuros*), crop fields (*Avena sativa*, *Secale cereale*), remnants of natural shrubland (*Cistus laurifolius*, *Erica cinerea*, *Juniperus communis*, *Rosa canina*) and *Quercus pyrenaica* woodland (Alday et al. 2010a).

After mining stopped, the open pit was filled with coal wastes from nearby mines and the surface was covered with 50-100 cm of fine-textured materials and a layer of cattle manure (30 t ha^{-1}). The fine-textured materials were a mixture of topsoil and sediments from deeper parts of the nearest opencast pit. This mixture had a clay loam texture, a pH value moderately alkaline (pH=8.2), organic matter content below 2% and a very sparse seed bank (González-Alday et al. 2008, 2009). In October 2003, the entire site was hydroseeded using a slurry containing soluble chemical fertilizer (150 kg ha^{-1}; 8N:15P:15K), and a seed mixture (210 kg ha^{-1}) of grasses and herbaceous legumes (81:19 by weight). The seed mixture included *Festuca* spp., *Avena sativa*, *Secale cereale*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Trifolium pratense*, *Lotus corniculatus*, *Medicago sativa* and *T. repens* in 9:3:3:2:2:2:1:1:1:1 proportions.

Vegetation sampling

As aspect is a major factor controlling vegetation development in Mediterranean climates (González-Alday et al. 2008; Alday et al. 2010a), this study investigated the development of soil and vegetation in three separate areas of different aspect: (1) a north-facing slope, (2) a south-facing slope, and (3) a flat area; both sloped areas were approximately 25º. In each of these areas three permanent plots of 20 m^2 were randomly located (n=9). Within each plot, eight 0.25 m^2 quadrats were positioned randomly in January 2004 and marked permanently (Alday et al. 2010a). The cover (%) of all vascular plant species present in each quadrat was estimated visually by the same observer every spring (May-June) for six years (2004-2009). The mean cover values of each species into the eight quadrats per plot were used to obtain an estimate of the cover of vascular plant species in each plot on each sampling occasion.

Soil sampling and analysis

In the spring of each year from 2004 to 2009 eight soil sub-samples were taken with a soil auger (diameter=8 cm; depth=10 cm) in the vicinity of each 0.25 m^2 quadrats. These sub-samples were pooled and thoroughly mixed to obtain a uniform sample of the soil characteristics for each plot (n=9). The soils were shallow and the sample depth included the developing A (organo-mineral) and the rooting layer of the herbaceous species present.

In the laboratory the soil samples were air-dried, sieved (≤2 mm mesh) and soil texture was analysed using the Bouyoucos-method (Day 1965). Soil chemical properties were then measured as follows: soil pH and electrical conductivity using a conductivity meter in a 1:2.5 soil:deionized water slurry (Allen 1989); total nitrogen concentration using the Kjeldahl-method (Bremner & Mulvaney 1982); available phosphorus concentration using the Olsen method (Olsen & Sommers 1982); total organic matter and carbon concentration using the Walkley-Black method (Walkley 1947); and finally exchangeable cations as calcium (Ca^{2+}), potassium (K^{+}) and magnesium (Mg^{2+}) with atomic absorption spectroscopy after extraction with 1N ammonium acetate (pH=7) (Allen 1989; Anderson & Ingram 1993).

Data analysis

All statistical analyses were implemented in the R software environment (version 2.10; R Development Core Team 2009), using the NLME package for LMM (Pinheiro et al. 2009) and the VEGAN package for multivariate analyses (Oksanen et al. 2010).

Change in soil parameters through time

Linear Mixed Models (LMMs) were used to evaluate soil parameters changes between areas an time. In this analysis, area (north-, south-facing, flat) was treated as categorical fixed factors, time was treated as a continuous fixed factor, and time nested within plot was included as random factor to account for temporal and spatial autocorrelation (Pinheiro & Bates 2000). Model simplification guidelines followed Crawley (2007) using the Akaike information criterion (AIC, Pinheiro & Bates 2000). The reduction of AIC (ΔAIC) was calculated as: $\Delta AIC=100\times(AICn-AICmin)/AICn$, where AICn and AICmin are AIC of the null and minimal adequate models respectively (Burnham and Anderson 2002). For each model the r^2 was calculated following the method proposed by Edwards et al. (2008). Models were fitted using REML method and data as percentages (%) were arcsin-transformed (arcsin ($\sqrt{x}/100$)). All values are reported as the mean ± standard error of the fixed factors, and the magnitude of the effects is calculated as the estimated difference from the flat area.

Floristic composition and soil variables

Initially the soil variables were analyzed using a Discriminant Analysis with Forward Selection procedure (DA-FS) to identify those that were most important in the maximization of the differences between the three areas (north-, south-facing, flat).

Ordination analysis was used to relate plant community species composition (log(x+1) transformed) to soil variables. A Detrended Correspondence Analysis (DCA) produced eigenvalues (λ) of 0.25, 0.10, 0.10 and 0.06, and gradient lengths (GL) of 2.21, 1.34, 1.50 and 1.00 for the first four axes respectively. The gradient lengths suggested that linear based ordination methods as redundancy analysis (RDA) were appropriate for subsequent analyses (Ter Braak & Šmilauer 2002). Using RDA the number of constraining soil variables (n=11) was further reduced by means of forward selection procedure based in AIC (Oksanen et al. 2010). The effects of the successional trends in the different plots were partialled out. Significance of the constraints was assessed by permutation tests using the reduced model with 199 permutations. Since significant test are biased because the model is selected for the best test performance (Oksanen et al. 2010), the variance explained for each constraint was calculated to aid the identification of the important soil variables. Only soil variables that were significant and explained more than 5% of the variance are discussed.

Vegetation structure and soil variables

Plant community diversity was assessed using the Shannon-Wiener index (H') and richness (S). Plant species were categorized by: (1) type (hydroseeded, ruderal and native), and (2) by functional groups and life-forms (annuals and perennials were split in grasses, legumes and forbs). The mean plant cover and richness of each type in each plot were then calculated, whereas for functional groups only plant cover was calculated.

Principal component analysis (PCA) was used to provide an integrated analysis of the patterns of variation for soil and vegetation structure variables and to evaluate successional trends. Twelve vegetation structural variables and ten soil variables were used in this analysis (see Table 2 for detailed list) and these were standardized before analysis to correct for different measuring units. In order to avoid autocorrelation some variables were excluded from the analysis, e.g.: lime fraction, total cover, native species richness, annual forbs and perennial legumes cover or total carbon.

Results

Soil development through time

The soil variables were separated into three groups based on the type of significant response to successional age. Group 1 (Fig. 1, Table 1) is comprised of three variables (pH, clay content and lime content) which all had negative linear slopes with respect to age and common intercept with respect to area. These variables decreased over the six years from 8.19±0.03, 27.88±0.82% and 26.12±0.71% to 7.74±0.09, 22±0.76% and 14±0.74% for pH, clay content and lime content respectively.

Group 2 comprised 5 variables with a continually increasing linear trend with succesional age and no significant differences between areas with a common intercept; these were the content of sand, organic matter, total carbon and nitrogen, and exchangeable Ca^{2+} concentration (Fig. 2, Table 1). These variables increased over the 6 years as follows: sand content showed an important increased from 46.67%±0.66 to 64%±0.57, organic matter increased 1% yr^{-1}, total carbon 0.6 % yr^{-1}, total nitrogen 0.07% yr^{-1} and the available calcium concentration increased slightly from 12.76%±0.42 to 19.5%±1.02 six years later.

Table 1. Parameters from the Linear Mixed Effects analysis of soil variables with respect to age since restoration. Two responses were noted: Group 1 showed negative relationships with age, and Group 2 showed positive relationships. All of these variables had a linear relationship and there was no significant effect of sample area (aspect effect).

Group	Variable	$\text{Slope}_{[1,44]}$	t	*p*-value	ΔAIC=%	r^2
1	pH	-0.08±0.02	-4.86	<0.001	74.9	0.38
	Clay content (%)	-0.82±0.25	-3.26	<0.01	1.2	0.18
	Lime content (%)	-2.83±0.24	-11.74	<0.001	18.0	0.72
2	Sand content (%)	3.5±0.24	14.84	<0.001	22.3	0.80
	Organic matter (%)	1.0±0.11	8.58	<0.001	17.2	0.62
	Total C (%)	0.60±0.06	8.75	<0.001	22.5	0.60
	Total N (%)	0.06±0.01	9.27	<0.001	73.0	0.65
	Exchangeable Ca^{2+}	1.13±0.16	6.99	<0.001	10.6	0.58

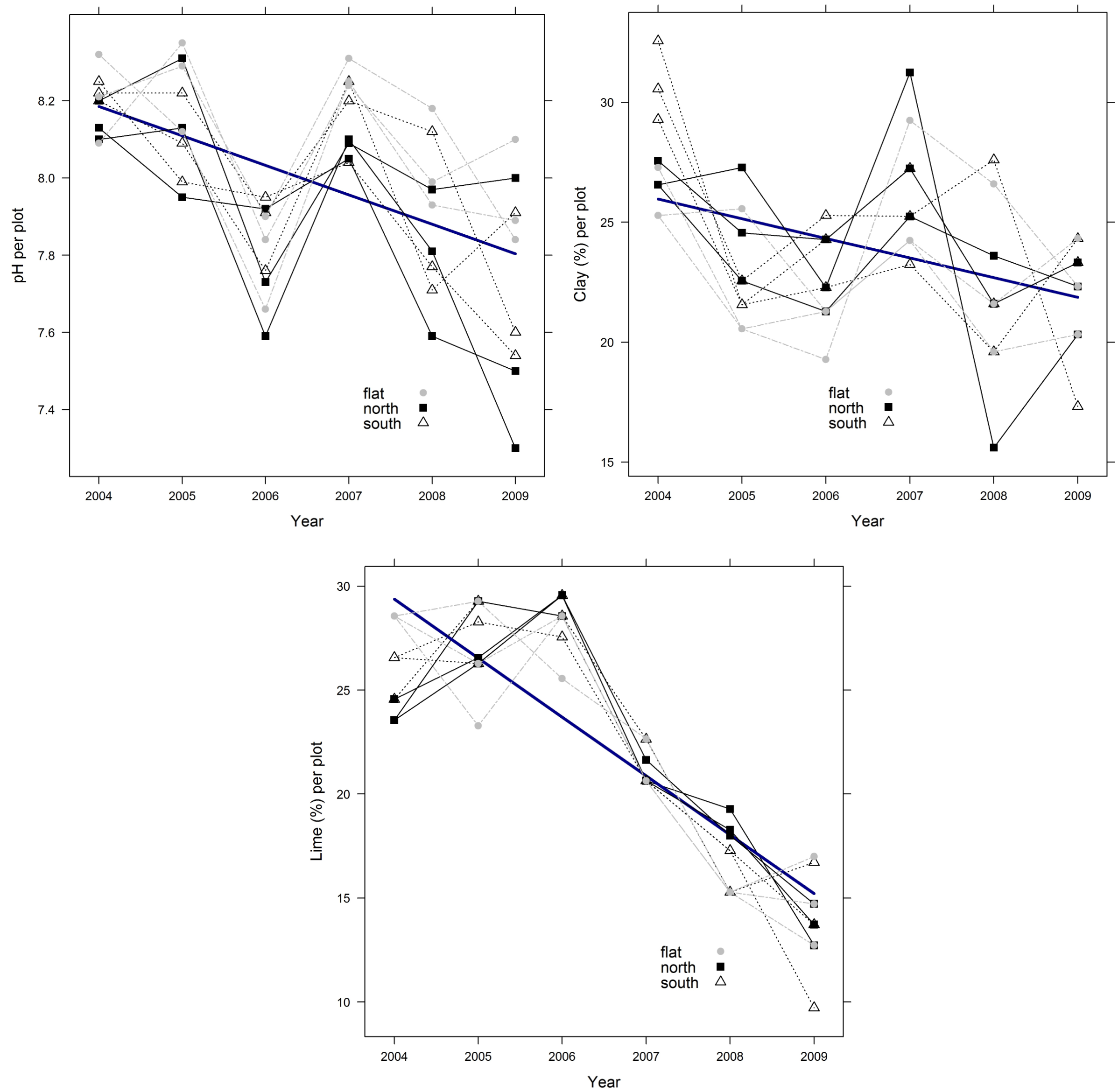

Figure 1. Decreasing soil variables (pH, clay and lime content) over time at 9 permanent plots distributed in three areas (flat, north- and south-facing slopes) at the restored ‘Pozo Sell’ mine. Blue line is the best fixed effect model.

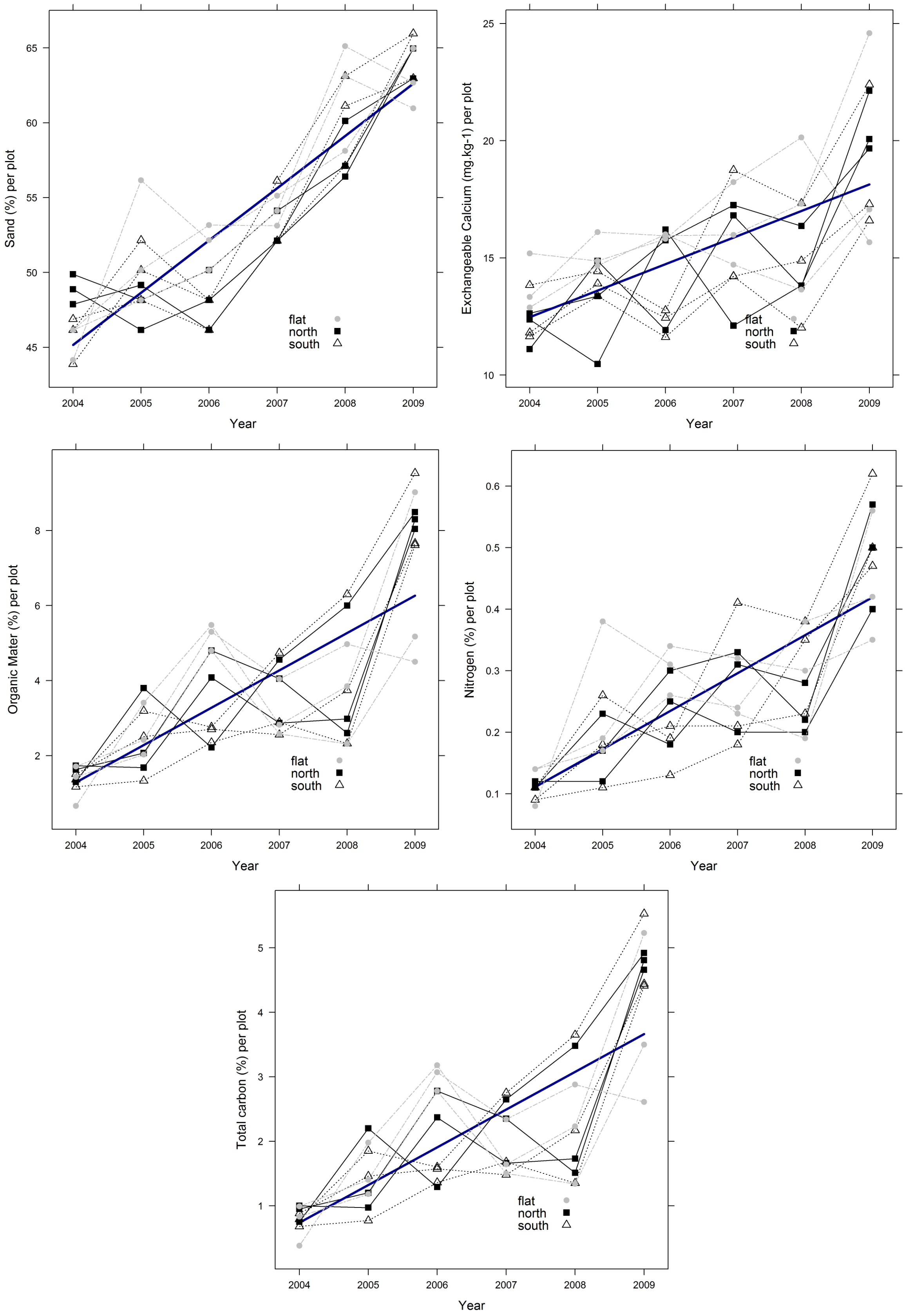

Figure 2. Increasing soil variables (sand content, Ca, organic matter, N, C) over time at 9 permanent plots distributed in three areas (flat, north- and south-facing slopes) at the restored ‘Pozo Sell’ mine. Blue line is the best fixed effect model.

Finally, Group 3 contained soil variables which showed not relationship with successional age, i.e. with stable values along the sequence (EC, P, Mg^{2+} and K^{+}, Table 2). Only, in the case of exchangeable potassium concentration significant differences were found between the three areas (Flat$_{[1,6]}$=142.16±12.9, t-value=11.03, $p<0.001$; North=42.26±13.84, t-value=3.05, $p<0.05$; South=47.18±13.84, t-value=3.41, $p<0.05$, ΔAIC=3.21%, $r^2=0.32$), suggesting lower concentrations of potassium in the flat area.

Table 2. Mean ± SE (n=3) of soil variables for each area (north-facing slope=N, south- facing slope =S and flat area=F) that showed no significant response to age.

Soil parameters	Area	2004	2005	2006	2007	2008	2009
EC (mmhos.cm^{-1})	F	0.12±0.01	0.22±0.04	0.38±0.03	0.13±0.01	0.14±0.01	0.36±0.01
	N	0.14±0.01	0.2±0.05	0.32±0.02	0.14±0.02	0.17±0.03	0.29±0.07
	S	0.12±0.01	0.24±0.04	0.34±0.03	0.15±0.01	0.19±0.04	0.19±0.04
P (mg.kg^{-1})	F	36±6.09	59.1±16.93	83.83±7.77	64.83±4.73	50.37±15.11	56.3±21.05
	N	51.7±1.52	46.86±10.42	67.7±17.51	92.33±22.46	35.4±5.16	68.47±4.93
	S	47.87±1.49	46.8±13.23	55.47±3.33	63.8±24.60	33.63±9.90	61.36±11.73
K (mg.kg^{-1})	F	113.72±9.53	182.3±1.59	166.53±17.72	128.78±13.36	134.6±11.70	128±26.95
	N	171.32±12.87	224.3±26.05	182.8±21.42	145.2±15.08	159.52±19.48	223.33±4.41
	S	154.3±5.99	167.5±31.21	255.75±14.80	169.54±19.50	190.94±29.21	198±12.50
Mg (mg.kg^{-1})	F	0.9±0.10	1.82±0.44	2.08±0.34	0.9±0.13	0.86±0.17	1.15±0.29
	N	1.53±0.01	1.97±0.31	2.12±0.33	1.25±0.12	1.18±0.06	1.84±0.17
	S	1.16±0.11	1.68±0.41	1.47±0.06	1.14±0.14	1.15±0.15	1.6±0.11

Floristic composition and soil variables

In total, 87 vascular plant species were recorded at the restored mine during the six-year period. All of the ten hydroseeded species were found, especially during the first 3 years, but their cover declined with age. The remaining 77 species included some early colonizers and ruderals (22 species; e.g. *Brassica nigra*, *Capsella bursa-pastoris*, *Lactuca* spp., *Malva sylvestris*) and native species (55 species; e.g. *Avenula sulcata*, *Helianthemun hirtum*, *Hieracium pilosella*, *Trifolium campestre*) which increased in cover with successional age.

Of the 11 soil parameters analyzed using the DA-FS only exchangeable potassium discriminated significantly between three areas (north-, south-facing, flat) (Wilk´s $\lambda=0.76$, $p<0.001$). Exchangeable potassium concentration was lower in the flat area than in the slopes, and there was no difference between the north- and south-facing areas. This analysis corroborates the results of the LMMs of the inexistence of differences among areas for the rest of soil parameters analyzed. The RDA ordination did not found any soil parameter with a significant important effect on plant community composition. The most influential soil parameters studied were sand content and pH, but they only represents the 2.9 and 3.4% respectively of the variation in plant species composition.

Vegetation structure and soil variables

The integrated analysis of soil parameters, vegetation structure variables and sampling dates using a PCA (Table 3, Fig. 3) explained 64.4% of the variations (axis 1=40%, axis 2=14.4%). Axis 1 was correlated positively with age, diversity (H', S), sand content, organic matter, ruderal richness and the cover of ruderal, native and annual species, and negatively with cover and richness of hydroseeded species, clay content and cover of perennial grasses. Axis 2 was correlated positively with pH and exchangeable cations (Ca^{2+}, K^{+}, Mg^{2+}) and negatively with total nitrogen. The PCA ordered the plots along the first axis according to their successional trajectories, increasing from left to right (Fig. 3b, c, d), whereas the second axis produced a separation within the plots with the same aspect (north, south and flat). Therefore, the first two axes seems to be responding to two relatively independent gradients: the main one associated with a successional gradient that showed a close relationship between vegetation structural changes and the changes in soil physical and organic related properties; and the second one related to soil bivalent cation (Ca^{2+}, K^{+}, Mg^{2+}) availability.

Table 3. Correlation coefficients of plot scores along axes 1 and 2 and the soil and vegetation variables used in the principal components analysis (PCA). In bold significant correlations at p<0.01 and r>0.50.

	Code	**Variable units**	**Axis 1**	**Axis 2**
Age	Age	Age since restoration (years)	**0.86**	0.10
Vegetation variables	Soil	Bare soil (%)	-0.08	-0.33
	CovH	Hydroseeded species cover (%)	**-0.89**	0.17
	RichH	Hydroseeded species richness	**-0.84**	0.09
	CovR	Ruderal species cover (%)	**0.70**	-0.10
	RichH	Ruderal species richness	**0.72**	0.13
	CovNa	Native species cover (%)	**0.75**	0.14
	Ga	Annual grasses cover (%)	0.43	0.06
	La	Annual legumes cover (%)	**0.62**	-0.24
	Fp	Perennial forbs cover (%)	**0.61**	-0.40
	Gp	Perennial grasses cover (%)	**-0.61**	0.21
	S	Richness	**0.93**	0.09
	H'	Shannon diversity	**0.86**	0.01
Soil variables	Sand	Sand (%)	**0.80**	0.12
	Clay	Clay (%)	**-0.61**	0.12
	pH	pH	-0.45	**0.50**
	EC	Electrical conductivity (mmhos.cm^{-1})	0.45	0.43
	OM	Organic matter (%)	**0.75**	**0.50**
	N	Total nitrogen (%)	0.40	**-0.60**
	P	Available phosphorus (mg.kg^{-1})	0.41	0.28
	K	Exchangeable potassium (mg.kg^{-1})	-0.11	**0.82**
	Ca	Exchangeable calcium (mg.kg^{-1})	0.37	**0.70**
	Mg	Exchangeable magnesium (mg.kg^{-1})	-0.06	**0.73**
		Eigenvalues	9.17	3.32
		Explained variance	40%	14.4%

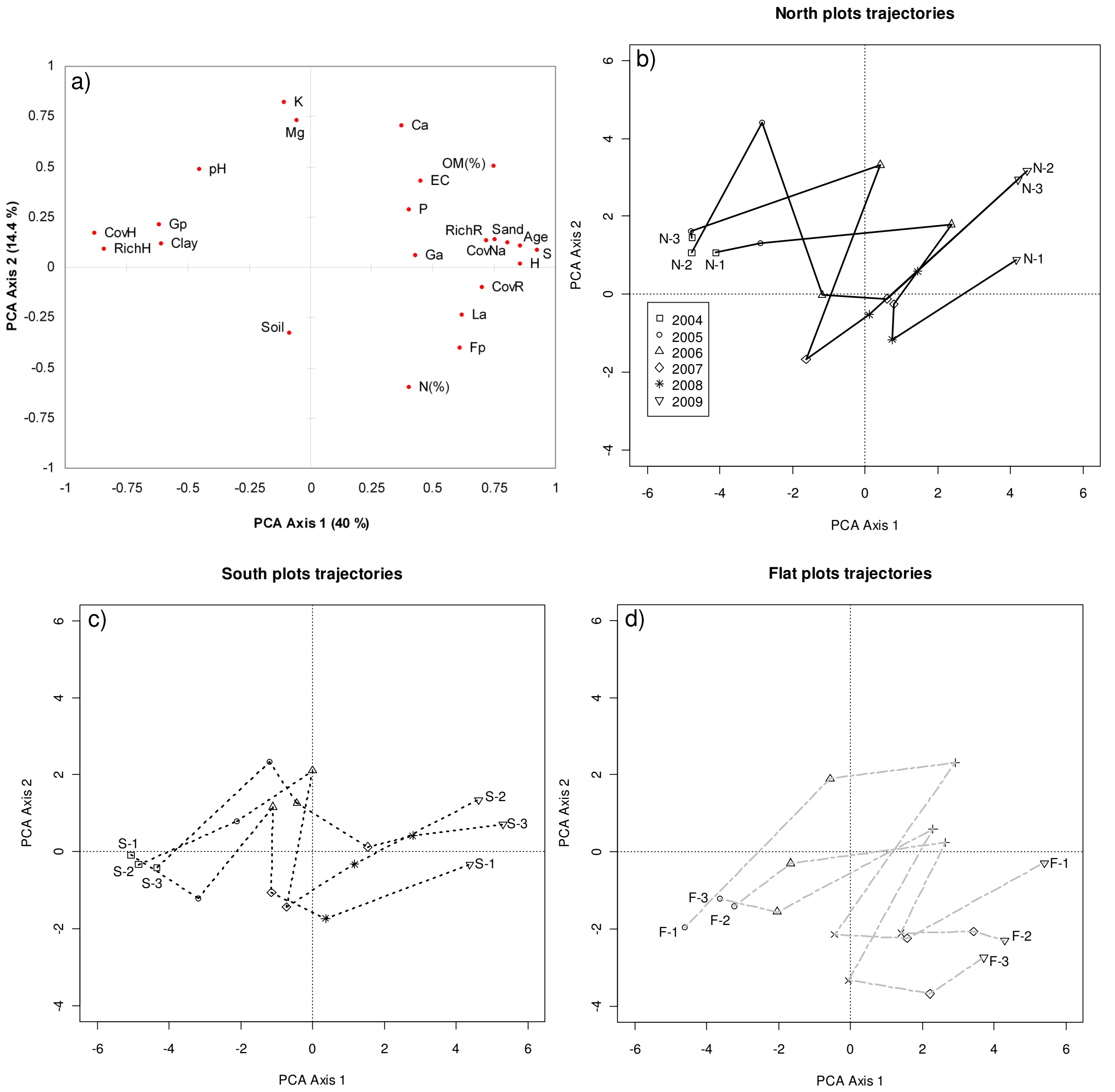

Figure 3. PCA ordination for the first two axes of soil and vegetation structure variables; data from 9 permanent plots monitored from 2004 to 2009 at the 'Pozo Sell' reclaimed open-pit coal mine at Palencia, northern Spain. (a) Variables ordination diagram; the distance of variables points from centre indicates the strength of the correlations between variables. (b), (c) and (d) Ordination of the plots (n=3 per area); points of the same plot are connected by successional vectors.

Discussion

Soil development through time

The influence of time on soil parameters have been shown in many successional studies (Jenny 1980; Marrs & Bradshaw 1993; Knops & Tilman 2000; Hodkinson et al. 2003). Our results for the first 6 years after the restoration of an open-cast coal mine under sub-humid Mediterranean climate showed that there was a general trend over time of increasing soil organic matter, total carbon and nitrogen, sand content and exchangeable calcium, but a reduction in soil pH, and clay and lime contents. More importantly, these changes in soil properties were independent of the three areas that differed aspects. As a result, age since restoration alone is, therefore, the agent driving change in these soil variables at least in short-term. This result differs from the vegetation succession where aspect has been shown to have an important influence on species colonization and subsequent change (Martínez-Ruiz et al. 2001; Martínez-Ruiz & Marrs 2007; González-Alday et al 2008; Alday et al. 2010a).

The increase in organic matter and total carbon and nitrogen with successional age is consistent with other studies on successional development on mineral wastes (Marrs et al. 1980a,b; Marrs & Bradshaw 1993; De Kovel et al. 2000; Banning et al. 2008; Frouz et al. 2008). However, here the accumulation of organic matter seems relatively rapid, increasing 1% year^{-1}, and reaching a mean value of 6% after 6 years. In other studies of post-agricultural landscape successions in Mediterranean climates organic matter increases was much slower, between 5-20% in 50 years (Cortez et al. 2007). This rapid accumulation of organic matter detected here may be caused by a combination of two factors: first, the manure layer applied before hydroseeding, but this would only account for the initial increase over the raw waste material; and second by rapid vegetation development with a high primary productivity, some of which is recycled into the soil organic matter (Marrs et al. 1980a; Frouz et al. 2008). On the other hand, the total carbon and nitrogen accumulation in the soil was produced at lower rates than organic matter (total carbon 0.6% yr^{-1} and total nitrogen 0.07% yr^{-1}), although being comparable with other post-mining landscapes (Šourková et al. 2005). The accumulation of carbon and nitrogen in the soil may be due to (1) the total nitrogen and carbon mineralization from soil organic matter (Dölle & Schmidt 2009), (2) the addition of nitrogen with the hydroseeding, and/or (3) because the rates of carbon and nitrogen accumulation in soil are related with the abundance of some plant types as grasses and legumes (García et al. 2007), which where the dominant plant types during the first 5 years in the studied mine (Alday et al. 2010a). Unfortunately the relative importance of each of these possible explanation sources remains unknown. In any case, the increase in these soil parameter is a very important result for soil dynamics restoration, since the accumulation of organic matter, total carbon and nitrogen are key factors in the activation of soil biological processes (Moreno-de las Heras 2009), providing food sources and habitat for soil fauna (Frouz et al. 2008). Particularly, it has been shown in various ecosystems that nitrogen is the most limiting resource during early succession (see De Kovel et al. 2000).

It is well known that clay and organic matter are the main soil bonding agents (Bronick & Lal 2005), and exchangeable calcium has a role in the stabilization of soil structure (Oades 1988). Our results showed that the increase in organic matter with restoration age was accompanied by an increase in sand content and exchangeable Ca^{+2} concentrations and a reduction in clay and lime contents. The decrease in clay content may have been brought about by small soil particles settling through the profile, and the lime may have been lost by leaching or through vegetation uptake (Oades 1988). As the lime was dissolved, the exchangeable Ca^{+2} concentrations would be expected to increase. As the clay and lime were removed, then the sand proportion would increase, which coupled with the increased organic matter would improve soil aeration and water-holding capacity in these clay-loam textured soils, further enhancing biological soil processes (Porta et al. 1994).

Soil reaction showed a decrease of 0.45 pH units during the 6 years; trends which appear common on mined sites. The variation is similar to a reduction of 1-1.5 pH units over 15 years on restored mines in the Czech Republic (Frouz et al. 2008), and larger if we compare with the slight reductions of 0.2 and 0.5 pH units over 36 and 100 years after abandonment in old-field successions in Germany and Pennsylvania respectively (Dölle & Schmidt 2009; Matlack 2009). The decrease in pH reported here might be caused by the greater increase of organic matter produced, which continuously extracted base cations from the soil minerals, releasing protons into the soil (van Bremen et al. 1984). In any case, the changes in soil reaction during succession depend on the initial pH and the buffering capacity of each soil (Dölle & Schmidt 2009); it seems that these new-formed soils have not much buffer capacity than previously developed soil.

An interesting result was that four soil variables (EC, available P, and exchangeable Mg^{2+} and K^{+}) did not show any successional trend, even though phosphorus and potassium were included as fertilizers within the hydroseeding slurry. It seems that the clay loam soil forming material used for restoration in this area maintained a sufficient amount of these elements for vegetation development.

Floristic composition and soil variables

Typically, successional studies of vegetation on mined lands have demonstrated that floristic compositional changes were conditioned by variation in edaphic factors (Prach et al. 2007; Moreno de las Heras et al. 2008). Similarly, Alday et al. (2010b) in a study of succession on restored coal wastes in the same geographical area as this research found that of the soil variables measured only pH was correlated with the floristic composition over the medium-term time-scale. In contrast, the results of this study showed that the compositional dynamics of vegetation were not conditioned by soil variables in short-term. The main cause may be that the soil-forming material was sufficiently good to prevent soil factors controlling species development, with the soil development proceeded at a similar rate on all areas (an exception of K^{+} in flat area). Therefore, these results provide support to the idea that compositional differences in short-term are mainly driven by a combination of stochastic (i.e. colonization chance (González-Alday et al. 2009) and climate (Alday et al. 2010a)) and abiotic factors (i.e. slope aspect (González-Alday et al. 2008), distance to surrounding species pool (González-Alday et al. 2009)), and in the first 3 years after hydroseeding by biotic competition between hydroseeded and native species (Alday et al. 2010c).

Vegetation structure and soil variables

In contrast to plant species composition, there was a clear relationship between some soil variables and vegetation structural/functional components during the short-term successional process. The soil variables could be viewed as two distinct groups based on the PCA results, and since these groups corresponded with different axes suggests that they vary independently. The first group can be understood in terms of accumulation of organic matter through time and increase of sand proportion. Only vegetation structural changes were correlated with this group, mainly because vegetation cover (plant biomass) helped in the accumulation of soil organic matter (Marrs et al. 1980a; Frouz et al. 2008). The accumulation of organic matter and increase in sand proportion led to increased soil aeration and water retention (Matlack 2009), these created better conditions for subsequent ruderal and native species establishment and development, increasing complexity of plant communities (increased species richness (S) and biological diversity index (H')). The second group was related to the concentration of exchangeable cations (Ca^{2+}, K^{+}, and Mg^{2+}) and their relation with soil pH. Although, calcium, potassium and magnesium are considered key factors for plant growth (Cañadas et al. 2010), our results showed that these cations were not related with structural changes during succession. Cations showed only a plot trend, possibly reflecting the soil cation differences at plot scale. In fact, soil cations have been reported as highly site-specific upon the initiation of succession (Cañadas et al. 2010).

Conclusions

The present work showed that age since restoration was the driving agent, at least in short-term, of soil changes on the new-formed soil for the restoration of a coal mine in the Palencia region, with its Mediterranean climate. Moreover, some soil changes as the accumulation of organic matter and sand content were correlated with vegetation structural/functional changes, mainly with vegetation cover (biomass production), which suggested that they may be involved in the mechanism that induce these soil changes, favoring the increase of plant community complexity in such mined areas. However, even whether some soil changes are produced, the levels of most stored parameters indicate that the studied soils are in the early stage of successional development (Šourková et al. 2005). In contrast, this study showed a lack of relationship between floristic compositional dynamics and soil parameters, which supported the idea that compositional differences in short-term are mainly driven by a combination of stochastic and abiotic factors. To determine the importance of these findings some supplementary experiments would be necessary, notably those dedicated to the determination of causes of soil element changes and their relationships with vegetation structural/functional components, as well as those dedicated to identify soil changes during long-term time scale.

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Chapter 7

Shrub colonization of a coal mine

Vegetation succession on restored coal wastes in Spain: the influence of soil and environmental factors

Josu G. Alday, Rob H. Marrs & Carolina Martínez-Ruiz

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Abstract

Question: How is vegetation succession on coal mine wastes under a Mediterranean climate affected by the restoration method used (topsoil addition or not)? How are plant successional processes influenced by local landscape and soil factors?

Location: Reclaimed coal mines in the north of Palencia province, northern Spain (lat 42º47'-42º50'N, long 4º32'-4º53'W).

Methods: In June-July of 2008, vascular plant species cover was monitored in 31 coal mines. The mines which had been restored using two restoration methods (topsoil addition or not) comprised a chronosequence of different ages from 1-40 years since restoration started. Soil and environmental factors at each mine were monitored and related to species cover using a combination of ordination methods and Huisman-Olff-Fresco modeling.

Results: Plant succession was affected by the restoration method used. Where topsoil was added, succession was influenced by age since restoration and soil pH. Where no topsoil was added, soil factors seem to arrest succession. The vegetation composition on the topsoiled sites showed a gradient with age, from the youngest mines with early colonizing species to older mines with an increase in woody species. The vegetation on the non-topsoiled sites was composed mainly of early-successional species. The response to age and pH of 37 species found on topsoiled mines were described.

Conclusions: Restoration of coal mines under this Mediterranean climate can be relatively fast if topsoil is added, with a native shrub community developing after 15 years. However, if topsoil is not used, it will take more than 40 years. For the topsoiled mines, the species found in the different successional stages were identified, and their tolerance to soil pH was derived. This information will assist future restoration projects in the area.

Key words: Restoration method; HOF modeling; topsoiling; age; pH; species responses; constrained ordination.

Abbreviations: AIC = Akaike Information Criterion; CCA = Canonical Correspondence Analysis; DCA = Detrended Correspondence Analysis; GAM = Generalized Additive Models; HOF = Huisman-Olff-Fresco models; MRPP= Multiresponse Permutation Procedure.

Nomenclature: Tutin et al. (1964-1980)

Introduction

Succession provides a temporal framework in which to understand ecological processes, such as species assembly (Walker & del Moral 2008), vegetation dynamics (Marrs & Bradshaw 1993) and ecosystem development (Odum 1969). At the same time, studies of vegetation succession can supply insights into solving practical problems in ecological restoration (del Moral & Walker 2007), e.g. the relative importance of biotic and abiotic conditions (Walker & del Moral 2008). Indeed, it has been suggested that ecological restoration should be considered as the practical implementation of management actions using successional concepts and processes to restore self-sustaining ecosystems on degraded lands (Walker et al. 2006; Hobbs et al. 2007). Within this general context, it is crucial to base restoration decisions and programs on a sound understanding of successional mechanisms and ecological processes (Prach et al. 2007a; Tischew & Kirmer 2007). It is, therefore, necessary to identify those abiotic and biotic factors that limit succession when it occurs (Walker et al. 2006; Walker & del Moral 2009).

Open-cast mining is one of the most severe disturbances produced by human activities; it generates highly localized impacts and anthropogenic habitats on sites where there is statutory or planning requirements to ensure vegetation restoration (Moreno-de las Heras et al. 2008). In northern Spain (León and Palencia) open-cast coal mining affects around 5,000 ha of land surface. The restoration approach to the land degraded during mining operations has evolved over the last 40 years. At the start of this period no management intervention was carried out, and vegetation had to develop through natural colonization. More recently, active restoration management has been implemented, initially through reshaping the landscape and improving the baseline soil-forming materials, and thereafter through the use of a more technical restoration with seed introduction. Usually herbaceous species have been seeded or hydroseeded (González-Alday et al. 2008) to surmount the lack of seeds in the soil bank (González-Alday et al. 2009). However these approaches have not always been successful in creating self-sustaining ecosystems. Therefore, a better understanding of mechanisms that govern successional processes after open-pit coal mining in this area is needed urgently to inform management decisions. These decisions should be based on both scientific knowledge (Prach et al. 2007a) and economic considerations (Bradshaw 2002). An initial challenge is to identify the soil and environmental factors that impede or restrict ecosystem succession (del Moral et al. 2009).

The aim of this study was, therefore, to characterize the early vegetation succession in restored coal mines in northern Spain in the forty years after abandonment, and relate this to potential successional constraints. Specifically, we tried to answer the following questions: (1) How is vegetation succession affected by the restoration method used (topsoil addition or not)? (2) How are successional processes influenced by local landscape and soil factors, and if so which are the most important? (3) Do individual species responses reflect these changes during succession and do they respond to local and landscape factors?

Methods

Site selection

Initially, historical information derived from mining company records (UMINSA, Unión Minera del Norte S.A.) was collated for all coal mines in the north of Palencia province, northern Spain (lat 42º47’-42º50’N, long 4º32’-4º53’W); data were abstracted on the type of restoration methods used and the time of implementation. Every coal mine was visited and only mines with at least two sides in contact with seral stages of *Quercus pyrenaica* woodland were selected; i.e. mines mainly surrounded by arable land were excluded. As a result, 31 open-cast coal mines were selected for detailed study. These sites ranged in size from 0.5 to 3 ha, and provided a chronosequence of elapsed time since abandonment of 1-40 years, which was further classified into three successional stages: young (1-10 years, n=10), middle (11-20 years, n=11), and late (>21years, n=10). Three different restoration methods were used at these sites: (1) topsoil addition followed by hydroseeding (Top-H; n=16; 1-25 years), (2) topsoil addition followed by natural regeneration (Top; n=10; 14-32 years); and (3) no topsoil addition and natural regeneration (No-Top; n=5; 15-40 years). The sites showed a sequence of treatments; generally the hydroseeded sites were the more recent, the topsoiled sites were intermediate and sites with natural regeneration were the oldest. The hydroseeding seed mixture included grasses and herbaceous legumes (81:19 by weight; 200 kg ha^{-1}), such as *Lolium perenne*, *Lotus corniculatus*, *Medicago sativa*, *Phleum pratense*, *Poa pratensis*, *Trifolium pratense* and *T. repens*.

Site description

The 31 coal mines selected for study were located relatively close together (within 30 km^2) to minimise geographic, altitudinal (1165-1419 m asl) and climatic variability. The climate is sub-humid Mediterranean, with an annual mean temperature of 9 ºC and average annual precipitation of 980 mm. Rainfall is distributed irregularly throughout the year; most rain falls during spring and autumn with a dry season in summer. The natural vegetation surrounding the studied mines is composed of *Q. pyrenaica* woodlands with vestiges of *Q. petraea*, remnants of natural shrubland (*Crataegus monogyna*, *Cytisus scoparius*, *Genista florida*, *Rosa canina*), and grasslands containing *Agrostis castellana*, *Arenaria erinacea*, *Arenaria montana*, *Bromus mollis* and *Vulpia myuros*.

Sampling

Sampling was carried out in June-July of 2008. At each mine 10 1×1 m quadrats were located randomly (Krebs 1999), and within each one the cover (%) of every vascular plant species was estimated visually by the same observer. The mean cover values of each species in ten quadrats per mine were used to obtain a uniform sample of the vascular plant cover of the mine. In addition, a range of landscape variables were characterized for each mine (Table 1).

At each mine, a soil sample was taken from each 1×1 m quadrat using a soil auger (diameter = 8 cm, depth = 10 cm). The ten sub-samples per mine were homogenised to obtain a uniform sample of the soil characteristics of the mine. Soil samples were air-dried, sieved (≤ 2 mm) and analysed for texture using the Bouyoucos-method (Day 1965). Soil chemical properties were measured as follows: organic matter using the k-dichromate oxidation method (Walkley 1947); soil pH and electrical conductivity using a conductivity meter in a 1:2.5 soil:deionized water slurry

(Allen 1989); total nitrogen concentration using the Kjeldahl-method (Bremner & Mulvaney 1982); available P concentration using the Olsen method (Olsen & Sommers 1982); exchangeable K by atomic absorption spectroscopy after extraction with 1N ammonium acetate (pH 7) (Allen 1989). The C/N ratio, cation exchange capacity (CeC), and exchangeable concentrations of calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), and sodium (Na) were determined using methods outlined by MAPA (1994).

Data analysis

All statistical analyses were implemented in the R software environment (version 2.10.1; R Development Core Team 2009), using the VEGAN (Oksanen et al. 2008) and GRAVY (Oksanen 2003) packages for multivariate analyses and HOF models, respectively.

Initial data exploration and the effect of restoration methods

Multivariate exploratory analysis was carried out to describe the plant community composition with a Detrended Correspondence Analysis (DCA) (λ_1=0.41, λ_2=0.26, λ_3=0.18, λ_4=0.11; GL_1= 3.92, GL_2=2.12, GL_3=2.15, GL_4=1.82). The plant species cover dataset was reduced by removing all species which only occurred in one sample, and a log-transformation (log(x+1)) was then applied. One no-topsoiled mine had a strong influence on the ordination, therefore, DCA was repeated excluding the no-topsoiled mine outlier (λ_1=0.35, λ_2=0.23, λ_3=0.15, λ_4=0.11; GL_1= 3.60, GL_2=2.04, GL_3=1.95, GL_4=1.97).

Thereafter, to test if the three restoration methods (topsoil, topsoil-hydroseeding and no-topsoil addition) shared the same primary gradients (hypothesis 1) two analytical approaches were used. First, different restoration methods were fitted onto DCA ordination using the “envfit” function with 1000 permutations (Oksanen et al. 2008). Their class centroids and bivariate standard error ellipses were then used to illustrate each restoration method on the biplots (Oksanen et al. 2008). Second, a multi-response permutation procedure (MRPP) was used over the first two DCA axes (function “mrpp” in VEGAN, using Euclidean distances and 1000 permutations) to examine the differences in floristic composition between topsoil, topsoil-hydroseeding and no-toposoil restoration methods (Oksanen et al., 2008). Bonferroni correction was used to adjust for the significance level of each contrast; here the critical probability level for detecting significance between contrasts was α=0.017.

Finally, a new DCA was produced using only topsoiled mines (n=26; λ_1=0.38, λ_2=0.23, λ_3=0.11, λ_4=0.09; GL_1=3.66, GL_2=2.15, GL_3=1.61, GL_4=1.18) to identify if the sequence of successional ages (young, middle and late) was established enabling the joint comparison of topsoiled mines. To aid interpretation the mines age and successional groups (young, middle and late) were fitted onto the DCA ordination plot using the “envfit” function with 1000 permutations. The compositional differences at middle successional age, where Top-H and Top mines mixed together, were also evaluated using MRPP as described above.

After inspection of the results from these analyses (Hypothesis 1; Figs 1, 2) and of the differences in physico-chemical soil variables between the topsoiled and non-topsoiled mines analysed using unbalanced ANOVAs (Table 1), the data were split into two groups: Group 1, the 26 topsoiled mines; and Group 2, the 4 non-topsoiled ones (eliminating the non-topsoiled outlier, a 15 years old mine). All subsequent analyses were applied to Group 1 only.

The low number of non-topsoiled mines considered (n=4) limits the use multivariate constrained ordinations. Therefore, we included only a brief description of the non-topsoiled mines community for tentative comparison.

Assessing effects of local soil factors and landscape factors on the succession

The effects of local landscape and soil factors on topsoiled mines succession were then evaluated using Canonical Correspondence Analysis (CCA). The most significant uncorrelated environmental variables from those available in Table 1 were selected using the forward selection approach with the AIC statistic as the selection criterion (Oksanen et al. 2008). Significance of the contrasts was assessed using permutation tests with the reduced model and 199 permutations. The large number of possible environmental variables (n=26) compared to the number of topsoiled mines (n=26) made selection difficult (Oksanen et al. 2008). Therefore selection was done within three subsets {age}, {landscape variables} and {soil variables}. The final model was fitted using only the variables that reduced the AIC within each set. In order to check if the response of constrained variables response was really linear and vectors were appropriate to draw over the CCA biplots, surface models of each constraining variables (age and pH) were fitted over the CCA ordination results using GAM models and the “ordisurf” function (Oksanen et al. 2008).

Species responses to age and pH

The cover of most important species were examined through time and over the pH gradient for the topsoiled mines (n=26) using Huisman–Olff–Fresco (HOF) models (Huisman et al. 1993). HOF models allow testing of alternative responses to ecological gradients using a hierarchical set of five response models, ranked by their increasing complexity (Model I, no species trend; Model II, increasing or decreasing trend to the maximum; Model III, increasing or decreasing trend below maximum attainable response; Model IV, symmetrical response curve; Model V, skewed response curve). HOF response models were fitted for species present in more than 11 mines (42% of topsoiled mines). The species cover data were transformed (arcsin(sqrt(x/100)) and a Gaussian error structure was used (Huisman et al. 1993). The AIC statistic was used to select the most appropriate model for each species.

Results

Community description and environmental variables

Two hundred and twenty three vascular plant species were recorded from the 31 coal mines. Of these 18 woody species and characteristics of the surrounding vegetation including *Crataegus monogyna*, *Cytisus scoparius*, *Genista florida*, *Quercus pyrenaica* and *Rosa canina* were found especially in mid- and late successional stages. The young successional stage was dominated by herbaceous species such as *Bromus mollis*, *Medicago polymorpha*, *Sonchus oleraceus*, *Trifolium campestre*, *Vulpia myuros*, and there was also a high cover of some species included in the hydroseeding mixture (e.g. *Lolium perenne*, *Trifolium repens*). At the same time, 61 species, including *Chamaespartium tridentatum*, *Lithodora diffusa* and *Silene conica* were found only once, and the 41% of this species group occurred only in the mid-successional stage.

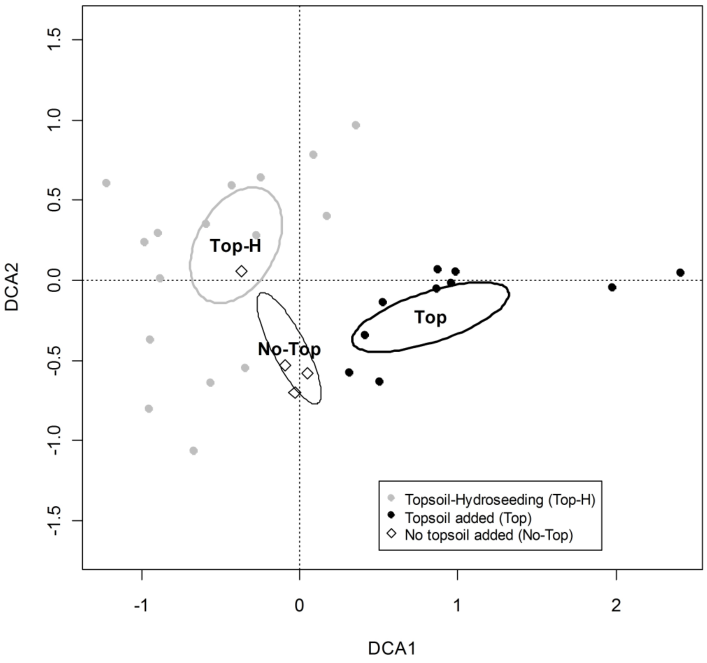

Figure 1. DCA ordination of floristic composition data from 30 coal mines in Palencia province, northern Spain. The positions of the three different restoration methods are illustrated as class centroids with overlain SE-ellipses for axis 1 and 2.

The influence of restoration methods (Hypothesis 1)

The DCA of mine sites is shown (Fig. 1). The three restoration methods were overlain on this biplot and they occupied different regions of the ordination plot. The hydroseeded topsoiled mines (Top-H, most of them from the young successional stage) appeared in the left hand area whereas the topsoiled mines (Top, most of them from middle- and late- successional stages) were in the right area of the plot. Surprisingly, the non-topsoiled mines (No-Top, most of them from the older sites) appeared in the central lower part of the plot, being more close to topsoiled young and middle sites than to older mines. Non-topsoiled mines showed significant compositional differences against Top-H (MRPP analysis, A=0.132, *p*=0.011) and Top (A=0.243, *p*=0.002). The composition of non-topsoiled mines was characterized by early-successional species (*Lactuca* spp., *Chondrilla* spp., *Cirsium arvense*, *Dianthus* spp., *Senecio jacobaea*, *Sonchus oleraceus*, *Vicia hirsuta*), and species adapted to dry conditions such as (*Sedum sediforme*, *Filago pyramidata*, *Minuartia mediterranea*). Simultaneously, there were significant differences on soil properties between non-topsoiled and topsoiled mines; physical properties (sand and clay composition) and chemical properties (Organic matter, C/N ratio, CeC, Mn, Fe, Na) (Table 1).

The second DCA for hydroseeded topsoiled (Top-H) and topsoiled mines (Top) grouped the mines along the first axis according to their successional age (Fig. 2). A close relationship was revealed between the first axis and the mines age (r^2=0.76, p<0.001). Young mines were plotted in the negative end of axis 1, middle mines from Top-H and Top appeared mixed in the central part showing no significant compositional differences (MRPP analysis, A=0.094, p=0.103), whereas the late successional mines were at the positive end.

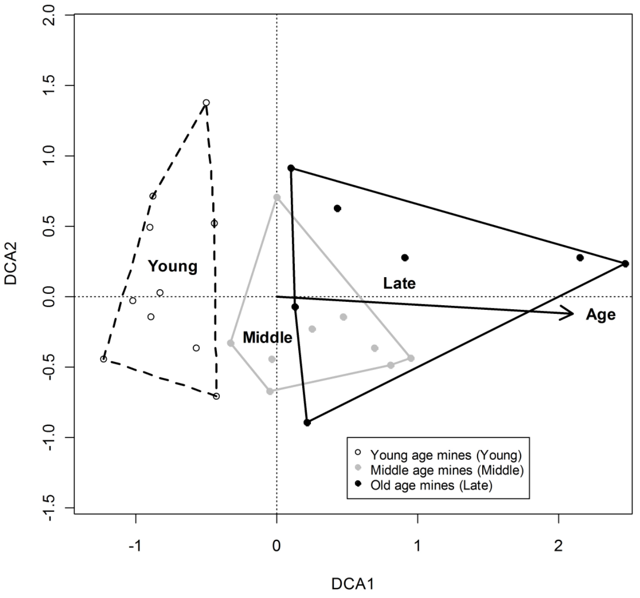

Figure 2. DCA ordination of floristic composition data from 26 topsoiled (Top-H and Top) coal mines in Palencia province, northern Spain. The three different successional stages are illustrated, and age since restoration fitted as passive variable over ordination.

Taken together these two analyses suggest two distinct groups present; the topsoiled mines (with and without hydroseeding) and the non-topsoiled mines.

Table 1. Environmental variables measured for each of 31 coal mines selected in Palencia province, northern Spain. Three sets of environmental variables were measured: (1) age since restoration was implemented; (2) landscape variables, measures of the processes that could affect restoration success from the surrounding landscape; and (3) local, site-specific variables of soil physico-chemical status. The asterisks in soil parameters indicate significant differences ($p<0.05$) between Top (topsoiled mines; n=26) vs. No-Top (non-topsoiled mines; n=5) restoration methods.

	Variable	**Units**	Median or mean (±S.E)	
			Top	**No-Top**
Age	Age since restoration was implemented	Years	15 (±2)	25 (±5)
Landscape environmental variables	Grazing	(y/n)	n	n
	Grazing Intensity	Number of faecal pellets in a randomly selected 100 m^2 plot	7(±2)	2 (±2)
	Grazing Animal	Faecal pellets of the most important species. Factor 3 levels: N = none; C = cow; S = sheep	N	N
	Runoff	Counting the number of rills and cover and then transformed in a ordered factor (0-4): 0= no runoff; 4> 75% of soil eroded	1	2
	Runoff type	Factor 3 levels: N = no; R = reger; C = landslide	R	C
	Border type	Factor 4 levels: N = nothing; W = woodland; G = grassland; S = shrubland	W	W
	Slope	º	18 (±1.9)	20.60 (±5.94)
	Altitude	Meters above sea level	1250 (±14.5)	1225 (±23.40)
	Orientation	Factor 5 levels: N, S, E, W, Flat	N	E
Local soil variables	Sand	%	63 (±1.18)	72 (±2.40)*
	Silt	%	16 (±0.5)	14 (±1.60)
	Clay	%	21 (±0.96)	14 (±1.80)*
	pH		6.60 (±0.24)	7.34 (±0.33)
	Electrical conductivity	mmhos.cm^{-1}	0.18 (±0.02)	0.11 (±0.02)
	Organic mater	%	5.54 (±0.54)	3.86 (±1.13)*
	Total nitrogen	%	0.32 (±0.03)	0.31 (±0.08)
	Available phosphorus	mg.kg^{-1}	11.01 (±2.05)	7.46 (±2.70)
	Exchangeable potassium	mg.kg^{-1}	175.36 (±13.73)	177 (±44.43)
	Exchangeable iron	mg.kg^{-1}	69.45 (±10.03)	23.60 (±5.04)*
	C/N ratio		12.90 (±0.82)	16.32 (±1.80)*
	Cation exchange capacity	meq.100g^{-1}	16.90 (±1.10)	12.84 (±1.54)*
	Exchangeable calcium	meq.100g^{-1}	11.18 (±1.11)	10.13 (±1.63)
	Exchangeable magnesium	meq.100g^{-1}	1.50 (±0.12)	1.57 (±0.52)
	Exchangeable manganese	meq.100g^{-1}	47.05 (±6.90)	16.54 (±5.04)*
	Exchangeable sodium	meq.100g^{-1}	0.02 (±0.01)	0.08 (±0.02)*

Assessing effects of environmental factors (landscape versus local soil) on succession (Hypothesis 2)

For the topsoiled mines, age and pH were the only significant explanatory variables included in the model; local landscape factors had no significant effect. These variables reduced the AIC of the null model from 128.2 to 127, and were significant (p=0.005). Age was the more influential factor on the course of vegetation succession (10% of the constrained inertia) followed by pH (7% of the constrained inertia). The CCA biplots (Fig. 3a,b) showed that age was correlated with the first axis, reflecting a gradient from the youngest mines with hydroseeded species (*Lolium perenne*, *Medicago sativa*) and early colonizing species (*Erodium cicutarium*, *Hordeum murinum*, *Medicago polymorpha*, *Papaver rhoeas*) on the right hand of the diagram, to the oldest mines with typical woody species (*Genista florida*, *Quercus pyrenaica*, *Rosa canina*) and late successional ones (*Viola riviniana*) on the left.

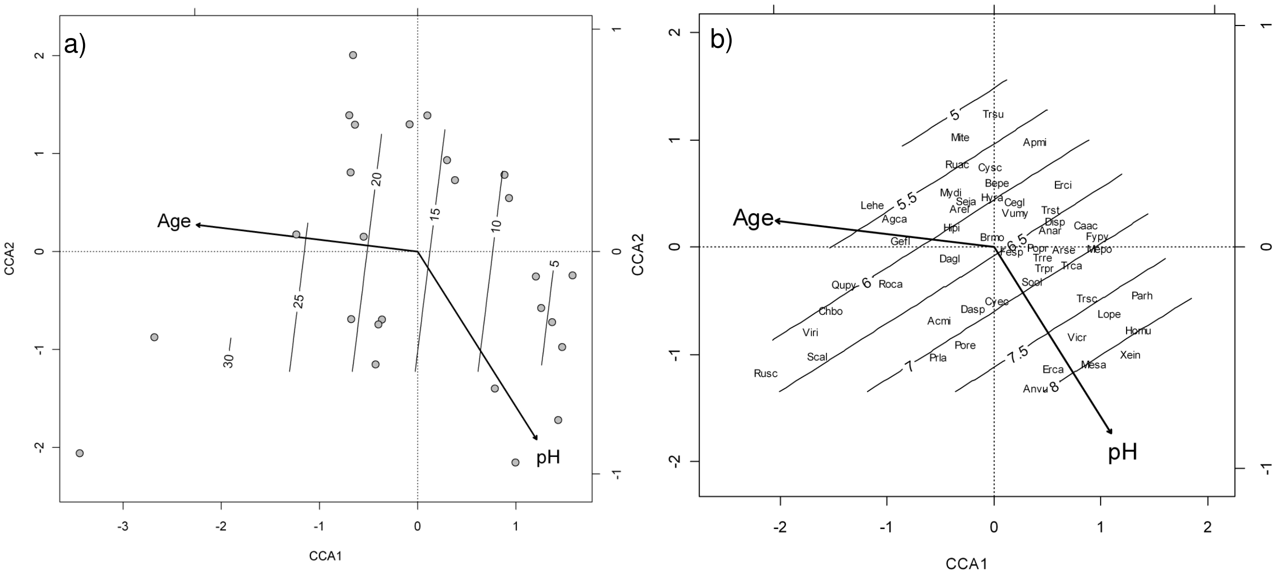

Figure 3. Constrained CCA ordination biplots of 26 coal mines in Palencia province, northern Spain, where topsoiling restoration method was used. The ordination was constrained on age since restoration and soil pH and their fitted surface responses are overlain. (a) Sites biplot with age since restoration fitted as a response surface; (b) Species biplot with soil pH fitted as a response surface. Species codes: Acmi = *Achillea millefolium*; Agca = *Agrostis castellana*; Anar = *Anthemis arvensis*; Anvu = *Anthyllis vulneraria*; Arel = *Arrhenatherum elatius*; Arse = *Arenaria serpyllifolia*; Apmi = *Aphanes microcarpa*; Bepe = *Bellis perennis*; Brmo = *Bromus mollis*; Caac = *Carduus acanthoides*; Cegl = *Cerastium glomeratum*; Chbo = *Chenopodium bonus-henricus*; Cyec = *Cynosurus echinatus*; Cysc = *Cytisus scoparius*; Dagl = *Dactylis glomerata*; Dasp = *Daucus* spp.; Disp = *Dianthus* spp.; Erca = *Eryngium campestre*; Erci = *Erodium cicutarium*; Fesp = *Festuca* spp.; Fypy = *Filago pyramidata*; Gefl = *Genista florida*; Hipi = *Hieracium pilosella*; Homu = *Hordeum murinum*; Hyra = *Hypochoeris radicata*; Lehe = *Lepidium heterophyllum*; Lope = *Lolium perenne*; Mepo = *Medicago polymorpha*; Mesa = *Medicago sativa*; Mite = *Micropyrum tenellum*; Mydi = *Myosotis discolor*; Parh = *Papaver rhoeas*; Popr = *Poa pratensis*; Pore = *Potentilla reptans*; Prla = *Prunella laciniata*; Qupy = *Quercus pyrenaica*; Roca = *Rosa canina*; Ruac = *Rumex acetosella*; Rusc = *Rumex scutatus*; Scal = *Scrophularia alpestris*; Seja = *Senecio jacobaea*; Sool = *Sonchus oleraceus*; Trca = *Trifolium campestre*; Trpr = *T. pratense*; Trre = *T. repens*; Trsc = *T. scabrum*; Trst = *T. striatum*; Trsu = *T. subterraneum*; Vicr = *Vicea cracca*; Viri = *Viola riviniana*; Vumy = *Vulpia myuros*; Xein = *Xeranthemum inapertum*.

The second axis was correlated negatively with pH; the mines with greater pH values and associated species (*Anthyllis vulneraria*, *Eryngium campestre*, *Vicia cracca*, *Xeranthemum inapertum*, all with Ellenberg R-values >7; Ellenberg et al. 1991) appeared at lower scores, whereas the mines with lower pH values and associated species (*Aphanes microcarpa*, *Lepidium heterophyllum*, *Micropyrum tenellum*, *Myosotis discolor*, *Trifolium subterraneum*, all with Ellenberg R-values <5; Ellenberg et al. 1991) at the positive end.

Species responses to age and pH in topsoiled sites

HOF-response models were calculated for the 37 most common species for the two significant explanatory variables (age and pH) in the topsoiled sites. Plots are presented for those species which produced HOF model types III-V (Figs 4,5). Species with the null model (Type I) are not presented, and no species exhibiting the Type II model were found.

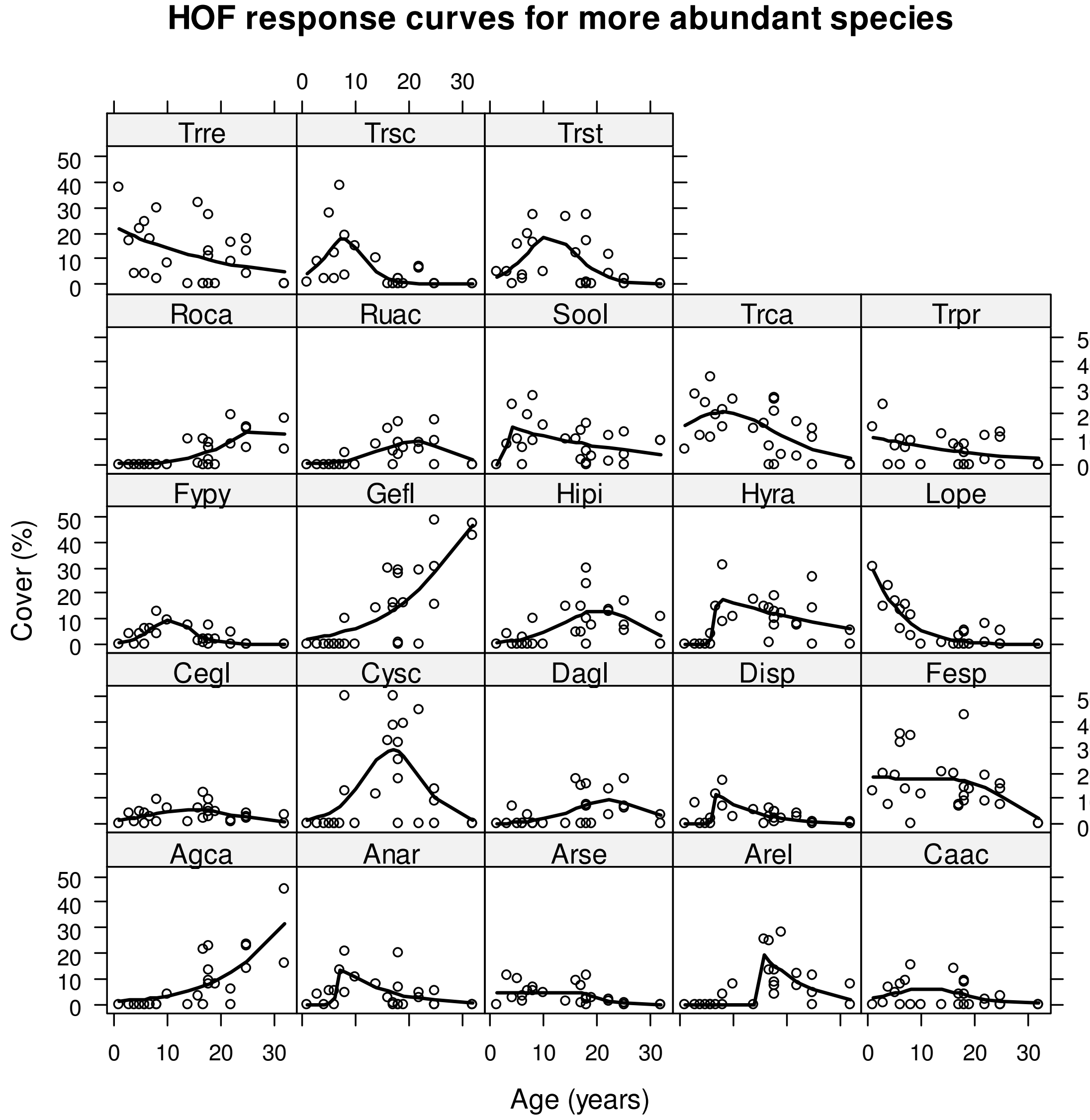

Figure 4. Significant response curves of the most abundant species with respect to age since restoration started at 26 topsoiled coal mines in Palencia province, northern Spain. For species identification see Fig. 3.

Four response groups were identified with respect to species response to age since restoration. Group 1 contained 14 species which showed no trend (HOF model I), which maintained stable cover values lower than 10%. The most important species in this group were *Bromus mollis* (10% of cover), *Vulpia myuros* (8.3% of cover), *Poa pratensis* (8% of cover), *Lotus corniculatus* (7% of cover) and *Plantago lanceolata* (6% of cover). Group 2 included two woody species (*Genista florida*, *Rosa canina*) and a grass (*Agrostis castellana*) increasing their cover through age according to HOF model III. Group 3 was the most abundant with 15 species; these species exhibited symmetrical or skewed response curves (HOF models IV, V) showing a gradual turnover of species from *Sonchus oleraceus*, *Trifolium campestre*, *T. scabrum*, *T. striatum*, which peaked in the young stages (<10 years), through *Arrhenatherum elatius*, *Cerastium glomeratum* and *Cytisus scoparius* which peaked between 10-20 years, to *Dactylis glomerata*, *Hieracium pilosella* and *Rumex acetosella* which had their optima between 20-30 years.

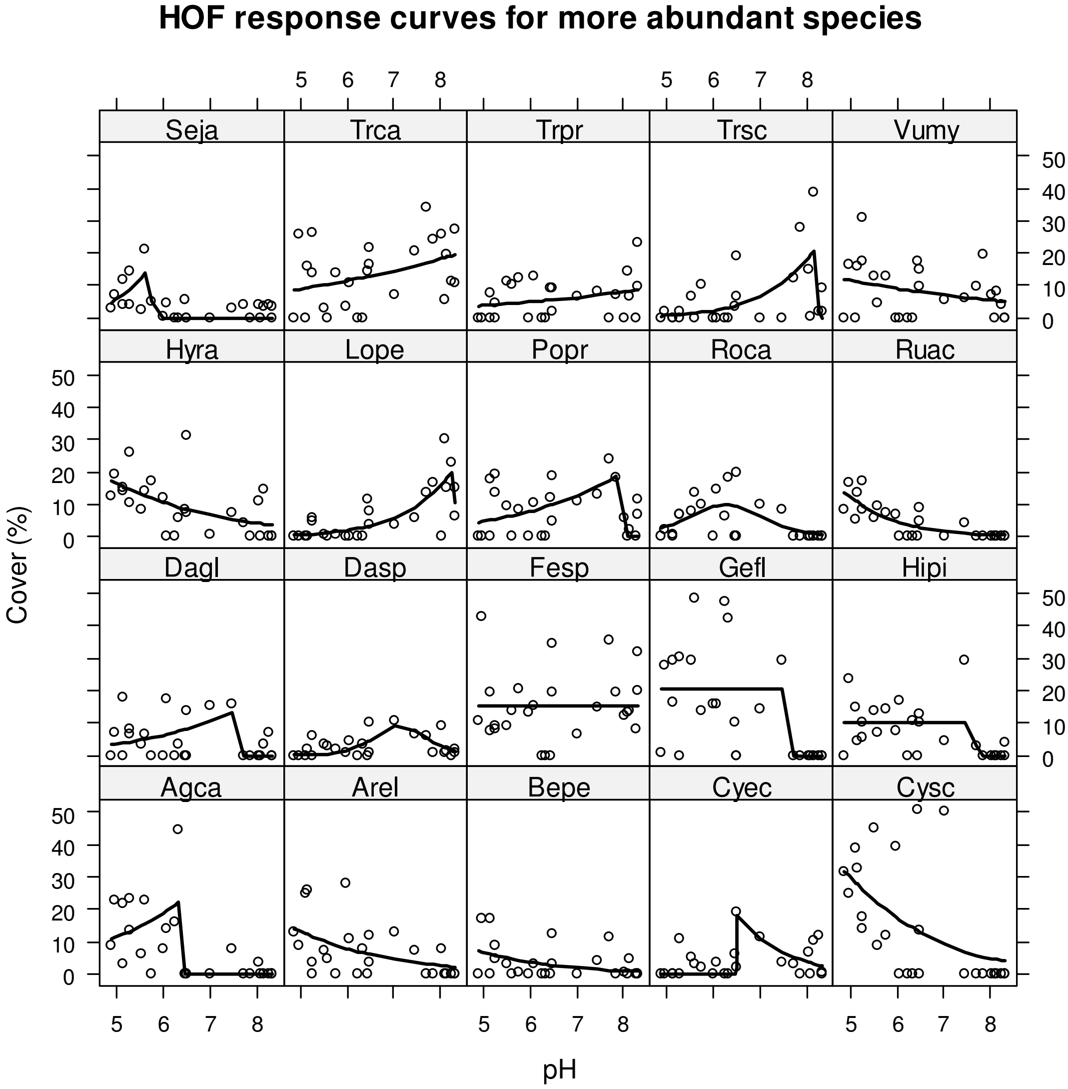

Figure 5. Significant response curves of the most abundant species with respect to soil pH at 26 topsoiled coal mines in Palencia province, northern Spain. For species identification see Fig. 3.

Finally, Group 4 showed a decreasing trend (HOF model III) with age, and this group included 5 herbaceous species that had greatest cover values in the early stages and a subsequent reduction with age. This group included three species (*Lolium perenne*, *T. pratense*, *T. repens*) typically included in the hydroseeding mixture, as well as *Arenaria serpyllifolia* and *Festuca* spp..

The species responses to pH gradient could also be separated in the same way into 4 groups. Group 1, contained 18 species showing no response to pH (HOF model I), including *Bromus mollis*, *Festuca* spp., *T. repens* and *Veronica arvensis*. Group 2 contained only two species (*T. campestre*, *T. pratense*) that showed an increasing trend with pH (HOF model III). Group 3 contained 12 species with an unimodal/skewed response; 11 species showed a skewed response (HOF model V) including *Dactylis glomerata*, *Poa pratensis*, *Senecio jacobaea*, but only *Rosa canina* showed unimodal one (HOF model IV); the optima for this group ranged from 5.5 to 8. Finally, Group 4 included six species that showed a decreasing trend with increasing pH (HOF model III), this group included *Arrhenatherum elatius*, *Hypochoeris radicata* and *Vulpia myuros*.

Discussion

The results presented here clearly show that the vegetation succession was affected by the restoration method applied (i.e. whether topsoil was added or not). Simultaneously, the environmental factors that influence successional processes differed between the restoration methods; the vegetation on topsoiled mines showed a trend with age and pH, whereas on non-topsoiled mines substrate-based constraints seemed to limit succession.

The influence of restoration method (Hypothesis 1)

An important result was that restoration method influenced vegetation succession. Particularly, the topsoiled mines (hydroseeded or not) shared the same primary gradients and the same direction of change in relation to age, whereas the older non-topsoiled mines were located among young- and mid-successional stages. This finding was expected given the importance of stable and fertile soil-cover to restore vegetation on mine wastes (Tordoff et al. 2000, Martínez-Ruiz & Marrs 2007). The vegetation on topsoiled mines included some native species, e.g. *Bromus mollis*, *Trifolium campestre*, *Vulpia myuros*, and showed an increase in native woody species with age, e.g. *Genista florida*, *Quercus pyrenaica*. These results are in agreement with previous studies that suggest topsoil spreading, especially under the extreme environmental conditions found on these mine sites, can enhance plant establishment by improving soil properties (Holl 2002), and therefore, increase the likelihood of re-establishment of native species of plants and micro-organisms (Martínez-Ruiz & Marrs 2007).

It is well known that coal mine wastes present hostile soil conditions for plant establishment in terms of structure, physico-chemical characteristics and instability (Felinks & Wiegand 2008). These aspects limit vegetation recovery by reducing the probability of the natural establishment of non-adapted native species (Prach et al. 2007b), thus slowing succession. The non-topsoiled mines conformed to this pattern; given that the constraints on succession on these mine wastes seem to be substrate-based, even though our analyses were based on few sites. Sand content and Na concentration have an impact on water holding capacity; a high sand content (here > 70%) will provide a low water-holding capacity and a high Na can increase clay dispersion, which will reduce water-holding capacity further (Southard 1999). The low water holding capacity will limit vegetation establishment and its effect will be accentuated by the regular and prolonged surface droughts in these Mediterranean areas (Blondel & Aronson 1999; Tordoff et al. 2000). At the same time, the lower organic matter, and Fe and Mn concentrations relative to the topsoiled mines are also factors that are likely to limit vegetation establishment and development (Bochet & García-Fayos 2004). Therefore, the vegetation of theses mines was characterized by early-successional species (e.g. *Cerastium* spp., *Chondrilla* spp., *Lactuca* spp.), those adapted to drought (e.g. *Filago pyramidata*, *Sedum sediforme*) and with a few woody species establishing after 20 years (e.g. *Cytisus scoparius*, *Thymus mastichina*).

The unbalanced design, caused by low number of non-topsoiled mines (n=4, after the elimination of one outlier) in comparison with topsoiled mines (n=26), reduced the strength of the evidence found. Therefore, further investigations are needed to quantify the relative importance of these findings. However, these results are in agreement with the hypothesis that improving soil conditions is important for improving plant establishment on coal wastes in Mediterranean ecosystems by overcoming physico-chemical constraints (Bochet et al. 2010).

Assessing effects of environmental factors (landscape versus local soil) on the succession (Hypothesis 2)

The environmental factors driving the course of succession at the topsoiled mines were age since restoration was implemented and soil pH. Similar conclusions have been found on restored sites in central Europe (Prach et al. 2007b) and on coal mines in central Spain (Moreno de las Heras et al. 2008). The course of the succession reflected a gradient of species turnover from the early-successional stages (*Arenaria serpyllifolia*, *Sonchus oleraceus*), with hydroseeded species (*Lolium perenne*, *Trifolium pratense*, *T. repens*) on young sites, to mid-successional stages (*Arrhenatherum elatius, Cerastium glomeratum*, *Cytisus scoparius*), and finally to late-successional stages where woody species (*Genista florida*, *Quecus pyrenaica*, *Rosa canina*) dominate. Analogous results of species turnover through age have been found in reclaimed coal mines in Virginia (USA), where the composition of the oldest reclaimed sites was approaching that of the adjacent natural vegetation (Holl & Cairns 1994). The vegetation of our study is approaching the composition of native vegetation within 32 years. Thus suggests that a longer time frame is needed to achieve our ecological restoration objective.

Soil pH in topsoiled mines determined successional patterns across stages of the same age, separating the most acidic soils from the neutral and basic ones. The influence of pH on vegetation is well known (Ellenberg et al. 1991), and is often a reliable predictor of species composition in succession (Christensen & Peet 1981). In our study, this is an important result since understanding species niche space with respect to pH will help with species selection for future restoration planning (Young et al. 2005), and suggests that manipulation of soil pH might be a useful tool for accelerating or directing succession (Prach et al. 2007b).

In other regions, studies of succession on mine sites have reported the importance of a range of environmental and landscape factors driving succession (Rehounková & Prach 2008; Moreno de las Heras et al. 2008). Surprisingly our results did not support this, because we did not find that local landscape factors had significant effect on successional process. In any case, these findings are consistent with the hypothesis of Rehounková & Prach (2006), who suggest that the role of the environmental factors in succession depends on the scales and the systems studied.

Species response to age and pH in topsoiled sites

A large number of species (mainly therophytes) showed no response to age or pH; these species are presumably adapted to disturbance produced by the annual climatic cycles of the Mediterranean region (Lawesson & Oksanen 2002; Alday et al. 2010). Some of these species were native ones, e.g. *Bromus mollis*, *Medicago lupulina* and *Veronica arvensis*, and we suggest that these species might provide a useful additional option for improving hydroseeding mixtures in the future, where we might expect these species to persist for a very long time, facilitating the subsequent establishment of later-successional species (Malkinson et al. 2003; Walker & del Moral 2009).

The species response to pH showed that species optima ranged from 5.5 to 8, and tolerances within this range were generally large indicating that these species are able to grow over a relative wide pH range (Wamelink et al. 2005). Some species showed optima at different parts of this range, for example *Vulpia myuros* had lower optima than *Trifolium pratense*. Information on the optima allows either species mixtures to be tailored to individual site conditions or conditions could be changed by liming to favour desired plant communities.

Conclusions

Wastes produced by surface coal mining in the Palencia region of northern Spain, under Mediterranean climate can, if topsoil is added, develop a native shrub community fairly quickly (ca. 15 years). However, it will take more than 40 years if topsoiling is not used. At the same time, these results identified the main species involved in the different stages of the colonization of coal mines in the region and will help guide future restoration strategies.

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Chapter 8

An Island of vegetation

Functional groups and dispersal strategies as guides for predicting vegetation dynamics on reclaimed mines

Josu G. Alday, Yésica Pallavicini; Rob H. Marrs & Carolina Martínez-Ruiz

Plant Ecology: In review (2010)

Abstract

In reclamation processes, there are two parameters particularly relevant: richness and plant cover. One common approach to explore the vegetation dynamics on reclaimed sites is to describe their patterns through time. We expand this approach by considering functional groups and dispersal strategies, as important components of ecosystem function and key traits for colonization. Here, we test if the species richness and cover of these traits change during succession in 26 reclaimed coal mines, and if these changes are related with variations in soil N, C:N and P. Species richness showed a skewed unimodal response through age since reclamation with a peak at 13 years. Functional groups richness showed a clear tendency in the order of species dominance starting from annuals, perennials and woody, whereas cover dominance was not similar to richness since perennials dominate along the sequence. Dispersal modes showed that zoochorus and anemochorus species were the most important on richness and cover. Soil variables were correlated with richness and cover of different functional groups, but only with zoochorus species. Our findings indicate that species response to disturbance are controlled in some part by species functional groups, whereas the colonization process are more dependent on seed sources from local species pool than on mines soil properties. Our results also highlight that the use of functional groups and dispersal strategies patterns improves the description and prediction of vegetation dynamics and allowed us to identify succesional stages. We discuss the implication of these findings for future reclamation approaches in similar areas.

Key words: Vegetation succession; life-forms; soil variables; diversity; nitrogen; phosphorus.

Introduction

Successional studies on restored sites are becoming a new framework in which identify important ecological processes and applications for restoration and reclamation (Pickett et al. 2008), and their importance is increasing due to accelerating land degradation in many areas of the world. Discoveries in areas such as site ameloriation (Bradshaw 1997), nutrient dynamics (Tordoff et al. 2000) and the identification of those biotic and abiotic factors limiting successional processes (Prach 2003) are supplying new information to guide ecological restoration and reclamation by manipulating succession (Walker & del Moral 2008). However, our knowledge of post-restoration successions could be improved if we elucidated the general causes of vegetation dynamics (Pickett et al. 2008), which are essential for solving practical restoration and reclamation problems through succession.

One of the fundamental objectives of mine reclamation is to facilitate vegetation establishment, improving the plant community and creating a self sustaining ecosystem (Holl 2002; Hobbs et al. 2007). Therefore, there are two parameters particularly relevant during reclamation processes: richness (a basic component of diversity; Magurran 2004) and plant cover (a measure of ecosystem functioning and structural complexity (Ruiz-Jaen & Aide 2005), which helps reduce erosion losses prevalent on unvegetated soils on slopes (Tordoff et al. 2000)). In addition, to understanding the general causes of vegetation dynamics on reclaimed sites, it is necessary to describe what patterns of richness and cover emerge through time (Pickett et al. 2008).

The richness and cover patterns through succession have been studied in detail in some Mediterranean old field successions (Tatoni et al. 1994; Bonet & Pausas 2004), suggesting an unimodal response of species richness with a maximum in early- or mid-succession, but plant cover and community structure continued to increase during succession becoming increasingly more complex, being modulated in some part by carbon accumulation and nitrogen and phosphorus levels (Walker & del Moral 2009). However, little is known about these relationships during land reclamation (Galvánek & Lepš 2008; Zhang & Dong 2010) and especially in Mediterranean climates (Martínez-Ruiz et al. 2007; González-Alday et al. 2008). An understanding of these local successional dynamics can greatly improve the chances for successful reclamation outcomes (Walker & del Moral 2008).

Open cast coal-mining affects around 5,000 ha of land surface in northern Spain (León and Palencia). Although, most of these areas have been subjected to reclamation approaches, they have not always been successful in creating self-sustaining ecosystems (Alday et al. 2010). Moreover, the direct introduction of some medium- and late-succesional native species to accelerate succession has failed, due to the lack of understanding of the ecological roles of these species and the shifts in relative species richness and cover of introduced plant groups (Gou 2001); this failure obviously wastes time and money (Suding & Hobbs 2008). An initial challenge, therefore, in order to manipulate succession better is to improve our knowledge about transfers between successional stages, and especially to determine the relative importance of functional groups and dispersal strategies during succession in order to manipulate succession better (Walker & del Moral 2009).

Here we use species functional groups, which are important components of ecosystem function (Young et al. 2005), and plant dispersal strategies to describe the changes in richness and cover during coal mine reclamation. The use of plant dispersal strategies allows the identification of dispersal dominance along succession, as a first step towards gaining knowledge of dispersal potential (Walker & del Moral 2008). Indeed, the successional changes in species through time might be limited by a lack of propagules dispersing into the site (Rehounková & Prach 2006), being dispersal limitations one of the factors that govern vegetation dynamics and important concern in reclamation (Young et al. 2005). It was hoped that this approach would lead to generic patterns that might inform reclamation work in other reclamation projects and link into studies of succession generally.

This study, therefore, characterized the change in richness and cover patterns of species that develop during the reclamation of coal mines in northern Spain in the thirty-two years after abandonment, and relate this to variations in three soil variables. Specially, we examined the following questions: (1) How does species richness change along restored mines succession? (2) How do functional groups (life-forms) and plant dispersal strategies richness and cover change during succession? and (3) Are richness and plant cover changes related to variations in some soil variables as C:N ratio, total nitrogen and available phosphorus?

Methods

Site description and selection

The study was carried out on 26 coal mines across the north of the Palencia province in northern Spain (lat 42º47'-42º50'N, long 4º32'-4º53'W). Historical documentation of restoration operations was derived for every coal mine in this region from mining company records (UMINSA), focusing particularly in the time since restoration work was implemented and the methods of restoration used. These 26 sites, which ranged in size from 0.5 to 3 ha, had been restored using a combination of topsoil addition lacking in seed-bank (González-Alday et al. 2009) and hydroseeding with a grassland species mixture (for further details see: Alday et al. 2010), providing a chronosequence of elapsed time since reclamation finished of 1-32 years.

The selected mines were relatively close together (within 30 km^2) so geographical and climatic variability was minimised. The altitude range was also relatively limited from 1165 to 1419 m asl. The climate is sub-humid Mediterranean, with an annual mean temperature of 9 ºC, an average annual precipitation of 980 mm, and with a dry season in summer. Soil texture for the mines was clay loam, with a mean pH values of 6.6±1 and organic matter content ranging from 2% to 10%. The natural vegetation close to these mines is composed of *Quercus pyrenaica* woodlands, remnants of natural shrubland (*Genista florida*, *Cytisus scoparius*) and different types of grassland containing *Bromus mollis*, *Vulpia myuros*, *Arenaria montana.*

Sampling and soil analysis

Sampling was done in June-July of 2008. At each mine, 10 1×1 m quadrats were located randomly and within each the cover (%) of all vascular plant species was estimated visually by the same observer. The mean values of cover and richness in ten quadrats per mine were used to obtain a uniform sample of the mine. A soil sample was taken from each 1×1 m quadrat using a soil auger (8 cm diameter, 10 cm depth). The ten sub-samples per mine were homogenised to obtain a uniform sample of the soil characteristics of the mine. Soil samples were air-dried, sieved (≤ 2 mm) and analysed for total nitrogen concentration using the Kjeldahl-method; available phosphorus concentration using the Olsen method; whereas the C:N ratio was determined using methods outlined by MAPA (1994).

Data analysis

For each site (n=26) we categorized all the species by (1) functional groups and life-forms (annuals, herbaceous perennials split in grasses and forbs, and woody; e.g. Foster & Tilman 2000) and by (2) dispersal strategies (anemochory, barochory, autochory, zoochory split in endo- and ectozoochory; e.g. Bonet & Pausas 2004). The main dispersal strategies were derived from field observation, and literature (Valbuena 1995; Bonet & Pausas 2004, González-Alday & Martínez-Ruiz 2007). For simplify the analysis, where a species exhibited more than one dispersal strategy only the main strategy was used here. However, since hydroseeded species were included artificially, they were excluded from the dispersion strategies analysis. The mean plant cover and richness of each functional group and dispersal mode in each site were then calculated.

Species richness and plant cover (total richness, richness and cover for each functional group and dispersal strategy) were modelled as a function of age since reclamation using linear and non-linear models. First, three types of lineal models were fitted (null, linear and quadratic) and the model that reduced the Aikaike Information Criterion (AIC) most, relative to the null model, was selected. However, when the linear models were not significant and descriptive plots indicated a relationship, non-linear models (Ricker curve and exponential models) were tested (Table 1, Crawley 2007). Species richness values were transformed before analysis to make the variance independent of the mean (Crawley 2007); most variables were transformed ($\sqrt{x}$), the exception being the richness of woody and endozoochorus species which were log-transformed ($\log(x+1)$). Only the best-fit models selected are presented here. The values were back-transformed to the original scale before plotting.

In order to determine possible relationships between patterns of richness and plant cover with variations in selected soil variables (C:N ratio, nitrogen and phosphorus), Pearson's correlation coefficients were calculated: data were standardized before the analysis, to correct for different scale of measuring units. All statistical analyses were implemented in the R software environment (version 2.10.0; R Development Core Team 2009).

Results

Richness

A total of 220 vascular plant species were recorded in the 26 studied mines. Species richness ranged from 7-8 species in the youngest (1 year) and oldest sites (32 years), to 28 in the 10 years mine. The relationship between species richness with respect to site age was a skewed unimodal one (Table 1, Fig. 1). Species richness increased quickly during the first 10 years, peaked and decreased slowly thereafter. The modelled peak was at 13 years where 22 species were predicted.

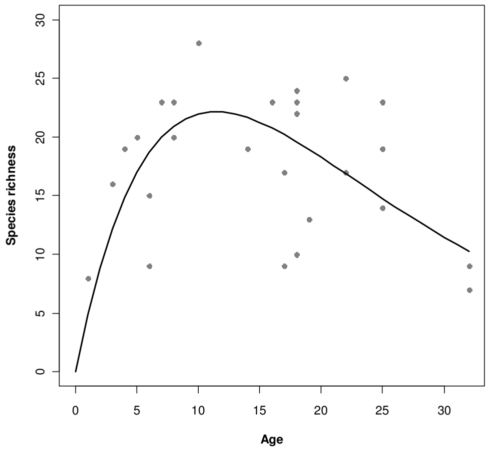

Figure 1. Plant species richness per mine along a successional reclamation gradient on coal mines in northern Spain. The parameters for the fitted line are available in Table 1.

Functional groups

From 220 plant species found, annuals dominated in number (48%) with cover values ranging from 3-64% (Fig. 2). Perennial species represented the 44% of the total, split in perennial grasses and forbs with 6% and 38% of the species respectively, whereas plant cover of perennial species ranged from 23-73%. Woody species were represented by 8% of the species and their cover values ranged from 0-41%.

The relationship between species richness and cover patterns differed with respect to age since reclamation gradient for each functional group (Table 1, Fig. 2). The richness of the functional groups showed a non-linear skewed pattern for annuals, log-normal model for woody (r^2=0.82), quadratic pattern for perennials (r^2=0.28) and forbs (r^2=0.29), and no trend for perennial grasses. There was a clear tendency in the order of species dominance along age gradient, with annuals being the most abundant in the early stage of reclamation, reaching a maximum of 12 species at 10 years. Perennials followed in importance with a maximum of 10 species at 16 years since reclamation, mainly because an increase of forbs and a maintenance of perennial grasses. The response of the perennials and forbs was much flatter than the sharp peak of the annuals. Whereas, woody species showed a slow increase, mainly at 15-25 years after restoration, in species number along age gradient. However in both cases the species number of annuals and perennials decreased with age.

The cover data showed similar responses to the richness data for annual species reaching a maximum of 42% at 10 years since restoration and decreasing latter to 10.7% at 32 years. However, the cover data for perennial grasses (r^2=0.17) showed a quadratic pattern and no significant relationship was found for forbs and total perennials. Moreover, the cover dominance along age gradient was not similar to richness. Perennials were the most important group covering the mines along the sequence (48%), forbs kept on 30%, whereas perennial grasses reached their minimum of 14% at 15-17 years since restoration, increasing latter to 26% at 32 years (Table 1,

Fig. 2). Woody species cover increased exponentially, especially during the last 10 years, becoming the dominant group at 32 years (51%), mainly composed by *Cytisus scoparius* and *Genista florida*.

Table 1. The parameters for the best-fit equations to explain the relationship between (a) richness and (b) cover of functional groups and dispersal modes in relation to age since reclamation. Values in parenthesis are standard errors of the parameters estimates and values in square brackets are 95% confidence intervals.

Non-linear models	**Parameter estimates**			
	a	b	Fitted models	r^2
Richness				
Total	1.15 (0.11) [0.92,1.41]	-0.08 (0.006) [-0.09,-0.07]	$y \sim a*age*e^{(b*age)}$	0.28
Annuals	0.94 (0.11) [0.72,1.21]	-0.10 (0.008) [-0.12,-0.08]	$y \sim a*age*e^{(b*age)}$	0.34
Anemochory	0.77 (0.06) [0.64,0.91]	-0.08 (0.005) [-0.07,-0.09]	$y \sim a*age*e^{(b*age)}$	0.48
Cover (%)				
Annuals	13.21 (2.92) [7.83,20.84]	-0.12 (0.02) [-0.08,-0.15]	$y \sim a*age*e^{(b*age)}$	0.25
Woody	-	0.13 (0.07) [0.11,0.13]	$y \sim e^{(b*age)}$	0.30
Autochory	-	0.08 (0.01) [0.04,0.09]	$y \sim e^{(b*age)}$	0.61

Linear models	**Parameter estimates**			r^2	*p*-value
	β_0	β_1 age	β_2 age^2		
Richness					
Perennials	2.31 (0.24) [1.82,2.80]	0.10 (0.03) [0.03,0.17]	-0.01 (0.001) [-0.005,-0.001]	0.27	0.010
Forbs	1.70 (0.25) [1.18,2.22]	0.12 (0.04) [0.05,0.19]	-0.004 (0.001) [-0.006,-0.001]	0.28	0.008
Grass	1.60 (0.05) [1.17,2.83]	-	-	-	-
Woody	-	0.04 (0.003) [0.03,0.04]	-	0.82	<0.001
Barochory	1.00 (0.20) [0.54,1.57]	0.10 (0.03) [0.04,0.15]	-0.003 (0.001) [-0.005,-0.01]	0.29	0.008
Autochory	-	0.15 (0.02) [0.10,0.20]	-0.004 (0.001) [-0.006,-0.002]	0.83	<0.001
Zoochory	6 (0.51) [4.53,6.63]	-	-	-	-
Ectozoochory	2 (0.31) [1.83,3.10]	-	-	-	-
Endozoochory	1.51 (0.21) [1.27,2.10]	-0.03 (0.01) [-0.05,-0.002]	-	0.15	0.036
Cover (%)					
Perennials	47.65 (2.66) [42.17,53.14]	-	-	-	-
Forbs	29.52 (2.20) [25.00,34.04]	-	-	-	-
Grass	27.56 (5.82) [15.53,39.60]	-1.69 (0.81) [-3.37,-0.02]	0.05 (0.02) [0.003,0.10]	0.17	0.110
Anemochory	40.73 (1.74) [37.15,43.31]	-	-	-	-
Barochory	-	3.51 (0.57) [2.33,4.70]	-0.10 (0.02) [-0.14,-0.05]	0.37	<0.001
Zoochory	42.01 (6.22) [29.18,54.85]	-0.87 (0.36) [-1.60,-0.13]	-	0.18	0.022
Ectozoochory	8.60 (1.40) [5.72,11.40]	-	-	-	-
Endozoochory	35.00 (5.40) [23.91,46.10]	-0.94 (0.31) [-1.57,-0.31]	-	0.25	0.005

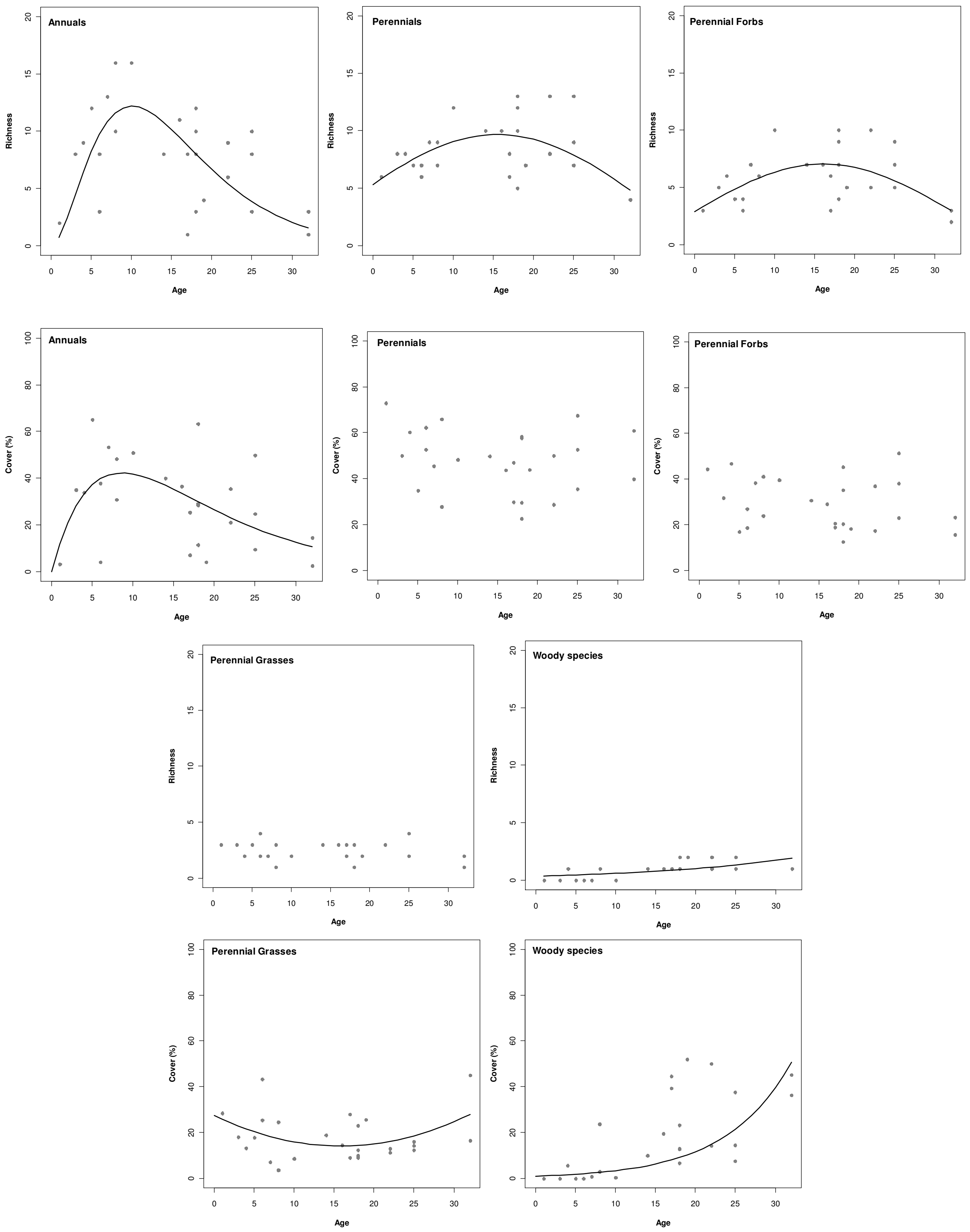

Figure 2. The response of different plant functional groups/life-forms as both richness and cover (%) along a successional reclamation gradient on coal mines in northern Spain. The parameters for the fitted lines are available in Table 1. Panels without a fitted line showed no significant relationship relative to the null model and included for completeness.

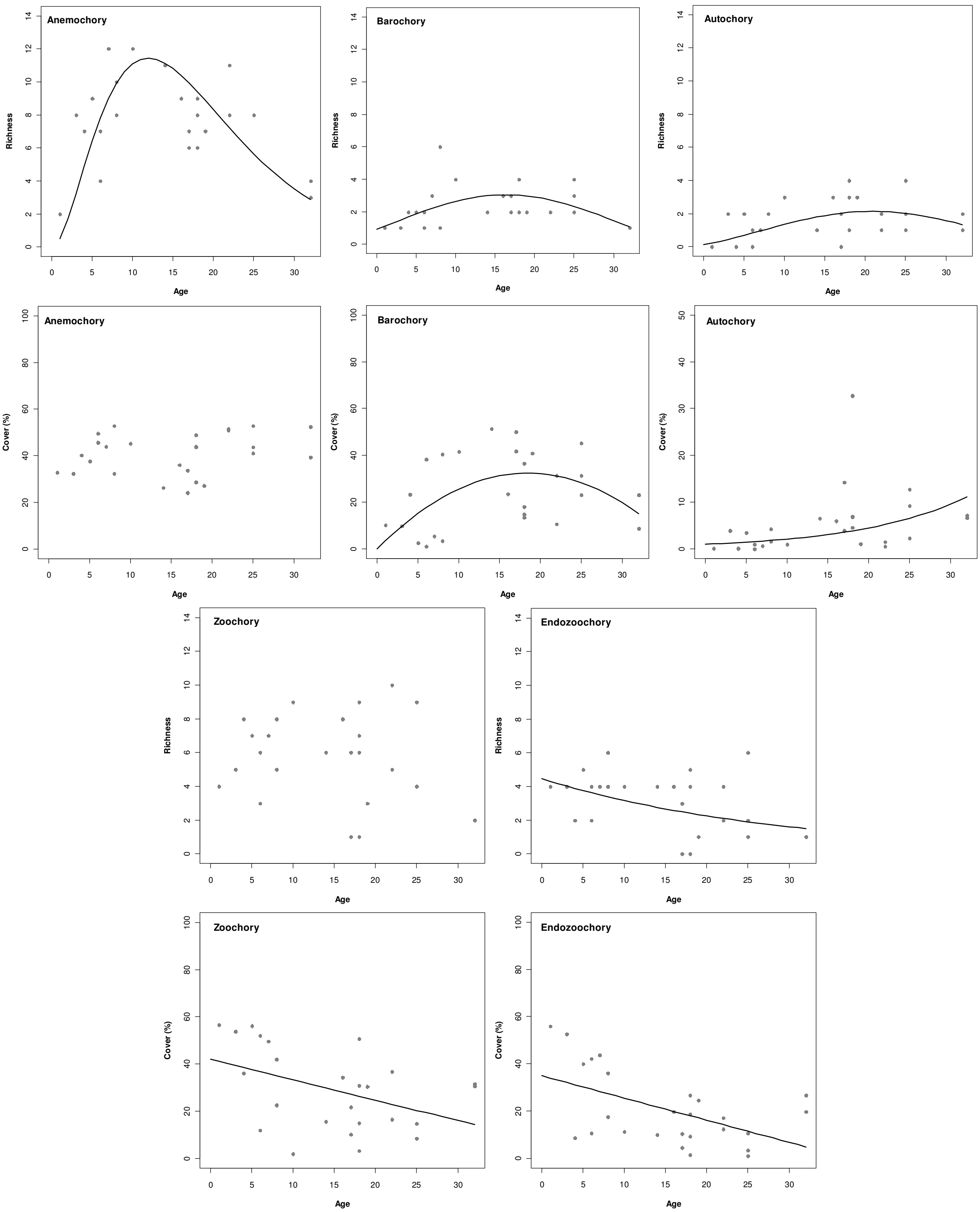

Figure 3. The response of different plant dispersal modes as both richness and cover (%) along a successional reclamation gradient on coal mines in northern Spain. The parameters for the fitted lines are available in Table 1. Panels without a fitted line showed no significant relationship relative to the null model and included for completeness. No significant relationship was fitted between richness and cover of species that exhibited ectozoochory, therefore these data are not presented.

Dispersal strategies

Most of the species found in the mines were dispersed by anemochory (37 of the 220 species recorded), and this group also dominated the cover (41%). Zoochorus species were 30% of the total, split into endozoochorus (14% of the total richness, 57% of cover) and ectozoochorus (16% of the total richness, 2% of cover). Barochory was the third dispersal mode represented by 17% of the species and cover values ranging from 1-51%, while autochorus species represented 16% with cover values ranging from 0-33%.

Species richness patterns differed between dispersal modes along the age since restoration gradient (Table 1, Fig. 3). A non-linear skewed relationship was found for the anemochorus species and quadratic relationship for the barochorus and autochorus species (r^2=0.29, r^2=0.83, respectively). In contrast, a linear response was detected for endozoochorus species (r^2=0.15), and there was no trend for zoochorus and ectozoochorus species. However, the species dominance of the dispersal agents across the age gradient varied. In the early stages, zoochorus species were the most abundant (<4 years, 6 species), mainly caused by 4 endozoochorus species. Thereafter there was an increase of anemochorus species which peaked at 13 years with 11 species and decreasing latter to just 3 species at year 32.

Dispersal strategies based on plant cover along the age gradient showed exponential relationship for autochory, quadratic for barochory (r^2=0.37), linear patterns for zoochory (r^2=0.18) and endozoochory (r^2=0.25), and no trend for anemochory and ectozoochory (Table 1, Fig. 3). No trend in cover dominance was found for anemochorus species because their cover dominated along the gradient (41%), except at year 1 when zoochorus species dominates (42%). Barochorus species reached their cover maximum (32%) at 18-20 years since restoration and autochorus cover increased exponentially, reaching the11% at 32 years since restoration.

Relationships of richness and cover with soil variables (Table 2)

The correlation analysis of functional groups showed that perennials and woody species richness were significantly positively correlated with total nitrogen. Perennials (both richness and cover) were significantly negatively correlated with C:N ratio, whereas woody species cover showed a positive correlation. Available phosphorus was only positively correlated with perennial grasses richness and cover of annuals.

Among the dispersal modes only zoochorus species showed significant correlations with the soil variables. Total nitrogen was correlated positively with zoochorus species richness and ectozoochorus plant cover. Soil C:N ratio was significantly negatively correlated with zoochorus and endozoochorus species richness. Finally, available phosphorus was significantly positively correlated with endozoochorus richness and plant cover of zoochorus and ectozoochorus species.

Table 2. Pearson correlation matrix between selected soil variables and the richness and cover of functional plant groups and dispersal modes; significant correlations (*p*<0.05) are shown in bold.

	P	C/N	N
Richness			
Annuals	0.29	-0.29	0.12
Perennials	0.15	**-0.48**	**0.45**
Forbs	0.00	**-0.39**	0.36
Grasses	**0.47**	**-0.42**	0.33
Woody	0.06	0.25	**0.47**
Anemochory	0.11	-0.29	0.24
Barochory	0.13	-0.07	0.18
Autochory	0.38	0.17	0.33
Zoochory	0.29	**-0.56**	**0.41**
Ectozoochory	0.04	-0.34	0.35
Endozoochory	**0.40**	**-0.53**	0.29
Cover (%)			
Annuals	**0.53**	-0.22	0.13
Perennials	-0.13	**-0.42**	-0.11
Forbs	-0.17	**-0.57**	-0.04
Grasses	0.01	0.07	-0.11
Woody	-0.33	**0.40**	0.15
Anemochory	-0.07	0.26	0.19
Barochory	-0.32	0.35	0.11
Autochory	0.14	0.17	0.30
Zoochory	**0.43**	-0.13	0.23
Ectozoochory	**0.40**	-0.03	**0.40**
Endozoochory	0.34	-0.16	0.06

Discussion

In this study, the patterns along a chronosequence of reclaimed coal mines in a sub-humid Mediterranean environment of Spain were different for plant cover and species richness for both functional groups and dispersal strategies. Moreover, the use of different functional groups and dispersal modes of species improved the description and prediction of the vegetation dynamics, by reducing the complexity of the successional processes (Gou 2001).

Species richness and functional groups

An important result was that total species richness peaked in the early-stages, 10-15 years after reclamation started. This early peak in total species richness is similar to those documented in other studies of old field successions in Mediterranean climates (Tatoni et al. 1994; Bonet & Pausas 2004), and is consistent with the resource-ratio hypothesis (Tilman 1985). The most apparent cause of this peak is the overlap or coexistence for a few years of pioneer species (mainly annuals) with species of more intermediate and mature stages such as perennial grasses and forbs and eventually woody species (Capitanio & Carcaillet 2008). However, there was a sequential replacement in functional groups richness dominance with age since reclamation, suggesting that species responses to disturbance are controlled in some part by species life traits as functional groups or life-forms (Picket et al. 2008; Walker & del Moral 2008).

As in most examples of succession, newly-created ecosystems on reclaimed coal areas are colonized by fast-growing annuals and some short-lived perennials (Foster & Tilman 2000), which are mainly dispersed by long distance dispersal modes as anemochory and zoochory (González-Alday & Martínez-Ruiz 2007). The cover of annuals increased up to 10 years after reclamation and was correlated with the high concentrations of available phosphorus found in the first years, generated by the use of fertilizers during the initial reclamation works (González-Alday et al. 2008). It is well known that the use of fertilizers can produce changes in the balance between species and functional groups (Walker & del Moral 2009). These annual species are generally displaced by perennials that tend to be more competitive for nutrients, especially soil nitrogen in degraded systems (Tilman & Wedin 1991). In fact, the peak of perennial species richness was achieved 16 years after reclamation, 4 years later than the annuals peak. This perennial richness pattern must be attributable mainly to forb richness unimodal response, since perennial grasses richness maintained along the sequence. Similar results have also been reported in restored sub-alpine meadows (Li et al. 2009).

Results from recent studies on replacement patterns on Mediterranean secondary succession have shown the same sequence in the order of functional groups richness and cover dominance (annuals, perennials and woody species; Bonet 2004; Bonet & Pausas 2004). Nevertheless, in our study the plant cover did not show the same dominance sequence as richness, in spite of annuals and woody species cover followed the classical patterns. This result ocurred because the perennial species maintained a greater stable cover along the sequence, probably because they were boosted through artificial introduction of some fast growing perennial grass species in the first years through hydroseeding (González-Alday et al. 2008), and phosphorus fertilization.

One important feature in this study was to determine the point at which woody species started to colonize the substrates after reclamation. After 8-10 years woody species colonized, these colonist were mainly legumes (*Cytisus scoparius* and *Genista florida*), which presumably would enhance the reclamation process by fixing atmospheric nitrogen, producing a large amount of soil organic matter and increasing nitrogen mineralization (Palaniappan et al. 1979), thus facilitating the invasion and development of late-successional species (Walker & del Moral 2009). This hypothesis is supported because of the positive correlation of woody richness and plant cover with total nitrogen and C:N ratio, and it has been reported in other studies of coal mine reclamation (Holl 2002). The results suggest that direct introduction of these woody leguminous species would accelerate successional development (Walker & del Moral 2009).

Dispersal modes

During the early stages of succession, dispersal and colonization processes may limit the establishment and growth of plant species (Walker & del Moral 2009), determining the structure of early-stage successional communities (Rehounková & Prach 2006). At the same time, anemochory and zoochory (main endozoochory) has been identified as the most important dispersal vectors in primary successions of mined areas (Kirmer et al. 2008), and especially in the early-successional stages (González-Alday & Martinez-Ruiz 2007). Our results are in agreement with these observations, as species that exhibit anemochory and endozoochory are most abundant on early stages, and they determine the ongoing species composition to some extent because the anemochorus species cover was the dominant along the sequence, whereas the endozoochorus species declined. Therefore, the peak of species richness could be also caused by the coexistence of different dispersal strategies (Bonet & Pausas 2004). Autochorus and barochorus species (mainly forbs and woody) increased in importance during the sequence, producing an increasing complexity in vegetation structure during the succession (Picket et al. 2008). The succession is, therefore, moving in the desired direction, towards shrub and forest community targets, and the reclamation can be judged successful.

One interesting result was that there were no significant correlations between anemochorus, autochorus and barochorus species richness or cover and any soil variables. Therefore, the pioneer colonization process is more dependent on seed sources from the local species pool from the area surrounding the mines than on those soil properties (Lepš et al. 2000). Seed dispersal has been shown to be more important than resource availability for plant establishment in other succesional models (Martineau & Saugier 2007). Nevertheless, the correlations showed by zoochorus species with available phosphorus and total nitrogen, greater at early and medium stages, may be more related to the behaviour of animal vectors than with plant species. It is well known that the influence of vertebrate herbibory, fundamental for zoochorus dispersal, declines with succession as the proportion of better-defended plant parts increase (Walker & del Moral 2009).

Conclusions

In conclusion, wastes produced by surface coal mines under sub-humid Mediterranean climate, showed a clear pattern of richness peak in the early stages (13 years), which could be explained through knowledge of the changes in either functional groups or dispersal strategies. However, the changes in cover patterns were not as clear as those described for richness. The use of functional groups and dispersal strategies patterns improves the description and prediction of vegetation dynamics and allowed us to identify the stages where it might be worth considering the introduction of alternative species, especially those species with very limited dispersal modes. Our results also highlight that species response to disturbance are controlled in some part by species functional groups, whereas the colonization process are more dependent on seed sources from local species pool than on mines soil properties. These are fundamental results for understanding the successional stage of the colonization of coal mines in the region, which helped to identify action points to guide future reclamation strategies (Table 3); clearly these needs to be tested by experiment. Nevertheless, the use of other grouping methods, as life spans and growth rates, could also reflects different underlying successional changes on mines, which could help to plan new reclamation approaches (Gou 2001).

Table 3. Examples of actions based on this results that could accelerate and solve problems at crucial stages of succession guiding future reclamation strategies.

Reclamation topic	Age since reclamation	Actions
Annual species	0-15	Favour their arrival and development for stabilize erosion. Phosphorus could enhance cover development.
Perennial native species	10-25	Introduce intermediate successional species, more competitive for soil nitrogen.
	>30	Introduce mature and shade tolerant species, favoured by shade and soil nutrients produced by shrubs.
Woody species introduction	>10	Direct planting of mature individuals or seed introduction. Focus on N-fixing species for increase nutrient availability especially organic matter and nitrogen to accelerate succession.
Seed dispersal limitations	0-30	Install natural seed traps (fences, trails, ploughs) to enhance seed capture from local species pool. Direct introduction of interesting species not found in local species pool.
	>20	Favour the introduction of interesting medium and mature successional species with limited dispersal mechanism.

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Chapter 9

No words

Synthesis

Short-term vegetation development: influential factors and processes

The study of an ongoing practical land restoration in the ‘Pozo Sell’ opencast coal mine during the initial 6 years provided information on restoration success at the establishment phase and about the factors and mechanisms that govern early-successional processes.

The initial cover of coal wastes with fine-textured materials and a layer of cattle manure provided an acceptable soil-forming material with soil characteristics favourable for plant establishment, growth and the development of a plant community, which prevented soil factors to control vegetation development during succession (Chapter 6). In contrast, the use of topsoil, stockpiled for 2 years and mixed with sediments from deeper parts of the opencast pit, diluted the amount of viable seeds available for restoration (Chapter 3). In such situation, the introduction of seeds to enable fast re-vegetation to prevent soil erosion is a need (Vallejo et al. 2006). At this mine hydroseeding was used for this purpose.

Hydroseeding of the ‘Pozo Sell’ mine included forage grass and legume species (Chapters 2 and 3), thus minimizing expense (Pensa et al. 2004). Hydroseeding was successful in the re-vegetation of this coal mine within two years providing soil protection (HSI>0.7), even though, the hydroseeded species were not part of the planned final target vegetation. Most hydroseeded species persisted for the whole experimental period, although they reduced in importance; only three species disappear (*Avena sativa*, *Secale cereale* and *Lotus corniculatus*; Chapter 5). At the same time, the developing seed bank contained a substantial component of hydroseeded species which will help these species persist. This may cause long-term problems for restoration of target community (Chapter 3), because plots with a high cover of hydroseeded species slowed colonization by native species (Chapter 5), while hydroseeded seeds in soil seed bank, depending on environmental changes (Leck et al. 1989), have the potential to produce changes in vegetation composition. Those points were not in agreement with the hypothesis that commercial seed mixtures act as facilitating species, growing fast the first years after hydroseeding and gradually vanishing as native species colonize (Mitchley et al. 1996; Merlin et al. 1999).

The lack of an initial soil seed bank means that native species must to colonize by dispersal from the surrounding vegetation (Chapter 3). Clearly, colonization is easier where the area to be restored is a short distance from seed source (Chapter 3), because seed colonization potential depends on its dispersal mechanisms and the distance that have to travel (Traba et al. 2003; Wagner et al. 2006). The studied areas had good colonization by native species, especially in the early stages by annuals with long dispersal mechanism (anemocory and zoochory; Chapters 4 and 5) and some ruderals. Through time the probability of target species arriving increased (Holl 2002), for *Astragalus sempervirens, Helianthemun hirtum, Ononis repens*. Once these native species arrive, they must establish in the presence of competition from the hydroseeded species, which might limit their growth (Chapter 5). The cover of native species during the first two years was low, and hydroseeded species dominated. Thereafter, the cover of native species increased sharply between 2005 and 2006, almost certainly assisted by the impact of an exceptionally dry year (Chapter 4). The extreme summer drought (stochastic factor) redirected the succession, native species increased and the succession was moved towards the target reference community (eg. Lanta & Lepš 2009; Chapter 5). It is likely that native colonizers were better adapted to recurrent disturbance produced by the drought prevalent in Mediterranean region than hydroseeded species (Lawesson & Oksanen 2002; Chapter 4).

On the basis of these results, we recommend the use of native species for future hydroseeding treatments of mine sites in similar areas because: (a) they are part of the target community (Chapter 5), (b) the development of a soil seed bank with non-target hydroseeded species will be reduced (Chapter 3), (c) the successional process between the time of colonization and when they start to dominate will be reduced (Chapters 4 and 5), and (d) the resultant plant community composed of native species should be better adapted to site climatic conditions (Chapter 4). Specifically, for future hydroseeding in similar areas we propose the use of a basic native mixture, aspect indifferent, composed mainly of grasses and legumes (*Bromus mollis, Trifolium campestre, T. striatum, T. scabrum* and *Vulpia myuros*) with the following species in lesser proportions: *Arenaria montana*, *Cerastium fontanum*, *Erodium cicutarium, Helianthemun hirtum* and *Minuartia mediterranea.* In any case, this proposed method needs to be tested to validate this proposal and to judge its success.

As well as stochastic factors (colonization chance and climate) and biotic competition between hydroseeded and native species, slope aspect, an abiotic factor, was shown to significantly influence the species composition and abundance during the early-establishment phase (Chapters 2, 4 and 5), but not the soil development (Chapter 6). Slope aspect conditioned 15-20% of the hydroseeded species cover, producing greater cover on north-facing slopes (Chapter 2). This effect was probably mediated through its effects on microclimate (Bennie et al. 2006) which reduced the water availability (Sternberg & Shoshany 2001). At the same time, topography (north-south-and flat area) conditioned the 5% of the floristic composition during the first 3 years, mainly through differences in the development of hydroseeded species (Chapter 3). However, 4-6 years after restoration was started, topography also conditioned the species composition, but mainly through differences in native species development (Chapters 4 and 5). In view of these results it seems that the combination of compositional responses to aspect (topography), age since restoration and climate variability create complex successional patterns, which reveal the frequency of multiple trajectories in early successions even in adjacent locations (del Moral 2007; Prach & Hobbs 2008; del Moral et al. 2010).

The species composition and vegetation structure (richness, diversity and cover) in the three restored mine areas (north, south and flat) was converging with the reference community over time, albeit at different speeds (Chapter 5). Over the six years period, vegetation cover (plant biomass) helped to accumulate soil organic matter (Marrs et al. 1980; Frouz et al. 2008) and nutrients into the soil (Chapter 6). The accumulation of organic matter and increase in sand proportion led to increased soil aeration and water retention (Matlack 2009), thus creating better conditions for subsequent native species establishment and development, increasing complexity of plant communities (increased species richness (S) and biological diversity index (H'); Chapter 6). Most of the native species that have colonized within the 6-year period were classified as widespread species with long distance dispersal mechanisms. As a consequence, reliance on this restoration approach may be adequate for species with good dispersal mechanisms, but may inappropriate for less common species or with limited dispersal abilities (Chapter 5).

Medium-term vegetation succession: processes and patterns

The medium-term succession (1-40 years) in coal mines depended on the restoration method used; especially whether topsoil was added or not (Chapter 7). In non-topsoiled mines, substrate base constraints, such as physico-chemical characteristics and instability, arrest succession leading to the formation of a community characterized by early-successional and species adapted to degraded habitats (Fig. 1). Strangely enough, this slow succession is produced in the older mines, i.e. those with a mean age of 25 years since abandonment. As a consequence of slow succession, the development of a native shrub community in such conditions will take more than 40 years (Chapter 7).

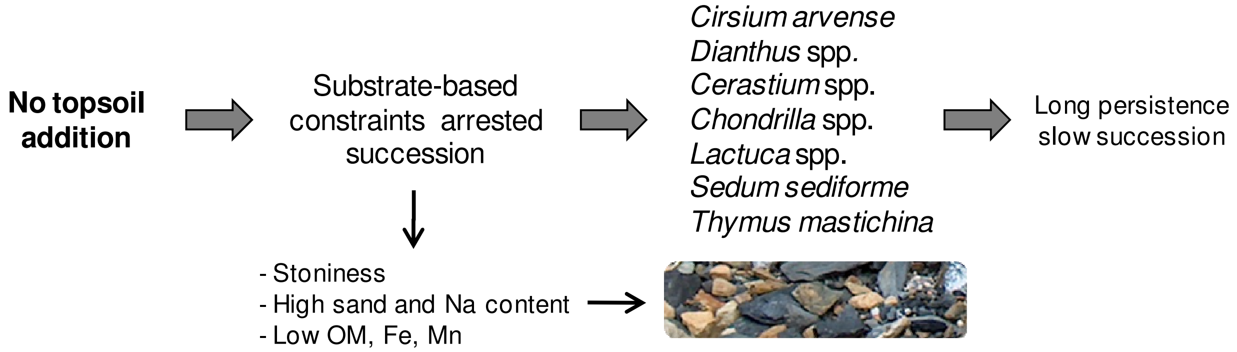

Figure 1. Succession series in non-topsoiled mines.

When mine wastes have been ameliorated through the addition of top-soil, the course of succession reflects the classical pattern of specie turnover from early-successional and hydroseeded species (i.e. *Lolium perenne*, *Trifolium pratense*, *T. repens*) on young sites, to mid-successional stages with perennial species as characteristics, and finally to late-successional stages where woody species begin to dominate (Chapter 7; Fig. 2). There was an increasing complexity in vegetation structure during succession (Picket et al. 2008). Although, the most abundant species during successional process showed no response to pH (i.e. *Bromus mollis*, *Festuca* spp.), or were able to grow over a relative wide pH range (i.e. *Vulpia myuros, Trifolium* *campestre*) indicating high growth plasticity (Wamelink et al. 2005). The presence of some species in these successional stages was modified by soil pH, specifically during early and medium stages (Chapter 7).

In the early stage of succession, herbaceous species dominate (Fig. 2). Mines are being colonized by fast-growing annuals and some short-lived perennials, most of which have long distance dispersal modes as anemochory and zoochory (Chapter 8). During this stage good establishment was found for *Bromus mollis*, *Festuca* spp., *Plantago lanceolata* and *Vulpia myuros* which maintained constant cover values through the succession. In contrast, the following species were dominant in this stage independent of soil pH; *Trifolium campestre*, *T. scabrum* (Chapter 7), while on acid-neutral soils appeared *Carduus acanthoides,* and *Anthyllis vulneraria* and *Xeranthemum inapertum* on basic soils.

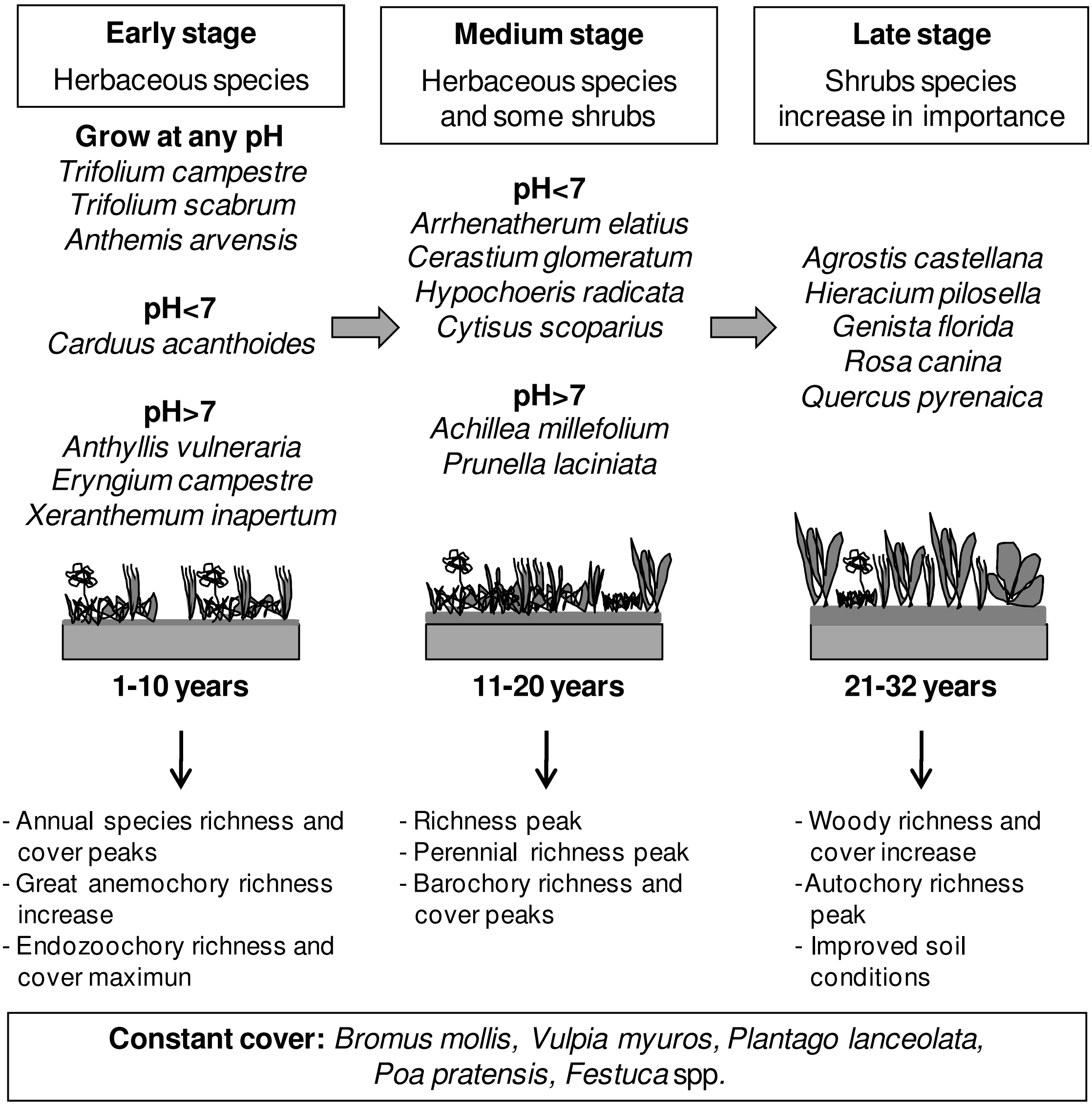

Figure 2. Succession series in top-soiled mines.

In the medium stage of succession, colonization of woody species started to become important with cover values nearly 40% at the end of stage (Chapters 7 and 8; Fig. 2). Species richness peaked between 10-15 years due to the coexistence for a few years of early-stage species (mainly annuals) with intermediate stage species (mainly perennials) (Capitanio & Carcaillet 2008). The colonization by woody legumes such as *Cytisus scoparius* and *Genista florida* enhanced the reclamation process by fixing atmospheric nitrogen, producing a large amount of soil organic matter and increasing nitrogen mineralization (Palaniappan et al. 1979). Then annual species were slowly displaced by perennials that tend to be more competitive for nutrients, especially soil nitrogen in degraded systems (Tilman & Wedin 1991). In fact, richness peak of perennial species was detected at 16 years after reclamation, 4 years later than the annuals peak (Chapter 8). *Arrhenatherum elatius, Cerastium glomeratum* or *Cytisus scoparius* become dominant on mines with a soil pH lower than 7, whereas *Achillea millefolium* and *Prunella laciniata* were important on basic soils (pH>7).

Finally, the late successional stage was characterized by an increase in woody species (*Genista florida, Rosa canina;* finishing with cover values nearly 70%), and the appearance of *Quercus pyrenaica* and some shade tolerant species (Fig. 2). The richness of species exhibiting autochory peaked in this stage and their cover increases exponentially (Chapter 8). The complexity of the vegetation structure increased, as well as the soil organic matter layer and nutrients concentrations, creating the adequate conditions for establishment of tree species (Chapter 7). The succession is, therefore, moving in the desired direction approaching the composition of native vegetation within 32 years. However, a longer time frame is needed to achieve the ecological restoration of target plant community.

Recommendations for restoration ecology

The restoration of mining landscapes is not easy task, mainly by the wide type of factors that conditioned its success. On the basis of our results three management suggestions could be make for the improvement of the restoration process in these mines:

1.- The use of topsoil to ameliorate the substrate base constraints of coal wastes. The topsoil spreading enhance plant establishment by improving soil properties, and therefore, increase the likelihood of re-establishment of native plants species. It would be preferable to conserve the topsoil prior to the mining operation, and thereafter use it in restoration process. However, topsoil must be unmixed with sediment from deeper layers; in this case top-soil will be a native species seed source during revegetation process.

2.- The use of native species for revegetation purposes. Native species are better adapted to local conditions and they will accelerate the successional process, reducing the time between colonization and when native species start to dominate. Nevertheless, the main problem for the use of native species in restoration is the difficulty to find them in higher amounts, since normally they are not included in the commercial mixtures. To surmount this problem maybe the recollection of native seeds from surrounding areas could be an option. Moreover, the use of plant clippings could be a viable alternative to traditional hydroseeding method (Kirmer & Manh 2001), although its effectiveness in this type of ecosystems needs to be tested.

3.- It is essential when planning restoration projects to accept that some abiotic factors such as topography (north-, south-facing or flat areas) and distance to seed sources will affect the outcome of succession. Topography need to be taken into account in the selection of species used for revegetation, particularly when commercial species are used by hydroseeding. In any case, different topographic structures required different management options, for example disturbances produced by summer droughts in Mediterranean climates have higher impact on south and flat aspects, and as a consequence they might require new revegetation actions in contrast to north aspects. On the other hand, whenever possible it is recommended that mined areas had some contact with native vegetation, which will favoured the native species colonization, increasing the colonization chance for species with limited dispersal abilities.

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Chapter 10

At the end of a long road

Conclusions-Conclusiones

Conclusions

Short term

1. The early growth dynamics of hydroseeded species were affected by aspect. Therefore, managers should take into account the aspect of slope when selecting species for use them in restoration, particularly by hydroseeding of commercial species. Otherwise short-term vegetation establishment will be substantially reduced under the more severe conditions on south-facing slopes.

2. The development of a seed bank is an important part of the restoration process. When the restoration area is close to natural communities the native species component of the seed bank increased. Management plans have to take in consideration the differences in seed bank development based on several characteristics of each site (distance to natural communities and topography). Otherwise seed bank differences may condition the future vegetation recovery, creating very different communities in very close areas.

3. The short-term vegetation establishment influences the successional development of restoration schemes. Stochastic factors as summer drought produced disturbances that can have a considerable effect on the floristic composition and diversity during restoration, which may be amplified by topography. In particular, topography will influence the persistence of hydroseeded species, and this in turn will influence the colonization of native species from the surrounding vegetation.

4. After 6 years of mine restoration, vegetation structure is approaching to the reference community. In contrast, species composition although is becoming similar, still differed considerably from the reference community, suggesting that much longer time-frame is needed to achieve the ecological restoration objective. At the same time, the increasing differences in species composition between areas and the different successional compositional rates indicate that within-area constraints appear to play an important part in species assembly and succession, producing diverse trajectories even in close locations.

5. Age since restoration was the driving agent, at least in short-term, of soil changes on the new-formed soil for the restoration. Moreover, the correlation of soil changes and vegetation structural/functional changes suggested that they may be involved in the mechanisms that induce these soil changes, favoring the increase of plant community complexity. In contrast, the lack of relationship between floristic compositional dynamics and soil parameters suggests that compositional differences in short-term are mainly driven by a combination of stochastic and abiotic factors.

Medium term

6. Vegetation succession was affected by the restoration method applied (i.e. whether topsoil was added or not). Simultaneously, the environmental factors that influence successional processes differed between the restoration methods; the vegetation on topsoiled mines showed a trend with age and pH developing a native shrub community fairly quickly (ca. 15 years), whereas on non-topsoiled mines substrate-based constraints limit succession, in this mines the development of native shrub community will take more than 40 years.

7. The use of functional groups and dispersal strategies patterns improves the description and prediction of vegetation dynamics and allowed to identify the stages where it might be worth considering the introduction of alternative species. At the same time, the species response to disturbance seemed to be controlled in some part by species functional groups, whereas the colonization process are more dependent on seed sources from local species pool than on mines soil properties.

Conclusiones

A corto plazo

1. La dinámica de crecimiento inicial de las especies hidrosembradas se vio afectada por la orientación. Por lo que la orientación de los taludes debe ser tenida en cuenta a la hora de seleccionar las especies para restaurar, en particular mediante hidrosiembra con especies comerciales. De lo contrario el establecimiento de la vegetación a corto plazo se reducirá sustancialmente en las condiciones más severas, en especial en las orientaciones sur.

2. Una parte importante dentro del proceso de restauración es la formación del banco de semillas. La distancia entre el área a restaurar y las comunidades naturales condiciona la proporción de especies nativas en el banco. Por tanto, los planes de gestión tienen que tener en cuenta las diferencias en el desarrollo del banco de semillas como consecuencia de las particularidades de cada sitio (distancia a las comunidades naturales y topografía). De lo contrario las diferencias entre bancos de semillas pueden condicionar la futura recuperación de la vegetación, mediante la creación de comunidades diferentes en áreas muy próximas.

3. El establecimiento de la vegetación a corto plazo influye en el desarrollo de la sucesión durante la restauración. Los factores estocásticos como la sequía estival producen perturbaciones que tienen un efecto considerable sobre la composición florística y diversidad, dichos efectos pueden ser amplificados por la topografía. En particular, la topografía influye en el mantenimiento de las especies hidrosembradas, lo que condiciona la colonización de especies locales procedentes de la vegetación circundante.

4. Seis años después de la restauración, la estructura de la vegetación se asemeja a la comunidad de referencia, mientras que la composición de especies, aunque se está aproximando, aún difiere considerablemente de ésta. Por tanto, se necesita mucho más tiempo para alcanzar el objetivo la restauración ecológica. Al mismo tiempo, las crecientes diferencias en la composición florística entre zonas y sus tasas de cambio sucesional indican que las limitaciones dentro de cada zona juegan un papel muy importante en el ensamblaje y sustitución de las especies, produciendo trayectorias sucesionales diversas incluso en lugares próximos.

5. La edad desde la restauración fue la causante, al menos a corto plazo, de los cambios en las propiedades del suelo. Por otra parte, la correlación entre los cambios del suelo y los cambios estructurales y funcionales de la vegetación indica que estos últimos están involucrados en los mecanismos que inducen cambios del suelo, favoreciendo el aumento de la complejidad de la comunidad vegetal. Por el contrario, la falta de relación entre la composición florística y los parámetros del suelo indican que las diferencias en composición a corto plazo se deben principalmente a la combinación de factores estocásticos y abióticos.

A medio plazo

6. La sucesión vegetal se vio afectada por el método de restauración aplicado, es decir si se añade tierra vegetal o no. Al mismo tiempo, los factores ambientales que influyen en los procesos de sucesión fueron diferentes según el método de restauración empleado. La vegetación de las minas recubiertas de tierra vegetal muestra una tendencia con la edad y el pH, desarrollando una comunidad arbustiva con bastante rapidez (aproximadamente en 15 años). Sin embargo, en las minas sin recubrir con suelo la sucesión se ve condicionada por las limitaciones del sustrato, en estas minas el desarrollo de una comunidad arbustiva necesitará más de 40 años.

7. El uso de grupos funcionales y mecanismos de dispersión para la descripción de los patrones sucesionales, mejora la descripción y predicción de la dinámica de la vegetación y permite identificar etapas en que valdría la pena considerar la introducción de especies alternativas. Al mismo tiempo, la respuesta de las especies a las perturbaciones parece estar controlada de algún modo por el grupo funcional al que pertenecen, mientras que el proceso de colonización depende más de la fuente de semillas que de las características edáficas de las minas.

Appendix

During the course of my PhD thesis, I had the opportunity to work on a research project about Mediterranean forests management. Then, I used the methodologies learned in mine sites successions to answers questions about the influence of different harvest intensities on *Pinus pinaster* communities. The most relevant publications are those:

[I] González-Alday, J., Martínez-Ruiz, C. & Bravo, F. 2009. Evaluating different harvest intensities over understory plant diversity and pine seedlings in a *Pinus pinaster* Ait. natural stand of Spain. *Plant Ecology* 201: 211-220.

[II] Alday, J.G., Martínez-Ruiz, C., Marrs, R.H. & Bravo, F. 2010. Influence of harvesting intensity on the floristic composition of natural Mediterranean Maritime pine forest. *Acta Oecologica* 36: 349-356.

Appendix I

Evaluating different harvest intensities over understory plant diversity and pine seedlings, in a *Pinus pinaster* Ait. natural stand of Spain

Josu González-Alday, Carolina Martínez-Ruiz & Felipe Bravo

Plant Ecology 201: 211-220 (2009)

Abstract

Although modern forestry takes into consideration the analysis of the effects of forest management on plant structure, diversity and seedlings, little is known about how those parameters respond to harvest techniques in the Mediterranean region. We investigated the effect of three different harvest intensities, respect to uncut controls, on understory plant species functional groups, richness, diversity and pine seedlings in a natural Maritime pine stand in Spain, three years after harvesting. The harvest treatments produced a reduction of the number of *Pinus pinaster* seedlings and woody species cover, and an increase of species richness (total and of annual species) and plant cover of annual species respect to control plots (CO). The Shannon diversity values showed no differences between treatments. These results emphasize that the tree harvest treatments analyzed are not suitable for the management of this *Pinus pinaster* stand. Otherwise, the reduction of pine seedling density by harvest treatments and the changes in richness and cover of functional groups would not induce the natural regeneration of this stand maintaining the understory plant layer.

Key words: Anthropogenic disturbance; herbaceous layer; Mediterranean ecosystem; silviculture; woody species.

Introduction

One of the major challenges for modern forestry is to combine conservation of biodiversity and ecosystem functioning with wood production and other values (Hummel 2003; Decocq et al. 2004; Nagai & Yoshida 2006; Newmaster et al. 2007). These general principles will obviously need to be achieved using adequate management practices (Kimmins 2004). It is generally assumed that management practices, and especially harvesting, modulate simultaneously the availability of different types of resources (e.g. light, water and soil nutrients; Decocq et al. 2004). As a result, understory species diversity and flora, which play a fundamental role in the structure and function of ecosystems (Roberts & Gilliam 1995; Newmaster et al. 2007), become quite affected (Hughes & Fahey 1991; Zenner et al. 2006). Therefore, the knowledge of the effects of different harvest disturbances on understory plant layer is an essential element to implement sustainable management of forest landscapes (Halpern & Spies 1995; Roberts & Gilliam 1995; Fahey & Puettmann 2007).

Maritime pine (*Pinus pinaster* Ait.) is a natural forest species characteristic of the western Mediterranean basin, mainly distributed over the Iberian Peninsula, France and Italy (Alía et al. 1996). Traditionally, *P. pinaster* in central Spain has been used for resin production and soil protection against mobile continental dunes (Bravo-Oviedo et al. 2007), with wood production as secondary objective. An important step towards ecologically sound wood production procedures is to test different management alternatives (i.e. harvest intensities) to induce the natural revegetation of these stands. At the same time, these alternatives should always contribute to maintain the landscape and ecological protection functions, mushrooms production and biodiversity of the stands (Oria de Rueda 2003), while sustainable wood and resin production is obtained.

The effect of forest management on plant diversity and flora is complex and more difficult to generalize than it was originally thought (Tárrega et al. 2006), underlining the importance of studying plant and diversity responses for different forest types and harvest techniques (Gilliam 2002). Moreover, most published studies concern managed forests in North America, whose history and tradition radically differ from Europe (Decocq et al. 2004), and particularly from the Mediterranean region (Scarascia-Mugnozza et al. 2000). The aim of this study, therefore, is to investigate the effects of three harvest intensities, relative to untreated control areas, on understory species richness, diversity, functional groups (life forms) and *P. pinaster* seedlings, and their relation to the remaining basal area and canopy cover of a natural maritime pine stand in semi-arid Mediterranean conditions in Spain, three years after harvest. We hypothesized that: (1) the number of *P. pinaster* seedlings in such semi-arid Mediterranean conditions would be reduced by harvest intensity, (2) the functional groups (annual and perennial herbs and woody species) cover and richness would be affected by harvest treatments, and (3) the understory plant richness and diversity would be markedly affected by harvest intensity.

Methods

Study area

This study was conducted in a flat natural Maritime pine forest located in the Segovia province (Cuellar, 757 m a.s.l.; 41º22'N, 4º29'W; Central Spain). The original stand density was 140 stems/ha, tree age ranges from 80 to 100 years and silvicultural practice is based on natural regeneration following a shelterwood system adapted to resin production. The climate is semi-arid Mediterranean, with a mean annual temperature of 11.2 ºC, a mean annual rainfall of 461 mm and dry period from the middle of June to the middle of September (M.A.P.A. 1987). The soils are sandy siliceous of Quaternary age (Junta de Castilla y León 1988), and the vegetation of the area is dominated by *Pinus pinaster* with some isolated trees of stone pine (*Pinus pinea* L.) and crop fields.

Treatments

About 16 continuous hectares of natural Maritime pine were selected in a ca. 15,000 ha of forest to delimit twelve 70 × 70 m permanent plots. To record the variation caused by silviculture treatments rather than to site variability, the selected hectares shared the same abiotic conditions, forest structure and vegetation composition. After plots were established two variables were recorded for all trees with diameter at breast height greater than 7.5 cm found inside the plots: diameter at breath height (DBH; cm), and crown diameters (m). The DBH and crown diameters were measured in order to obtain the basal area (BA) and the canopy cover (%) as informative parameters of the light conditions for ground vegetation (Härdtle et al. 2003). Three levels of harvest intensity with three replicates for each one were applied over nine of the permanent plots: (1) 25% of basal area removed (close plots, H25), (2) 50% of basal area removed (open plots, H50), and (3) 100% of basal area removed (clear-cut plots; CC). All treatments were randomly allocated on these nine plots, whereas the remaining three permanent plots were used as control units (CO) not receiving any treatment during the study. Harvesting was carried out manually with handsaw once all trees selected for cutting were marked according to the basal area removal criteria. The trees were harvested using a silvicultural criterion to facilitate the natural regeneration, i.e. trees showing disease or physical damage were removed first, followed by the smaller trees and finally by others with larger diameters, to increase the amounts of low- and mid-story shade. Moreover, harvesting was designed to distribute residual overstory canopies as uniform as possible inside every particular harvest plot (H25, H50). The sampling of DBH and crown diameters was carried out in summer 2003, whereas the harvest operations were made in autumn 2003.

Understory vegetation sampling

To sample understory vegetation in each of 12 permanent plots, 20 quadrats of 1 × 1 m were placed using simple random sampling design (Krebs 1999). However, in order to evade edge effect the first 10 m from the plot edge were avoided. In each quadrat, the cover (%) of all vascular plant species present and the number of *P. pinaster* seedlings (criteria = maximum 3-years old) were estimated visually by the same observer in May 2006.

Data analysis

Diversity of understory plant communities was assessed using the Shannon index (H') (Shannon & Weaver 1949) with logs to base 2, and its two components, richness (S) and evenness (J') (Pielou 1969). Shannon diversity and richness were calculated both on two scales, similar to Tárrega et al. (2006): (i) on small scale (per quadrat or m^2), alpha diversity or microcosmic diversity (Magurran 2004); and (ii) on a community scale for each plot (4,900 m^2), plot gamma diversity or macrocosmic diversity (from the joint consideration of the 20 samples carried out for each study plot). Evenness, however, was calculated only on a community scale. By using the comparison of both types of diversity, beta diversity or spatial heterogeneity was calculated: S_β by the Whittaker (in Magurran 2004) formula, $S_\beta = (S/S_\alpha) - 1$, and H'_β as the difference between H' and the average of H'_α (Margalef 1972). The number of *P. pinaster* seedlings is referred to the total number of seedlings in the 20 quadrats of each plot.

To evaluate the significance of different harvest treatments, relative to a controls, on the number of *P. pinaster* seedlings, functional groups cover (annual herbs, perennial herbs and woody species), species richness (S), evenness (J') and diversity values (H'_α, H', H'_β, S_α and S_β), one-way analyses of variance (ANOVA) were applied followed by Tukey's HSD tests to enable pairwise comparisons of means ($p < 0.05$). In all cases, the inspection of residuals was carried out to check for normality and homoscedasticity. Nevertheless, when variables not meet normality and variance assumptions data were transformed using arcsine squared-root transformation for binomially distributed variables (i.e. plant cover) and squared-root transformation for count data (i.e. richness) (Zar 1996).

In order to determine possible relationships among the 13 variables analyzed, a Pearson's correlation matrix was constructed considering: canopy cover, basal area, number of *P. pinaster* seedlings, number of woody species, number of perennial and annual herbs and J', H'_α, H'_β, H', S_α, S_β and S. A Principal components analysis (PCA) was used to summarise the relationships among treatments and the variables as a whole. Data for the 13 variables used in PCA were standardized prior to analysis to correct for different measuring units.

Results were expressed as mean ± standard error and all statistical computations were implemented in the R software environment (version 2.7.0; R Development Core Team 2008).

Results

P. pinaster seedlings

The density of *P. pinaster* seedlings found in the plots was lower than 3.3 seedlings/m^2, however significant differences among harvest intensities were found ($F_{[3,8]} = 23.4$, $p < 0.001$; Fig. 1). Untreated control plots (CO) showed the greatest number of seedlings (66 ± 13.5), clear cut plots (CC) the lowest (1 ± 0.58), and H25 and H50 treated plots an intermediate number of them (16 ± 8.5 and 8 ± 1.8, respectively).

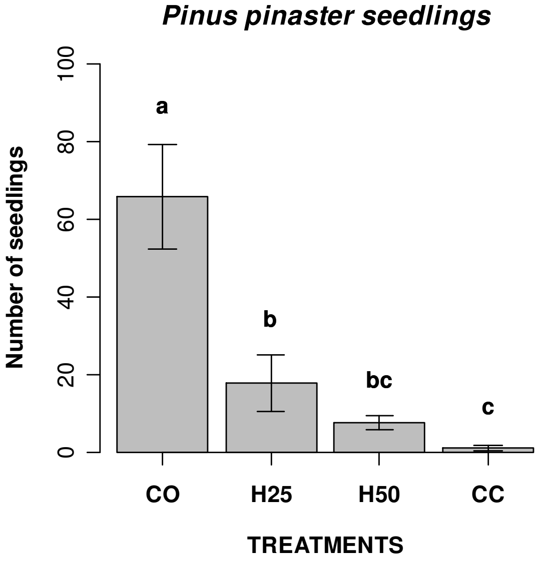

Figure 1. Comparison of the number of *P. pinaster* seedlings (per 20 m^2) among treatments (mean ± SE). CO: control plots; H25: 25% of basal area removed (close plots); H50: 50% of basal area removed (open plots); CC: 100% of basal area removed (clear cut). Different letters above the bars indicate significant differences ($p<0.05$) by Tukey's test.

Functional groups (life forms)

Annual species dominated, in number and cover, the understory plant communities in the four treatments (Fig. 2). Annual cover was similar in the tree treated areas (H25, H50 and CC), ranging between 37 and 41%, and significantly greater than in the untreated CO ($F_{[3,8]}$ = 16.59, p<0.001; Fig. 2a). On the contrary, the cover of woody species was significantly greater in the CO than in the CC and open plots (H50) ($F_{[3,8]}$ = 7.01, p = 0.013), where it hardly reached a 2%. The cover of perennial herbs only differed between the CC and open plots (H50) ($F_{[3,8]}$ = 5.36, p = 0.026).

Annual species number significantly varied with harvest intensity. Clear cut plots (CC), with maximum values (41 ± 1.78), followed by open plots (H50; 31 ± 0.33) showed significantly greater values than control plots (CO) ($F_{[3,8]}$ = 23.21, p<0.001; Fig. 2b). Perennial species number was also significantly greater in the clear cut plots (CC) than in the rest ($F_{[3,8]}$ = 7.3, p = 0.011; Fig. 2b), whereas the number of woody species did not differ with harvest intensity ($F_{[3,8]}$ = 0.58, p = 0.647).

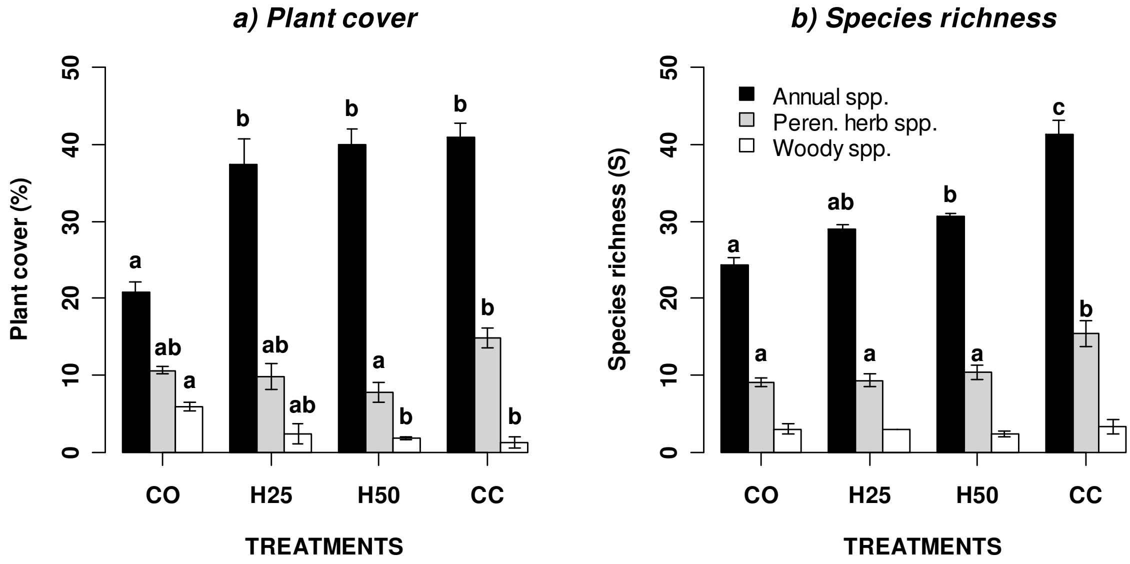

Figure 2. Comparison of annual, perennial herbaceous and woody species cover and richness among treatments (mean ± SE). See Methods or Figure 1 caption for treatment description. Different letters above the bars indicate significant differences among treatments (p<0.05) by Tukey's test.

Richness and diversity

The small scale richness (S_α) varied between 12 and 17 species/m^2 in control and clear cut plots, respectively, but not differed significantly among treatments ($F_{[3,8]}$ = 2.54, p = 0.130; Fig. 3a). In contrast, species richness on a community scale (S), which ranged between 37 and 62 species per treatment, showed significantly greater values in clear cut plots (CC) than in the remainder ($F_{[3,8]}$ = 16.86, p < 0.001; Fig. 3a). In spite of that, Shannon diversity index, which showed high values in the four treatments (H' always above 4.2; Fig. 3b), did not differ significantly with harvest intensity ($F_{[3,8]}$ = 0.28, p = 0.835), due to a reduction of evenness, though not statistically significant, in the treated plots (H25, H50 and CC) in comparison with controls (CO; Fig. 3d). There were no significant differences in spatial heterogeneity among harvest intensities (Fig. 3c).

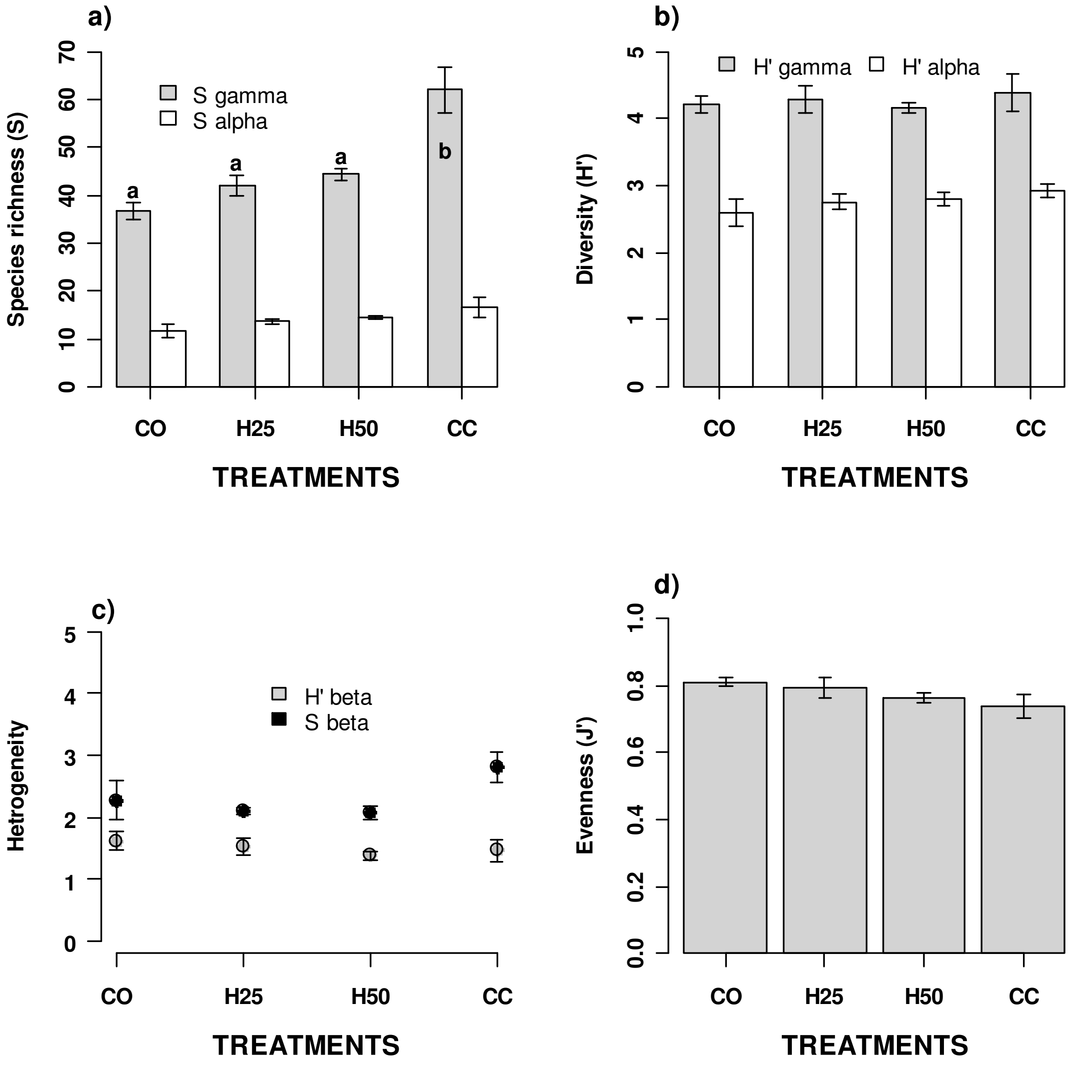

Figure 3. Comparison of different richness (a), Shannon diversity (b), heterogeneity (c) and evenness (d) values among treatments (mean ± SE). See Methods or Figure 1 caption for treatment description. Different letters above the bars indicate significant differences among treatments ($p<0.05$) by Tukey's test.

Relationship between variables

The correlation analysis carried out to determine the relationship among the 13 variables analyzed (Table 1) showed that basal area, canopy cover and *P. pinaster* seedlings were negatively correlated with different richness values (S, S_α and S_β), as well as to the number of perennial and annual herbs. However, in general those parameters were not correlated with diversity values (H', H'_α, H'_β). The Shannon diversity (H') showed a significant positive correlation with H'_α, H'_β and J', and with the number of perennial herbs and woody species. The number of *P. pinaster* seedlings was negatively correlated with annual species number and positively with basal area.

The PCA performed for the joint comparison of all the variables produced an ordination of plots with the two first axes accounting for 78% of the total variance. The first component explained 55% of variance and was strongly positively correlated with S, S_α, H'_α, and number of perennial and annual herbs, on the contrary it was strongly negatively correlated with basal area, canopy cover and number of *P. pinaster* seedlings (Table 2). The second component explained an

Table 1. Pearson correlation matrix between richness, diversity, functional groups richness (As: annual species number; Ps: perennial species number; Ws: woody species number), number of *P. pinaster* seedlings (Pp), canopy cover (Cc) and basal area (Ba). In bold type significant correlations at $p<0.05$.

	S_γ	S_α	S_β	H'_γ	H'_α	H'_β	J'	As	Ps	Ws	Pp	Cc	Ba
S_γ	1												
S_α	**0.85**	1											
S_β	0.41	-0.13	1										
H'_γ	0.53	**0.71**	-0.20	1									
H'_α	**0.60**	**0.83**	-0.30	**0.66**	1								
H'_β	0.06	0.04	0.05	**0.60**	-0.21	1							
J'	-0.32	0.00	-0.57	**0.63**	0.15	**0.66**	1						
As	**0.94**	**0.72**	0.50	0.26	0.52	-0.21	-0.57	1					
Ps	**0.92**	**0.79**	0.37	**0.59**	**0.59**	0.14	-0.19	**0.79**	1				
Ws	0.46	0.54	-0.07	**0.72**	0.33	**0.58**	0.42	0.28	0.36	1			
Pp	**-0.67**	**-0.73**	0.01	-0.26	**-0.65**	0.35	0.38	**-0.72**	-0.55	0.03	1		
Cc	**-0.83**	-0.52	**-0.69**	-0.19	-0.29	0.06	0.55	**-0.86**	**-0.79**	-0.12	0.50	1	
Ba	**-0.86**	**-0.59**	**-0.61**	-0.19	-0.40	0.18	**0.58**	**-0.90**	**-0.81**	-0.10	**0.60**	**0.98**	1

additional 23% and only showed positively correlation with diversity (H' and H'_β), evenness (J') and woody species number (Table 2). In the ordination diagram, the first axis ordered the sites according to their treatment, increasing harvest intensity from the left to the right hand (Fig. 4). Controls (CO) were located on the left hand associated with greater basal area and lower richness. Close plots (H25) were located near controls and open plots (H50) in intermediate position. However, clear cuts (CC) appeared on the right hand without basal area and greater species number. The second axis was related to diversity gradient, increasing diversity, evenness and woody species number to the positive end, and produced a separation between plots within the same treatment.

Table 2. Correlation coefficients of plot scores along axes 1 and 2 and the 13 variables used in the principal components analysis (PCA). In bold type significant correlations at $p<0.01$.

	Axis 1	**Axis 2**
S_γ	**0.98**	0.12
S_α	**0.82**	0.46
S_β	0.43	-0.55
H'_γ	0.45	**0.87**
H'_α	**0.61**	0.46
H'_β	-0.06	**0.65**
J'	-0.41	**0.87**
Annual species number	**0.96**	-0.16
Perennial herbs species number	**0.90**	0.18
Woody species number	0.32	**0.72**
Number of *Pinus pinaster* seedlings	**-0.74**	0.04
Canopy cover	**-0.88**	0.28
Basal area	**-0.93**	0.29
Eigenvalues	8.17	3.47
Explained variance	55%	23%

Discussion

The results illustrate that three harvest treatments applied over a natural stand of Maritime pine in Spain influenced on species richness, annual herbs and woody species cover, and reduced the number of *P. pinaster* seedlings. These results were in agreement with previous studies that have documented how overstory alterations conditioned the post-disturbance response of understory vegetation (Ramovs & Roberts 2003).

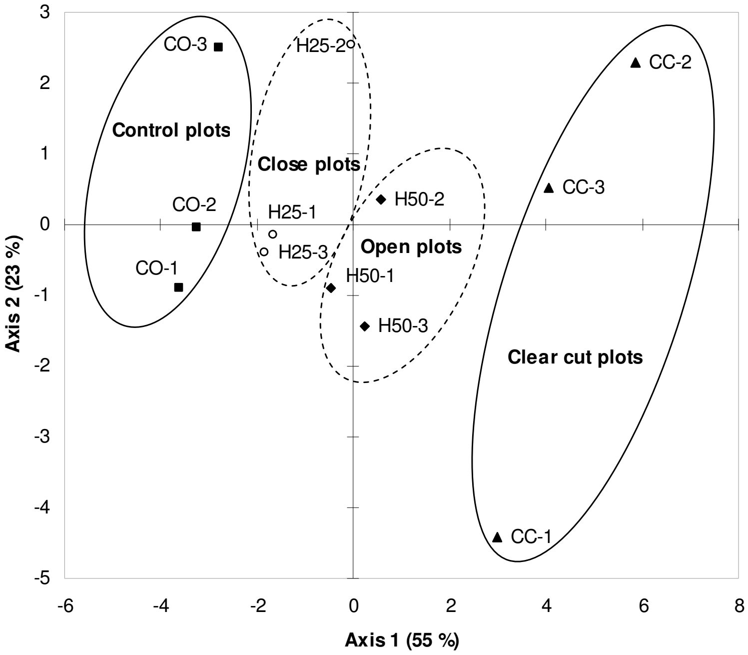

Figure 4. First two axes of the PCA ordination of different harvest intensity plots. See Methods or Figure 1 caption for treatment description. The number after the treatment abbreviation indicates the number of replicate.

P. pinaster seedlings

An important result was that the three harvest intensities reduced the number of *P. pinaster* seedlings in comparison with control plots; thus the first hypothesis is accepted. The reduction in the number of established seedlings from control to clear cut plots was correlated positively with basal area and negatively with annual species number. Therefore, this reduction may be caused by a combination of factors: (1) a decrease of seed inputs caused by the elimination of trees in treated plots in comparison with controls; (2) a reduction of canopy cover, which undoubtedly changed understory microclimate (Aussenac 2000), increasing the radiation intensity during summer and reducing the water availability for seedlings and their viability (Castro et al. 2004; Gómez-Aparicio et al. 2005; Calvo et al. 2008) and (3) competition for water and nutrients between coniferous seedlings and annual species (Peltzer et al. 2000), since annuals were able to dry up the upper soil layer leading to seedling mortality, especially during the early period of seedling development (Sternberg et al. 2001). Indeed, those factors could be highly emphasized by the intense summer droughts detected in the study area at 2004–2006 periods. Especially over treated plots, because temperature and moisture stress are lower in the presence of an overstory cover (Pérez & Moreno 1998; Aussenac 2000). The relative importance of each of these possible explanations required further investigation.

In any case, the density of seedlings found in this stand three years after harvest is very low, even in controls (3.3 seedlings/m^2), compared with 8 seedlings/m^2 recommended to ensure natural regeneration (Luis-Calabuig et al. 2002). Therefore, artificial reintroduction of seeds or seedlings may be a suitable option to increase the seedling density (Pausas et al. 2004), with the objective of facing up to the survival loss caused by inter-specific competition (Eshel et al. 2000), and water availability by summer droughts (Gómez-Aparicio et al. 2005), which would become normal in this area in near future as a consequence of climate change (Intergovernmental Panel on Climate Change (IPCC) 2007).

Functional groups (life forms)

The harvest treatments, in comparison with controls, influenced the richness of annual and perennial herbs and plant cover of annual herbs and woody species, thus the second hypothesis is partially accepted.

Different studies have reported that harvesting increases potential growing space in the understory (Newmaster et al. 2007), and the relative availability of resources (Fredericksen et al. 1999), especially light (Zenner et al. 2006), improving the conditions for establishment of early colonizer species (Newmaster et al. 2007). Not surprisingly, our results provided similar patterns, with an increase of annual species richness and cover along the harvest intensity gradient (from controls to clear-cuts).

In these semi-arid Mediterranean forests, with three months of summer drought, harvesting generates habitats with a strong seasonal stress and with understory vegetation dominated by annuals. Under these conditions, perennial species establish themselves with difficulty compared to annuals whose life cycle is adapted to this seasonal stress (Madon & Médail 1997). At the same time, and as we said previously, the pine seedling establishment may be reduced by the great cover development of annual species through inter-specific competition (Eshel et al. 2000).

On more disturbed plots (clear-cut), with greater solar radiation intensity during summer, species richness of perennial herbs showed greater values than on control plots. This may be caused because the new established species were characteristic of Mediterranean open sites (e.g. *Cynodon dactylon* or *Armeria arenaria*), in accordance with previous research findings in recent clear-cut stands (Roberts & Gilliam 1995; North et al. 1996).

Woody species showed an opposite pattern, maintaining their species richness and decreasing in cover along the harvest intensity gradient (from controls to clear-cuts). Woody species were more abundant in sites with higher tree cover, as in control and close (H25) plots, than in clear-cut and open (H50) plots. It is possible that the partial shade provided by trees may alleviate the harsh environmental factors prevailing under full-sun environments (Alrababah et al. 2007), enhancing the woody species growth. However, under the most severe treatments, although woody species richness was similar to control plots, the physical destruction of existing woody species by the harvest operations (Newmaster et al. 2007), linked to the marked seasonal stress may cause their cover reduction.

The different responses of annual and perennial herbs, and woody species richness along the harvest treatments supports the hypothesis of Peet's (1978), who found different response patterns of plant species richness for different structural groups (woody and herbs).

Richness and diversity

The influence of harvest is clear only in the case of richness; therefore the third hypothesis is partially accepted. Three years after harvesting, understory plant richness was higher in treated plots than in controls, although differences were significant only for the most severe disturbance treatment (clear-cutting). At the same time, plant richness had negatively significant relationship with basal area, suggesting an increase in richness as harvest intensity increases, as observed in similar studies in temperate forest (Fredericksen et al. 1999; Götmark et al. 2005; Zenner et al. 2006). Harvesting increased species richness because of the colonization of annuals and some perennial herbs (Swindel et al. 1983; Götmark et al. 2005), which were favored by the modification of the stand habitat-conditions (Jobidon 1990).

Despite the positive influence of harvesting on species richness showed in this study, no differences with control plots on the understory Shannon diversity values were found, as in other studies in temperate forest (Gilliam et al. 1995; Gilliam 2002; Krzic et al. 2003). The relative high Shannon diversity values reached under all treatments indicated that plant communities after harvesting were not dominated by just a few species (Krzic et al. 2003). On the contrary, these results did not suggest that an increase in harvest intensity did not influence the understory species layer. Peltzer et al. (2000) found that plant diversity did not change when increasing the intensity of silvicultural disturbances, but a higher number of herb species appeared. These results are consistent with our findings of increasing annual and perennial herbs richness with harvest intensity. The Shannon index (H') is affected by species richness and evenness (Westman 1990). As previously explained, richness increased as harvest intensity increases, whereas evenness decreased, resulting in no changes in the Shannon diversity index (H'). This suggest that in control plots the relative abundance of species is more similar than in treated plots (H25, H50 and CC), in which some of new species tend to be relatively uncommon or rare (Small & McCarthy 2002).

The separation between different harvest treatments was clearly connected with basal area, canopy cover and *P. pinaster* seedlings reduction, and with the increase of richness (S and S_α) and herbs richness (annual and perennial). This indicates that the elimination of tree cover favoured the establishment of new herbs species, which increased their cover by the addition of more species, rather than by the growth increase of a few of them (Gilliam 2002). In contrast, diversity (H' and H'_β), evenness and woody species number were related with differences between plots of the same treatment, rather than with differences between harvest treatments. These results emphasize the difficulty in making general conclusions of the effects of harvest treatments (disturbances) on diversity, supporting the conclusions of Gilliam (2002) and Tárrega et al. (2006).

Conclusions

Our results emphasize that the tree harvest treatments assessed are not suitable for the management of this *P. pinaster* stand. Otherwise, the reduction of pine seedling density and the changes in richness and cover of functional groups by harvest treatments would not induce the natural regeneration of this stand, maintaining the understory plant layer. Managers must realize that even controls would have problems to ensure natural regeneration, because the pine seedling density reached in three years is not enough to guarantee it. Therefore, further investigations are needed to assess seedling establishment limiting factors, the effectiveness of reintroduction of pine seeds or seedlings and other silvicultural alternatives (i.e. single tree selection or nurse plant strategies) to achieve adequate management practices, including wood production, with respect to ecosystem functioning.

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Appendix II

Influence of harvesting intensity on the floristic composition of natural Mediterranean Maritime pine forest

Josu G. Alday, Rob H. Marrs, Carolina Martínez-Ruiz & Felipe Bravo

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Abstract

Understorey plant species composition is an important part of forest ecosystems and its conservation is becoming an increasingly frequent objective in forest management plans. However, there is a lack of knowledge of the effect of timber harvesting on the characteristic understorey species in the Mediterranean region. We investigated the effects of three different harvest intensities on the short-term dynamics of understorey vegetation in a natural Maritime pine forest in Spain, and compared the results with uncut controls. Clear-cutting induced both qualitative and quantitative differences with respect to the controls, but intermediate levels of harvesting (25% and 50% removal) induced only quantitative differences. Harvesting reduced the frequency and cover of 56% of characteristic forest species, but only 22% showed an increase. Of the most abundant plant families only the *Fabaceae* showed a significant response with respect to harvesting intensity. Our findings suggest that Light- and Medium-harvest regimes are better management options than clear-cutting if the aim is to conserve the understorey vegetation.

Key words: Anthropogenic disturbance; herbaceous layer; ISA=Indicator species analysis; silviculture; species response.

Nomenclature follows Aizpuru et al. (1999) and Castroviejo et al. (1986–2009).

Introduction

Disturbance plays an important role in structuring natural communities, as it is likely to influence both community composition and population persistence (Vandvik et al. 2005). Forest management treatments, especially harvesting, are examples of a large-scale disturbance that could be expected to impact on species composition and forest structure (Bengtsson et al. 2000). As there is now increasing societal demand to obtain multiple outputs from silvicultural systems, where timber production and the conservation of woodland understorey species are integrated (Kimmins 2004), there is a need for developing harvesting protocols that maintain woodland species composition (Burke et al. 2008).

The changes in environmental conditions and habitat loss provided by silvicultural treatments (as clear-cutting or shelter-wood; Cavallin & Vasseur 2009) influence the distribution of forest herbaceous species leading to differences in species composition, especially in pine forests of Mediterranean region (Torras & Saura 2008). Disturbance-sensitive species may be unable to recover after harvest, and they may decline, or even become locally extinct; this decline has been demonstrated in both deciduous and conifer harvested forests in Canada (de Graaf & Roberts 2009). Knowledge of the response of the understorey layer to different harvest disturbances is, therefore, an important requirement for developing sustainable forest management practices, which are integrated with conservation programs (Kimmins 2004; Uytvanck & Hoffmann 2009).

Pinus pinaster Ait. (Maritime pine) is a natural forest species in the western Mediterranean basin, distributed mainly through the Iberian Peninsula, France and Italy (Alía et al. 1996). In central Spain, this natural forest has been traditionally managed mainly for resin production, with timber extraction as a secondary objective (González-Alday et al. 2009). Currently, resin extraction is low and, coupled with the low economic value of the timber; the management policy has been re-focussed towards the development of multi-functional forests, where there is an increased emphasis on preserving the structural and functional attributes of the ecosystem for nature conservation purposes (Leone & Lovreglio 2004). Management strategies are, therefore, being developed to consider commercial activity (resin and timber) in conjunction with maintaining landscape and ecological protection functions. These new functions included carbon stocks (Bravo et al. 2009), mushroom production and the conservation of understorey species (Pérez-Ramos et al. 2008), which must also include the conservation of endemic species, and the singular genetic diversity of *Pinus pinaster* (de Lucas et al. 2009). With the need to regenerate the forest naturally, foresters recognize the necessity for an improved knowledge of the ecology of the understorey vegetation and how it responds to forest harvesting. The use of different tree harvesting intensities as a means of enhancing natural regeneration is one of the management alternatives being considered for implementation.

The inclusion of forest conservation objectives in management strategies requires information about the understorey vegetation performance (D'Amato et al. 2009). However, the response of forest understorey species to forest management practices, and tree harvesting in particular, has not been well-studied (Burke et al. 2008; Torras & Saura 2008). This is especially true for the Mediterranean region, which is a biodiversity hotspot (Blondel & Aronson 1999). The objective of this study was to assess the initial impact (first three years) of tree harvesting intensity on the understorey species composition of a natural Maritime pine forest in semi-arid Mediterranean conditions in Spain. We hypothesized that: (1) the harvest treatments would induce quantitative and qualitative changes in species composition in relation to uncut control areas; (2) the treatments would affect characteristic forest species more than other species, reducing both their cover and frequency; and (3) the treatments would influence the cover of the most abundant families (*Asteraceae*, *Fabaceae*, *Poaceae*, etc). A further aim was to identify indicator species that would be typical of the different harvesting treatments. Ultimately, we want to better understand the forest dynamics and to be able to assess the importance of tree harvesting on understorey component.

Methods

Study area

This study was conducted in a natural, maritime pine forest in Segovia province in Central Spain (41º22'N, 4º29'W). The site is at an elevation of 760 m asl, and experiences a semi-arid

Mediterranean climate with a mean annual temperature of 11.2 ºC, a mean annual rainfall of 461 mm, and a dry period between mid-June to mid-September (M.A.P.A., 1987). The soils have a sandy-siliceous texture and are of Quaternary age (Junta de Castilla y León 1988). The vegetation of the area is dominated by *Pinus pinaster* with some isolated trees of *Pinus pinea* (Stone pine), and a few crop fields (González-Alday et al. 2009). The baseline tree density was 140 stems/ha, and tree age ranged from 80 to 100 years; the current site silvicultural practice is to allow natural regeneration following a shelter-wood system adapted for resin production (González-Alday et al. 2009).

Harvest treatments

Sixteen continuous hectares of natural maritime pine forest with similar abiotic conditions, forest structure and vegetation composition (González-Alday et al. 2009) were selected for study within a 20,000 ha forest. Twelve 70 m × 70 m treatment plots were delimited. To these plots three replicates of four harvesting treatments were applied randomly in autumn 2003. The four treatments were: (1) no harvesting, the experimental control (coded H0); (2) light harvesting (25% of basal area removed, coded H25); (3) moderate harvesting (50% of basal area removed, coded H50); and (4) complete harvesting (100% of basal area removed as a clear-cut treatment, coded H100). A three-step harvesting procedure was adopted: (1) all trees that needed to be felled to achieve the basal area reduction criteria were marked; (2) the selected trees were felled by chainsaw; (3) all harvested timber was removed from the plot. The use of chainsaws to harvest the timber followed by low-intensity removal is common practice in European conservation forestry management, where the aim is to minimise site disturbance. This is the usual practice in the Mediterranean region (Serrada et al. 2008). Accordingly, the disturbance induced in this study will be much less than that produced by clear-cutting operations in intensively-managed forests, where mechanical treatment through the use of harvesters and skidders is practiced.

Sampling the understorey vegetation

The central 50 m x 50 m area within each treatment plot (n=12) was delimited as the sampling area to avoid edge effects. In each sampling area, 20 quadrats (1 m × 1 m) were located randomly and the cover (%) of all vascular plant species was estimated visually by the same observer in May 2006, three years after treatment. Only vascular plants were assessed because the understorey vegetation of these dry xeric woodlands does not have a large bryophyte or macro-lichen component (Oria de Rueda 2003).

Data Analysis

All statistical analyses were implemented in the R software environment (version 2.7.2; R Development Core Team, 2008), using the VEGAN package for multivariate analyses (Oksanen et al. 2008) and the LABDSV package for indicator species analysis (Roberts 2007). Attribution of plant species as characteristic *Pinus pinaster* forest species was based on Ruiz de la Torre (1996).

Multivariate analysis was used to test and quantify the effects of the harvest treatments on the floristic composition of the field layer plant community in the short term. Hypothesis 1 was tested using Permutational Multivariate Analysis of Variance (PMAV); here distance matrices were used to examine and quantify the differences in floristic composition between treatments (Oksanen et al. 2008). Bray and Curtis and Jaccard distance measures were used to test quantitative and qualitative differences, respectively. The overall differences between harvest treatments (α=0.05) and all pair-wise comparisons were tested with respect to the control (H0). Bonferroni correction was used to adjust for the significance level of each contrast (Sokal & Rohlf 1995); here the critical probability level for detecting significance between contrasts was α=0.017.

Detrended Correspondence Analysis (DCA) was then performed to describe the plant community using the cover data for each plant species that were present in more than four sampled quadrats. To aid interpretation the harvest treatments were fitted onto the DCA ordination plot using the VEGAN ENFIT function and 1000 permutations (Oksanen et al. 2008). Standard deviational ellipses of each treatment were then used to illustrate the position of each harvest treatment on the biplots (Oksanen et al. 2008). In order to describe more clearly the response of forest characteristic species to harvest treatments and to help in their classification, response surface models of each species cover values were fitted over DCA ordination results by GAM models using the VEGAN ORDISURF function (Oksanen et al. 2008).

Univariate analysis was also performed with ANOVA and generalized linear models (GLM) to evaluate the significance of harvest treatments relative to controls, on the cover and frequency of characteristic species and most abundant families (Hypothesis 2 and 3). Cover data (%) were transformed (arcsin(√x/100)) before ANOVA to meet normality and variance assumptions (Crawley 2007). ANOVA's were only calculated if the characteristic species was present in more than 50% of the plots. Tukey's HSD tests were used to enable pairwise comparisons of means (α=0.05). The effect of harvest treatments on frequency (the number of quadrats occupied by each species) was modelled using GLM with a Poisson error distribution and a logarithmic link function (Crawley 2007). The model simplification guidelines for count data with categorical explanatory variables of Crawley (2007) were used.

Dufrene-Legendre indicator species analysis (ISA) was then used to determine which, if any, understorey species were representative of a particular harvest treatment (Dufrene & Legendre 1997). This procedure combines information about frequency and abundance of species among treatments and assigns an indicator value (IV), which indicates the affinity of each species to each treatment (0 no affinity and 1 perfect affinity; Roberts 2007). For each species, the significance of indicator value (IV) was tested using a Monte Carlo simulation procedure with 1000 permutations. Only species with an indicator value >0.10, and $p<0.05$ are discussed.

Results

One hundred and seven understorey vascular plant species were encountered in the study. Eighteen species characteristic of the sandy *Pinus pinaster* community (Ruiz de la Torre, 1996), including *Lavandula pedunculata*, *Helichrysum italicum*, *Corynephorus canescens* and *Sedum amplexicaule* were found in uncut controls (H0). The most frequent understorey species (i.e. occurring in more than 80% of the total sampled quadrats) were *Hypochoeris maculata*, *Micropyrum tenellum* and *Vulpia myuros*. Thirteen species, including *Medicago polymorpha, Leontodon taraxacoides*, *Rumex crispus* and *Thymus mastichina* were found only once, and most of these species were in the clear-cut plots (H100).

Effects of harvesting on species composition (Hypothesis 1)

All harvesting treatments influenced species composition significantly relative to the uncut controls (PMAV quantitative data: all contrasts with 1000 permutations; $p<0.012$; Table 1), and accounted for 45% of the variance in the species data. Re-analysis using the qualitative data set showed overall differences between treatments ($F=2.02$, $p<0.001$; Table 1), and accounted for 43% of the variance in the data. However, here only the clear-cut plots showed significant differences from controls.

Table 1. Comparison of tree harvesting intensity on the understorey floristic composition of natural maritime pine communities under semi-arid Mediterranean conditions in Spain using Permutational Multivariate Analysis of Variance with 1000 permutations. Quantitative and qualitative analyses are presented based on different Indices. Pair-wise contrasts with respect to the uncut controls are also shown; significance was assessed (*) after Bonferroni correction with α<0.017.

Type of analysis	Index	Harvest treatment/ contrasts	*F*-value	*p*-value
Quantitative	Bray & Curtis	All treatments	2.16	0.003*
		Control (H0) vs Light harvesting (H25)	1.88	<0.001*
		Control (H0) vs Medium harvesting (H50)	3.02	0.012*
		Control (H0) vs Complete harvesting (H100)	4.05	<0.001*
Qualitative	Jaccard	All treatments	2.02	0.001*
		Control (H0) vs Light harvesting (H25)	1.25	0.308
		Control (H0) vs Medium harvesting (H50)	1.94	0.169
		Control (H0) vs Complete harvesting (H100)	6.35	0.016*

The DCA produced eigenvalues (λ) of 0.52, 0.40, 0.27 and 0.28, and gradient lengths (GL) of 3.95, 4.21, 3.41 and 3.31 for the first four axes respectively. The fit of harvest treatments to this ordination was significant ($p<0.001$). The species plot (Fig. 1a) showed that the overall distribution of species reflected the harvest treatments applied. The biplot coupled with SD-ellipses (Fig.1b) indicated that the four harvesting treatments occupied different regions of the ordination space. The uncut controls (H0) were located on the upper left hand quadrant and contained *P. pinaster* and *Sedum amplexicaule*; the clear-cut treatment (H100) occupied the lower right hand quadrant and contained a great number of species including grassland weeds *Cynodom dactylon*, *Geranium molle* and *Avena fatua*; and the two intermediate harvesting treatments (H25 and H50) were located between these two extremes with considerable overlap. The H25 treatment ellipse contained *Aira caryophyllea* and *Festuca* spp. as distinctive species, whereas H50 contained *Mibora minima*, *Corrigiola litoralis* and *Ornithopus pinnatus*.

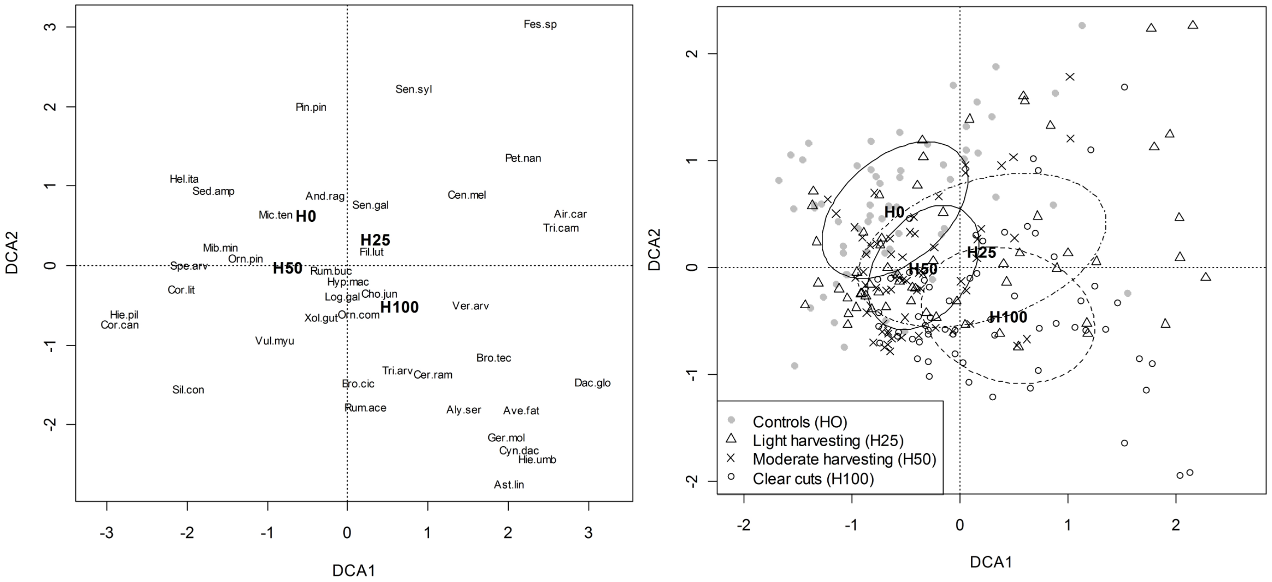

Figure 1. DCA ordination for the first two axes of understorey plant species in natural maritime pine stands under semi-arid Mediterranean conditions in Spain subject to four harvesting intensity treatments. (a) Species in relation to harvest intensity; and (b) plot positions within each harvest treatment with their SD ellipses. Harvest treatment codes: H0=Control; H25=Light harvesting; H50=Medium harvesting; H100=Clear-cut. Species codes are shown in Table 3.

Effects of harvesting on characteristic forest species (Hypothesis 2)

Fourteen species characteristic of *Pinus pinaster* forest showed significant treatment effects on either their frequency or cover. From these results, three types of response to harvest treatments were observed (Table 2) and one example of each type is illustrated in Fig. 2 using plant cover isolines. The three responses types were: (1) Species with a reduced cover or frequency as a result of harvesting. This group contained nine species including both shrub and herbs, e.g. *Helichrysum italicum*, *Lavandula pedunculata, Corynephorus canescens* and *Senecio sylvaticus* (Fig. 2a). (2) Species that maintained their cover and frequency over all the treatments. This group contained four species, but only one showed a significant response (*Andryala ragusina,* Fig. 2b). (3) Species that showed a significant increase in cover or frequency as a result of harvesting. This group contained four herb species, *Hypochoeris maculata*, *Vulpia myuros*, *Xolantha guttata* and *Ornithopus compressus* (Fig. 2c).

Effects of harvesting on the most abundant plant families (Hypothesis 3)

The harvest treatments only showed a significant effect relative to the control on the *Fabaceae* ($F_{[3,8]}$=5.34, p=0.026), where legume cover was greatest in the clear-cut treatment (H100, 18.1±3.2%), intermediate in the H25 and H50 treatments (H25=16.5±4.3%; H50=8.8±1.0%), and lowest in the controls (H0=4.6±0.9). Effects on harvesting on the *Poaceae*, *Asteraceae* and other families (*Caryophyllaceae*, *Geraniaceae, Crassulaceae*) were not significant.

Table 2. The effects of four harvesting intensity treatments on the frequency and mean cover values (± SE) of representative species found in a natural maritime pine communities under semi-arid Mediterranean conditions in Spain. Harvesting treatments codes: H0=Control; H25=Light harvesting; H50=Medium harvesting; H100=Clear-cut. Frequency data were analysed using GLM and cover data were analyzed using ANOVA. Significant differences are indicated by asterisks; letter "a" letter after cover values indicate significant differences vs. control plots (p<0.05) determined using the Tukey HSD test. *Cor. canescens*= *Corynephorus canescens*.

	Frequency					Cover				
	H0	H25	H50	H100	χ^2	H0	H25	H50	H100	$F_{[3,8]}$
Reduced										
Cor canescens	10	4	4		15.01*	0.50±0.18	0.10±0.08	0.12±0.07	-	-
Halimium umbellatum	12	6			28.81*	0.30±0.12	0.07±0.05	-	-	-
Helichrysum italicum	21	10	11	3	18.89*	5.30±1.10	2.20±0.78	2.20±0.70	0.35±0.26a	5.38*
Lavandula pedunculata	1	1	1		1.33	0.42±0.42	0.03±0.03	0.16±0.16	-	-
Malcomia triloba	9	6			22.48*	0.26±0.12	0.33±0.17	-	-	-
Mibora minima	15	2	12		31.85*	0.66±0.22	0.10±0.08	1.05±0.39	-	-
Pinus pinaster	47	19	21	4	73.83*	2.85±0.57	0.65±0.20a	0.29±0.09a	0.26±0.25a	12.38*
Sedum amplexicaule	51	40	40	18	41.76*	8.18±0.84	2.91±0.53a	2.36±0.43a	0.31±0.11a	13.06*
Senecio sylvaticus	22	16	3		50.25*	2.12±0.46	1.06±0.40	0.02±0.17	-	-
Spergularia arvensis	25	10	18	6	19.82*	1.06±0.27	0.31±0.13a	0.50±0.14	0.12± 0.07a	7.00*
Maintained										
Andryala ragusina	24	50	45	26	37.08*	1.24±0.33	1.95±0.31	2.03±0.54	0.21±0.05	1.60
Jasione montana	9	9	14	16	3.96	0.87±0.42	0.27±0.17	0.27±0.13	0.12±0.06	2.73
Lupinus angustifolius	14	13	16	25	7.12	0.92±0.33	1.07±0.35	0.60±0.18	1.30±0.30	0.18
Micropyrum tenellum	37	40	48	36	7.05	3.60±0.80	7.20±1.25	10.12±1.07	3.10±0.72	3.40
Increased										
Hypochoeris maculata	44	43	49	53	6.76	3.32±0.53	1.73±0.32	2.85±0.54	10±1.50a	4.12*
Ornithopus compressus	24	47	44	49	29.44*	1.06±0.27	5.24±0.69	3.22±0.54	5.75±1.03a	10.94*
Vulpia myuros	36	40	49	55	21.19*	6.87±1.28	10.19±1.53	15.40±1.67a	15.30±1.75a	4.36*
Xolantha guttata	13	38	26	25	22.10*	0.36±0.18	2.18±0.47	0.80±0.30	1.80±0.47a	5.59*

Determination of the understorey species associated with the different harvest treatments

The ISA identified six indicator species for the control and intermediate harvesting treatments (H0, H25, H50) and 21 indicators for the clear-cut treatment (Table 3). Therefore, 20% of the total understorey species sampled (n=107) were indicators of the clear-cut treatment, and if the 12 indicator species detected in the H25 and H50 harvest treatments are included, 31% were indicative of a harvest effect.

Species indicative of the control treatment included species which reduced their cover and frequency with harvest intensity, e.g. *Pinus pinaster* seedlings, *Sedum amplexicaule* and *Senecio sylvaticus*. Likewise, both intermediate harvest treatments were identified by two *Pinus pinaster* community species (H25=*Andryala ragusina* and *Xolantha guttata*; H50=*Micropyrum tenellum* and *Mibora minima*). The indicator species of the clear-cut treatment (H100) included a large number of generalists and ruderals (e.g. *Rumex acetosella*, *Erodium cicutarium*, *Avena fatua*, *Cerastium ramossisimum, Geranium molle*), with just three *Pinus pinaster* characteristic species (*Hypochoeris maculata*, *Ornithopus compressus*, *Vulpia myuros*).

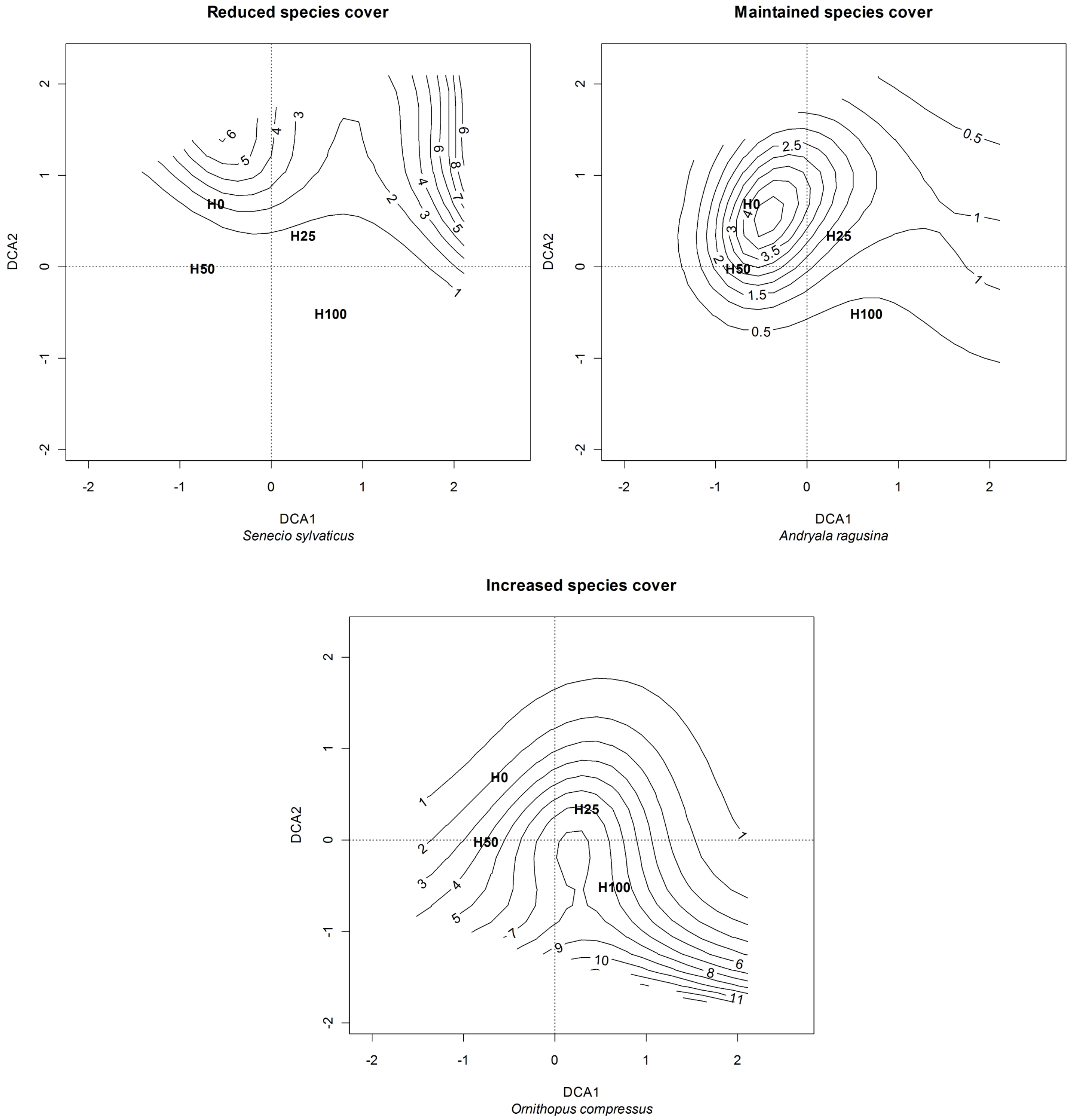

Figure 2. Examples of the three identified responses (Table 2) to harvest intensity treatments in natural maritime pine stands under semi-arid Mediterranean conditions in Spain. The isolines values represent the predicted plant cover of each species fitted by GAM on the DCA ordination of understorey species compositions (Fig. 1). The three responses with respect to harvesting are: (a) Reduction: plant cover isolines decreased their values moving away from H0 (*Senecio sylvaticus*), (b) Maintenance: similar values over the treatments (*Andryala ragusina*), and (c) Increased: plant cover isolines values increased as they approached to H50 and H100 (*Ornithopus compressus*). Harvest treatment codes: H0=Control; H25=Light harvesting; H50=Medium harvesting; H100=Clear-cut.

Table 3. Indicator species in the understorey natural maritime pine communities under semi-arid Mediterranean conditions in Spain for each of the four harvesting intensity treatments. Only species with significant indicator values >0.10 are presented. Late-successional species characteristic of *Pinus pinaster* communities were denoted as (✓), whereas early successional species as (E) and medium-successional species as (M).

Harvest treatment	Species codes	Species	Species Characteristic	Indicator value	*p*-value
Uncut controls (H0)	Pin.pin	*Pinus pinaster*	✓	0.55	0.001
	Sed.amp	*Sedum amplexicaule*	✓	0.51	0.001
	Sen.syl	*Senecio sylvaticus*	✓	0.24	0.001
	Spe.arv	*Spergularia arvensis*	✓	0.22	0.001
	Hel.ita	*Helichrysum italicum*	✓	0.19	0.001
	Cor.can	*Corynephorus canescens*	✓	0.10	0.015
Light harvesting (H25)	And.rag	*Andryala ragusina*	✓	0.30	0.009
	Xol.gut	*Xolantha guttata*	✓	0.27	0.003
	Hie.pil	*Hieracium pilosella*	M	0.25	0.001
	Air.car	*Aira caryophyllea*	M	0.17	0.001
	Fes.sp	*Festuca* sp.	E-M	0.10	0.024
	Dac.glo	*Dactylis glomerata*	E	0.10	0.001
Medium harvesting (H50)	Mic.ten	*Micropyrum tenellum*	✓	0.34	0.001
	Rum.buc	*Rumex bucephalophorus*	M	0.26	0.019
	Orn.pin	*Ornithopus pinnatus*	M	0.19	0.005
	Cor.lit	*Corrigiola litoralis*	M	0.12	0.001
	Mib.min	*Mibora minima*	✓	0.12	0.017
	Log.gal	*Logfia gallica*	M	0.11	0.044
Clear-cut (H100)	Hyp.mac	*Hypochoeris maculata*	✓	0.49	0.001
	Rum.ace	*Rumex acetosella*	E	0.41	0.001
	Sen.gal	*Senecio gallicus*	E	0.36	0.001
	Ero.cic	*Erodium cicutarium*	E	0.33	0.001
	Orn.com	*Ornithopus compressus*	✓	0.31	0.002
	Vul.myu	*Vulpia myuros*	✓	0.29	0.001
	Tri.arv	*Trifolium arvense*	E	0.27	0.001
	Bro.tec	*Bromus tectorum*	E	0.26	0.001
	Cer.ram	*Cerastium ramossisimum*	E	0.24	0.019
	Cho.jun	*Chondrilla juncea*	E	0.20	0.015
	Pet.nan	*Petrorhagia nanteuilii*	E	0.20	0.001
	Cyn.dac	*Cynodon dactylon*	E	0.19	0.001
	Hie.umb	*Hieracium umbellatum*	E	0.15	0.001
	Tri.cam	*Trifolium campestre*	E	0.14	0.007
	Sil.con	*Silene continhoi*	M	0.13	0.017
	Ver.arv	*Veronica arvensis*	E	0.12	0.010
	Aly.ser	*Alyssum serpyllifolium*	E	0.12	0.001
	Ger.mol	*Geranium molle*	E	0.11	0.001
	Ast.lin	*Asterolinon linum-stellatum*	E-M	0.10	0.002
	Ave.fat	*Avena fatua*	E	0.10	0.001
	Cen.mel	*Centaurea melitensis*	E	0.10	0.004
	Fil.lut	*Filago lutescens*	E-M	0.10	0.001

Discussion

Harvesting trees had a significant impact on the short-term vegetation dynamics in the natural maritime pine forests in Spain. Harvesting produced clear changes in the composition of understorey species, and especially on the characteristic species of these forest communities including members of the *Fabaceae*. Disturbance caused by the increasing severity of harvest treatment provide new opportunities for early successional species to colonise and hence for sites to develop differences in species composition and distribution (Burke et al. 2008; Torras & Saura 2008).

Effects of harvesting on species composition (Hypothesis 1)

The understorey species composition was influenced both quantitatively and qualitatively by harvest treatment; therefore, Hypothesis 1 is accepted. The greatest differences in species composition from the controls (H0) were found in the clear-cut treatment (H100). The clear-cut treatment had a much reduced cover and frequency of species characteristic of these woodland communities (Pérez-Ramos et al. 2008), and a much greater abundance of early successional species such as *Avena fatua*, *Erodium cicutarium*, *Geranium molle* and *Rumex acetosella*. The drastic effects of clear-cutting on environmental conditions produced new homogeneous open areas that promoted the colonization of common herbaceous species characteristic of disturbed sites (Torras & Saura 2008; Zang & Ding 2009), with increased light and substrate availability (Burke et al. 2008). Not surprisingly, similar patterns have been documented under different forest types and indeed other ecosystems. For example, in temperate deciduous forest in France selective cutting systems had a deleterious negative impact on true forest species, and increased the dominance of early successional species (Decocq et al. 2004). A similar short-term increase in ruderal species has been described after (a) clearing cork oak forest in the Iberian Peninsula (Pérez-Ramos et al. 2008), and (b) in clear-cut treatments in conifer and deciduous forest in Canada (de Graaf & Roberts 2009). Therefore, whilst this may be a general effect after forest management, it is an important result for the implementation of conservation management in the natural Maritime pine forests in Spain. Clear-cutting, even when implemented sensitively, will produce an undesirable ecological outcome for the conservation of the characteristic understorey species of these ecosystems.

A particularly noteworthy result was that the compositional differences between the control treatment (H0) and the intermediate levels of harvesting (H25, H50) were only quantitative. Two types of change were detected. Firstly, a group of species had a reduced cover after harvesting, these included characteristic species such as *Helichrysum italicum, Sedum amplexicaule*, *Senecio sylvaticus*, *Spergularia arvensis*. Secondly, a group that increased as a result of harvesting, this group included characteristic species such as *Andryala ragusina*, *Micropyrum tenellum, Xolantha guttata*, and *Aira caryophyllea* and *Hieracium pilosella*. Collectively, these results suggest that the light and medium harvesting intensities (H25, H50) are adequate to maintain the species complement in this ecosystem at least for the three years after harvest. Similar results have been found in oak-pine forest in Maine (Schumann et al. 2003). Moreover, as harvest intensity increases there appears to be an increased impact on understorey species composition. Low levels of harvesting (<50%), did not affect the qualitative component of species composition; essentially the forest appears resilient to these partial harvesting intensities. These conclusions mirror those from woodlands in south-western Ontario Canada, after a partial harvest (Burke et al. 2008). However, when the forest was completely cleared there was substantive damage to the species composition with both quantitative and qualitative components affected (Cayuela et al.

2008). Presumably, somewhere between a 50-100% harvest there is a shift towards damaging the ecosystem qualitatively, but we do not know this threshold level yet. Further research is needed to quantify this threshold in more detail.

Effects of harvesting on characteristic forest species (Hypothesis 2)

The harvesting treatments reduced the cover and frequency relative to the control treatment of 56% of the characteristic species, whereas 22% of species either maintained or increased their cover and frequency. Thus, Hypothesis 2 is partially accepted.

The species that were affected negatively by harvesting treatments were mainly the indicator species of control plots (H0), which have an affinity for shade (Aizpuru et al. 1999; Castroviejo et al. 1986-2009). It is reasonable, therefore, to hypothesise that their reduction after harvesting can be attributed to the sensitivity of these species to microclimate change, such as increased light (Suding 2001), a reduction in suitable microhabitats (D'Amato et al. 2008) and competitive displacement by early-successional colonizers (Roberts & Gilliam 2003; D'Amato et al. 2009). It is also possible that the two shrubs *Helichrysum italicum* and *Lavandula pedunculata* were damaged physically during the harvesting operations (González-Alday et al. 2009). These results were in agreement with Decocq et al. (2004) who found a negative impact of silvicultural practices on characteristic forest species in temperate deciduous woodland in France.

In contrast, *Andryala ragusina*, *Jasione montana*, *Lupinus angustifolius* and *Micropyrum tenellum* were not affected by harvesting, possibly because they are better adapted to high light intensities (L-values=7, Ellenberg et al. 1991). However, most of these species had greater frequency and cover in the Light- and Medium-harvest treatment (H25, H50). These results suggest that some form of intermediate disturbance generates suitable canopy conditions and its associated microhabitats for the maintenance of these species (Kimmins 2004). Indeed *Andryala ragusina* and *Micropyrum tenellum* were indicator species of Light harvesting (H25) and Medium harvesting (H50) respectively.

The species that were affected positively by harvesting were *Hypochoeris maculata*, *Vulpia myuros*, *Xolantha guttata* and *Ornithopus compressus*, most likely through a combination of factors. These species tend to require light conditions (L-values >8, Ellenberg et al. 1991) and they appear to thrive in newly-created, dry microhabitats (Ruiz de la Torre 1996; D'Amato et al. 2009). It is also likely that these species are able to disperse over long distances, as they have appropriate dispersal mechanisms such as anemochory and zoochory. It is, therefore, important to consider the life-history strategy of each species involved and any interactions between species and between species and their environment (Cavallin & Vasseur 2009), because impacts on individual species may go unnoticed in community-level analyses (Loya & Jules 2008).

Effects of harvesting on the most abundant plant families (Hypothesis 3)

The harvest treatments, in comparison with controls, only influenced the plant cover of the *Fabaceae*; thus Hypothesis 3 is partially accepted. Many studies on understorey species have shown an increased plant cover after tree removal, as Moore et al. (2006) over Ponderosa pine forest in USA, and especially of *Fabaceae* and *Poaceae* after forestry management in *Pinus pinaster* woodlands in Spain (Pérez & Moreno 1998). Our results for the *Fabaceae* are consistent with this general pattern since legumes cover increased with harvest intensity. The lack of response to harvest treatments of other families (*Poaceae, Asteraceae*, *Caryophyllaceae*, *Geraniaceae*) may be attributable to the short-term period since harvest, which restricted species recruitment (Cadenasso & Pickett 2001), and the response of these other families, which were composed mainly of opportunistic species (Pérez & Moreno 1998), will increase in the longer-term.

Conclusions

For conservation management, the Light- and Medium-harvest treatments (H25 and H50) were better conservation options than the clear-cut treatment, even though all had a significant impact on understorey community composition. In the partial harvest treatments (H25 and H50) most of the characteristic species survive, at least in the short-term. The species that are least able to tolerate disturbance were reduced and this affects the conservation value and regeneration potential of the developing vegetation. If management of these forests includes a conservation objective, it is essential to consider the different responses of understorey vegetation to harvesting in the management planning phase, and the selection of harvesting intensities. Otherwise substantive and expensive restoration action may be needed to return the understorey vegetation to its original state (Duffy & Meier 1992). Clearly, further investigations are needed to assess the longer term effect of these harvest treatments on understorey vegetation, because of the well-know resilience of Mediterranean ecosystems (Pérez-Ramos et al. 2008). At the same time, will be interesting to analyze the exact causes of the different species' responses (plant traits), and the potential value of alternative silvicultural practices, such as single tree selection. Our results point the way for the development of better multi-use forest management strategies, where conservation of biodiversity can be integrated with maintaining the landscape and ecological protection functions of these woodland ecosystems, whilst still producing a sustainable supply of resin and timber.

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