Restoration of gypsum habitats affected by quarrying: Guidance for assisting vegetation recovery

Restauración de hábitats de yesos afectados por la explotación de canteras: Guía para asistir la recuperación de la vegetación

Tesis Doctoral

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Chapter 5

Ballesteros M, Cañadas EM, Foronda A, Fernández-Ondoño E, Peñas J, Lorite J. 2012. Vegetation recovery of gypsum quarries: Short-term sowing response to different soil treatments. *Applied Vegetation Science* **15**: 187-197.

Chapter 6

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Chapter 7

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Chapter 8

Ballesteros M, Ayerbe J, Casares M, Cañadas EM, Lorite J. 2017. Successful lichen translocation on disturbed gypsum areas: A test with adhesives to promote the recovery of biological soil crusts. *Scientific Reports* **7**: 45606.

Summary

Summary

Gypsum soils in drylands support important habitats for conservation of unique specialised flora that must be preserved. Gypsum habitats are destroyed, fragmented and degraded by human activities, and several limitations challenge the recovery of gypsum vegetation. However, the restoration of vegetation after disturbance has only been partially addressed. The aim of this thesis is to study several methods to assist in the recovery of gypsicolous vegetation affected by quarrying in SE Spain under Mediterranean conditions. We assessed habitat current conditions and studied local native plant communities to establish references for restoration, addressed the effect of gypsum on plant development, determined the suitability of various soil treatments and revegetation methods, and explored the potential of lichen translocation to recover gypsum biological soil crusts. We focused on characteristic gypsicolous species included in the habitat of Community interest 1520 'Iberian gypsum vegetation, *Gypsophiletalia*' affected by quarrying in centre-west Granada province (SE Spain). In Chapter 1 we determined the distribution, abundance and response to disturbance of the narrow endemic *O. tridentata* subsp. *crassifolia* to assess its conservation status. Habitat depletion from quarrying, assuming the projected exploitation plan suggests this subspecies should be categorized as Vulnerable and that its recovery and the ecological restoration of altered areas are required. In Chapter 2 and 3 we tested the effect of gypsum at different stages of plant development under controlled conditions, with the final aim of gaining insight into the propagation of a selection of native species for habitat-restoration purposes. Gypsum improved the efficiency in propagation of *O. tridentata* subsp. *crassifolia* and other important species in gypsum habitats. In Chapters 4, 5 and 6 we assessed the suitability of planting and sowing methods on four substrates (raw gypsum, gypsum spoil, topsoil or marls) alone or combined with surface treatments (organic matter addition or organic blanket overlays). Raw gypsum has higher cost but remarkable benefits for gypsophiles, particularly for *O. tridentata* subsp. *crassifolia,* what must be considered in the design of restoration plans. Topsoil increases plant cover, but should not be routinely used to restore gypsicolous vegetation. The conclusion is reached that gypsum spoil is the most recommendable bedding material for the general habitat restoration due to its low cost, wide availability, and satisfactory establishment of target vegetation. Building on this finding, in Chapter 7 we assessed the suitability of three hydroseeding methods to restore gypsicolous vegetation on quarry spoil slopes considering the effect of slope and aspect. Hydroseeding with wood fibre is recommendable in most situations, alternatives being the cheaper but less effective paper mulch on shallow slopes, or the more expensive paper mulch + blanket on steep slopes in case of high erosion risk. Shallow and southern-steep slopes are more suitable for the recovery of gypsum vegetation by hydroseeding, compared to northern-steep slopes where target species are outcompeted by non-target species. In Chapter 8, we tested how a selection of adhesives could improve translocation of a representative gypsum lichen-species to quarry spoils in rainfall-simulation and field experiments. We found making quarry-spoils wet allowed thalli to remain

longer in place after translocation without compromising lichen vitality. This thesis contributes to a better restoration of gypsicolous vegetation affected by quarrying and improves the understanding of plant life on gypsum that will help to develop future programs for the management of gypsum habitats.

Resumen

Resumen

Los suelos de yesos en zonas áridas son a menudo hábitats de interés para la conservación que albergan flora única especializada que ha de ser protegida. Los hábitats de yesos son alterados frecuentemente por la actividad humana y su recuperación puede revestir serias dificultades. Sin embargo, las medidas para restaurar la vegetación gipsícola han sido poco estudiadas. El objetivo de esta tesis es estudiar varios métodos para recuperar la vegetación gipsícola afectada por la minería en condiciones mediterráneas en el sureste ibérico. Para ello, evaluamos el hábitat y sus comunidades vegetales para establecer referencias apropiadas para la restauración, estudiamos el efecto del yeso en el desarrollo de especies autóctonas, determinamos la aplicabilidad de varios sustratos y métodos de revegetación, y exploramos el potencial del método de traslocación para restaurar la costra biológica del suelo. Este estudio se centra en especies características del hábitat de interés comunitario 1520 'Vegetación gipsícola ibérica, *Gypsophiletalia*' afectado por la minería en el centro-oeste de la provincia de Granada (SE de España). En el Capítulo 1 determinamos la distribución, abundancia y respuesta a la alteración del endemismo *O. tridentata* subsp. *crassifolia* y determinamos su estado de conservación. La perdida del hábitat ocasionada por la minería, asumiendo el plan de explotación proyectado, sugieren que esta subespecie ha de catalogarse como Vulnerable, siendo necesaria su recuperación y la restauración ecológica de las áreas degradadas. En los capítulos 2 y 3 evaluamos el efecto del yeso sobre distintas fases del desarrollo vegetal en condiciones controladas, con el objetivo de mejorar la propagación de una selección de especies para restaurar el hábitat. El yeso mejoró el desarrollo de *O. tridentata* subsp. *crassifolia* y otras especies características del hábitat. En los capítulos 4, 5 y 6 evaluamos la idoneidad de los métodos de siembra y plantación sobre cuatro sustratos (yeso bruto, rechazo de yeso, rescate o margas) solos o con tratamientos superficiales (adición de materia orgánica o colocación de mantas orgánicas). El yeso bruto tiene un coste superior pero favoreció notablemente a los gipsófitos, en particular a *O. tridentata* subsp. *crassifolia*, lo que ha de considerarse en el diseño de futuros planes de restauración. El rescate aumenta la cobertura vegetal, pero no debe recomendarse rutinariamente para restaurar la vegetación gipsícola. El rechazo de yeso es en conclusión el mejor material para la restauración general del hábitat debido a su bajo coste, disponibilidad y capacidad para establecer la vegetación objetivo. Basándonos en este resultado, en el Capítulo 7 evaluamos la idoneidad de tres métodos de hidrosiembra para restaurar la vegetación gipsícola en terraplenes de cantera considerando el efecto de la pendiente y la orientación. La hidrosiembra con fibra de madera es recomendable en la mayoría de las situaciones, siendo el mulch de pasta de papel una alternativa más económica pero menos eficiente en pendientes suaves, o el mulch de pasta de papel + manta orgánica más caro pero útil en pendientes fuertes con riesgo de erosión. Las pendientes suaves en general y las fuertes con orientación sur son más favorables para establecer la vegetación gipsícola mediante hidrosiembra, comparado con las pendientes fuertes orientadas al norte, donde las especies objetivo son desplazadas por especies generalistas. En el Capítulo 8, testamos en campo y en simulaciones de

lluvia en interior cómo una selección de adhesivos podía mejorar la traslocación sobre rechazo de una especie de liquen representativa del hábitat. Descubrimos que humedecer el rechazo permite que los talos permanezcan más tiempo en el lugar de traslocación sin comprometer su vitalidad. Esta tesis mejora el conocimiento sobre la recuperación de la vegetación gipsícola afectada por la minería y contribuirá a desarrollar mejores programas futuros de restauración y manejo de los hábitats de yeso.

Introduction

Introduction

Gypsum soils: Important habitats for plant conservation

Gypsum is a rock and soil-forming mineral composed of calcium sulphate dihydrate ($CaSO_4 \cdot 2H_2O$); (Herrero *et al.*, 2009). Gypsum is originated mainly by chemical precipitation of calcium sulphate in highly saline sea or lake water (Withington and Jaster, 1960; Pérez-López *et al.*, 2011). Gypsum-bearing soils cover an estimated area between 1 and 2.7 million km^2 worldwide (Eswaran and Gong, 1991; Boyadgiev and Verheye, 1996); (Fig 1, left). These soils are mainly in arid and semi-arid climates where low rainfall prevents the leaching of the gypsum accumulated (Porta, 1998). Gypsum soils are especially important in North and East Africa, the Mediterranean Basin, the Middle East, SW of Asia, Australia and SW of USA and México (Gil de Carrasco and Ramos-Miras, 2011). The largest gypsum outcrops in Europe are in Spain, occupying between 4.2 and 7.2% of the country mainly in the eastern half (Macau and Riba, 1962; Escavy *et al.*, 2012); (Fig. 1, right).

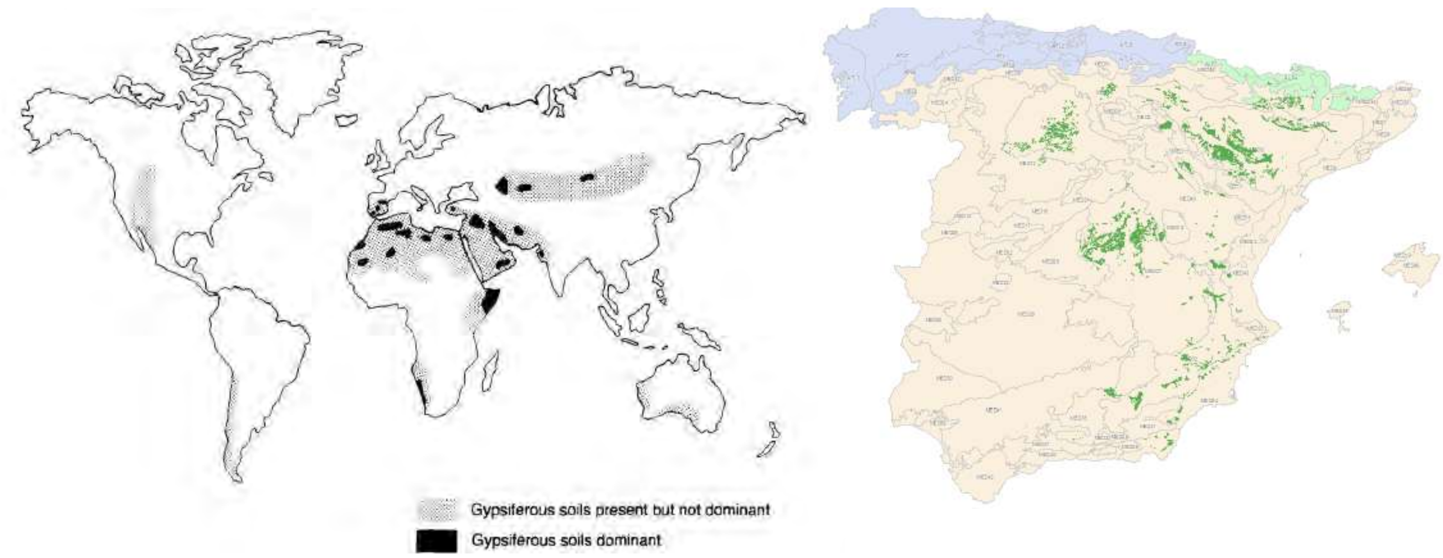

Figure 1. World map of gypsum soils (Boyadgiev and Verheye, 1996; **left**), and gypsum habitats in Spain (Escudero, 2009; **right**). Gypsum habitats in Spain distribute in the Duero and Ebro basins, the Tagus basin and nearby areas of La Mancha and Madrid, and the Guadalquivir basin and nearby areas in Granada and Almería, spreading towards Murcia and Valencia.

Gypsum soils are often important habitats for plant conservation supporting a highly diverse and unique flora (Parsons, 1976; Meyer, 1986; Guerra *et al.*, 1995; Akpulat and Celik, 2005; Mota *et al.*, 2011a). Gypsum habitats are typically low cover scrubs on gypsum soils in arid and semiarid areas where gypsophiles are abundant (Escudero *et al.,* 2009). Gypsophiles are plants exclusive to gypsum soils (Johnston, 1941; Parsons, 1976) which have a range of strategies to cope with harsh climatic and soil conditions (Mota *et al*., 2011a; Escudero *et al.*, 2015). Plants that grow both on and off gypsum soils are gypsovags (Escudero *et al.*, 2015) and together with gypsophiles are denominated gypsicolous species through this thesis. The stressful conditions in these habitats generally result in poor plant productivity and sparse vegetative cover. Although generally perceived as depauperate, gypsum plant communities typically have high plant diversity and harbour a high proportion of plant gypsum-endemic species, many of them threatened at different spatial scales (Mota *et al.,* 2011a). For example, in Spain, 77 plant species are endemic to gypsum soils, and more than 50% are threatened or endangered (Mota *et al.*, 2011a). Therefore, the species and the habitat that occupy have been considered valuable for conservation at the international, national or regional level (e.g. Gómez-Campo, 1987; European Commission, 1992; Cabezudo *et al.*, 2005; Moreno, 2008). Environmental policies such as the habitat directive 92/43/EEC have considered the Iberian gypsum vegetation as a conservation priority (1520, 'Iberian gypsum vegetation, *Gypsophiletalia*'). However, these habitats are often destroyed, fragmented or degraded due to human activities (Pueyo and Alados, 2007a), quarrying being one of the most drastic causes of disturbance. Quarrying activities generally inflict heavy impact at both landscape and plant community level, leading to soil loss, topographical alteration and vegetation removal (Pulido *et al.*, 2004; Dana and Mota, 2006; Castillejo and Castelló, 2010). Thus, gypsum quarries typify the conflict of interest between mining and conservation (e.g. Mota *et al.*, 2003, 2004; Dana and Mota, 2006).

Plants living on gypsum: A wide field for research

The ecology of plants living on gypsum is under-represented in the scientific literature compared to other edaphic specialists associated to singular substrates (i.e. serpentine, calcareous and saline soils); (Escudero *et al.*, 2015; Palacio and Escudero, 2014). Research on this matter has been conducted with unequal efforts worldwide being Spain, with only 0.3% of the global extent of gypsum soils, responsible of 66% of the available studies (Escudero *et al.*, 2015). Great efforts have been placed on studying the diversity and phytosociology of gypsum plant communities (Rivas-Goday *et al.*, 1956; Braun-Blanquet and de Bolós, 1957; Rivas-Goday and Esteve-Chueca, 1965; Rivas-Goday and Rivas-Martínez, 1968; Rivas-Martínez and Costa, 1970; Lázaro, 1984; Loidi and Fernández-González, 1994; Díez-Garretas *et al.*, 1996; Loidi and Costa, 1997; Dana, 2000; Marchal *et al.*, 2008). Ecological and physiological aspects of gypsicolous species have also been extensively studied (e.g. Escudero *et al.*, 1997, 1999, 2000; Caballero *et al.*, 2003, 2008; Olano *et al.*, 2005; Pueyo *et al.*, 2007, 2008; Pueyo and Alados, 2007a, 2007b; Castillejo *et al.*, 2011; Ochoa-Hueso *et al.*, 2011; Saiz *et al.*, 2014), some with a particular focus on determining the mechanisms behind the strategies of plants to live on gypsum soils (Merlo *et al.*, 1998; Palacio *et al.*, 2007, 2014a, 2014b; Bolukbasi *et al.*, 2015). Biological soil crusts (BSCs), playing a critical role in dryland ecosystems worldwide (Rosentreter and Belnap, 2003), have also received recently attention on gypsum habitats (Gutiérrez and Casares, 1994, 2011; Martínez *et al.*, 1994; Pintado *et al.*, 2005; Maestre *et al.*, 2010, 2011; Castillo-Monroy *et al.*, 2011a, 2011b; Sancho *et al.*, 2016). The distribution of gypsophilous flora has also been studied to inform conservation policies (Martínez-Hernández *et al.*, 2011). Recently, two comprehensive monographies have devoted to the relationship between vegetation and gypsum soils at different levels (Mota *et al.*, 2011a; Escudero *et al.*, 2015). However, despite the great advances made so far in the understanding of plant life in gypsum soils and the ecology of gypsum habitats, few scientific studies have addressed how to recover gypsicolous vegetation after disturbance and restoration measures remain largely unexplored.

Disturbances and limitations for the recovery of gypsicolous vegetation: Quarrying in gypsum habitats

Several natural and anthropogenic disturbances cause the loss, fragmentation and degradation of gypsum habitats, rendering them permanently or temporarily unable to support gypsicolous vegetation. Agriculture, urbanisation, communication networks, quarrying, afforestation, burning, trampling, grazing and climatic factors are the most important sources of disturbance (Matesanz *et al.*, 2007, 2009; Pueyo *et al.*, 2008; Quintana-Ascencio *et al.*, 2009; Cañadas *et al.*, 2010; Mota *et al.*, 2011b; Vicente-Serrano *et al.*, 2012; Chiquoine *et al.*, 2016). The recovery of gypsum habitats after disturbance is slow due to regional and local processes controlled by climate, substrates and landscape structure. Successional patterns on gypsum substrates seem to not differ greatly from other areas under semi-arid-type climate, where primary succession may require several decades to reach the pre-disturbance state (Fowler, 1986; Dana and Mota, 2006). A singularity of gypsophiles is that they are inherently restricted to discrete gypsum “edaphic islands” (Schenk, 2013). These islands are often separated several kilometres within the fragmented landscape by other substrates (e.g. limestones or marls), or by farming and urban developments (Johnston, 1941; Moore and Jansen, 2007; Pueyo and Alados, 2007b). The low connectivity among these islands together with the low dispersal abilities of gypsophiles limit propagule arrival to colonise the disturbed sites (Escudero *et al.*, 1999, 2000; Moore and Jansen, 2007; Martinez-Duro *et al.*, 2010; Schenk, 2013). Together with connectivity and dispersal limitations, vegetation recovery is confronted with other problems inherent to mining sites, making recovery especially challenging (Dana 2000, Mota *et al.*, 2003; Dana and Mota, 2006); (Fig. 2).

Figure 2. Abandoned gypsum quarry in Granada (Ventas de Huelma), Spain.

Gypsum quarrying damages important habitats for plant conservation (Mota *et al.*, 2004, 2011b). Gypsum is an industrial mineral in global demand (Herrero *et al.*, 2013) and its extraction causes drastic environmental changes affecting both vegetation and soil (Pulido *et al.*, 2004; Mota *et al.*, 2011b). Spain is one of the main gypsum producers in the world (Escavy *et al.*, 2012; Herrero *et al.*, 2013). One of the first quarrying steps to ease mineral extraction is to remove the vegetation and topsoil. Often, few unaltered vegetation patches remain as efficient sources of propagules to recolonise the quarried site. In addition, quarrying alters both topography and soil properties, leaving a wealth of newly exposed surfaces (i.e. gypsum bedrock, wastes) unsuitable for plant establishment (Bradshaw, 2000; Pulido *et al.*, 2004). Physical and chemical limitations (e.g. compaction, instability or chemical issues) can make areas barren for decades until these conditions improve. In turn, suitable substrates in the post-quarrying environment can be colonised earlier by generalist species with more efficient dispersal abilities causing priority effects that hinder the establishment of gypsicolous species. The limited understanding of the dynamics of gypsicolous communities makes difficult to envisage successful restoration strategies despite mining companies are often compelled to conduct restoration programs. Thus, restoration efforts must identify and address how these limitations affect vegetation recovery and test whether manipulating site abiotic and biotic conditions can speed up the recolonisation process to arrive at a desired species composition and hence state (Hobbs, 2004).

Restoration of gypsum disturbed areas

Ecological restoration is a process that seeks to 'assist recovery' of a natural or semi-natural ecosystem rather than impose a new direction or form upon it (McDonald *et al.*, 2016). Projects that focus solely on reinstating some form of ecosystem functionality without seeking to also recover a substantial proportion of the native biota found in an appropriate native reference ecosystem would be best described as rehabilitation (McDonald *et al.*, 2016). Approaches to managing gypsum disturbed areas have considered generally two different goals: increasing the natural value of the disturbed site by recovering native plant communities (i.e. restoration), or improving ecosystem productivity or protection against erosion (i.e. rehabilitation; Marqués *et al.*, 2005; Castillejo and Castelló, 2010). Although both goals are complementary, they have rarely been addressed together in restoration programs. Multi-purpose restoration practices ignoring appropriate local reference models have led to inappropriate selection of species to restore gypsum habitats (Matesanz *et al.*, 2007). The occurrence of gypsum substrates associated to some degree of salinity has made the halophyte *Atriplex halimus* be used in the rehabilitation of gypsum areas (Marqués *et al.*, 2005; Castillejo and Castelló, 2010). However, this is not a characteristic species of gypsum habitats and may hinder the establishment of valuable gypsicolous communities (Castillejo and Castelló, 2010; Mota *et al.*, 2011b).

Despite the interest of encouraging the development of gypsicolous communities, few experiments have been designed to direct the trajectory of vegetation change and improve conservation value of disturbed sites. Spontaneous succession in gypsum disturbed environments may be a useful, low-cost restoration tool in many situations (Bradshaw, 1997; Prach and Hobbs, 2008). However, the potential of spontaneous succession is strongly conditioned by site-specific conditions, sometimes proving limited for the restoration of the original species in the medium- to long-term (25-70 years; Dana 2000, Martín *et al.*, 2003; Mota *et al.*, 2003, 2004; Dana and Mota, 2006). Active restoration methods have included common approaches, such as the introduction of native and non-native species by sowing, hydroseeding or planting, in combination with the management of the available substrates (Mota *et al.*, 2004; Matesanz *et al.*, 2007; Castillejo and Castelló, 2010). These substrates normally are spoil materials derived from quarrying involving problems such as instability, compaction or chemical imbalance that, especially under dry conditions, become restrictive factors for vegetation recovery. In addition, despite biological soil crusts are characteristic features in gypsum habitats they are normally ignored in restoration projects (see Bowker, 2007). Both spontaneous succession and active restoration have directly or indirectly reported environmental filtering due to low availability and arrival order of propagules, the soil quality and the interactions between species strongly condition the outcomes of restoration, highlighting the importance of site-specific restoration (Dana and Mota, 2006).

Thesis aim: Assisting the recovery of gypsicolous vegetation disturbed by quarrying

In this thesis, the main aim was assisting the recovery of gypsicolous vegetation affected by quarrying. Throughout this thesis we will analyse several aspects to assist in the recovery of gypsicolous species included in the habitat of Community interest 1520 'Iberian gypsum vegetation, *Gypsophiletalia*', (European Commission, 1992) affected by quarrying in SE Spain under Mediterranean conditions, including: a) the assessment of the local native plant communities to establish appropriate references for restoration, b) the effect of gypsum at the initial stages of plant development, c) the suitability for restoration of various soil treatments and revegetation methods, and d) the potential of lichen translocation to recover gypsum lichenic crusts. The specific objectives of this thesis are addressed in the following chapters as described in Table 1.

Table 1. Thesis aim and specific objectives and chapters in which these are addressed.

Thesis aim: Assist the recovery of gypsicolous vegetation affected by quarrying.	
Specific objectives:	**Chapters**
Assess the conservation status of the narrow endemic *Ononis tridentata* subsp.*crassifolia*.	**1**
Test the effect of gypsum to improve native species propagation.	**2**, **3**
Assess the suitability of various substrate management methods.	**4**, **5**, **6**, **7**
Evaluate the applicability of common revegetation methods (planting, sowing and hydroseeding).	**4**, **5**, **6**, **7**
Establish appropriate references for restoration based on local native plant communities.	**6**
Test the effect of slope and aspect on vegetation establishment.	**7**
Explore the potential of lichen translocation to recover gypsum lichenic crusts.	**8**
Contribute to a better understanding of plants living on gypsum.	**1-8**

One key to improved aims in restoration is to understand the nature of the target (Walker and del Moral, 2003). Restoring an ecosystem requires appropriate reference local models, identifying how the species that occupy the habitat respond to environmental changes, and an understanding of the various outcomes commonly achieved through different restoration methods (Walker and del Moral, 2003; Palma and Laurance, 2015; McDonald *et al.*, 2016). To address the ecological restoration of gypsum habitats is crucial to identify and describe appropriate reference local models to accordingly set restoration goals (McDonald *et al.*, 2016). This is especially relevant when endemic species occurring in the area are affected. Thus, in **Chapter 1** we focused on the narrow endemic *O. tridentata* subsp. *crassifolia* (evaluated as Data Deficient in the Red List of the vascular flora of Andalusia; Cabezudo *et al.*, 2005) restricted to gypsum outcrops in south-east Spain (centre-west Granada province), where its habitat quality is declining because of human activities. We determined its distribution, abundance, response to disturbance due to quarrying, ploughing, overgrazing and afforestation, and finally assessed its conservation status.

Given the close link between vegetation and the soil where it occurs, an important aspect in restoration is to understand how plants respond to the available substrates. Debate exists about how chemical and physical properties of gypsum soils influence plant development, and the effect of gypsum through the life cycle of plants is poorly understood (Parsons, 1976; Merlo *et al.*, 1998; Palacio *et al.*, 2007). Whether gypsum imposes restrictions or have favourable effects at the different life stages of plants has been controversial and needs to be clarified (Duvigneaud and Denaeyer-De Smet, 1966; Ruiz *et al.*, 2003; Herrero *et al.*, 2009). In **Chapters 2 and 3** we determined the effect of gypsum at different stages of plant development under controlled conditions, with the final aim of gaining insight into the propagation of a selection of native species for habitat-restoration purposes in and beyond our study area. In **Chapter 2** we assessed whether gypsum could chemically influence seed germination of 24 taxa occurring in gypsum and limestone substrates grouped according to their ability to inhabit gypsum areas (i.e. gypsophiles, gypsovags, and calcicoles). Three levels of gypsum solution and one control treatment of distilled water were tested. In **Chapter 3** we tested whether gypsum added to a standard nursery growing medium (peat) can improve seedling performance of gypsicolous species (i.e. gypsophiles and gypsovags) and, therefore, optimize the seedling production for outplanting purposes. We used four treatments according to the proportions, in weight, of gypsum:standard peat.

Additionally, to restore an ecosystem, understanding the outcomes achieved through common restoration methods is essential (Palma and Laurance, 2015). However, the applicability of common revegetation methods such as planting, sowing, hydroseeding have not been reported in the scientific literature for gypsum habitats, and whether useful information derived from restoration actions exists, it has remained elusive and largely confined to unpublished private technical reports. In **Chapter 4, 5 and 6**, we determined the suitability under field conditions of planting and sowing on four substrates potentially useful to restore gypsicolous vegetation. The substrates were raw gypsum, gypsum spoil, topsoil on gypsum spoil, and topsoil removal (marls). These substrates were used as bedding materials alone (Chapter 4) or in combination with surface treatments (Chapters 5 and 6). In **Chapter 4** we tested the effectiveness of planting to establish three native gypsophiles identifying the bedding materials that maximized plant performance. In **Chapter 5 and 6** we determined the effectiveness of sowing to establish a wider suite of native gypsicolous species on the same materials, and in combination with soil surface treatments. In **Chapter 5** we evaluated the initial vegetation establishment of the target species in the first year, and in **Chapter 6** we evaluated the effect of soil management on vegetation successional dynamics five years after sowing and compared the outcomes against local plant communities in the target habitat and in an unrestored quarry used as references.

The topography determines the establishment and development of vegetation in gypsum habitats (Pueyo and Alados, 2007b), and is a crucial factor that influences the effectiveness of restoration measures (Alday *et al.*, 2010). In **Chapter 7**, building on the findings of previous chapters, we investigated the suitability of three hydroseeding methods to restore gypsicolous vegetation on quarry spoil slopes testing the effect of slope and aspect.

Another important aspect in gypsum habitats are BSCs, considered one of the most remarkable biotic components in these areas, with lichens being more abundant in late successional stages (Belnap and Lange, 2003; Lalley and Viles, 2008; Gutiérrez and Casares, 2011). Despite BSCs are important in the dynamics and regeneration of dryland ecosystems (Bowker *et al.*, 2010; Maestre *et al.*, 2011), there are hardly any experiences of active management of this component (Bowker, 2007). Limitations such as the slow growth of their components, low reproduction rates, the destruction of propagules and habitat together with the lack of a clear restoration methodology make the recovery of BSCs especially challenging (Belnap and Eldridge, 2003; Maestre *et al.*, 2006; Bowker, 2007; Chiquoine *et al.*, 2016; Zhao *et al.*, 2016). In **Chapter 8**, we explored the restoration of biological lichenic crusts evaluating the translocation of *Diploschistes diacapsis,* a representative species of gypsum lichen communities affected by quarrying. We tested how a selection of adhesives could improve thallus attachment to the substrate (quarry spoil) and affect lichen vitality in rainfall-simulation and field experiments.

The **General Discussion** briefly summarizes the main findings and conclusions, indicating implications of chapters 1 to 8. Limitations of the study and suggestions for further research are considered.

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Material and Methods

Materials and Methods

Study area and target habitat

The study area is in centre-west Granada province (SE Spain), in "El Temple" region (Fig. 1). The area is in the Neogene sedimentary basin of Granada; the dominant materials being marl, clay and conglomerates with lime and gypsum deposited in the late Miocene (Aldaya *et al.*, 1980). The main gypsum outcrops are located in the S of La Malahá, Escúzar and Ventas de Huelma, with other smaller outcrops occurring to the SW (near Bermejales reservoir), N of Granada city (near Alfacar) and to the W (near Cacín). Gypsum habitats distribute mainly between 720-1000 m asl. and have an occupation area of 4.7 km^2 and extension of presence of 337 km^2 (although these might be bigger, Lorite *et al.*, 2011).

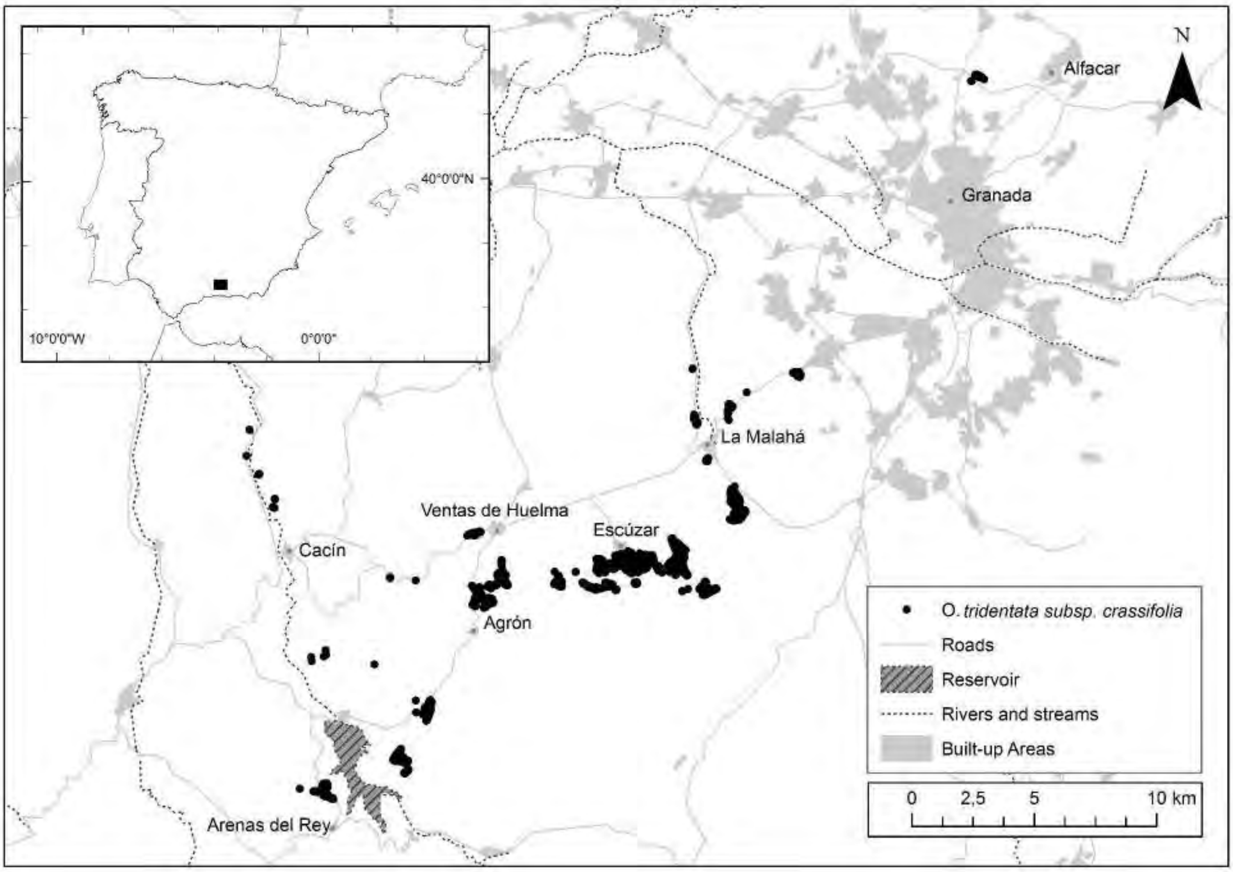

Figure 1. Gypsum habitat in the area represented by presence records of *Ononis tridentata subsp. crassifolia* recorded in our surveys (habitat occupancy is moderately bigger). The rectangle in the inset indicates the location of the main figure in southern Spain.

The climate is continental Mediterranean, with relatively cold winters, hot summers, and four months of water deficit. The mean annual temperature is 15.1 °C, with an average monthly minimum temperature in January of 7.6 °C and maximum of 24.2 °C in August. The annual rainfall, occurring mainly in winter, averages 420 mm. The area is bioclimatically under dry-semiarid mesomediterranean thermotype and biogeographically in the Granadian-Almijarensean sector, Betic province (Valle *et al.*, 2005). The dominant soils in the area are Calcaric Regosols (Aguilar *et al.*, 1992). The dominant soils in the gypsum outcrops are Gypsiric Leptosols (Aguilar *et al.*, 1992; IUSS Working Group WRB, 2015).

The vegetation of the area is a mosaic of fields with cereal crops and olive (*Olea europaea*) and almond orchards (*Prunus dulcis*) and scattered patches of native plants growing over gypsum outcrops. Native plants characteristic in the unaltered area include species exclusive to gypsum soils (gypsophiles) and non-exclusive species of gypsum substrates (gypsovags), and the habitat is described as 1520, 'Iberian gypsum vegetation, *Gypsophiletalia*' (European Commission, 1992), (Fig. 2). Gypsophiles include *Helianthemum squamatum*, *Lepidium subulatum* and the narrow endemic *Ononis tridentata* subsp. *crassifolia* (Fig. 3). Other species in the area with affinity to gypsum soils are *Reseda stricta* subsp. *stricta* and *Frankenia thymifolia* and the therophytes *Campanula fastigiata* and *Chaenorhinum grandiflorum* subsp. *carthaginense* (Mota *et al.*, 2011a). *Gypsophila struthium,* frequent in other gypsum outcrops in Granada (Guadix-Baza basin), does not occur in the study area (Lorite *et al.*, 2011). Gypsovags include *Rosmarinus officinalis*, *Stipa tenacissima*, *Helianthemum syriacum*, *Helianthemum violaceum, Thymus zygis* subsp. *gracilis* (Fig. 3), *Teucrium capitatum* subsp. *gracillimum* and *Coris monspeliensis* (Marchal *et al.*, 2008). Open areas between native plants are often occupied by a well-developed biocrust community characterised by lichens such as *D. diacapsis, Acarospora placodiiformis*, *A. nodulosa* var. *reagens*, *Buellia zoharyi*, *Squamarina lentigera, S. cartilaginea*, *F. desertorum*, *F. fulgens, F. poeltii*, *F. subbracteata*, *Psora decipiens,* and *P. saviczii* (Gutiérrez and Casares, 2011; Ibarz, 2012); Fig. 4).

Figure 2. Gypsum habitat in Granada (Escúzar), Spain.

The phytosociological association *Helianthemo squamati-Ononidetum crassifoliae* Marchal and Lendínez 2008 has been described for these communities (Marchal *et al.*, 2008). When gypsum is less abundant these communities often contact formations associated to the potential domain of basophilic Holm oak forests (*Paeonio-Querco rotundifoliae* S.), such as alpha-grass steppes (*Thymo gracilis-Stipetum tenacissimae* Perez-Raya and Molero 1988) and thyme-scrubs (*Thymo gracilis-Lavanduletum lanatae* Pérez-Raya and Molero 1988); (Marchal *et al.*, 2008). In deposit areas of materials originated by the washing and erosion of the surrounding gypsum hills, these scrubs are replaced by halophyte species (*Soncho crassifolii-Juncenion maritimi* Rivas-Martínez 1984); (Marchal *et al.*, 2008).

Gypsophile species: Plants exclusive to gypsum soils

Ononis tridenta subsp. *crassifolia* (Boiss.) Nyman

Helianthemum squamatum (L.) Dum. Cours.

Lepidium subulatum L.

Gypsovag species: Plants occurring in both gypsum and non-gypsum soils

Helianthemum syriacum (Jacq.) Dum. Cours.

Stipa tenacissima L.

Thymus zygis L. subsp. *gracilis* (Boiss.) R. Morales

Rosmarinus officinalis L.

Figure 3. Target species characteristic in the habitat 1520, 'Iberian gypsum vegetation, *Gypsophiletalia*' used in revegetation experiments (Chapters 4, 5, 6 and 7).

Figure 4. Soil crust community on gypsum soil dominated by the lichen *Diploschistes diacapsis* in a gypsum scrub community in the study area in Granada, Spain.

Experimental area

Our experimental area is next to an active quarry located in Escúzar, Granada (37° 2' 57'' N, 3° 45' 30'' W; 950 m asl).

The plots for planting (Chapter 4) and sowing experiments (Chapter 5 and 6) were built in October 2009 on an area 0.45 ha on a cereal old-field consisting of marls, providing materials derived from the mineral extraction. These materials were chosen based on their potential applicability for restoration. We prepared four flat plots (15 m x 60 m) on the old-field, each consisting of a bedding material (see properties in Chapter 4, Appendix A), as follows:

(1) marls (M) also named topsoil removal (TR) herein, using the site substrate, previously removing the topsoil (c. 30 cm) and thus its seed bank (coming from the pre-existing cereal crop);
(2) gypsum spoil (GS), providing a layer of gypsum mine spoil (0.5 m) derived from gypsum extraction;
(3) topsoil addition (TA), placing a layer of topsoil (ca. 10 cm) retrieved from the natural habitat (after 8 months of stockpiled storage), on top of a gypsum spoil layer (0.5 m);
(4) raw gypsum (RG), consisting of a layer (0.5 m) of coarse gypsum (i.e. the same material used in the factory to be processed).

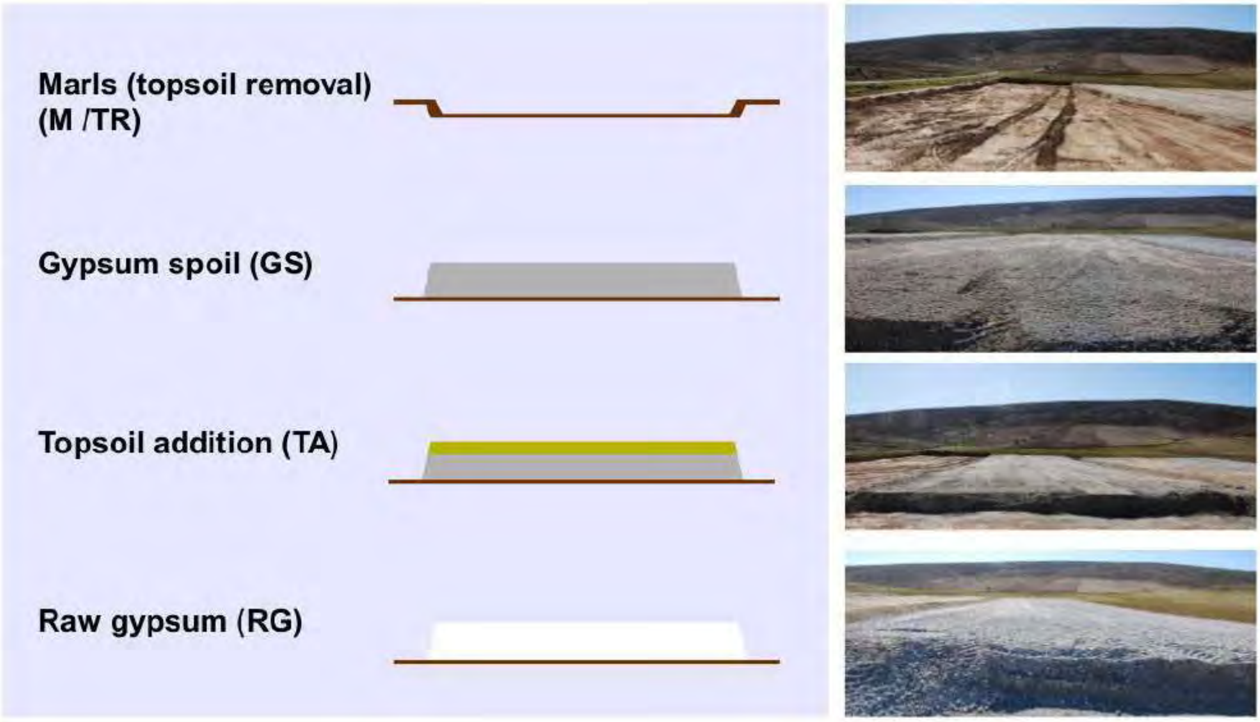

Figure 4. Schematic section and view of the bedding materials used in experiments in Chapters 5, 6 and 7.

Experimental slopes for hydroseeding experiments (Chapter 7) were built in October 2011 on an area of 0.7 ha using gypsum spoil (see properties in Chapter 7, Table S1), derived from gypsum extraction (Fig. 5; left). The plots for the lichen translocation experiment (Chapter 8) were established on a near conditioned flat area of 0.2 ha consisting of bare gypsum spoil as well (Fig. 5; right). This material was chosen on the basis of planting and sowing experiments (Chapters 4, 5 and 6).

Figure 5. View of the slopes experimental area where different surface treatments can be observed (**left**). Gypsum spoil area conditioned for lichen translocation experiments (**right**).

Further information, including details relevant to each chapter of this thesis concerning the study site, target species, experimental design, vegetation sampling and data analyses is presented in the Material and Methods section of each chapter or as Appendices.

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Chapter 1

Conservation status of the narrow endemic gypsophile *Ononis tridentata* subsp. *crassifolia* in southern Spain: effects of habitat disturbance.

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Oryx 47 (2013), 199-202.

Conservation status of the narrow endemic gypsophile *Ononis tridentata* subsp. *crassifolia* (southern Spain): effects of habitat disturbance

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Abstract

Ononis tridentata L. subsp. *crassifolia* (Leguminosae) is a narrow endemic plant restricted to gypsum outcrops in south-east Spain. Its habitat and populations are currently threatened by anthropogenic disturbance. Because of the paucity of information concerning its distribution, abundance and response to disturbance, we assessed its status and evaluated the impacts of quarrying, ploughing, grazing and afforestation. Distribution and population size were estimated by field surveys, censuses and mapping. We measured cover, plant volume, fruit and seed production, seed predation and seedling recruitment to asses any effects of disturbance. The species' area of occupancy is 1.6 km^2 and its extent of occurrence 337 km^2, in 29 habitat patches and 16 populations between 705 and 1,125 m altitude, and its population size estimated as 531,605 individuals. Quarrying, ploughing, overgrazing and afforestation negatively affected the species, in this order. We recommend this subspecies be categorized as Vulnerable following IUCN criteria. A species recovery plan is required, and the ecological restoration of altered areas would mitigate negative effects on the species, improving the overall conservation of gypsum habitats.

Keywords: Conservation status, grazing, gypsophile, gypsum quarrying, habitat disturbance, narrow endemic, *Ononis tridentata*, ploughing

Gypsum outcrops harbour rare and endemic flora restricted to this substrate (Mota et al., 2004, 2011), which is often negatively affected by human disturbances, especially quarrying (Mota et al., 2003, 2004). *Ononis tridentata* subsp. *crassifolia* (Dufour ex Boiss.) Nyman (Leguminosae) is a rare subspecies with a narrow distribution restricted to gypsum outcrops in south-east Spain (centre-west Granada province), where its habitat quality is declining because of anthropogenic disturbance (Ballesteros et al., 2011). The *Ononis tridentata* complex is endemic to gypsum outcrops in Spain and Morocco (Supplementary Fig. S1). Other than taxonomic studies (Devesa & López, 1997) there is little information on the biology, distribution and conservation status of *O. tridentata* subsp. *crassifolia*. It is categorized on the Red List of the vascular flora of Andalusia as Data Deficient (Cabezudo et al., 2005). For species in this category, proper assessment is vital to determine their current situation, often revealing threats involving a genuine change of status (e.g. Good et al., 2006). Following a preliminary assessment as Near Threatened (Ballesteros et al., 2011), the aims of this study were to (1) assess the subspecies' conservation status, (2) quantify the impact of human disturbance, and (3) recommend conservation measures.

The distribution of *O. tridentata* subsp. *crassifolia* was first determined using biodiversity databases, including the Global Biodiversity Information Facility (GBIF, 2008) and Anthos (Castroviejo et al., 2008), herbarium records (GDA and GDAC), and available literature (Devesa & López, 1997; Marchal et al., 2008). Neighbouring areas potentially suitable for the subspecies were identified based on the ecology of known populations and aerial photographs. Field surveys were conducted during 2008–2011. Presence records were mapped, using *Quantum GIS v. 1.7.0* (Quantum GIS Development Team, 2011), to establish the subspecies' known extent of occurrence (EOO) and area of occupancy (AOO; IUCN, 2001). We estimated population size by counting all reproductive individuals in a total of 142 linear transects of 25 x 2 m (50 m^2) throughout the subspecies' known range, and extrapolating average density to AOO. All the information available was then used to assess the status of *O. tridentata* subsp. *crassifolia* using IUCN criteria (2001, 2011).

The impact of habitat disturbance was determined using qualitative data (IUCN/SSC, 2001), available literature (Cabezudo et al., 2005; Escudero, 2009), information on land use (REDIAM, 2008) and earlier observations (Ballesteros et al., 2011). Areas with four types of disturbance were considered: afforestation (areas forested with *Pinus* *halepensis* c. 15 years ago), ploughed land gypsophile shrubland ploughed for cultivation but abandoned c. 15 years ago), topsoil removal (area quarried, with topsoil removed 2 years ago), and overgrazing (by free-ranging goats and sheep). These were compared with apparently undisturbed areas. Three uniform plots per disturbance type were selected (3 plots x 5 treatments = 15 plots), at least 100 m far from each other, avoiding environmental differences between selected plots (aside from habitat disturbance).

Cover was estimated using five intercept point transects of 25 x 2 m (50 m^2), with three contact points per m (75 per transect), in each plot. All reproductive individuals were recorded and recruitment was estimated by counting all seedlings along five transects of 10 x 2 m (20 m^2) per plot. To determine size structure we randomly selected 30 reproductive individuals per plot (a total of 450 individuals) and estimated their volume as the volume of a semi-spheroid (Lorite et al., 2010). To examine the effect of disturbance on the fitness of these selected individuals we counted the number of fruits produced at the end of July. To examine any effects of habitat disturbance on seeds, 10 ripe fruits were collected from 10 individuals per plot (i.e. a total of 1,500 fruits), dissected, and the number of well-formed, aborted and predated seeds counted. Well-formed seeds, by plot, were separated in 5 subsets of 5 seeds and weighed to a precision of ± 0.1 mg, and the mean weight per plot calculated.

We estimated the total number of individuals and the confidence interval by bootstrapping the average density from censuses (n = 10,000, $P < 0.05$) and calculating it for the AOO. To evaluate the effect of habitat disturbance on the plant parameters examined, we fitted Generalized Linear Models, with a normal function for continuous variables, Poisson error distribution and log as a link function on count data, and binomial error distribution and logit as the link function for proportions. For some analyses, we performed a multiple-comparison test with the Bonferroni correction. For all statistical analyses we used *R v. 2.13.0* (R Development Core Team, 2011).

Using c. 20,000 presence records of the species we estimated AOO to be 1.6 km^2 and EOO to be 337 km^2 (Ballesteros et al., 2011). The average density, from censuses (n = 142) and computed by bootstrapping (n = 10,000), was 0.33 m^{-2} (0.29–0.37), at $P < 0.05$). The estimate of the population is at least 531,605 (467,831–584,564 individuals, $P < 0.05$). We located the species in 29 habitat patches, forming 16 populations (defined as a patch or cluster of patches separated by at least 500 m, i.e. a pollinator's foraging distance, Gathmann & Tscharntke, 2002; Fig. 1). Moreover, there is a projected habitat depletion due to quarrying. Assuming a 39.94% of habitat loss in the next 60 years (63.9 ha of projected habitat loss due to the quarrying activity, unpublished Environmental Impact Assessment), it would involve the loss of 212,310 individuals (between 186,840–233,460 individuals, $P < 0.05$).

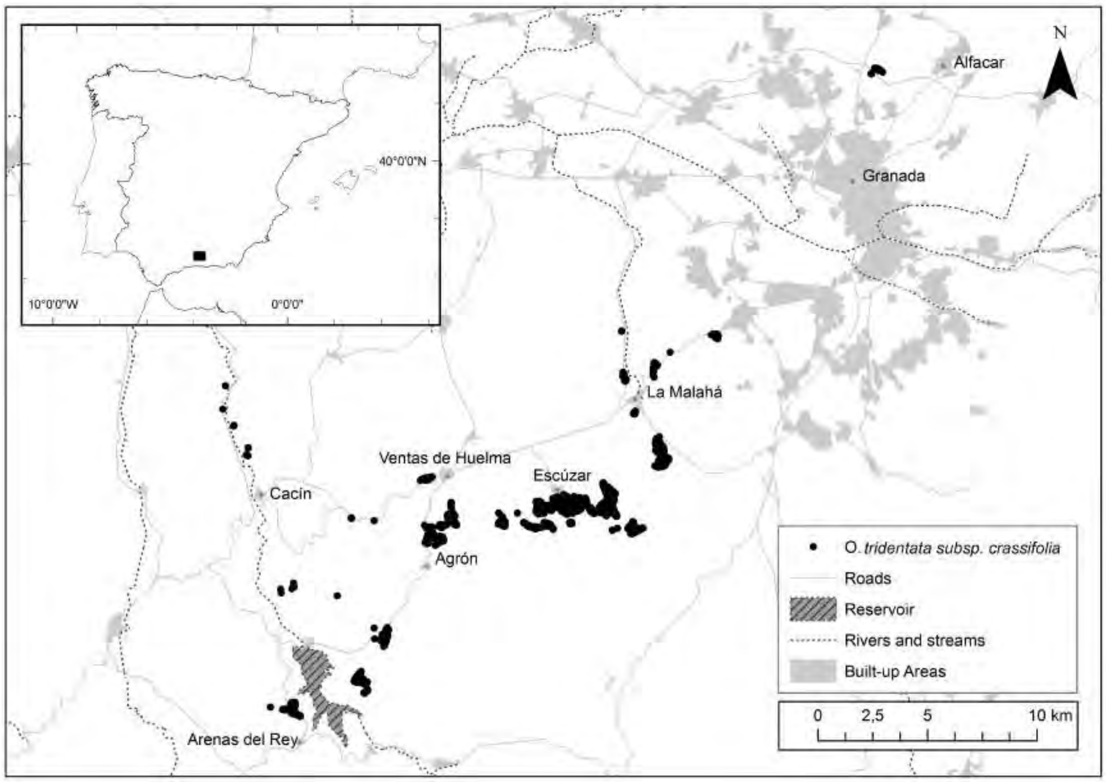

Figure 1. Presence records of *Ononis tridentata* subsp. *crassifolia* recorded in our surveys. The rectangle in the inset indicates the location of the main figure in southern Spain.

Our findings suggest that *O. tridentata* subsp. *crassifolia*, should be categorized as Vulnerable, with criteria A3cd; D2 criteria (IUCN, 2001, 2011): a projected population reduction, a decline of AOO, EOO and quality of the habitat (c), and actual or potential exploitation levels (d), and an AOO of < 20 km^2 (D2).

The results show that all the disturbances examined have negative consequences for the species (Table 1): in descending order, topsoil removal, ploughing, overgrazing, and afforestation. The number of seeds per fruit was similar between areas, although the predation rate differed significantly between areas and was greatest in undisturbed sites. This could be related to vegetation cover (Meiss et al., 2010), which was highest in undisturbed sites. There were no significant differences in seed weight between areas.

Table 1. Parameters evaluated for *Ononis tridentata* subsp. *crassifolia* under different habitat disturbance types (mean values ± SE). Different letters indicate significant differences in the post hoc test after Bonferroni correction at P < 0.05. GLM results; *Df*.: Degrees of freedom, χ^2: Chi-square values, *P*: p-values in bold show significant differences at p<0.05 between disturbance types.

	Undisturbed	Afforestation	Overgrazing	Ploughed land	Topsoil removal	Df	χ^2	P
Cover (%)	15.56 ± 2.71 ^{a}	9.15 ± 1.85 ^{a}	9.66 ± 1.44 ^{a}	2.82 ± 0.52 ^{b}	2.31 ± 0.85 ^{b}	4	242.61	**< 0.001**
Plant volume (dm^3)	135.16 ± 12.92 ^{b}	234.98 ± 32.33 ^{a}	38.56 ± 4.20 ^{c}	95.81 ± 13.52 ^{bc}	29.74 ± 4.67 ^{c}	4	23.25	**<0.001**
Seedling density (m^{-2})	0.07 ± 0.03 ^{ab}	0.32 ± 0.15 ^{a}	0.02 ± 0.01 ^{b}	0.04 ± 0.02 ^{ab}	0.08 ± 0.03 ^{ab}	4	139.09	**<0.001**
Fruit production (%)	63.05 ± 3.22 ^{a}	48.16 ± 3.43 ^{b}	38.89 ± 3.72 ^{b}	41.84 ± 3.92 ^{b}	73.45 ± 4.06 ^{a}	4	1436.74	**<0.001**
Seeds per individual	103 ± 21 ^{ab}	158 ± 32 ^{a}	57 ± 9 ^{b}	68 ± 12 ^{b}	105 ± 16 ^{ab}	4	1488.99	**<0.001**
No. of seeds per fruit	0.94 ± 0.07	1.03 ± 0.08	1.09 ± 0.09	1.05 ± 0.07	1.35 ± 0.08	4	2.23	0.6928
Predated seeds (%)	67.42 ± 3.20 ^{a}	63.69 ± 3.45 ^{ab}	54.31 ± 3.86 ^{ab}	50.31 ± 3.75 ^{b}	36.45 ± 3.02 ^{c}	4	2362.10	**<0.001**
Empty seeds (%)	24.96 ± 3.00 ^{b}	24.02 ± 3.20 ^{b}	29.59 ± 3.64 ^{b}	33.39 ± 3.57 ^{b}	47.57 ± 3.15 ^{a}	4	2119423	**<0.001**
Well-formed seeds (%)	7.62 ± 1.69 ^{b}	12.29 ± 2.30 ^{ab}	16.10 ± 2.74 ^{ab}	16.31 ± 2.72 ^{ab}	15.98 ± 2.19 ^{a}	4	877594	**<0.001**
Mean seed weight (mg)	6.3 ± 0.7	7.2 ± 0.5	6.6 ± 0.5	6.6 ± 0.6	7.3 ± 0.3	4	0.00	1

The major threat to *O. tridentata* subsp. *crassifolia* is habitat destruction and fragmentation because of human activities, as for most narrow endemic species (e.g. Fenu et al., 2011; Peñas et al., 2011). In general all of the disturbances we examined have negative consequences for the species, except afforestation (where the pre-existing individuals were not removed during plantation). In studies of other species afforestation has been found to be detrimental (Maestre et al., 2004). As expected, quarrying and ploughing create the greatest disturbance. Mining has been shown to have great impacts on gypsicolous flora (e.g. Mota et al., 2003, 2004). Livestock may enhance seed germination by digestive tract effects on hard-coated seeds (Baskin & Baskin, 1998) and by eliminating interspecific competition or creating micro niches for seedling colonization, but may injure seedlings by trampling and browsing (e.g. Hobbs, 2001), as we observed in this study.

Our recommendations for the conservation of *O. tridentata* subsp. *crassifolia* are similar to those generally made for plants of gypsum habitats (Escudero, 2009): minimize the risk of direct destruction and fragmentation by controlling human activities and land-use.

Impacts could be reduced by identifying areas with good quality habitat for the species and then designing reserves composed of patches of habitat (Mota et al., 2011). The negative impacts of quarrying necessitate a recovery programme; we have found that the species responds to restoration, either as planting (authors, unpubl. data) or sowing with the appropriate soil preparation (Ballesteros et al., 2012). The habitat of *O. tridentata* subsp. *crassifolia* is included in the EU Habitat Directive (92/43/EEC) as a priority for conservation, and therefore no additional measures are needed in this respect. Our results will be put at the disposal of the regional government and taken into account to develop proper measures to restore the vegetation of gypsum habitats in the area.

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Biographical sketches

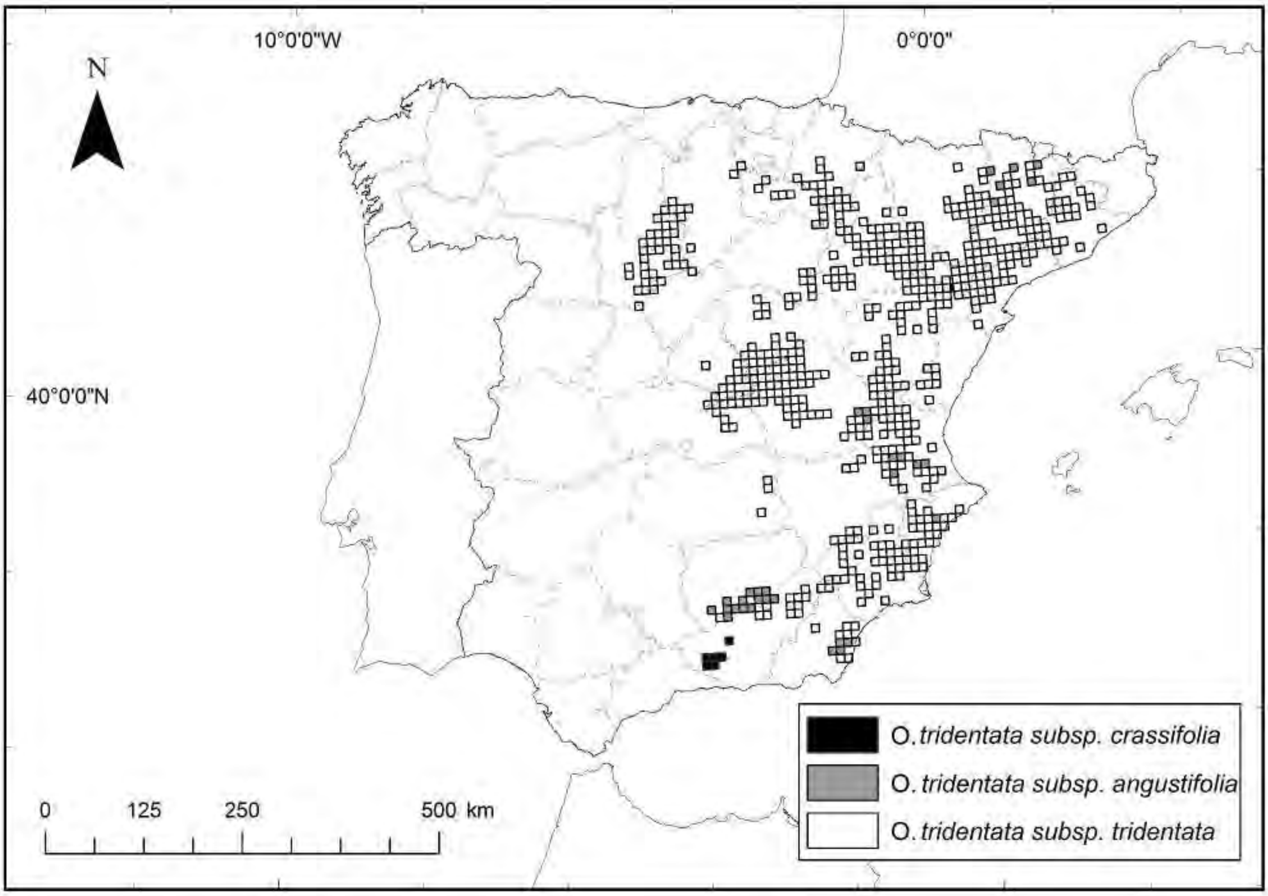
The research of the authors is devoted to the conservation of threatened and endemic flora and the ecological restoration of disturbed areas in Mediterranean environments. Miguel Ballesteros research interests currently focus in developing measures to recover gypsum habitats affected by quarrying in SE Spain. Ana Foronda research interests include biodiversity conservation and ecological restoration in areas affected by quarrying activities. Eva María Cañadas is interested in plant-environment relationships, applied to both flora conservation and habitat restoration. Julio Peñas is interested in biogeographical patterns and conservation of endemic vascular-plants in the Western Mediterranean region. Juan Lorite has worked on conservation biology and restoration ecology, with special focus in high mountain areas. Currently he leads several projects regarding the conservation and restoration of gypsicolous habitats affected by quarrying.

Short Communication

Conservation status of the narrow endemic gypsophile *Ononis tridentata* subsp. *crassifolia* in southern Spain: effects of habitat disturbance

MIGUEL BALLESTEROS, ANA FORONDA, EVA MARÍA CAÑADAS, JULIO PEÑAS and JUAN LORITE

SUPPLEMENTARY Fig. S1 Distribution of the *Ononis tridentata* complex, including *O. tridentata* subsp. *crassifolia*, in Spain, in UTM grid cells of 10 x 10 km^2. Modified from Mota et al. (2011) and completed with information from the GBIF (2008) and Anthos (Castroviejo et al., 2008) databases.



Ononis tridentata subsp. *crassifolia*

Habitat disturbance in the study area caused by quarrying (top left), ploughing (top right), grazing (bottom left) and afforestation (bottom right).

Chapter 2

Does gypsum influence seed germination?

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Does gypsum influence seed germination?

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Abstract

Flora inhabiting gypsum outcrops in arid environments shows a high level of specialization. However, the processes involved are still unclear, specifically at the key stage of germination. Here, to assess whether gypsum could chemically influence seed germination, we tested the germination of species according to three functional groups: gypsophiles, gypsovags and calcicoles. A total of 24 taxa were selected, all occurring in gypsum and limestone substrates, under semi-arid and dry Mediterranean climate. Three levels of gypsum ($CaSO_4 \cdot 2H_2O$) solution (low=0.5 g/l, medium=1 g/l, and high=2.4 g/l) and one control treatment of distilled water were tested. Results depended on the particular species rather than on the functional group. We found that gypsum favored germination in some species (e.g. *Lepidium subulatum* and *Gypsophila struthium*), whereas significant negative effects appeared for only one species (*Rosmarinus officinalis*). In contrast, most of the species studied responded neutrally to gypsum solutions. Our results suggest that chemical features of gypsum could offer an advantage at the germination stage for certain species, rather than posing a constraint for seed germination.

Keywords: Germination rate; gypsum solution; gypsophile; gypsovag; calcicole.

1. Introduction

Gypsum outcrops host specialized flora that appears to be more distinctive in arid and semi-arid regions (Parsons, 1976; Meyer, 1986; Akpulat and Celik, 2005; Mota et al., 2011). Plant species growing exclusively in gypsum soils are called gypsophiles, while others, occurring regularly in both gypsum and non-gypsum soils are called gypsovags (Meyer, 1986). Nevertheless, many species that live in nearby habitats rarely colonize gypsum (e.g. many calcicolous plants). In this context, there is a great debate concerning the physical and chemical constraints of the flora inhabiting these areas, as well as about whether gypsophiles are refugees or specialists of gypsum substrates (Parsons, 1976; Meyer, 1986; Escudero et al., 1999, 2000; Romao and Escudero, 2005; Palacio et al., 2007).

In gypsum soils, plants encounter physical limitations such as irregular moisture distribution, high resistance to root penetration, or formation of physical soil crust (Romao and Escudero, 2005). Several studies, directly or indirectly, link gypsophily with physical factors (e.g. Meyer et al., 1992; Escudero et al., 1999). Furthermore, in gypsum soils, the plant development could be restricted chemically from excess of sulfur and calcium (Duvigneaud and Denaeyer-De Smet, 1966; Ruíz et al., 2003), or from a nutritional impoverishment caused by the exchange of calcium for other ions retained in the soil such as nitrogen, phosphorous, and potassium (Guerrero Campo et al., 1999). Despite certain evident chemical constraints of gypsum soils for plants, the way in which gypsum chemically influences the life cycle of plants is poorly understood (Parsons, 1976; Merlo et al., 1997; Palacio et al., 2007).

Germination, a key stage in the life cycle of plants, is largely determined by temperature, water availability, and light, but also by other environmental factors such as salinity (Pujol et al., 2000). Many studies examine the influence of more soluble salts than gypsum (e.g. NaCl, $CaCl_2$) on germination (e.g. Song et al., 2005; Tobe et al., 2003), demonstrating that higher salinity levels usually lower the percentage of seed germination and delay the onset of the germination, or completely inhibit the process (Pujol et al., 2000). Research on these issues is prolific and, although the germination of species inhabiting gypsum environments has been the subject of some studies (e.g. Escudero et al., 1997), the effect of gypsum on seed germination has hardly been studied (Merlo et al., 1997).

In this paper, we tested the effect of gypsum at different concentrations on seed germination for a set of species classified in three functional groups according to their ability to inhabit gypsum areas: gypsophiles, gypsovags, and calcicoles. Our aim is to assess whether gypsum could influence seed germination, as well as a better understanding of the plants living on this particular geological substrate.

2. Material and methods

2.1. Species selection and seed collection

Twenty-four taxa (Table 1) were selected and assigned to functional groups according to their edaphic preference as follows: gypsophile, for plants restricted to gypsum soils; gypsovag, for plants that occur regularly on both gypsum and non-gypsum substrates; and calcicole, for plants confined to, or most frequently found in, calcium-rich ("lime") habitats (following Mota et al*.,* 2011). Seeds were collected in gypsum, limestone, or both substrates in the south-eastern Iberian Peninsula (37.17°N, 2.84°W), under semi-arid and dry Mediterranean climate (rainfall averaging 200 to 600 mm). Seeds were harvested from at least 50 individuals per species in natural populations from July to October 2009. Seeds were cleaned discarding any visually malformed seed, and stored in darkness in paper bags under room conditions (c. 20°C and c. 30% relative humidity) until the germination tests were started (November 2009).

Species by functional group	**Abbreviations**
Gypsophiles	
Coris hispanica Lange	Ch
Gypsophila struthium L. subsp. *struthium*	Gs
Helianthemum squamatum (L.) Dum. Cours.	Hsq
Lepidium subulatum L.	Ls
Ononis tridentata subsp. *crassifolia* (Boiss.) Nyman	Otc
Ononis tridentata L. subsp. *tridentata*	Ott
Santolina viscosa Lag.	Sv
Teucrium turredanum Losa & Rivas Goday	Tt
Gypsovags	
Frankenia thymifolia Desf.	Ft
Helianthemum syriacum (Jacq.) Dum. Cours.	Hsy
Helianthemum violaceum (Cav.) Pers.	Hv
Lygeum spartum L.	Lsp
Pinus halepensis Mill.	Ph
Rosmarinus eriocalyx Jord. & Fourr.	Re
Rosmarinus officinalis L.	Ro
Stipa tenacissima L.	St
Calcicoles	
Cistus albidus L.	Ca
Cistus clusii Dunal	Cc
Digitalis obscura L.	Do
Lavandula lanata Boiss.	Lln
Lavandula latifolia Medik.	Llt
Phlomis lychnitis L.	Pl
Santolina chamaecyparissus L.	Sc
Thymus mastichina (L.) L. subsp. *mastichina*	Tm

Table 1. Study species assigned to functional groups by ecological preference.

2.2. Seed-germination test

We treated seeds with three levels of gypsum solution (low=0.5 g/l, medium=1 g/l, and high=2.4 g/l of calcium sulfate 2-hydrate [$CaSO_4 \cdot 2H_2O$] solution) and one control treatment with distilled water. Levels of solution were based on the maximum solubility of gypsum (2.4 g/l in water at 20ºC, Meyer, 1986). Petri dishes 100 mm in diameter were prepared with a layer of sterile glass beads covered with a disk of filter paper. Afterwards, 25 ml of the three solutions or distilled water were added. The whole set-up was pasteurized before the seeds were placed in the Petri dishes. Seeds were previously imbibed for 12 h and afterwards disinfected against mould with a 2% solution of commercial sodium hypochlorite for 2 min and subsequently washed with distilled water. Five replicates of 25 seeds per level treatment and species (25 seeds x 5 replicates x 4 level treatment x 24 species) were tested in a germination chamber (ASL ± 0.1ºC) maintained at 20ºC and under 16 h light/ 8 h darkness. Germination, identified as visible radicle protrusion, was recorded for 60 days. The solutions were replenished when needed to avoid water restriction, replacing filter-paper disks to avoid an increase in the gypsum concentration.

Some species were pretreated to enhance seed germination: *Helianthemum* seeds were mechanically scarified by abrasion between two sheets of fine-grit sandpaper (Pérez-García and González-Benito, 2006), *Cistus albidus* seeds received a dry-heat pretreatment of 5 min at 100°C (Escudero et al., 1997), and *Ononis tridentata* seeds were immersed in distilled water boiled at 100ºC and left to cool in the water to room temperature (c. 23ºC) for 12 h (Escribá and Laguna, 2006).

2.3. Statistical analysis

We evaluated the effect of the gypsum solutions on the seed-germination rate and germination speed (as T_{50}, being the time in days needed for manifestation of half of the final germination level) by functional group, fitting Generalized linear-mixed models (GLMM), including gypsum treatment as fixed factor and species as random factor. To estimate model parameters the Laplace approximation of likelihood was used (see Bolker et al. 2009). Generalized Linear Models (GLMs) were used to model effect of gypsum treatment by species. Models were fitted specifying a binomial error distribution and logit as the link function in the case of the germination rate, and Poisson error distribution and log as a link function in the case of T_{50}. All the statistical analyses were performed using the R statistical package (R Development Core Team, 2010).

3. Results

Analysis showed significant effects of gypsum on seed germination by functional group (Table 2). The three levels of gypsum solution had significant positive effect on the germination of gypsophile group. For gypsovag species, only the lower gypsum concentration showed a significant negative effect on seed germination. The group of calcicoles was notable in that more seeds germinated at the highest gypsum concentration (Table 2).

Table 2. Generalized Linear Mixed Model results by species group for the effect on seed germination of gypsum treatment (fixed factor). Species were included as random factor. ([1]) *SG* species group, *G* gypsophiles, *GV* gypsovags, *C* calcicoles. ([2]) *GT* gypsum treatment. Mean values (±SE) by species group and treatment are also provided.

SG[1]	**Generalized Linear Mixed Model results**					**GT**[2]	**Mean values (% ± SE)**
		Estimate	**SE**	**z value**	**Pr(>|z|)**		
G	Intercept	0.3680	0.6775	0.543	0.5870	Control	60.80±2.62
	Low	0.2831	0.0619	4.570	<0.0001	Low	57.00±5.39
	Medium	0.3145	0.0620	5.068	<0.0001	Medium	60.70±5.59
	High	0.1362	0.0615	2.214	0.0269	High	61.10±5.34
GV	Intercept	0.1632	0.3697	0.441	0.6589	Control	53.50±3.98
	Low	-0.1167	0.0504	-2.317	0.0205	Low	51.20±4.27
	Medium	-0.0127	0.0504	-0.252	0.8009	Medium	53.25±4.40
	High	-0.0178	0.0504	-0.354	0.7237	High	53.15±4.04
C	Intercept	0.0660	0.2251	0.293	0.7693	Control	50.46±4.01
	Low	0.1455	0.0470	3.097	0.0019	Low	54.50±3.50
	Medium	0.3447	0.0472	7.297	<0.0001	Medium	59.00±3.26
	High	0.4258	0.0474	8.983	<0.0001	High	60.80±2.62

Regarding the effects of gypsum solutions on germination by species, we found a significant response for some of them. However, for most species, we identified neither positive or negative significant effects, or did not follow a pattern (Figure). Gypsum solutions favored the germination of some gypsophile species (Figure, chart A). In particular, a significantly higher number of *Lepidium subulatum* seeds germinated in Petri dishes with gypsum (at any concentration) than without it. The seeds of *Gypsophila struthium* subsp. *struthium* germinated at a lower proportion under control conditions (90.4±3.49). The medium level of gypsum promoted the germination rate of *Helianthemum squamatum* (44.0±3.35) while the highest level of gypsum caused the lowest total germination (32.0±1.79) and reduced the germination speed. Also, at the highest gypsum level the fewest *Coris hispanica* seeds germinated (68.0±3.35), while the highest germination rate for this species was reached under control conditions (74.4±3.71).

For the gypsovag group, we found a positively significant effect of certain gypsum concentrations on *Pinus halepensis* and *Lygeum spartum* (Figure, chart C). The highest germination rate of *P. halepensis* seeds was reached at the medium level of gypsum (85.6±5.88), significantly more seeds germinating than in control (68.8±5.28). *L. spartum* germinated better at medium and high gypsum levels. On the contrary, we identified a negative effect on *Rosmarinus officinalis* seeds, which germinated faster (14.72±0.51 days) and at a higher proportion (76.0±3.10) under control conditions.

Only the seeds of *Lavandula* genus showed a significant effect of gypsum in the calcicolous group (Figure, chart D). In particular, *L. latifolia* seeds almost failed to germinate without gypsum (3.2±2.33), but they germinated at a high rate at medium (68.0±7.48) and high (69.6±0.98) gypsum levels.

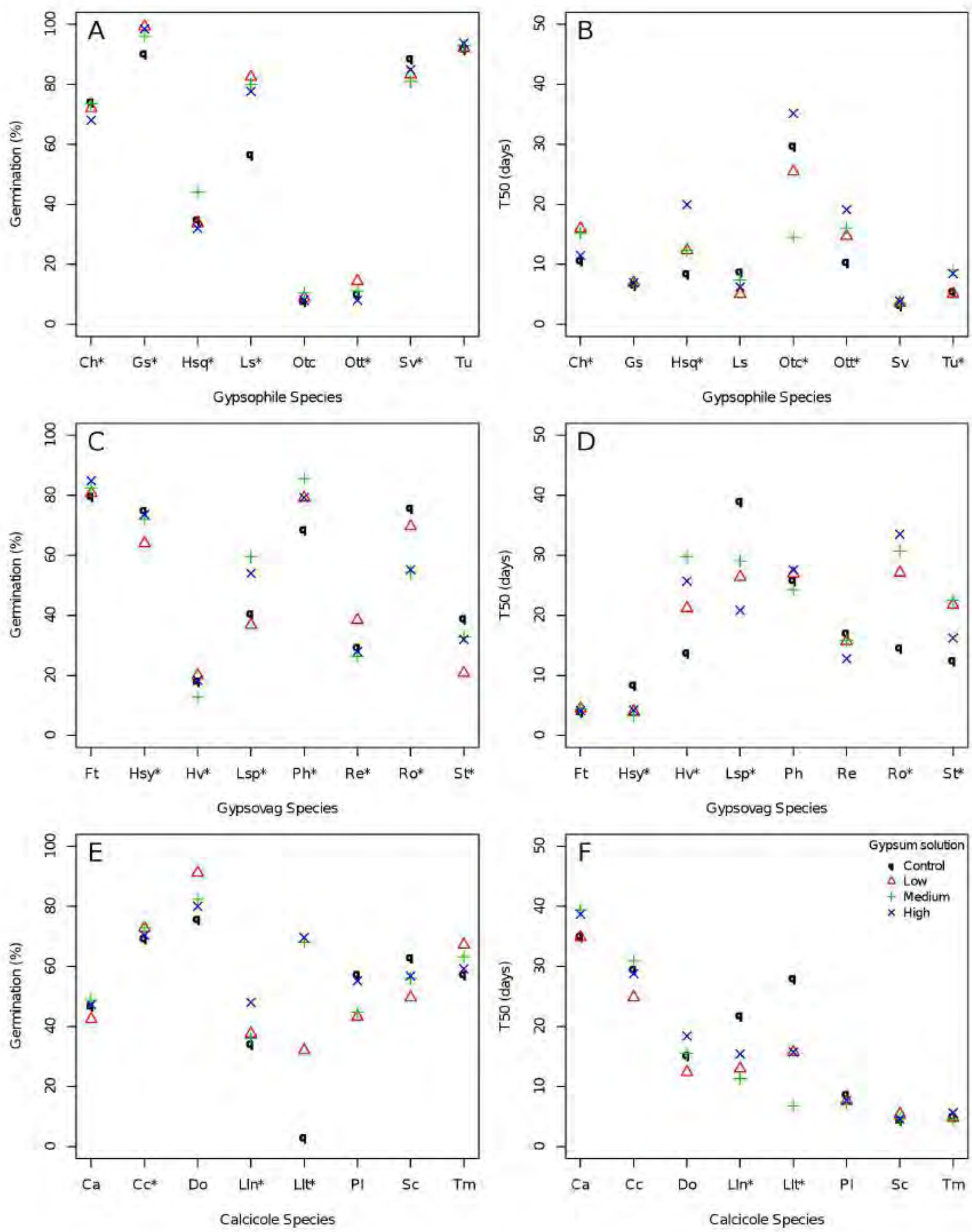

Figure. Mean values for the effect of gypsum treatment on germination (%) and T50 (days) by species and functional group. T50: time in days needed for manifestation of half of the final germination. Species abbreviations are given in Table 1. Asterisks (*) for the species on x axis indicate significant differences between treatments at p<0.05, according to the GLM performed. Legend is given in F.

4. Discussion

According to our results, for most species gypsum does not pose a chemical constraint at the seed germination stage. In this sense, we detected a negative response to gypsum solutions for only one species, among the 24 studied species. In contrast, salts more soluble than gypsum have been shown in many studies to exert an inhibitory effect on germination (Pujol et al., 2000; Song et al., 2005). The effect of salts such as NaCl on seed germination has been attributed to both osmotic stress and ion toxicity (Song et al., 2005). However, the lack of a negative response to gypsum solutions in most species studied is consistent with Herrero and Porta (2000), suggesting gypsum causes negligible osmotic stress and ion toxicity in seed germination. Specifically, sulfates are less toxic than chlorides probably because sulfate is a macronutrient involved in the synthesis of cell-detoxification molecules, whereas the chloride ion is a micronutrient (Léon et al., 2005). Moreover, calcium ions (Ca^{2+}) could alleviate the toxic effects of other salt components on seed germination (Tobe et al., 2003; Zehra et al., 2012). In our study, the germination analyses by functional group and gypsum treatments showed positive noteworthy effects only in the seeds of the calcicoles. This result appears to be due mainly to the strong response of *Lavandula latifolia* to the presence of Ca ions in the solution. Calcium is not only tolerated by some calcicoles but even required by others (Clymo, 1962).

In addition, we identified a clear response to the gypsum treatments in some species. Specifically, our results suggest that some gypsophile species, such as *Lepidium subulatum* and *Gypsophila struthium* subsp. *struthium,* or *Helianthemum squamatum,* at specific concentrations, could be favored during germination by the presence of gypsum. Also, Merlo et al. (1997) found that certain gypsum concentrations improved the germination of two gypsophile species. Therefore, it may be a specialization sign supporting the "specialist" model, since the soil would provide a chemical advantage for the emergence of certain gypsophiles. Consistent with this fact, other authors (Duvigneaud and Denaeyer-De Smet, 1966; Ruiz et al., 2003) pointed out other adaptations of some gypsophile species to the chemical components of gypsum soils at other life stages. Moreover, the germination of some gypsovags (i.e. *L. spartum* and *P. halepensis*) is favored by certain gypsum concentration (especially at 1 g/l). In this sense, the role of gypsum solutions at specific concentration would aid seed germination of some species, being useful to select appropriate conditions to promote seedling production for restoration purposes. In particular, gypsum has been found of key importance when preparing the substrate on which to perform sowings for recovery gypsum habitats (e.g. Ballesteros et al., 2012).

By contrast, we identified a negative effect of gypsum on *Rosmarinus officinalis* seeds, which germinated faster and at a higher proportion under control conditions. This gypsovag species could be favored at other stages of the cycle, developing strategies to accumulate or exclude some toxic elements characteristic of gypsum soil (Palacio et al*.,* 2007). As an example, Romao and Escudero (2005) described a similar behavior for *Teucrium capitatum,* another gypsovag, the performance of which is hindered only in some phases by gypsum soil.

Nevertheless, the chemical features of gypsum do not seem to have a determinant effect on the germination for the overall species. The presence or absence of certain plants in gypsum outcrops may be determined by other life stages, other factors, or a combination thereof. Thus, some previous studies on the growth and survival of *L. subulatum* and *H. squamatum* in gypsum soils (Escudero et al*.,* 1999, 2000) proposed the "refuge" model for these species linking gypsophily with some physical properties of the surface crust. Subsequently, Romao and Escudero (2005) suggested that at least for *H. squamatum*, there is an intermediate strategy: it primary refuges because it can penetrate gypsum crusts at the emergence stage, but it has also evolved adaptive strategies to perform better in such soils. Recently, Palacio et al. (2007), studying leaf chemical composition, suggested that regionally dominant gypsophiles (such as *Gypsophila struthium*, *Lepidium subulatum, Helianthemum squamatum,* and *Ononis tridentata*) might fit the 'specialist' model, being specifically adapted to gypsum, whereas both gypsovags and narrow-gypsophile endemics might fit the 'refuge' model, being stress-tolerant species that find refuge on gypsum soils to escape competition. This statement agrees with our results on germination, while we found positive effects of gypsum on regionally dominant gypsophiles (specifically *Gypsophila struthium*, *Lepidium subulatum,* and *Helianthemum squamatum*)*,* we found negative or neutral effects of gypsum solution on narrow-gypsophile endemics (*Coris hispanica* and *Teucrium turredanum*, respectively).

No specific physiological mechanism seems adequate to explain the original flora characteristic of peculiar soil parent material (Gankin and Major, 1964). To face adverse environments, some species have developed specialized structures or mechanisms and therefore are specialists, while other species are simply able to tolerate or resist harsh conditions. This behavior is not a characteristic only of the species itself, but also of a particular life stage. The studies published to date on gypsophily suggest that it is closely linked to physical as well as chemical factors. Specifically, we found that while dissolved gypsum has no effect on germination for many species, for some widespread gypsophile species in the Iberian Peninsula, such as *Lepidium subulatum* and *Gypsophila struthium,* the presence of gypsum could represent an advantage at the germination stage.

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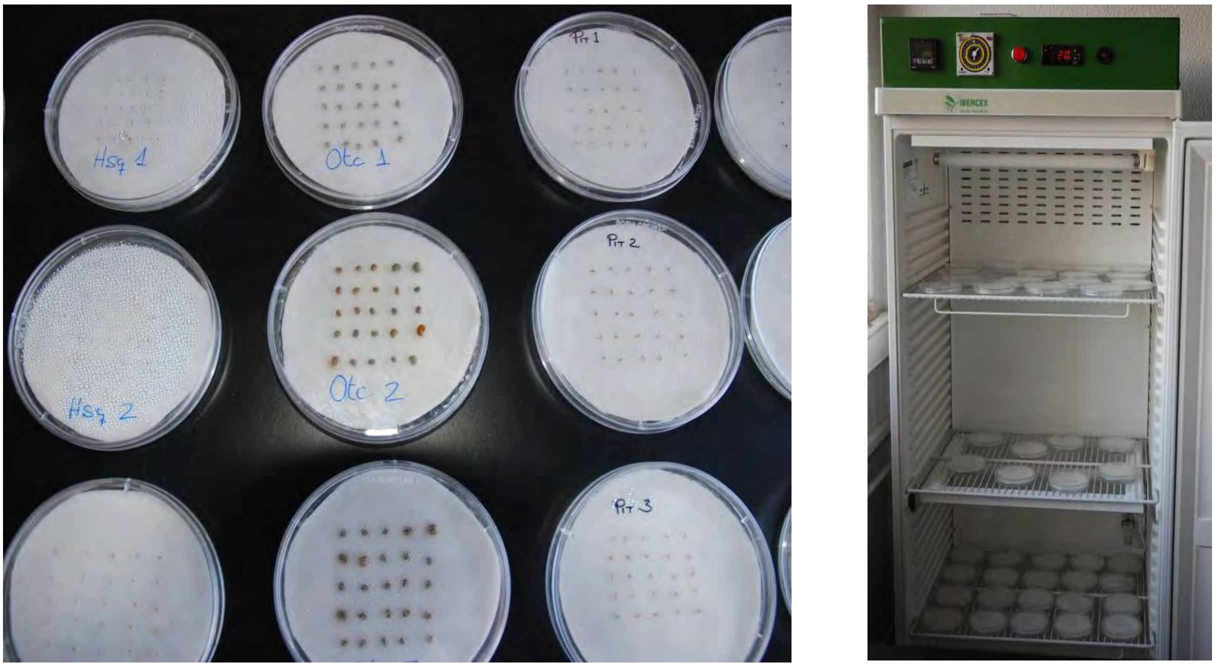
Seeds of *Stipa tenacissima* (left) and *Reseda stricta* (right).

Seeds of various species inbibed before being placed on petri dishes.



Petri dishes with seeds of various species (left) and the germiantion chamber used in the experiment (right).

Chapter 3

Enhancing seedling production of native species to restore gypsum habitats.

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Enhancing seedling production of native species to restore gypsum habitats

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Abstract

Gypsum habitats are widespread globally and are important for biological conservation. Nevertheless, they are often affected by human disturbances and thus require restoration. Sowing and planting have shown positive results, but these actions are usually limited by the lack of native plant material in commercial nurseries, and very little information is available on the propagation of these species. We address this issue from the hypothesis that gypsum added to a standard nursery growing medium (peat) can improve seedling performance of gypsum species and, therefore, optimise the seedling production for outplanting purposes. We test the effect of gypsum on emergence, survival, and growth of nine native plant species, including gypsophiles (exclusive to gypsum) and gypsovags (non-exclusive to gypsum). We used four treatments according to the proportions, in weight, of gypsum:standard peat (G:S), i.e. high-g (50G:50S), medium-g (25G:75S), low-g (10G:90S), and standard-p (0G:100S).
Our results showed that the gypsum treatments especially benefited the emergence stage, gypsophiles as group, and *Ononis tridentata* as a taxon. In particular, the gypsum treatments enhanced emergence of seven species, survival of three species, and growth of two gypsophiles, while the use of the standard peat favoured only the emergence or growth of three gypsovags. Improving emergence and survival at the nursery can provide a reduction of costs associated with seed harvesting, watering,

and space, while enlarging seedlings can favour the establishment of individuals after outplanting. Thus, we suggest adding gypsum to standard peat for propagating seedlings in species from gypsum habitats, thereby potentially cutting the costs of restoring such habitats. Our assessment enables us to provide particular advice by species. In general, we recommend using between 25 and 50% of gypsum to propagate gypsophiles, and between 0 and 10% for gypsovags. The results can benefit not only the production of widely distributed species commonly affected by gypsum quarrying, but also of narrow and threatened endemic species that require particularly efficient use of their seeds. In addition, our study highlights the importance of using appropriate growing media to propagate plants characteristic of special substrates for restoration purposes.

Keywords: Growing medium, gypsum treatment, gypsophiles, gypsovags, gypsum species, seedling production

1. Introduction

Gypsum soils are widespread, with more than100 million ha worldwide, almost exclusively in arid and semi-arid regions (Boyadgiev and Verheye, 1996). These soils host very rare and narrow endemic flora that includes many endangered species, making them priority sites for biological conservation (Anonymous, 1992; Parsons, 1976; Mota et al., 2011; Sosa and De-Nova, 2012). However, gypsum habitats are often impacted by human disturbances such as quarrying, ploughing or grazing (Al-Harthi, 2001; Mota et al., 2004; Pulido-Bosch et al., 2004; Pueyo and Alados, 2007; Ballesteros et al., 2013). Therefore, recovery plans for these environments need to be addressed, and proactive measures need to be considered (Ballesteros et al., 2012, 2014), because natural succession has proved inefficient over the short term (Mota et al. 2003, 2004; Dana and Mota, 2006).

The recovery of gypsum areas has been satisfactorily approached through hydroseeding (Matesanz and Valladares, 2007), sowing (Ballesteros et al., 2012) or outplanting (Sharma et al., 2001; Blignaut and Milton, 2005; Ballesteros et al., 2014). Nonetheless, one of the main problems in restoring these environments is the lack of native plant material (seeds and seedlings), even though some studies report that this is a key factor (e.g. Matesanz et al., 2006). Thus, despite the successful use of outplanting as a restoration technique for gypsum habitats (e.g. Ballesteros et al., 2014), it is difficult to find seedlings of native species for gypsum substrates (gypsum species, hereafter) in commercial or public nurseries. In fact, little information is available for producing these native species. In addition, many of the gypsum species are narrowly endemic and/or endangered species and require specific harvesting efforts and efficient use of their seeds, for which the development of effective propagation methods constitutes a priority. In this sense, testing methods are required in order to enhance the emergence and survival of seedlings. Moreover, promoting early growth of seedlings during the nursery phase is particularly relevant for better outplanting performance (Kormanik, 1986; Thompson and Schultz, 1995; Jacobs et al., 2005).

In this context, we studied seedling production in gypsum species, starting from the premise that most of these are highly specialized in gypsum substrates. In this regard, several field experiments have demonstrated that the selection of a suitable substrate, composed mainly of native gypsum, effectively contributes to the success in sowing and outplanting (Ballesteros et al., 2013, 2014). Also, other experiments evidence that the presence of gypsum in the growth medium can be a key factor for gypsum species at the initial stages (e.g. Escudero et al., 1999, 2000; Cañadas et al., 2014), but this has never been verified for seedling production. Thus, we hypothesised that the addition of gypsum to a standard growing medium could enhance seedling performance and, therefore, the production of native plants in the recovery of gypsum habitats. To test this, we designed a manipulative factorial experiment to produce seedlings of nine gypsum species in a growth chamber, adding different gypsum proportions to a nursery growing medium commonly used for plant production (peat). We monitored three key stages in plant production: emergence, survival, and early growth. Therefore, in this study, we determine whether gypsum treatments affect seedling performance, with the final aim of gaining insight into the propagation of gypsum species for habitat-restoration purposes.

2. Materials and methods

2.1. Target species and seed collection

Nine characteristic species of the EU priority habitat "Iberian gypsum vegetation, *Gypsophiletalia"* (Anonymous, 1992) were selected, including gypsophile (i.e. restricted to gypsum soils) and gypsovag plant species (i.e. occurring commonly on both gypsum and non-gypsum substrates; *sensu* Meyer, 1986). The gypsophiles were *Helianthemum squamatum* (L.) Dum. Cours. (*Cistaceae*), *Lepidium subulatum* L. (*Brassicaceae*), *Gypsophila struthium* L. subsp. *struthium* (*Caryophyllaceae*), *Ononis tridentata* L. subsp. *crassifolia* (Dufour ex Boiss.) Nyman (*Leguminosae*), and *Santolina viscosa* Lag. (*Asteraceae*). The first three gypsophiles are widely distributed in gypsum outcrops in the Iberian Peninsula and some localities in North Africa, and the last two are narrow endemic species restricted to specific gypsum outcrops in southeastern Iberian Peninsula and considered threatened (Vulnerable; Cabezudo et al., 2005; Ballesteros et al., 2013). The four remaining species were gypsovags: *Helianthemum syriacum* (Jacq.) Dum. Cours. (*Cistaceae*), *Frankenia thymifolia Desf.* (*Frankeniaceae*), *Rosmarinus officinalis L. (Lamiaceae), Stipa tenacissima* L. (*Poaceae*), all with a Mediterranean distribution (see Blanca et al., 2009 and Mota et al., 2011 for further details on the selected species).

Seeds were collected in gypsum outcrops in south-eastern Spain (37.17°N, 2.84°W), under a semiarid and dry Mediterranean climate (rainfall ranging from 200 to 500 mm). Seeds were harvested from at least 50 individuals per species in natural populations. Subsequently, seeds were cleaned, discarding any visually malformed seed, and stored in darkness in paper bags under ambient conditions (c. 20°C and c. 30% relative humidity) until the experiment started.

2.2. Experimental design

We performed a manipulative experiment in a full factorial design including two factors: species (specified above) and gypsum treatments. To apply gypsum treatments, we prepared four different mixtures of standard nursery growing medium, i.e. peat (composition: organic matter= 85.4 %, pH=6-7, N=260 mg/kg, P=389 mg/kg, K=2000 mg/kg, Mg=678 mg/kg, Fe=15 mg/kg) and powdered gypsum ($CaSO_4 \cdot 2H_2O$). According to the gypsum:standard peat (G:S) proportions in weight, we established four treatments, called: high-g (50G:50S), medium-g (25G:75S), low-g (10G:90S), and standard-p, (0G:100S, which represents the control treatment, because it is customarily used to propagate nursery plants).

Fifty replicates (6 cm x 5.6 cm x 8 cm) per treatment and species were prepared (50 pots x 4 treatments x 9 species = 1800 pots), and in each replicate ten seeds of the same species were sown. The pots were placed in a completely randomized array, in a growth chamber on three aluminium tables equipped with controlled spray-irrigation systems set to water every three days.The chamber was kept at 25ºC (ETN® thermostat, Carrier España, S.L.), under 14 h light/ 10 h darkness (FAEBER® lighting system,TIGER®, including 400w E40/ES OSRAM® lights, and a MicroRex D11 timer, LEXIC, LEGRAND®), reproducing favourable conditions for optimal plant development in the habitat (photoperiod and temperature from June to September).

2.3. Data collection

Pots were monitored for 21 weeks recording weekly emergence and survival. We visually checked cotyledon protrusion for emergence and marked the first seedling to emerge in each pot, or a randomly selected one if several seedlings emerged the same week (first individual, hereafter), for survival monitoring. Following the same criteria, a second seedling was marked to ensure that enough individuals were available to assess growth, in case of early death of the first individual. When each pot had two seedlings, new emerging plants were immediately clipped after recording emergence. The second marked seedling in each pot was also clipped after 4 weeks if the first individual survived, in order to avoid competition between seedlings.

After 21 weeks, the seedlings were harvested and washed with distilled water. Subsequently, we separated the shoots from roots and dried them in an oven (70ºC for 48 h). We weighed the samples in a precision scale (0.0001 g), after stabilization at room temperature, recording shoot and root biomass separately. These data were used to evaluate gypsum effects on growth.

2.4. Data analyses

The effect by species of gypsum treatments on emergence (measured as the percentage of emerged seedlings and as the time to emergence of the first individual) and growth (in terms of shoot and root biomass) was modelled by fitting generalized linear models (GLMs). Emergence was modelled by specifying a binomial error distribution and logit-link function for the percentage of emerged seedlings, and a poisson error distribution and a log-link function for the time to emergence of the first individual. The growth data were submitted to logarithmic transformation. To assess the effect of the different gypsum treatments on seedling survival, we fit Cox proportional hazard models by species as well as the Kaplan-Meier function to plot differences in survival among treatments (R “survival” package; Therneau, 2013). Despite that pots were monitored for 21 weeks, only individuals that emerged before the ninth week were used to assess the time to death in the survival analysis, ensuring an individual monitoring of 12 weeks at least (first week being the week of emergence). Also the biomass of the surviving individuals emerged before the ninth week was used to evaluate gypsum effects on growth.

3. Results

3.1. Emergence

Gypsum proved to have a significant effect on emergence for most species, with at least one gypsum treatment being positive compared to the standard-p for all gypsophiles and two gypsovags (Tables 1 and 2, Appendix A; Table A.1). In particular, emergence of the two threatened endemic species (*O. tridentata* and *S. viscosa*) was significantly higher in any of the gypsum treatment than in standard-p. The highest emergence rate of *G. struthium* was recorded in medium-g while high-g negatively influenced emergence. Moreover, the highest number of emerged seeds was found in high-g for *F. thymifolia*, medium-g for *L. subulatum*, and low-g for *H. squamatum* and *H. syriacum*. Standard-p was a better treatment for emergence only in the case of *S. tenacissima* and *R. officinalis.* Gypsum treatments had no effect on the emergence time of the first individual in any case (Appendix A: Table A.2).

Table 1. Summary of the results by stages, species, and treatments. Treatments according to weight proportions of gypsum:standard peat; High-g (50G:50S), Medium-g (25G:75S), Low-g (10G:90S), Standard-p (0G:100S).

Species	Gypsum level	Mean Emergence (% ± SE)	Survival (%)	Mean shoot biomass (mg± SE)	Mean root biomass (mg± SE)
Ononis tridentata subsp. *crassifolia*	Standard-p	12.6 ± 1.7	20.7	18.3 ± 1.8	7.9 ± 0.9
	Low-g	17.1 ± 2.2	51.6	32.1 ± 1.8	17.3 ± 1.2
	Medium-g	17.3 ± 1.9	76.3	36.1 ± 7.1	18.1 ± 3.5
	High-g	17.4 ± 1.4	83.3	147.8 ± 32.5	43.5 ± 7.2
Gypsophila struthium subsp. *struthium*	Standard-p	54.4 ± 3.2	81.6	128.6 ±16.0	28.1 ± 4.0
	Low-g	54.0 ± 2.6	86	125.1 ± 15.8	24.1 ± 3.4
	Medium-g	56.6 ± 2.5	84	119 ± 16.7	30.0 ± 4.8
	High-g	41.8 ± 3.4	72.9	123.9 ± 14.5	29.2 ± 3.1
Helianthemum squamatum	Standard-p	44.8 ± 3.0	42.6	3.5 ± 0.4	2.4 ± 0.3
	Low-g	48.8 ± 2.2	42.9	4.4 ± 0.4	1.9 ± 0.2
	Medium-g	46.8 ± 2.4	60	4.1 ± 0.3	2.3 ± 0.2
	High-g	47.4 ± 3.0	78	4.5 ± 0.4	1.8 ± 0.2
Lepidium subulatum	Standard-p	22.6 ± 2.1	25	30.7 ± 11.4	4.9 ± 1.5
	Low-g	15.8 ± 2.3	41.9	10.8 ± 3.2	3.1 ± 0.9
	Medium-g	29.4 ± 3.4	24.4	18.9 ± 10.4	3.4 ± 1.8
	High-g	22.4 ± 2.3	16.7	5.8 ± 1.0	2.9 ± 0.7
Santolina viscosa	Standard-p	41.2 ± 2.6	95.9	15.3 ± 2.5	7.3 ± 1.2
	Low-g	43.8 ± 3.1	97.9	11.4 ± 2.0	5.8 ± 0.8
	Medium-g	60.0 ± 3.7	95.9	13.8 ± 2.3	6.0 ± 0.7
	High-g	56.6 ± 3.0	94.0	11.4 ± 2.3	4.3 ± 0.6
Helianthemum syriacum	Standard-p	78.6 ± 3.1	91.8	5.0 ± 0.1	2.4 ± 0.5
	Low-g	81.8 ± 1.9	80	7.0 ± 0.0	2.3 ± 0.2
	Medium-g	78.0 ± 2.9	91.8	7.1 ± 0.3	2.9 ± 0.3
	High-g	72.4 ± 3.1	82	3.8 ± 0.1	1.2 ± 0.1
Frankenia thymifolia	Standard-p	30.0 ± 3.1	26.2	11.9 ± 3.2	5.9 ± 1.1
	Low-g	47.2 ± 2.6	58.8	7.9 ± 2.2	1.7 ± 0.4
	Medium-g	30.0 ± 2.9	38.6	1.4 ± 0.4	0.5 ± 0.1
	High-g	57.8 ± 2.9	44.9	0.7 ± 0.2	0.3 ± 0.1
Rosmarinus officinalis	Standard-p	51.8 ± 3.2	91.8	32.5 ± 5.3	17.3 ± 1.7
	Low-g	44.0 ± 2.9	100.0	25.1 ± 3.7	15.6 ± 1.8
	Medium-g	38.0 ± 3.3	97.8	26.1 ± 5.4	13.7 ± 1.5
	High-g	50.0 ± 3.9	93.0	21.8 ± 2.4	11.5 ± 0.9
Stipa tenacissima	Standard-p	22.8 ± 2.6	93.2	25.6 ± 3.0	13.8 ± 1.8
	Low-g	15.2 ± 2.0	94.3	27.6 ± 2.6	14.6 ± 1.3
	Medium-g	11.2 ± 2.0	100.0	29.0 ± 3.8	16.0 ± 3.1
	High-g	15.8 ± 2.9	93.3	24.3 ± 1.9	13.3 ± 1.3

Table 2. Summary of gypsum treatment effects on emergence, survival, shoot growth and root growth by species. Treatments according to weight proportion of gypsum:standard peat; H/High-g (50G:50S), M/Medium-g (25G:75S), L/Low-g (10G:90S), standard-p (0G:100S). Sign of gypsum treatment effect compared to standard-p: (+) positive, (-) negative, (ns) no significant effects, according to GLMs and Cox proportional hazard model (see Appendix A for additional information). ([a]): The number of stages (emergence, survival, growth) favoured by the most beneficial treatment appears in brackets; (*) indicates marginally significant effects.

	Emergence			Survival			Shoot growth			Root growth			Most beneficial treatment [a]
	L	M	H	L	M	H	L	M	H	L	M	H	
O. tridentata	+	+	+	+	+	+	+	+	+	+	+	+	**High-g (3)**
H. squamatum	+	+	+	ns	+*	+	+	+	+	ns	ns	ns	**High-g (3)**
G. struthium	ns	+	-	ns	ns	ns	ns	ns	ns	ns	ns	ns	**Medium-g (1)**
L. subulatum	-	+	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**Medium-g(1)**
S. viscosa	+*	+	+	ns	ns	ns	ns	ns	-	ns	ns	-	**Medium-g (1)**
H. syriacum	+	ns	-	ns	ns	ns	ns	ns	-	ns	ns	-	**Low-g (1)**
F. thymifolia	+	ns	+	+	+*	+	-	-	-	-	-	-	**Low-g (2)**
R. officinalis	-	-	-*	ns	ns	ns	ns	ns	ns	ns	-	-	**Standard-p (1)**
S. tenacissima	-	-	-	ns	ns	ns	ns	ns	ns	ns	ns	ns	**Standard-p (1)**

3.2. Survival

Gypsum treatments positively affected the survival of three species after 12 weeks (Tables 1 and 2, Fig. 1, Appendix A: Table A.3). In particular, the survival of *O. tridentata* subsp. *crassifolia* and *F. thymifolia* seedlings proved significantly higher with any of the gypsum treatments than in standard-p. Thus, *O. tridentata* survival rose from 20.7% in standard-p to 83.3% in the high-g. *F. thymifolia* survival was 26.2% in standard-p but increased to 58.0% in the low-g. The highest survival values for *H. squamatum* seedlings were recorded in high-g (78.0%), while the lowest survival (42.6%) was in standard-p. Also, significant differences among treatments were found for *L. subulatum*, although differences between the highest survival in low-g (41.9%) and standard-p (25%) were not significant. For the remaining five taxa, the survival was high in both standard-p and gypsum treatments (higher than 72.9% in all cases), with no significant effects among treatments.

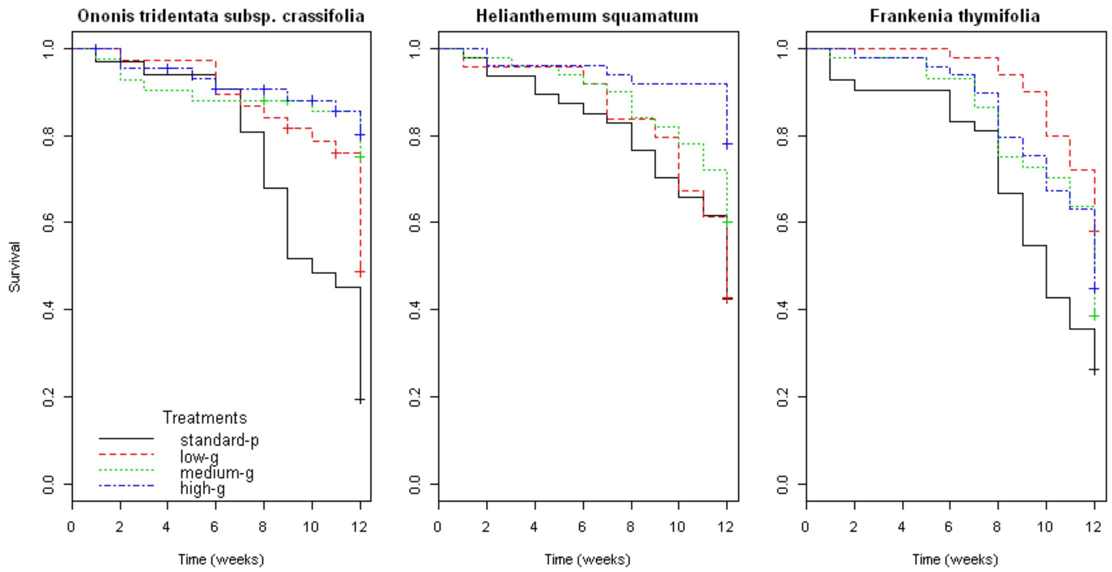

Figure 1. Kaplan-Meier survival curves representing species survival over 12 weeks for each treatment. Only the plots for species in which the treatment had significant effect on the survival are shown.

3.3. Early growth

Gypsum had a significant effect on seedling growth for some of the species (Tables 1 and 2, Appendix A: Table A.4). In particular, we found no negative effects of gypsum on early growth in plants of the gypsophile group, except for *S. viscosa* at high-g. By contrast, gypsum had a significantly positive effect on *O. tridentata* growth, with the effect of high-g being particularly positive on shoot and root. Shoot growth of *H. squamatum* was also significantly higher in all gypsum treatments than in the standard-p. Concerning the gypsovag group, no significant positive effects of gypsum were found. On the contrary, the effect of gypsum treatments on *F. thymifolia* growth was negative. *H. syriacum* growth was significantly lower at high-g than in standard-p, but medium and low-g did not negatively affect growth. In addition, medium-g and high-g reduced root growth of *R. officinalis* compared to standard-p, and no significant response was recorded for *S. tenacissima.*

4. Discussion

Our results reveal that gypsum treatments had positive effects on seedlings for most of the target species at some of the stages studied (i.e. emergence, survival and/or growth). Gypsum treatments especially favoured the performance of gypsophiles, while the use of standard peat without gypsum benefited only emergence or growth of three gypsovags (Table 2).

We found that emergence was the most affected stage, when gypsum positively influenced most of the species (seven of nine) while the standard peat favoured only the emergence of two gypsovags. Our results on emergence partially agree with a previous germination study (Cañadas et al., 2014), and the differences could be related to substrate, germination chamber, and type of gypsum treatments (e.g. Boeken et al., 2004; Golle et al., 2010). Regarding survival, we found that gypsum treatments favoured three species while no species benefited by growing in the standard peat. Moreover, gypsum also enhanced growth of two gypsophiles but did not bolster the growth of any gypsovag. Our results are in contrast to those obtained by Boukhris and Lossaint (1975), who stated that gypsophiles grew equally well in soils with high sulphur content and in commercial soils; however our result are only comparable to a certain extent given sulphur is just one of the elements forming gypsum.

Overall, more positive effects of gypsum were found for gypsophiles than for gypsovags, suggesting that effects depend not only on the growing medium properties but also on the ecological strategies of species. In line with our results, different ecological strategies in gypsum species have been linked to plant groups in some studies (i.e. widely distributed gypsophiles, narrowly distributed gypsophiles, and gypsovags; e.g. Palacio et al., 2007; Cañadas et al., 2014; Escudero et al., 2014; Palacio et al., 2014). In particular, Palacio et al., (2014) evidenced gypsophiles have special mechanisms to live in gypsum soils, such as the ability to accumulate S and Ca, whereas gypsovags are only stress tolerant plants without specialized chemical adaptations that can regulate the uptake of these elements. This specialization could explain the better performance of some of the gypsophiles tested in gypsum treatments However, the functioning of gypsum species and the habitat that they occupy is still not fully understood and further studies are needed in this regard (Escudero et al., 2014).

Certainly, our results revealed that the addition of gypsum to a standard peat is advantageous to seedling performance and, therefore, to optimise production of native species for gypsum-habitat restoration. In seedling production, the harvested seeds can provide greater efficiency if emergence and survival are optimised, which could reduce harvesting costs or problems arising from low availability of seeds. Also other inputs influencing costs of plant production, and therefore of restoration plans, such as space and water could be optimised. In this respect, at least one of the gypsum treatments favoured emergence in seven of the nine species studied as well as the survival in three species, whereas the standard treatment benefited only the emergence of two gypsovag species and did not enhance the survival of any of the species.

In addition, the seedlings of two species (*O. tridentata* and *H. squamatum)* were larger in all of the gypsum treatments than in standard-p. Size is a reliable, easy-to-use indicator of seedling quality (Jacobs et al., 2005; Renou-Wilson et al., 2008; Oliet et al., 2009; Close et al., 2010), and using high-quality seedlings is a key factor in establishing plantations (e.g. Wilson and Jacobs 2006), especially under arid Mediterranean conditions (e.g. Cortina et al., 2006; Oliet et al., 2009; Jiménez et al., 2014). Despite that this issue has not been resolved for gypsophile seedlings in planting, under natural conditions the largest seedlings of *H. squamatum* and *L. subulatum* also showed the highest survival rate (Escudero et al., 1999, 2000). Therefore, the field performance after the planting of species such as *O. tridentata* and *H. squamatum* could be enhanced if seedlings are grown after adding gypsum to the standard peat. However, seedling performance in the field will also depend on other factors such as shoot-to-root ratio, stem diameter, and physiological condition of seedlings (e.g. Ritchie et al., 2010).

Results by species enable us to provide particular suggestions to optimise the production of each species (Table 2), which is feasible because it involves only the addition of gypsum to standard peat in the initial phase. The results are particularly relevant for the two endemic and threatened taxa studied, i.e. *O. tridentata* subsp. *crassifolia* and *S. viscosa*. Gypsum treatments enhanced the emergence of both species, which is especially important for *O. tridentata*, the seeds of which are often difficult to harvest, highly depredated (Ballesteros et al., 2013), and have low germination rates (Cañadas et al., 2014). Furthermore, emerged seedlings of *O. tridentata* showed higher survival rates in medium-g and high-g, and all gypsum treatments favoured seedling growth in comparison to standard-p, the high-g treatment being particularly favourable. In addition, emergence, survival, and growth for the gypsophile *H. squamatum* were also benefited by the high-g. This result agrees with Escudero et al. (1999), who found that *H. squamatum* was able to grow in the field on a wide variety of soils, although its survival rate and growth were higher on genuine gypsum soils. We also found that medium-g favoured the emergence of *L. subulatum* and *G. struthium,* while other stages were not significantly influenced by gypsum. Thus, we suggest sowing *O. tridentata* subsp. *crassifolia* and *H. squamatum* using the high-g (because it benefits the three stages studied), and *S. viscosa, G. struthium,* and *L. subulatum* using the medium-g (because it favoured emergence). Regarding the gypsovag group, seedling production of *F. thymifolia* and *H. syriacum* could be also enhanced using the low-g, because it favoured their emergence and *F. thymifolia* survival. Conversely, for species such as *R. officinalis* and *S. tenacissima,* we suggest using a non-amended standard peat, because it yielded the best emergence.

5. Conclusions

Our results reveal that the addition of gypsum to a standard nursery growing medium benefited seedling performance in most of the tested species. This constitutes the first approach to the testing of methods to produce seedlings of gypsum species for restoration purposes. In particular, the gypsum treatments especially benefited emergence as a stage, gypsophiles as a plant group, and *O. tridentata* as *a taxon*. Altogether, seven of nine species benefited from the gypsum treatments to improve emergence and/or survival, implying better use of the available seeds and a reduction in costs associated with seed harvesting, watering or space. Furthermore, larger seedlings of two species resulted after using gypsum, which could favour the establishment in the field of individuals after outplanting. Thus, we suggest applying gypsum treatments to improve efficiency in the propagation of gypsum species, which would cut the costs of gypsum-habitat restoration plans. The results regarding plant performance by species enable us to provide particular suggestions to optimise the cultivation of each species, which are feasible to apply. In general, we recommend using a standard peat mixed with 25-50% of gypsum by weight to propagate gypsophiles, while using solely the standard peat, or 0-10% of gypsum, to propagate gypsovags. The results may benefit not only the production of widely distributed species commonly affected by gypsum quarrying, but also narrow and threatened endemic species such as *O. tridentata* subsp. *crassifolia*, which require a particularly efficient use of its seeds. Finally, our study highlights the importance of using appropriate growing media to propagate plants characteristic of special substrates when planning restoration measures.

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Appendix A. Supplementary data

Table A.1. Analysis of deviance and summary of the GLM showing effects of gypsum levels on percentage of emergence by species. Treatments according to weight proportions of gypsum:standard growing medium; High-g (50:50), Medium-g (25:75), Low-g (10:90), Standard-t (0:100).

Species	Deviance Resid.	Resid. Dev	P(>|Chi|)		Estimate	Std. Error	z value	Pr(>|z|)
O. tridentata c.	63.292	2732.2	1.163e-13 ***	(Intercept)	-1.93680	0.04262	-45.448	<0.0001
				Low-g	0.36126	0.05703	6.334	<0.0001
				Medium-g	0.36913	0.05648	6.536	<0.0001
				High-g	0.37926	0.05664	6.696	<0.0001
G. struthium	270.14	3821.4	< 2.2e-16 ***	(Intercept)	0.17646	0.02839	6.214	<0.0001
				Low-g	-0.01611	0.04014	-0.401	0.6881
				Medium-g	0.08909	0.04025	2.213	0.0269
				High-g	-0.50745	0.04035	-12.575	<0.0001
H. squamatum	16.612	3095.3	0.0008492 ***	(Intercept)	-0.20875	0.02844	-7.341	<0.0001
				Low-g	0.16075	0.04011	4.007	<0.0001
				Medium-g	0.08058	0.04015	2.007	0.04475
				High-g	0.10466	0.04014	2.608	0.00912
L. subulatum	267.71	4120.9	< 2.2e-16 ***	(Intercept)	-1.23104	0.03381	-36.407	<0.0001
				Low-g	-0.44215	0.05145	-8.594	<0.0001
				Medium-g	0.35500	0.04590	7.734	<0.0001
				High-g	-0.01147	0.04790	-0.239	0.811

S. viscosa	520.22	4540.0	< 2.2e-16 ***	(Intercept)	-0.35570	0.02873	-12.380	<0.0001
				Low-g	0.10642	0.04047	2.629	0.00855
				Medium-g	0.76117	0.04073	18.688	<0.0001
				High-g	0.62125	0.04049	15.342	<0.0001
H. syriacum	130.51	4716.4	< 2.2e-16 ***	(Intercept)	1.30098	0.03448	37.730	<0.0001
				Low-g	0.20187	0.05032	4.012	<0.0001
				Medium-g	-0.03531	0.04852	-0.728	0.467
				High-g	-0.33659	0.04680	-7.193	<0.0001
F. thymifolia	1167.9	4153.4	< 2.2e-16 ***	(Intercept)	-8,47E+02	3,09E+01	-27.46	<0.0001
				Low-g	7,35E+02	4,19E+01	17.55	<0.0001
				Medium-g	-2,98E-07	4,36E+01	0.00	1
				High-g	1,16E+03	4,21E+01	27.60	<0.0001
R. officinalis	238.12	5115.7	< 2.2e-16 ***	(Intercept)	0.07203	0.02830	2.545	0.0109
				Low-g	-0.31319	0.04016	-7.799	<0.0001
				Medium-g	-0.56158	0.04062	-13.825	<0.0001
				High-g	-0.07203	0.04001	-1.800	0.0718
S. tenacissima	251.43	4344.4	< 2.2e-16 ***	(Intercept)	-1.21964	0.03371	-36.182	<0.0001
				Low-g	-0.49936	0.05184	-9.632	<0.0001
				Medium-g	-0.85083	0.05610	-15.166	<0.0001
				High-g	-0.45355	0.05138	-8.828	<0.0001

Table A.2. GLM Analysis of deviance showing effects of gypsum levels on time to emergence by species.

Species	Deviance	Resid. Df	Resid. Dev	P(>|Chi|)
O. tridentata c.	3.1423	154	328.32	0.3702
G. struthium	2.7828	194	85.147	0.4263
H. squamatum	5.6045	193	100.14	0.1325
L. subulatum	1.2937	159	71.216	0.7306
S. viscosa	1.6251	193	40.323	0.6537
H. syriacum	0.9726	194	23.420	0.8079
F. thymifolia	1.6212	181	22.634	0.6546
R. officinalis	4.5858	186	183.72	0.2048
S. tenacissima	1.2599	135	25.448	0.7387

Table A.3. Summary of Cox proportional hazard models for mortality of the nine target species with different treatments.
Coef exp (significantly negative values indicate that the gypsum treatment reduces the hazard rate and thus increases the survival regarding standard treatment, while positive values imply a trade-off), standard error (SE), the Wald test statistics (indication of the relative importance of the effect), and the significance of the regression coefficients.Treatments according to weight proportions of gypsum:standard growing medium; High-g (50:50), Medium-g (25:75), Low-g (10:90), Standard-t (0:100).

											95% CI for exp.	
Species	**n**	**nº events**	**Wald test**	**P value**	**Gypsum treatment**	**Coef exp**	**Exp(coef)**	**SE(coef)**	**Z Value**	**Pr(>|z|)**	**Lower**	**Upper**
O. tridentata c.	158	61	30.03	**9.23e-004**	Low-g	-0.7991	0.4497	0.3106	-2.572	0.0101*	0.24464	0.8268
					Medium-g	-1.6281	0.1963	0.3777	-4.310	1.63e-05***	0.09363	0.4116
					High-g	-1.8602	0.1556	0.4093	-4.544	5.51e-06***	0.06978	0.3472
G. struthium	198	37	3.03	0.3863	Low-g	-0.3231	0.7239	0.5040	-0.641	0.521	0.2696	1.944
					Medium-g	-0.2052	0.8145	0.4859	-0.422	0.673	0.3142	2.111
					High-g	0.3930	1.4813	0.4337	0.906	0.365	0.6331	3.466

H. squamatum	197	81	15.34	**0.001547**	Low-g	-0.03683	0.96384	0.26976	-0.137	0.89142	0.5680	1.6354
					Medium-g	-0.51071	0.60007	0.29517	-1.730	0.08359.	0.3365	1.0702
					High-g	-1.26095	0.28338	0.35822	-3.520	0.00043***	0.1404	0.5719
L. subulatum	163	121	8.44	**0.03856**	Low-g	-0.4358	0.6467	0.2917	-1.494	0.135	0.3651	1.146
					Medium-g	-0.1393	0.8700	0.2429	-0.573	0.566	0.5404	1.400
					High-g	0.3589	1.4318	0.2425	1.480	0.139	0.8901	2.303
S. viscosa	197	8	0.91	0.8228	Low-g	-0.680374	0.506427	1.224755	-0.556	0.579	0.04592	5.585
					Medium-g	0.006382	1.006403	1.000005	0.0006	0.995	0.14176	7.145
					High-g	0.401664	1.494309	0.912874	0.440	0.660	0.24969	8.943
H. syriacum	198	30	2.67	0.4446	Low-g	0.9028	2.4665	0.5917	1.526	0.127	0.7734	7.866
					Medium-g	0.5700	1.7682	0.6268	0.909	0.363	0.5176	6.041
					High-g	0.8452	2.3286	0.6010	1.406	0.160	0.7170	7.562
F. thymifolia	185	106	13.92	**0.00301**	Low-g	-1.0318	0.3564	0.2836	-3.638	0.00027***	0.2044	0.6214
					Medium-g	-0.4797	0.6189	0.2640	-1.817	0.06915.	0.3689	1.0383
					High-g	-0.6169	0.5396	0.2639	-2.337	0.01943*	0.3217	0.9052
R. officinalis	190	9	1.71	0.634	Low-g	-1.988e+01	2.319e-09	1.029e+04	-0.002	0.998	0.00000	Inf
					Medium-g	-1.327e+00	2.652e-01	1.118e+00	-1.187	0.235	0.02964	2.373
					High-g	1.042e-01	1.110e+00	7.072e-01	0.147	0.883	0.27754	4.438
S. tenacissima	139	7	0.05	0.9969	Low-g	-2.022e-01	8.170e-01	9.129e-01	-0.221	0.825	0.1365	4.889
					Medium-g	-1.948e+01	3.464e-09	1.164e+04	-0.002	0.999	0.0000	Inf
					High-g	-3.583e-02	9.648e-01	9.129e-01	-0.039	0.969	0.1612	5774

Table A.4. Summary of the GLM for growth by species. Gypsum treatments: Low-g (low gypsum proportion), Medium-g (medium gypsum proportion), High-g (high gypsum proportion).

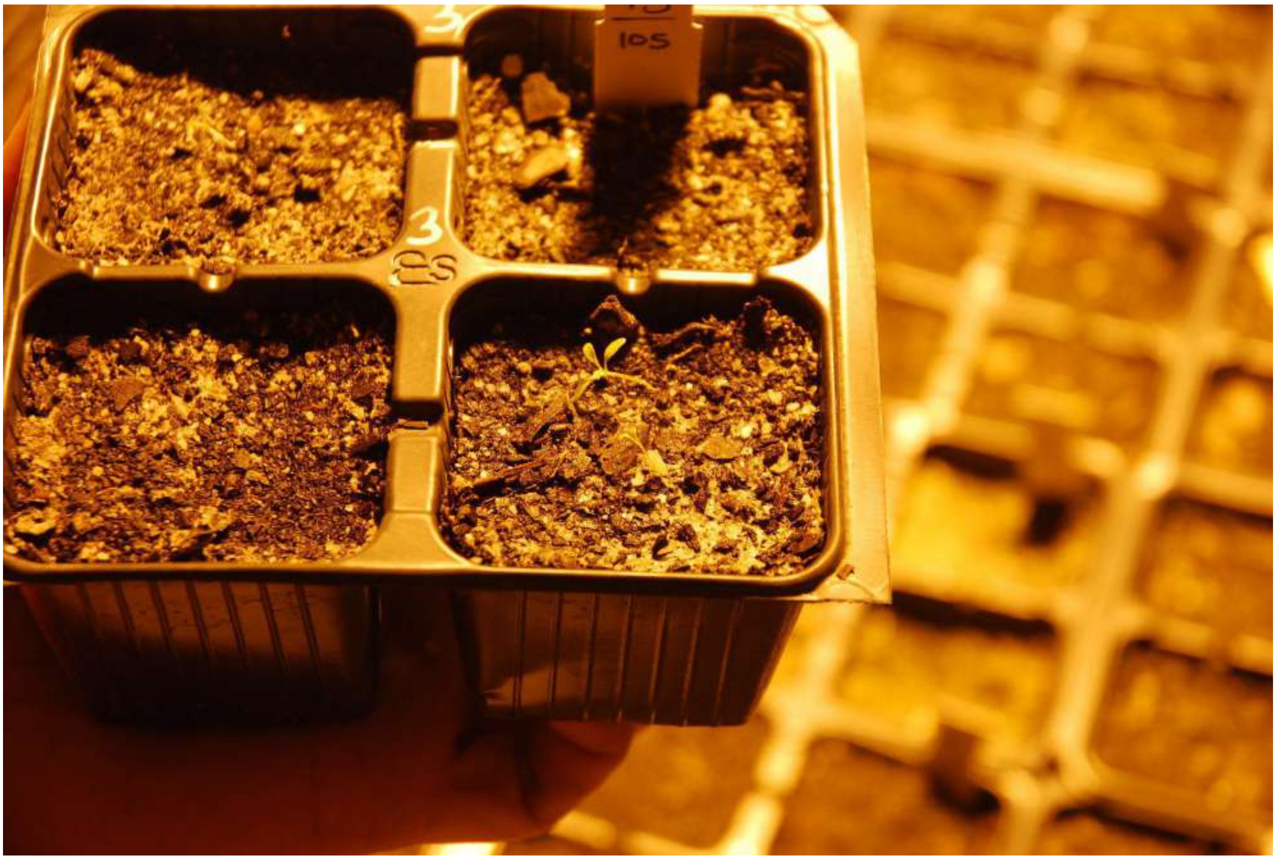
		Shoot growth				Root growth			
		Estimate	**Std. Error**	**t value**	**Pr(>|t|)**	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
O. tridentata c.	(Intercept)	-4.0715	0.2036	-19.997	<0.0001	-4.9596	0.1790	-27.702	<0.0001
	Low-g	**0.5834**	**0.2523**	**2.312**	**0.0227**	**0.8266**	**0.2219**	**3.726**	**0.000314**
	Medium-g	**0.5263**	**0.2494**	**2.111**	**0.0372**	**0.7041**	**0.2193**	**3.211**	**0.001751**
	High-g	**1.4214**	**0.2414**	**5.889**	**<0.0001**	**1.3981**	**0.2122**	**6.587**	**<0.0001**
G. struthium	(Intercept)	-2.57151	0.15814	-16.261	<0.0001	-4.03130	0.13136	-30.688	<0.0001
	Low-g	0.03297	0.22365	0.147	0.883	-0.09976	0.18578	-0.537	0.592
	Medium-g	-0.07061	0.22365	-0.316	0.753	0.07220	0.18578	0.389	0.698
	High-g	0.09884	0.22597	0.437	0.662	0.28823	0.18770	1.536	0.126
H. squamatum	(Intercept)	-5.92802	0.09307	-63.693	<0.0001	-6.54025	0.12254	-53.374	<0.0001
	Low-g	**0.39660**	**0.13092**	**3.029**	**0.00279**	0.16507	0.17144	0.963	0.337
	Medium-g	**0.25754**	**0.12896**	**1.997**	**0.04726**	0.24347	0.16890	1.441	0.151
	High-g	**0.36035**	**0.12896**	**2.794**	**0.00574**	0.01255	0.16890	0.074	0.941
L. subulatum	(Intercept)	-4.3117	0.3370	-12.795	<0.0001	-5.79394	0.34941	-16.582	<0.0001
	Low-g	-0.7599	0.4422	-1.718	0.0922	-0.67593	0.46417	-1.456	0.1521
	Medium-g	-0.7725	0.4680	-1.651	0.1053	-0.85955	0.48524	-1.771	0.0831
	High-g	-0.9564	0.5696	-1.679	0.0996	0.02722	0.62178	0.044	0.9653
S. viscosa	(Intercept)	-4.83061	0.16924	-28.544	<0.0001	-5.4364710	0.1529997	-35.533	<0.0001
	Low-g	-0.21858	0.23934	-0.913	0.362	-0.1776709	0.2163742	-0.821	0.41257
	Medium-g	-0.02143	0.23934	-0.090	0.929	-0.0005577	0.2163742	-0.003	0.99795
	High-g	**-0.49989**	**0.23934**	**-2.089**	**0.038**	**-0.6712729**	**0.2163742**	**-3.102**	**<0.0001**

		Shoot growth				Root growth			
H. syriacum	(Intercept)	-6.5242	0.1460	-44.689	< 0.0001	-6.5242	0.1460	-44.689	<0.0001
	Low-g	0.1000	0.2145	0.467	0.64161	0.1000	0.2145	0.467	0.64161
	Medium-g	0.2249	0.2765	0.813	0.41753	0.2249	0.2765	0.813	0.41753
	High-g	**-0.6673**	**0.2116**	**-3.154**	**0.00198**	**-0.6673**	**0.2116**	**-3.154**	**0.00198**
F. thymifolia	(Intercept)	-4.9044	0.3484	-14.078	<0.0001	-5.5713	0.2747	-20.281	<0.0001
	Low-g	**-1.0296**	**0.4345**	**-2.369**	**0.0203**	**-1.5376**	**0.3426**	**-4.488**	**<0.0001**
	Medium-g	**-2.3920**	**0.4660**	**-5.133**	**<0.0001**	**-2.6256**	**0.3675**	**-7.145**	**<0.0001**
	High-g	**-2.9719**	**0.4561**	**-6.515**	**<0.0001**	**-2.9858**	**0.3597**	**-8.301**	**<0.0001**
R. officinalis	(Intercept)	-3.8702	0.1202	-32.196	< 0.0001	-4.27392	0.09055	-47.201	<0.0001
	Low-g	-0.2355	0.1700	-1.386	0.167	-0.10086	0.12805	-0.788	0.4318
	Medium-g	-0.2713	0.1700	-1.596	0.112	**-0.28991**	**0.12805**	**-2.264**	**0.0247**
	High-g	-0.1998	0.1700	-1.175	0.241	**-0.31094**	**0.12805**	**-2.428**	**0.0161**
S. tenacissima	(Intercept)	-3.84980	0.08441	-45.608	< 0.0001	-4.54149	0.11093	-40.939	<0.0001
	Low-g	0.12087	0.12012	1.006	0.316	0.16292	0.15888	1.025	0.307
	Medium-g	0.12683	0.12345	1.027	0.306	0.03591	0.16224	0.221	0.825
	High-g	0.03515	0.12640	0.278	0.781	0.06685	0.16612	0.402	0.688

Table A.5. Analysis of deviance and summary of the GLM showing effects of gypsum levels on volumetric water content.

Deviance Resid.	Resid. Dev	P(>|Chi|)		Estimate	Std. Error	z value	Pr(>|z|)
137.97	876.94	2.2e-16 ***	(Intercept)	2.71281	0.02643	102.651	< 2e-16 ***
			Low-g	0.10821	0.03597	3.008	0.00263 **
			Medium-g	0.34080	0.03450	9.879	< 2e-16 ***
			High-g	0.26382	0.03530	7.473	7.86e-14 ***

Sowing in a experiment to produce seedlings of nine gypsum species in a growth chamber, adding different gypsum proportions to a nursery growing medium commonly used for plant production (peat).



Seedling of *Lepidium subulatum* growing under controlled light, water and temperature conditions.

Chapter 4

Central role of bedding materials for gypsum-quarry restoration: An experimental planting of gypsophile species.

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Central role of bedding materials for gypsum-quarry restoration: an experimental planting of gypsophile species

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Abstract

The loss of the original soil and mineral resources caused by quarrying activities represents a major challenge for the restoration of singular flora associated with specific substrates. In particular, the rare and original gypsum flora is severely affected by quarrying, and identifying the best measures to recover it is decisive for its conservation. In this paper, we evaluate the efficacy that planting with several contrasting bedding-materials has for the recovery of three native gypsophile species in gypsum habitats affected by quarrying. With this aim, in a disturbed gypsum area in SE Spain, we experimentally planted one-year-old nursery-grown plants of *Helianthemum squamatum*, *Lepidium subulatum*, and *Ononis tridentata* subsp. *crassifolia,* employing four bedding materials potentially useful for restoration: raw gypsum, gypsum spoil, topsoil on gypsum spoil, and marls. Plant performance was evaluated in terms of survival, growth, and the production of flowers, fruits, and seeds. High survival was achieved in all the treatments, demonstrating the excellent response of these species to planting. However, bedding materials had a significant effect on plant performance, with raw gypsum and gypsum spoil being the options that most benefited growth and production (in terms of flowers, fruits, and seeds). Remarkable results were achieved in raw gypsum, although gypsum spoil appears to be the most reasonable option for restoration, given its low cost, wide availability, and potential to recover disturbed gypsum environments. By contrast, common measures such as the use of topsoil should not be routinely recommended for the recovery of gypsum vegetation. Hence, our study shows the importance of identifying the most appropriate measures when specialized flora is the object of restoration and thus will contribute to the development of strategies for the conservation of gypsum habitats affected by quarrying.

Keywords: Bedding material, gypsophile, gypsum habitat, planting, quarry spoil, restoration techniques.

1. Introduction

Restoration of the native vegetation in mining areas usually poses a major problem due to several limitations, with soil loss and the alteration of the original topography being some of the most drastic disturbances (Bradshaw and Chadwick, 1980; Clemente et al., 2004). Consequently, additional substrates are required when the recovery of the former plant community is the goal of restoration (Oliveira et al., 2011). Common practices include the use of raw spoils generated by mining to backfill the disturbed area (Carrick and Krüger, 2007). These materials are often regarded as difficult substrates for vegetation recovery due to several limiting factors, such as low nutrient content or poor structure (Singh et al., 2002). Thus, the application of the topsoil retrieved from the pre-mined area and/or the addition of organic amendments to the spoil are widely used to overcome these limitations (Ghose, 2004; Kundu and Ghose, 1994; Martínez-Ruiz and Fernández-Santos, 2005). However, severe changes in the original soil properties may result in contrasting situations for plant development. While the use of raw spoil may constitute a harsh environment and slow down plant-cover regeneration (Alday et al., 2011), the use of topsoil or amendments may promote the establishment of undesirable species (e.g. generalist-colonizers) at the expense of the native vegetation, and consequently hinder the restoration of the former habitat (Ballesteros et al. 2012; Castillejo and Castelló, 2010; Nair et al., 2000). In this context, the selection of starting materials determines the success of restoration processes (Bradshaw, 2000), and is particularly decisive for the recovery of singular flora associated to specific substrates, as reported for copper, serpentine or gypsum soils (Ballesteros et al., 2012; O'Dell and Claassen, 2009; Whiting et al., 2004).

Specifically gypsum areas, which harbor rare and original flora worldwide (Escudero et al., 1999; Meyer 1986; Parsons, 1976), are often affected by quarrying and represent a major challenge in the restoration of disturbed singular environments (Mota et al., 2011). In this context, previous works have demonstrated the limitations that natural succession has for the recovery of gypsum vegetation over the short or middle term (e.g. Ballesteros et al., 2012; Dana and Mota, 2006; Mota et al., 2003, 2004). Thus, several studies have based their approach on the management of substrates and species in accordance with the restoration goals. In this sense, previous works in arid gypsum areas have reported satisfactory revegetation with environment-adapted species planted on byproducts (e.g. spoil or tailings) from gypsum mining (Blignaut and

Milton, 2005; Rao and Tarafdar, 1998; Sharma et al., 2001). Other works in Mediterranean areas have approached the rehabilitation of disturbed gypsum soils by applying topsoil and/or organic amendments, showing that the establishment of undesirable vegetation can hinder the restoration of native gypsophile species (Ballesteros et al. 2012; Castillejo and Castelló, 2010; Marqués et al., 2005). Moreover, the study of the short-term response of sowing native gypsophile species under several bedding materials and soil surface treatments have demonstrated that the measures applied may strongly influence the restoration process (Ballesteros et al., 2012).

Identifying the best restoration techniques is crucial to recover gypsum vegetation. In particular, the Iberian gypsum vegetation is severely affected since Spain is one of the main gypsum producers worldwide (Craig et al., 2007). Despite that gypsum habitats are considered a priority for conservation at the European level (Anonymous, 1992), large areas are disturbed, affecting the singular local flora (e.g. Ballesteros et al., 2013, 2012; Mota et al., 2004). Consequently, there is a need to develop specific measures to restore these environments. In this sense, the recovery of the flora has mostly been approached relying on unaided natural succession (Dana and Mota, 2006; Mota et al., 2004, 2003), or using active restoration techniques such as hydroseeding (Matesanz and Valladares, 2007) or sowing (Ballesteros et al., 2012). Sowing may constitute an advantageous method in restoration projects. Under ideal conditions, it could be useful to provide high plant density and cover in a natural-like distribution at a lower cost than planting. By contrast, planting can provide more efficient seed use, more resistant plants and faster establishment at the expense of increasing propagation and planting labor costs. However, although previous works have reported the benefits of planting in disturbed quarry areas (Singh et al., 2002), and specifically for species inhabiting gypsum environments (Blignaut and Milton, 2005; Sharma et al., 2001), there is no scientific literature available that tests the applicability of planting for the restoration of gypsum specialists.

Therefore, given the importance of substrates and species, the aim of this study is to improve the restoration of Iberian gypsum habitats affected by quarrying by (1) testing the applicability of planting as a method to establish three characteristic gypsophile species and (2) identifying the bedding materials that maximize plant performance.

2. Material and Methods

2.1. Site description

The study was performed in an experimental area set on a cereal old field consisting of marls next to an active quarry in Escúzar (Granada, SE Spain; 37º 2' N, 3º 45' W) at 950 m asl. The climate type is continental Mediterranean, with relatively cold winters, hot summers, and four months of water deficit. The mean annual temperature is 15.1°C, with an average monthly minimum temperature in January of 7.6°C and maximum of 24.2°C in August. Annual rainfall averages 421.1 mm, occurring mainly in winter. The area is in the Neogene sedimentary basin of Granada, the dominant substrates being lime and gypsum from the late Miocene, the latter in combination with marls (Aldaya et al., 1980). The predominant soils in the gypsum outcrops are gypsisols (Aguilar et al., 1992). The vegetation of the area is a mosaic of fields with crops and orchards (cereals and almond and olive trees) and scattered patches of native plants growing over gypsum outcrops. The native vegetation is included in the Habitat Directive as 1520, "Iberian gypsum vegetation, *Gypsophiletalia*" (Escudero, 2009), and is characterized by plants exclusive to gypsum soils (gypsophiles), such as *Helianthemum squamatum* (L.) Dum. Cours., *Lepidium subulatum* L. or *Ononis tridentata* subsp. *crassifolia* (Dufour ex Boiss.) Nyman. In addition, there are also other frequent non-exclusive species of gypsum substrates such as *Stipa tenacissima* L., *Rosmarinus officinalis* L., *Helianthemum syriacum* (Jacq.) Dum. Cours. and *Thymus zygis* L. subsp. *gracilis* (Boiss.) R. Morales (according Marchal et al. 2008).

2.2. Target species

Three characteristic species of the gypsum habitat were selected for experimental planting including: *H. squamatum* (*Cistaceae*) and *L. subulatum* (*Brassicaceae*), both widely distributed in gypsum outcrops in the Iberian Peninsula and some localities in North Africa (see Mota et al., 2011), and *O. tridentata* subsp. *crassifolia* (*Leguminosae*), a narrow endemic restricted to gypsum outcrops in SE Spain (CW Granada province) and considered under threat (Vulnerable, VU, according to Ballesteros et al., 2013).

2.3. Field experiment

Four materials reproducing potential options for plant reintroduction that mimicked possible post-quarrying conditions were set up in the experimental area (see Ballesteros et al., 2012). The bedding materials included: (1) marls (M), using the substrate in the area to recreate a scenario where gypsum rock had been completely eliminated, and where the old-field topsoil (c. 30 cm) and thus its seed bank had been removed; (2) gypsum spoil (GS), providing a layer of gypsum mine spoil (0.5 m) generated from gypsum quarrying; (3) topsoil (T), placing a layer of topsoil (ca. 10 cm) retrieved from the natural habitat (after 8 months of stockpiled storage), on top of a gypsum spoil layer (0.5 m); and (4) raw gypsum (RG), consisting of a layer (0.5 m) of coarse gypsum. While raw gypsum is the material used for industrial processing, gypsum spoil is a by-product with no commercial value generated during the quarrying. Supplementary information on the properties of the materials and the moisture content are provided in Appendix A and B, respectively.

In February 2011, we planted on each material one-year-old nursery-grown plants of *H. squamatum* (50 ind. x 4 bedding treatments= 200 individuals), *L. subulatum* (50 ind. x 4 bedding treatments= 200 individuals), and *O. tridentata* subsp. *crassifolia* (33 ind. x 3 bedding treatments= 99 individuals). Due to the low plant availability of the latter species, we ruled out planting in the marls treatment, for being considered *a priori* the least suitable for restoration. Plants were set 0.75 m apart. The spontaneous flora was eliminated by clipping aboveground biomass in all materials in February 2011 and April 2012 to reduce potential competition from other species. Plants were produced in a nursery, using 250 cm^3 plastic pots filled with a mixture of commercial substrate (organic matter = 85.4%, pH 6-7, N 260 $mg \cdot kg^{-1}$, P 389 $mg \cdot kg^{-1}$, K 2000 $mg \cdot kg^{-1}$, Mg 678 $mg \cdot kg^{-1}$, Fe 15 $mg \cdot kg^{-1}$) and gypsum in proportions of 75 and 25%, respectively. All plants were obtained from seeds manually harvested in patches of natural vegetation in the study area between June and September 2009.

To evaluate species performance with respect to bedding material, we monitored the survival of each individual on a monthly basis from February 2011 to June 2012. In addition, plant growth was estimated by measuring differences in plant volume between three sampling dates (July 2011, April 2012 and June 2012), using the equation for the volume of a semispheroid [$V= (4/3\ \pi\ r^2\ h)\ /2$], where r is the plant radius and h is the plant height (Lorite et al., 2010). Flower production was estimated by counting the flowers in three randomly taken flowering stems per plant at flowering peak (June 2012). Afterwards, the flower average was multiplied by the number of flowering stems per plant to estimate the number of flowers per plant. Fruit production was estimated following the same procedure at peak fruiting (July 2012). Seed output was estimated by counting well-formed seeds in 10 fruits from 30 plants per species and treatment (10 fruits x 30 plants per species x bedding material; 1200 fruits, respectively, for *L. subulatum* and *H. squamatum,* and 900 fruits for *O. tridentata* subsp. *crassifolia*). Seed production per individual was calculated by multiplying its fruit yield by average seed set for each species.

2.4. Statistical analysis

The effect of bedding treatment on overall species mortality and global growth over time was modeled fitting Generalized Linear Mixed Models (GLMMs), including bedding material as a fixed factor and species as a random factor. In particular, mortality was modeled using the "lmer" function, (R "lme4" package; Bates et al., 2012), specifying a binomial error distribution and logit-link function. Survival differences between bedding materials were assessed for each species using Kaplan-Meier survival curves (R "survival" package; Therneau, 2013). Plant growth was modeled, using the "lme" function (R "nlme" package; Pinheiro et al., 2011). Model parameters were estimated using the Laplace approximation of likelihood (Bolker et al., 2009). To evaluate the effect of bedding treatment by species on plant growth, production of flowers, fruits, and seeds, and on the average seed production per fruit, we fitted Generalized Linear Models (GLMs), assuming a Poisson error distribution and log-link function. Multiple comparisons were made in all analyses using the R "multcomp" package (Hothorn et al., 2008).

3. Results

3.1. Survival

Overall survival over the first 17 months (February 2011-June 2012) , considering all species and bedding treatments was 87.2 %, with the highest survival in marls (94%), followed by gypsum spoil (88.7%), raw gypsum (88%) and topsoil (79.7%). Despite the high overall survival, significantly more deaths occurred in topsoil (Table 1). This was mainly due to *H. squamatum,* which showed significantly lower survival in this treatment, since no differences were found for *L. subulatum* or *O. tridentata* subsp. *crassifolia* when the effect of the bedding-materials was compared within species (Fig. 1).

Table 1. Effect of bedding treatment on overall plant survival, considering all species, evaluated fitting a Generalized Linear Mixed Model (GLMM). Bedding treatment, as fixed factor and species as random factor. Random effects are expressed in the last row of the table.

Survival	**Estimate**	**SE**	**Z value**	**Pr(>|z|)**
Intercept	2.7352	0.8925	3.065	0.00218
Raw gypsum	-0.0816	0.4034	-0.202	0.83978
Topsoil	-0.8055	0.3742	-2.153	0.03132
Marls *	0.8640	0.5263	1.642	0.10068
Random effects	Variance: 2.04		SD: 1.428	

* The marls treatment was not applied to *Ononis tridentata* subsp. *crassifolia*.

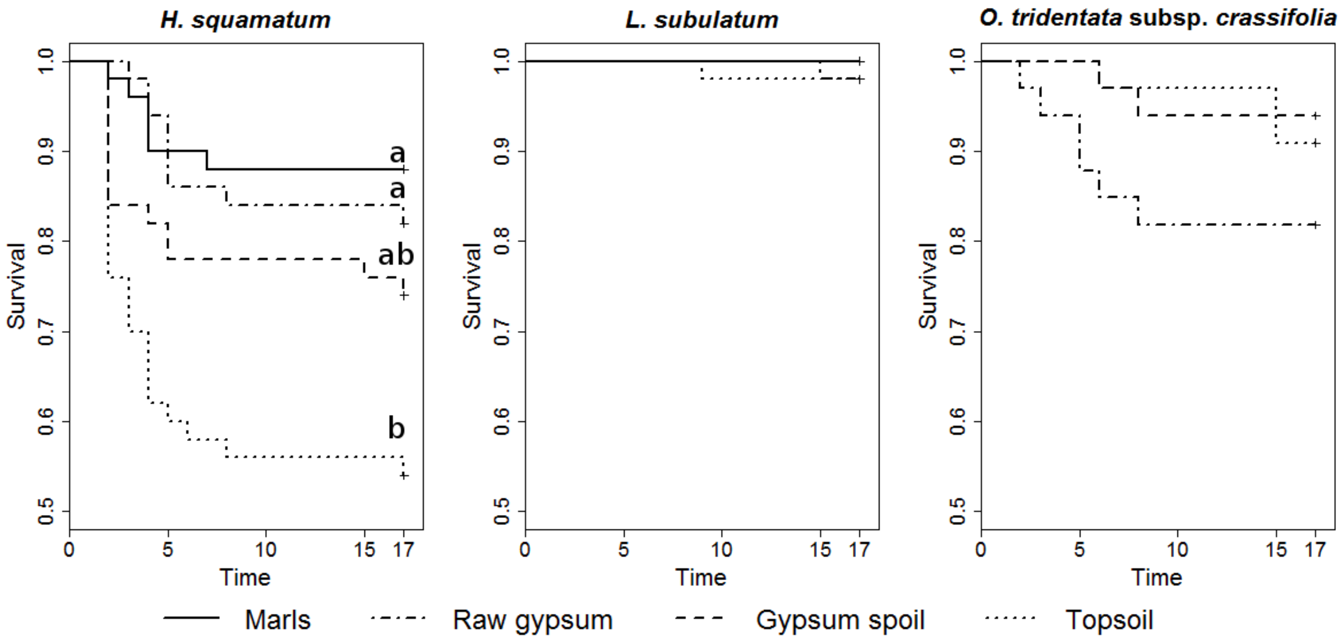

Fig. 1. Kaplan-Meier survival curves representing species survival over time (from February 2011 to June 2012) for each bedding material. Different letters represent statistically significant differences between groups (p<0.05).

3.2. Growth

Bedding material significantly affected overall plant growth when all species were considered together (Fig. 2a and Table 2). Significant differences were found between treatments in each sample period (Table 2). The first volume measures taken in July 2011 showed significant positive effect only in gypsum spoil. Nevertheless, measures taken in April 2012 and June 2012 showed significant positive effects in both gypsum spoil and raw gypsum treatments, with the highest overall plant growth under the raw gypsum treatment. This pattern recurred in all species individually (Fig. 2 bcd, and Table 3 expressing the last measures taken in June 2012). In the other treatments, plants had significantly lower growth, the lowest being in the marls for *L. subulatum,* and in topsoil for *H. squamatum* and *O. tridentata* subsp. *crassifolia* (Fig. 2bcd).

Table 2. Effect of bedding treatment on plant growth (volume) in the three sampling periods (July 2011, April 2012 and June 2012) considering all species, evaluated fitting a Generalized Linear Mixed Model (GLMM). Bedding treatment, as fixed factor and species as random factor. Random effects for each period are expressed in the last row of the table.

Model effects on plant volume

	July 2011				April 2012				June 2012			
	Estimate	**SE**	**z value**	**Pr(>|z|)**	**Estimate**	**SE**	**z value**	**Pr(>|z|)**	**Estimate**	**SE**	**z value**	**Pr(>|z|)**
Intercept	7.66980	0.54798	14.0	**<0.0001**	8.52329	0.51237	16.6	**<0.0001**	10.23510	0.29562	34.6	**<0.0001**
Raw gypsum	-0.45485	0.00280	-162.6	**<0.0001**	0.32896	0.00149	220.9	**<0.0001**	0.71973	0.00064	1120.9	**<0.0001**
Topsoil	-1.00019	0.00334	-299.6	**<0.0001**	-1.11411	0.00230	-484.2	**<0.0001**	-1.57803	0.00130	-1212.7	**<0.0001**
Marls *	-1.27678	0.00594	-214.9	**<0.0001**	-2.20861	0.00462	-478.3	**<0.0001**	-2.54901	0.00239	-1065.8	**<0.0001**
Random effects	Variance: 0.90083		SD: 0.94912		Variance: 0.78756		SD: 0.88745		Variance: 0.26217		SD: 0.51202	

* The marls treatment was not applied to *Ononis tridentata* subsp. *crassifolia*.

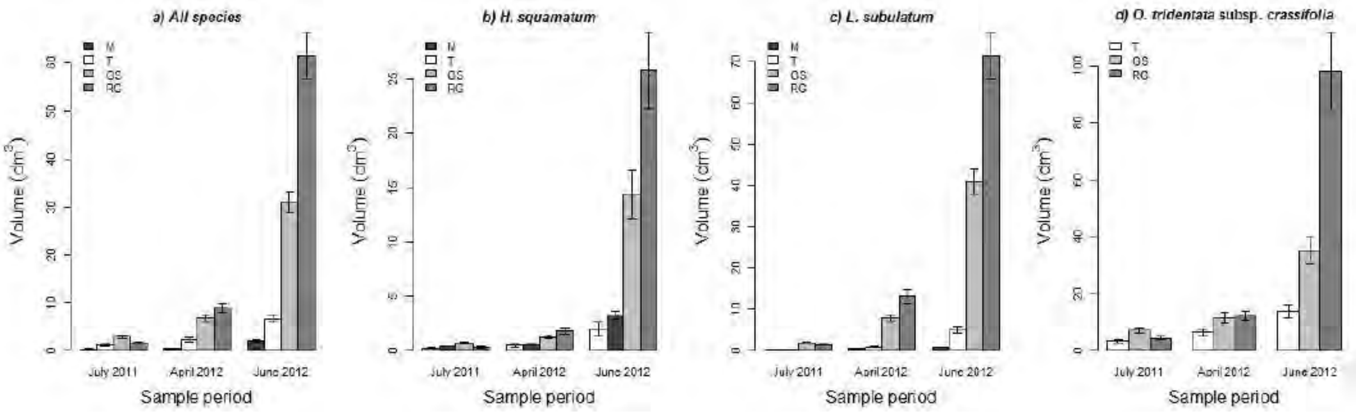

Fig. 2. Plant volume (mean±SE) of the three species assayed measured in three sample periods (July 2011, April 2012, and June 2012) for each bedding material: M, marls; T, topsoil; GS, gypsum spoil; RG, raw gypsum.

3.3. Flower, fruit, and seed production

Bedding materials had significant effects on flower, fruit, and seed production in all species (Table 3). Flower and fruit production differed significantly between all treatments, proving higher in raw gypsum for all species, followed by gypsum spoil. Comparing marls and topsoil treatments (the overall worst treatments), *H. squamatum* produced more flowers and fruits in marls, while the opposite was true of *L. subulatum*, which performed better in topsoil. *O. tridentata* subsp. *crassifolia,* registered the worst results for topsoil. Fruits yielded similar seed sets within species among treatments, except for *H. squamatum,* having more seeds per fruit in the gypsum spoil and less in topsoil. The number of seeds per plant also differed significantly between treatments, being the highest in raw gypsum and gypsum spoil for all species, followed by marls in *H. squamatum*, or topsoil in *L. subulatum* and *O. tridentata* subsp. *crassifolia*, (Table 3).

				Bedding materials							
				Raw gypsum		Gypsum spoil		Topsoil		Marls	
Species	Parameters	X^2	p	N	mean±se	N	mean±se	N	mean±se	N	mean±se
H. squamatum	Vol. (dm^3)	1246	**<0.0001**	41	25.76±3.49a	37	14.38±2.25b	27	1.99±0.66d	44	3.24±0.37c
	Flowers	315445	**<0.0001**	41	5366.85±827.76a	37	2487.16±422.47b	27	217.82±63.78d	44	378.68±49.24c
	Fruits	187657	**<0.0001**	41	3640.66±681.58a	37	2189.11±361.04b	27	198.93±64.17d	44	455.81±65.21c
	Seeds	723566	**<0.0001**	41	13069.96±2446.88a	37	8636.38±1424.35b	27	492.58±158.89d	44	1588.01±227.18c
	Seeds/Fruit	8.6912	**0.0337**	30	3.59±0.14ab	31	3.95±0.14a	21	2.48±0.19b	31	3.48±0.12ab
L. subulatum	Vol. (dm^3)	6341.1	**<0.0001**	49	71.33±5.68a	50	40.90±3.00b	48	4.94±0.78c	50	0.86±0.09d
	Flowers	4129794	**<0.0001**	49	42505.39±4475.53a	50	19115.30±1955.29b	48	1453.44±329.70c	50	47.42±13.84d
	Fruits	3594211	**<0.0001**	49	37466.88±4806.23a	50	16422.94±1888.93b	48	1428.42±292.08c	50	51.32±16.98d
	Seeds	5940637	**<0.0001**	49	61595.55±7901.44a	50	26523.05±3050.62b	48	2202.53±450.37c	50	71.28±23.58d
	Seeds/Fruit	0.50554	0.9177	25	1.64±0.05	20	1.62±0.06	31	1.54±0.04	18	1.39±0.11
O. tridentata subsp. *crassifolia*	Vol. (dm^3)	2219.8	**<0.0001**	27	98.10±13.67a	30	35.11±4.51b	30	13.78±2.36c	-	-
	Flowers	153001	**<0.0001**	27	3557.85±547.83a	30	448.94±86.01b	30	88.93±24.25c	-	-
	Fruits	89981	**<0.0001**	27	2012.04±313.38a	30	236.58±47.19b	30	36.63±12.68c	-	-
	Seeds	125130	**<0.0001**	27	2784.66±433.71a	30	320.80±63.99b	30	49.93±17.28c	-	-
	Seeds/Fruit	0.007517	0.9962	25	1.38±0.05	25	1.36±0.10	12	1.36±0.09	-	-

Table 3. GLM results and mean values (± SE) of the parameters evaluated to assess the performance of the three species on each bedding material. Parameters: Vol.=Volume (expressed in dm^3), flower, fruit, and seed production per individual, and seeds per fruit. Different letters indicate significant differences ($p<0.05$) in the Tukey's *post hoc* test.

4. Discussion

The high survival rate found in our study, with 87.2% of plants alive at the end of the study period, suggests that planting on the tested bedding materials is an efficient technique to achieve good establishment for all the species. Thus, considering all species together, the response to each material proved very satisfactory, with survival rates as high as 79.7% in the worst case (topsoil). Species individually responded very favorably to planting, with good survival rates, except for significantly worse performance of *H. squamatum* in topsoil (54% survival). Similarly, moderate or good survival have been achieved in other species planted on degraded gypsum soils (i.e. ~ 45% in Spain, Rincón et al., 2007) or gypsum spoil (i.e. 68% in South Africa; Blignaut and Milton, 2005, or up to 85% in the India; Sharma et al., 2001). Planting may be especially advantageous in quarry spoils, since planting tasks (e.g. site preparation, digging holes, etc.) can help to overcome common problems for plant development on these materials (e.g. crusting or compaction), and introducing nursery-grown plants raised to a size adequate to cope with adverse conditions (e.g. low water availability during the summer) can help to achieve good survival and establishment. Specifically, Escudero et al. (2000, 1999) reported that survival was size-dependent for *H. squamatum* and *L. subulatum* (i.e. larger plants having a better chance of surviving), and it is probably why low survival has been reported for seedlings emerging in nature (i.e. <40% for *H. squamatum,* and 47.3% for *L. subulatum*; Escudero et al., 1999, 2000) or under greenhouse conditions (i.e. <25% both species, Matesanz and Valladares, 2007). In contrast, the three species tested in our study achieved similar survival to our plantings when sown on the same materials (>70%, Ballesteros et al. 2012), pointing out the need of comparing both restoration approaches in depth. However, since plantings in our study have achieved high survival rates in all the bedding materials, the choice for restoration should also take into account the capacity of materials to encourage species growth and production.

In this sense, raw gypsum and gypsum spoil were the most beneficial materials for growth, flowering, fruiting, and seed production of all gypsophiles in comparison to topsoil or marls. Significantly larger and more productive plants found on these materials indicated advantageous effects on species development. The greater plant size and production on these materials would likely be due to resource availability (e.g. substrate chemical composition or water availability during the summer). Particularly, these gypsum-rich materials may have reproduced some of the features that gypsophiles find beneficial in their natural habitat. Consistent for most materials in our study, gypsum or sulfur content has been claimed by some authors to increase growth and development of gypsophiles (Meyer, 1986; Ruiz et al., 2003). However, it does not seem conclusive to explain the lower performance in topsoil (Appendix A). Therefore, other factors would have to be modulating plant response. In this regard, water availability during the summer drought has been used to explain active summer growth and summer flowering phenology of most gypsophiles (Escudero, 2000, 1999; Meyer, 1986). However, there was more water only in the top 10 cm of raw gypsum and gypsum spoil compared to topsoil during the most active growing and reproductive periods (April to July), (Fig. 2, Appendix B), making it difficult to attribute results only to this factor. In addition, competition has been reported to affect plant performance on this material (Ballesteros et al., 2012), but since aboveground biomass was eliminated in our study, gypsophiles response would rather be modulated by the edaphic properties of the material. Nevertheless, although the reasons for better performance on raw gypsum and gypsum spoil are not conclusive, both materials have proved to greatly benefit gypsophiles, so that their use in restoration would be very positive to recover the three species tested in areas affected by quarrying.

By contrast, topsoil and marls proved to be less beneficial for gypsophiles. Since smaller and less productive plants were recorded on these materials, their use suggests only limited benefits for the introduction of the target species by planting. Restoration guidelines for mining-disturbed areas (e.g. Department of Industry, Tourism and Resources, 2006; Department of Minerals and Energy Western Australia, 1996; Jorba and Vallejo, 2010, etc.) and many authors have recommended the use of topsoil to provide a seed bank and to enhance soil properties for improved plant development and revegetation (e.g. Castillejo and Castelló, 2010; Ghose, 2004; Tormo et al. 2007). However, this treatment did not enhance species performance in our study, and its effectiveness at assisting the unaided (neither planting nor sowing) restoration of the whole gypsum community would need to be demonstrated. In this sense, previous studies have reported competition when applying topsoil for gypsum-quarry restoration, hindering the development of gypsophile species (Ballesteros et al. 2012; Castillejo and Castelló, 2010). However, since the competition was eliminated in our study, the poor response of gypsum species would rather be related to topsoil edaphic properties. Similarly, marls proved less appropriate as a potential alternative to enhance the performance of the three study species in a potential scenario where gypsum had been depleted, since this treatment showed the worst growth and production results in general. Thus, the restoration of the disturbed area would be better undertaken by the application of a layer of raw gypsum or gypsum spoil jointly with the planting of gypsum species.

The planting of gypsophiles on raw gypsum and gypsum spoil may benefit not only restoration by reintroducing new individuals into the disturbed area, but may also start seed-bank buildup and increase species opportunities for establishment from their own seed. Accordingly, the choice for restoration should also be based on the capacity of these substrates to ensure the regeneration of the target species, as well as the diversity of the gypsum plant community over the long term. In this regard, despite raw gypsum produced a larger number of seeds in our study, previous sowing tests have shown low recruitment compared to gypsum spoil under the same conditions (Ballesteros et al., 2012). These results suggest that if higher recruitment could be achieved by a smaller but more efficient seed bank, gypsum spoil could constitute an equal or better option to encourage the long-term establishment of these species. Additionally, gypsum spoil has also proved the most beneficial option for the restoration of other desirable scrub species occurring in gypsum habitats in the area (e.g. *Stipa tenacissima, Helianthemum syriacum, Thymus zygis* subsp*. gracilis, Rosmarinus officinalis*) when sown on this material (Ballesteros et al., 2012). Moreover, the use of gypsum spoil in restoration is of particular interest since this material constitutes an inexpensive byproduct of the quarrying operation, produced in large quantities, and used commonly to fill quarry pits before vegetation recovery is attempted, in contrast to the industrial value and the consequent low availability of raw gypsum for restoration. Thus, despite the remarkable success achieved with raw gypsum, the many advantages of gypsum spoil suggest it is the most suitable option to conduct vegetation-restoration works in disturbed gypsum areas affected by quarrying.

Sowing has also proved beneficial for the establishment of gypsophiles in gypsum disturbed environments (Ballesteros et al. 2012; Matesanz and Valladares, 2007), and probably the reintroduction of species such as *H. squamatum* and *L. subulatum* would be more cost effective using this method, since seed harvest at peak fruiting would provide enough seeds for restoration purposes. By contrast, the seeds of the threatened and narrow endemic *Ononis tridentata* subsp. *crassifolia* are often difficult to harvest, highly predated (Ballesteros et al., 2013) and with low germination rates (Cañadas et al., 2014). Thus, propagating this vulnerable species in a nursery would be particularly beneficial for the efficient use of seeds collected for restoration, and given the satisfactory results achieved by planting in our study, this approach could effectively encourage its establishment. Therefore, the choice for restoration method should be based on a sound analysis considering the specific objectives of the project, the availability of seeds and bedding materials, and the cost-effectiveness of each approach (i.e. Gilardelli et al. 2014).

Restoration studies in abandoned quarries (gypsum, limestone, marble, etc.) have demonstrated the strong potential of these areas for biodiversity conservation (e.g. Davis et al., 1979; Gentili et al., 2010; Mota et al., 2004). Restoration of abandoned gypsum quarries constitute an opportunity for the conservation of specialized and/or endangered species, and should be aimed at creating suitable environments to settle sustainable populations and minimizing the risks of fragmentation of areas of high conservation value in gypsum habitats (Ballesteros et al., 2012, 2013, Dana and Mota, 2006; Mota et al. 2011).

5. Conclusion

In conclusion, the restoration of the gypsophiles *Helianthemum squamatum*, *Lepidium subulatum*, and *Ononis tridentata* subsp. *crassifolia* can successfully be undertaken by planting on bedding materials such as raw gypsum and gypsum spoils. Plantings on these materials achieved good survival as well as enhanced growth and seed production, proving their utility to conduct the restoration. However, gypsum spoil should be recommended, given its low cost, wide availability, and potential for the recovery of the areas affected by gypsum quarrying. Plant-cover regeneration must be evaluated over the long term to guarantee the restoration success and confirm the ecological and economic viability of using these materials over time. By contrast, topsoil and marls were less advantageous for the reintroduction of the study species by planting. Finally, this work highlights the importance of conducting specific research to identify the most beneficial measures for the restoration of conspicuous flora and vegetation in disturbed areas, and specifically those inhabiting gypsum habitats.

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Appendices

Appendix A. Mean values (±SE) of the edaphic variables of the bedding materials studied. Different letters indicate statistically significant differences in the *post hoc* test after Bonferroni correction at $p<0.05$. Five samples were randomly collected on each bedding material (20 in total) at 0-30 cm depth. Analyses were conducted following the methodology in Mañares et al. (1998) and MAPA (1994).

Variable	Raw gypsum	Gypsum spoil	Topsoil addition	Topsoil removal
Gravel (>2mm) (%)	55.38 ± 1.42 a	31.72 ± 2.44 b	7.74 ± 2.60 c	2.37 ± 0.30 c
Sand (2-0.05 mm) (%)	31.26 ± 2.21 a	32.55 ± 3.33 a	39.89 ± 3.07 a	5.01 ± 0.48 b
Coarse silt (0.05-0.02 mm) (%)	5.76 ± 0.73 b	11.89 ± 1.60 ab	16.43 ± 4.84 a	5.45 ± 0.68 b
Fine silt (0.02 mm) (%)	62.98 ± 1.57 b	55.56 ± 4.29 b	43.68 ± 2.73 c	89.54 ± 0.91 a
pH	7.91 ± 0.02 a	8.04 ± 0.01 a	7.90 ± 0.06 a	7.99 ± 0.05 a
CEC (meq/100g)	8.23 ± 0.32 b	7.40 ± 0.42 b	9.24 ± 0.21 b	20.11 ± 1.30 a
Ca^{2+} (mg/l)	34.68 ± 0.97 b	39.73 ± 1.33 a	39.68 ± 0.54 a	35.00 ± 0.84 b
Mg^{2+} (mg/l)	9.81 ± 0.41 a	4.40 ± 0.61 b	3.24 ± 0.30 b	0.83 ± 0.03 c
Na^{+} (mg/l)	1.95 ± 0.34 a	1.97 ± 0.26 a	1.90 ± 0.21 a	1.62 ± 0.36 a
K^{+} (mg/l)	3.74 ± 0.45 a	1.18 ± 0.13 b	1.44 ± 0.10 b	0.65 ± 0.40 b
S^{2-} (mg/l)	2664.62 ± 61.98 a	2352.00 ± 73.33 b	2358.86 ± 98.65 b	1999.20 ± 43.13 c
Organic carbon (%)	0.33 ± 0.07 b	0.42 ± 0.12 b	1.03 ± 0.07 a	0.92 ± 0.17 a
Organic matter (%)	0.56 ± 0.13 b	0.73 ± 0.20 b	1.78 ± 0.12 a	1.59 ± 0.29 a
N (%)	0.03 ± 0.00 c	0.03 ± 0.00 c	0.06 ± 0.01 b	0.10 ± 0.01 a
$CaCO_3$ (%)	30.45 ± 1.13 b	23.97 ± 0.36 c	23.37 ± 0.74 c	52.52 ± 1.32 a
Gypsum (%)	87.18 ± 1.12 a	88.22 ± 1.92 a	93.56 ± 1.39 a	27.30 ± 11.73 b
Quartz (%)	0.58 ± 0.12 b	0.52 ± 0.11 b	0.82 ± 0.36 b	5.06 ± 1.08 a
Calcite (%)	0.48 ± 0.16 b	3.82 ± 0.78 b	3.44 ± 0.44 b	46.56 ± 7.43 a
Dolomite (%)	8.18 ± 0.29 a	3.92 ± 0.68 b	0.38 ± 0.38 c	0.00 ± 0.00 c
Phyllosilicates (%)	2.30 ± 1.02 b	1.78 ± 1.09 b	0.66 ± 0.50 b	18.54 ± 3.90 a

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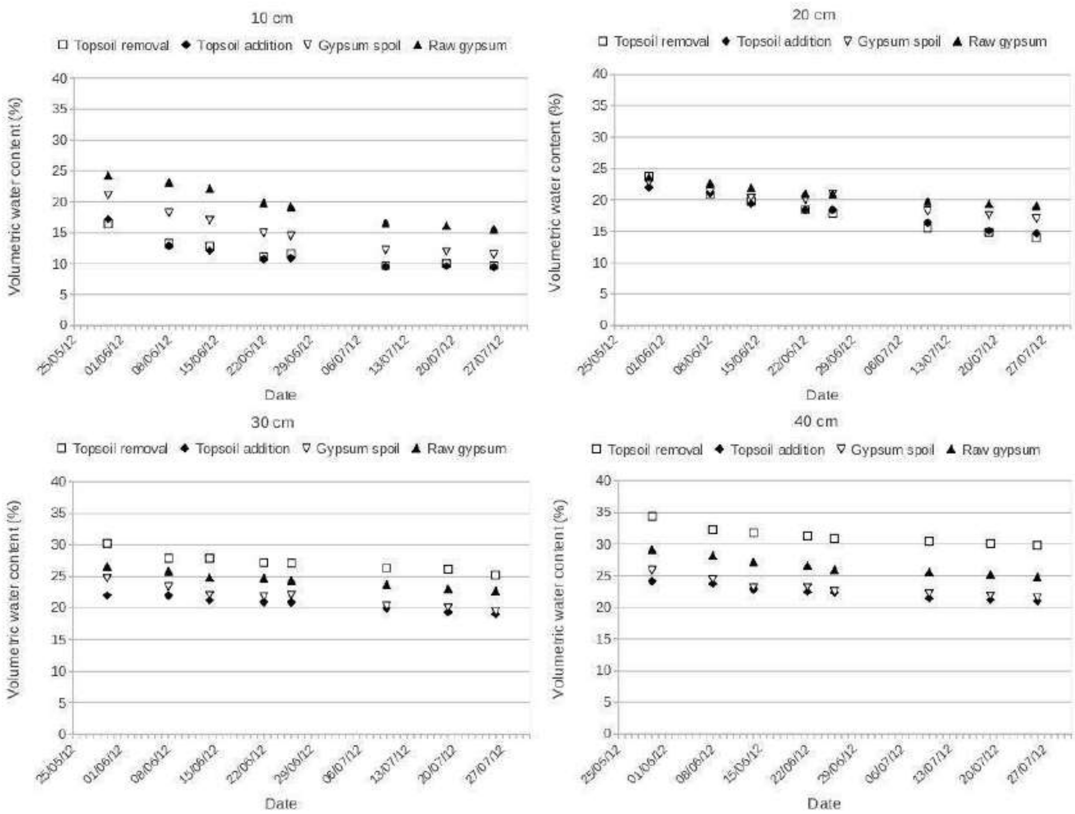
Appendix B. Volumetric soil-water content (%) on the bedding materials from May to July 2012, monitored in Ballesteros et al. 2014 at 10, 20, 30, and 40 cm depth in four access tubes using a Profile probe PR2 and a HH2 moisture meter (Delta-T Devices). Bedding materials: RG, raw gypsum; GS, gypsum spoil; TA, topsoil addition; TR, topsoil removal.



Gypsophile species used in the planting experiment



Ononis tridenta subsp. *crassifolia* *Lepidium subulatum* *Helianthemum squamatum*



Plantings of *O. tridenta* subsp. *crassifolia, L. subulatum* on the raw gypsum treatment (left and center respectively) and *H. squamatum* on the topsoil removal treatment (right).

Planting experiment (already finished) on raw gypsum in June, 2015. *H. squamatum* (left) and *L. subulatum* (right).

Chapter 5

Vegetation recovery of gypsum quarries: Short-term sowing response to different soil treatments.

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Vegetation recovery of gypsum quarries: Short-term sowing response to different soil treatments

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Abstract

Question: How does the sowing of native-species under different soil treatments contribute to the recovery of gypsum habitats affected by quarrying in Mediterranean environments?

Location: Mediterranean gypsum outcrops in Granada (SE Spain; 37º 2' N, 3º 45' W).

Methods: We conducted an experimental sowing of native perennial species from gypsum habitats (both gypsophiles and gypsovags) considering two factors: bedding materials and surface treatments. For bedding material, we used: gypsum spoil, topsoil addition on gypsum spoil, raw gypsum, and topsoil removal. The surface treatments were: control, sowing, sowing plus organic matter, and sowing plus an organic blanket. We made five replicates per combination treatment (80 plots in total, of 25 m^2 each). The sowing was performed in November 2009. All subplots were monitored to estimate density, richness, survival, growth of seedlings, and herbaceous biomass, in two monitoring periods (July and October).

Results: No gypsophiles or gypsovags were found in the control plots (no sowing nor surface treatment), and therefore natural succession proved ineffective in the first year. On the contrary, sowing showed very satisfactory result, especially on gypsum spoil, where mean density was of more than 15 individuals/m^2. This result is noteworthy as this material remains at the end of gypsum-mining activity. Spreading topsoil over gypsum spoil proved to be no more positive, since it provided not only seeds of target species but also of competitor species. Also, with regard to herbaceous plant species, this treatment favored a highly significant increase of biomass. The organic blanket increased plant density, whereas the addition of organic matter had significant positive effects on the survival and growth of the seedlings. The global high survival rate should be noted, especially on the gypsum spoil treatment.

Conclusions: We highlight the importance of implementing recovery measures in gypsum habitats. A proper selection of seed mixture and density, as well as the use of gypsum spoil (the most favorable bedding material, according the results), are sufficient to ensure the presence of the key species. Both technical solutions tested, organic blanket installation and organic-matter addition, improved the results in terms of the density, survival, and growth of the seedlings.

Keywords: Ecological restoration, gypsophiles, gypsum habitat, seed mixture, restoration techniques, bedding material, surface treatment.

Abbreviations

BM = bedding material;

TR = topsoil removal;

GS = gypsum spoil;

TA = topsoil addition;

RG = raw gypsum;

ST = surface treatment;

C = control;

S = sowing;

SO = sowing plus organic matter addition;

SB = sowing plus organic blanket.

Introduction

Gypsum outcrops have a scattered distribution in arid and semiarid areas in the world, covering about 100 million ha (Boyadgiev & Verheye 1996). Due to their particular chemical and physical properties, they harbor original flora with a high degree of rare and endemic taxa (Parsons 1976; Meyer 1986; Guerra et al. 1995; Mota et al. 2004; Akpulat & Celik 2005; Moore & Jansen 2007). Consequently, this habitat type is included in the European Habitat Directive (Anonymous 1992) as a priority for conservation. In turn, many characteristic plant species are under different degrees of threat and thus are included in red lists and red books (e.g. Gómez-Campo 1987; Cabezudo et al. 2005; Moreno et al. 2008) and are protected by international, national or regional legislation (e.g. Anonymous, 2003).

However, gypsum is also a major economic resource for mining (Al-Harthi 2001; Mota et al. 2003, 2004; Pulido-Bosch et al. 2004). Quarrying activities generally inflict heavy impact at both the landscape and community level, leading to soil loss, topographical alteration, and vegetation removal (Bradshaw 1997, 2000; Correia et al. 2001; Milgrom 2008). Thus, gypsum quarries typify the conflict of interests between mining and conservation (e.g. Mota et al. 2004).

The recovery of the areas at the end of mining activities by means of natural succession has shown poor results, especially in substrates under unfavorable conditions (Bradshaw 2000). In fact, gypsum outcrops occur usually in arid environments (Parsons 1976) where natural succession processes are particularly slow (Fowler 1986). Moreover, vegetation development is severely restricted by inherent gypsum features, such as physical (e.g. high soil compaction) or chemical (ion imbalance or toxicity) constraints (Meyer 1986; Meyer et al. 1992; Merlo et al., 1998; Escudero et al. 1999, 2000; Guerrero-Campo et al., 1999; Romao & Escudero 2005; Palacio et al. 2007; Pueyo et al. 2007; Drohan & Merkler 2009, Herrero et al. 2009). As a result, plants of gypsum environments have a low natural colonizing power, as has been showed in previous studies (Mota et al., 2003, 2004).

Despite the many papers dealing with ecological issues in gypsum areas (e.g. Escudero et al. 1999, 2000; Caballero et al. 2003; Pueyo & Alados 2007) few works are available on ecological restoration. Some studies deal with the ecological regeneration on gypsum outcrops by means of natural succession (Mota et al. 2003, 2004; Dana & Mota 2006), but research applying methodologies to recover the flora and vegetation of these areas has yielded few conclusions for restoration projects. Marqués et al. (2005) focused on the combined use of organic amendment and revegetation to reduce erosion in gypsic soils. Castillejo & Castelló (2010) suggested that gypsum-quarry rehabilitation in semiarid environments can be accelerated by using organic amendments to improve physical (structure) or chemical (nutrient content) soil properties, although these authors did not use characteristic species of gypsicolous habitats. Matesanz & Valladares (2007) studied the combination of native species with commercial fast-growing species, typical in the hydroseeding mixtures, to revegetate gypsum slopes under Mediterranean conditions. They highlighted the need for further studies focusing on the suitability of using herbaceous species tolerant of gypsum soils. In this context, many issues remain unknown and new approaches are needed to provide technical solutions for the ecological restoration of gypsum quarries.

This work presents a field experiment that seeks to develop measures to contribute to the recovery plan of a gypsum quarry under Mediterranean conditions. As a requirement to authorize mining, the company that operates the quarry is compelled to recover the native species regarded in the habitat of Community interest 1520 “Iberian gypsum vegetation, Gypsophiletalia”, (Habitat Directive, 92/43/EEC). Given the close link between vegetation and the soil where it occurs (Parsons 1976; Kazakou et al. 2008; Mota et al. 2008) the cornerstone for restoration is firstly to recover the specific substrate. The foreseen restoration plan includes to fill the pits created during the extraction with gypsum spoil, and placing on top the topsoil removed and preserved at the beginning of the operation. Thus, it seems appropriate to test the performance of the native species on these materials. We have opted for sowing, as it has been suggested as an economical and reliable method to propagate plant in restoration works (e.g. Jochimsen 2001; Bochet et al. 2010; Novák and Prach 2010) and results in a random distribution and natural-looking vegetation (Ghose 2004). In addition, the properties of the materials where to conduct the sowing may be enhanced by technical solutions, including surface treatments to increase the organic matter and nutrient content, or protecting the soil from seed removal or erosion (Muzzi et al. 1997; Vetterlein and Hüttl 1999).

The aims of the study are: a) to improve the restoration of the most characteristic native species in the study area included in the habitat of Community interest 1520 “Iberian gypsum vegetation, Gypsophiletalia”; b) to test the applicability of sowing to revegetate after quarrying operation, and c) to test the performance of gypsum native species under different combinations of bedding materials generated by quarrying, and soil-surface treatments (organic-matter addition or organic-blanket laying).

Methods

Site description

The experimental area is located in a gypsum outcrops area in Escúzar (Granada, SE Spain; 37º 2' N, 3º 45' W) at 950 m asl. The climate type is continental Mediterranean, with relatively cold winters, hot summers, and four months of water deficit. The mean annual temperature is 15.1°C, with an average monthly minimum temperature in January of 7.6 °C and maximum of 24.2 °C in August. Annual rainfall averages 421.1 mm, occurring mainly in winter. The area is in the Neogene sedimentary basin of Granada, the dominant substrates being lime and gypsum from the late Miocene, the latter in combination with marls (Aldaya et al. 1980). The predominant soils in gypsum outcrops are gypsisols (Aguilar et al. 1992). The vegetation of the area is a mosaic of scattered patches of natural plants growing over gypsum outcrops, surrounded by fields with crops (almonds, olive trees and cereals).

Target habitat

The aim of the study is to test measures to recover the most characteristic species of the habitat included in the European Habitat Directive (92/43/EEC) as 1520 “Iberian gypsum vegetation, Gypsophiletalia”. Specifically, in the study area the target habitat is characterized by three gypsophile species: *Ononis tridentata* subsp. *crassifolia* (local endemic), and *Helianthemum squamatum* and *Lepidium subulatum* (widespread in gypsum outcrops of the Iberian peninsula). In addition, are also frequent other non exclusive species of gypsum outcrops (gypsovags) such as *Stipa tenacissima, Helianthemum syriacum, H. violaceum, Thymus zygis* subsp. *gracilis, Teucrium capitatum subsp. gracillimum, Rosmarinus officinalis, Hippocrepis bourgaei,* and *Fumana thymifolia* (according Marchal et al., 2008).

Experimental design

The quarry to restore was under exploitation at the time of our study. Therefore an experimental area was set on a cereal old field consisting on marls next to the quarry (see site description for further details), using the materials generated during gypsum extraction~~,~~ to mimic possible post-quarrying conditions. The sowing experiment considered two factors: bedding material and surface treatment. Four flat plots (15 m x 60 m), each provided with a bedding material, were prepared over the experimental area, including: (i) topsoil removal (TR), removing the upper 30 cm to eliminate the topsoil, and thereby the seed bank within; (ii) gypsum spoil (GS), displaying a 0.5-m-thick layer of the byproduct obtained after gypsum is processed in the quarry; (iii) topsoil addition (TA), placing a layer of topsoil (c. 10 cm), previously retrieved from the natural habitat, on top of a 0.5-m-thick gypsum-spoil layer; and (iv) raw gypsum (RG), consisting of a 0.5-m-thick layer of the coarse gypsum used in the factory to be processed. The projected recovery plan includes filling quarry pits with gypsum spoil and placing habitat topsoil on top at the end of the activity. Therefore GS and TA treatments represent the most likely options to perform the restoration works. TR and RG represent extreme situations regarding gypsum content (i.e. having TR the lowest and RG the highest gypsum content respectively).

Each plot was divided into 20 subplots (5 m x 5 m), where the surface treatments were randomly applied. The surface treatments were: (i) Control (no sowing or surface treatment) (C); (ii) Sowing (S); (iii) Sowing plus organic-matter addition (SO); and (iv) Sowing plus organic blanket (SB). The organic matter was added in the form of commercial substrate (organic matter= 85.4 %, pH=6-7, N=260 mg/kg, P=389 mg/kg, K=2000 mg/kg, Mg=678 mg/kg, Fe=15 mg/kg) at 160 l/subplot. The organic blanket was a natural biodegradable net made of straw and alpha grass (*S. tenacissima*). Five replicates per combination treatment were performed (4 bedding-material types x 4 surface treatments x 5 replicates = 80 subplots in total).

For sowing, seeds of the most characteristic species of the target habitat in the study area were selected. The nearest natural seed source to the experimental area was more than 300 m far. Seeds were manually harvested in the surrounding area between June and September 2009. Due to its disperse flowering and availability we included fewer *Ononis* seeds in the mixture. The sowing was performed in November 2009, using 500 seeds per square meter. The proportion used was 60% of gypsophiles species: *Helianthemum squamatum* (180 seeds/m^2), *Lepidium subulatum* (120 seeds/m^2), *Ononis tridentata* subsp. *crassifolia* (6 seeds/m^2); and 40% of gypsovag species: *Helianthemum syriacum* (50 seeds/m^2), *Rosmarinus officinalis* (50 seeds/m^2), *Stipa tenacissima* (50 seeds/m^2) and *Thymus zygis* subsp. *gracilis* (50 seeds/m^2). Gypsovag species are widely commercialized (unless *H. syriacum*), what would facilitate the implementation of the future restoration plan.

Data collection

All subplots were monitored to estimate perennial-plant density, species richness, as well as survival and growth of target species, in two monitoring periods, July and October 2010. For the determination of density and richness, a set of 15 random samples in quadrats of 0.5 m X 0.5 m were taken per subplot, counting all individuals per species in each quadrat. We recorded both sown species as well as other spontaneous perennial species (chamaephytes and hemicryptophytes). For monitoring survival and growth, we marked and measured 30-40 seedlings per sown species in each combination of soil-mixture and soil-surface treatment (depending on seedling availability; see Table 3). In addition, to estimate herbaceous biomass, the above-ground part was harvested in two samples of 0.5 m X 0.5 m per subplot, oven dried 48 h at 70°C and weighed using a precision scale (0.1 mg).

Statistical analysis

To evaluate differences in plant density, species richness, herbaceous biomass, and plant growth with respect to each of the bedding materials (BM), surface treatments (ST), and their combination (BM x ST), we fitted Generalized Linear Models (GLMs), assuming a Poisson error distribution and log link function. Regarding density and richness, we used data recorded in October of both total perennial species and only target species. To assess the effect of the monitoring period, we used only target species, since the presence of other perennial species was very low in general (except in the topsoil-addition treatment).

The data of monitored seedlings (only when more than 10 seedlings per species and treatment were available; see Table 3) were used to analyze survival and growth. For survival analysis, a non-parametric Wilcoxon test was computed to check the effects of the treatments. We analyzed both total survival and survival by species. Moreover, the effect of treatments on overall growth as well as on growth by species was analyzed using GLM. All the statistical analyses were performed using JMP 7.0 (SAS Institute).

Results

Plant density and richness

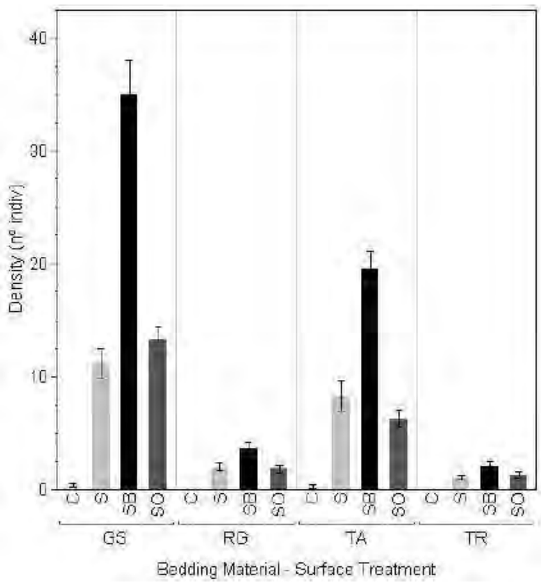
There were significant differences in the performance of bedding material (BM), surface treatments (ST), and their combined effect, on both plant density and species richness, although in the case of target species the interaction between BM and ST had no effect on richness (Table 1). The results were very similar whether considering all perennial species or just the target species. The presence of other perennial plants was only higher (although it was not significant) in the topsoil-addition treatment, due to colonizing hemicryptophytes (such as *Picnomon acarna, Onopordum nervosum, Carthamus lanatus* or *Centaurea calcitrapa*). For this reason, we present the results only for target species.

Table 1. Summary of the GLM testing the effect of soil treatments on density and richness of total perennial species or only target species.

		Total perennial species				Sown species			
		Density		Richness		Density		Richness	
Source	**DF**	**χ2**	**P**	**χ2**	**P**	**χ2**	**P**	**χ2**	**P**
Bedding material (BM)	3	3008.6115	0.0000	1922.7606	0.0000	572.2521	<.0001	112.7458	<.0001
Surface Treatment (ST)	3	1781.5965	0.0000	1076.9627	<.0001	2396.2708	0.0000	387.2644	<.0001
BM X ST	9	506.13922	<.0001	230.7508	<.0001	84.15706	<.0001	7.9321	0.5410

For the duration of the present experiment, topsoil addition (TA) and, especially quarry gypsum spoil (GS), were the bedding materials that performed best. In both cases the organic blanket (SB) enhanced species richness and density, whereas the addition of organic matter had no significant effect on the sowing in either case (GS and TA). The density exceeded 35 individuals/m^2 for the most effective treatment combination (GS+SB). On the contrary the option of no sowing (control) proved ineffective (Fig. 1). Plant density by species was also favored on gypsum spoil combined with organic blanket (see Fig. 2). In this option, the density was also high for most of the sown species (between 7.09±1.04 and 8.85±1.15 individuals/m^2). The density was lower for *O. tridentata* subsp. *crassifolia*, *H. syriacum*, and *S. tenacissima*, even in the more effective treatments.

A



B

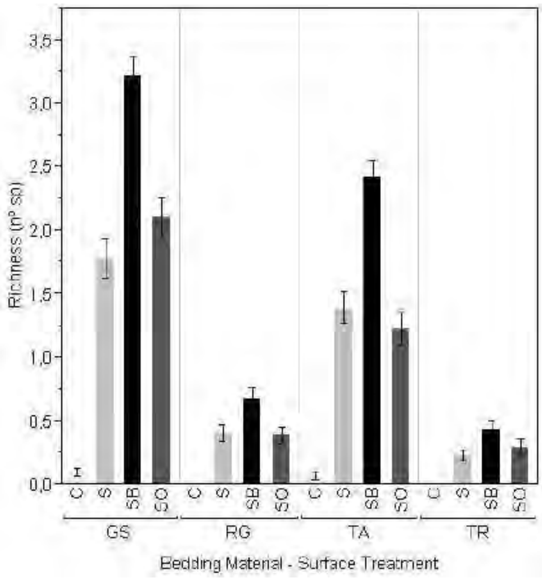


Fig. 1. Mean density (±SE) (nº individual/m^2) (A) and mean richness (±SE) (nº species/0.25 m^2) (B) of all target species by soil treatment. Bedding material: GS (gypsum spoil), RG (raw gypsum), TA (topsoil addition), TR (topsoil removal); Surface Treatment: C (control), SB (sowing plus organic blanket), SO (sowing plus organic matter), S (sowing).

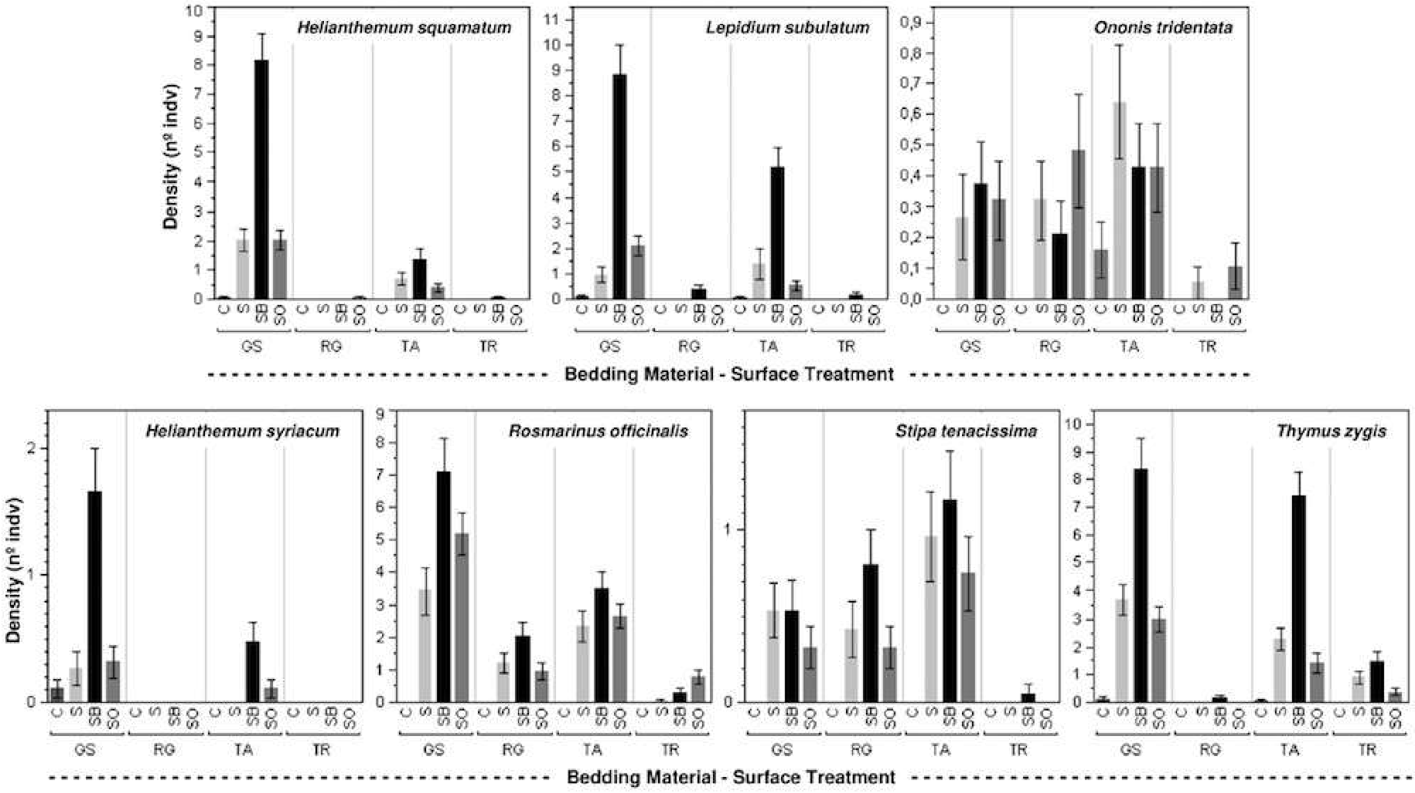


Fig. 2. Mean density (±SE) (nº individual/m^2) of target species by soil treatment. Bedding material: GS (gypsum spoil), RG (raw gypsum), TA (topsoil addition), TR (topsoil removal); Surface Treatment: C (control), SB (sowing plus organic blanket), SO (sowing plus organic matter), S (sowing).

The monitoring period influenced plant density for target species in relation to both bedding material (χ^2=51.8339, P <0.0001) and surface treatment (χ^2=19.9610, P=0.0002), increasing the density during the summer in all treatments except in raw gypsum. The greatest increase was found in the gypsum-spoil bedding material (Fig. 3A) and sowing plus organic-blanket surface treatment (Fig 3.B). A combination of these three factors (monitoring period, bedding material, and surface treatment) also had a significant effect on plant density (χ^2 =265.8673, P <0.0001) but not on target species richness. Monitoring period affected richness only in relation to bedding material (χ2 =8.2891, P <0.0404; Fig. 3.C).

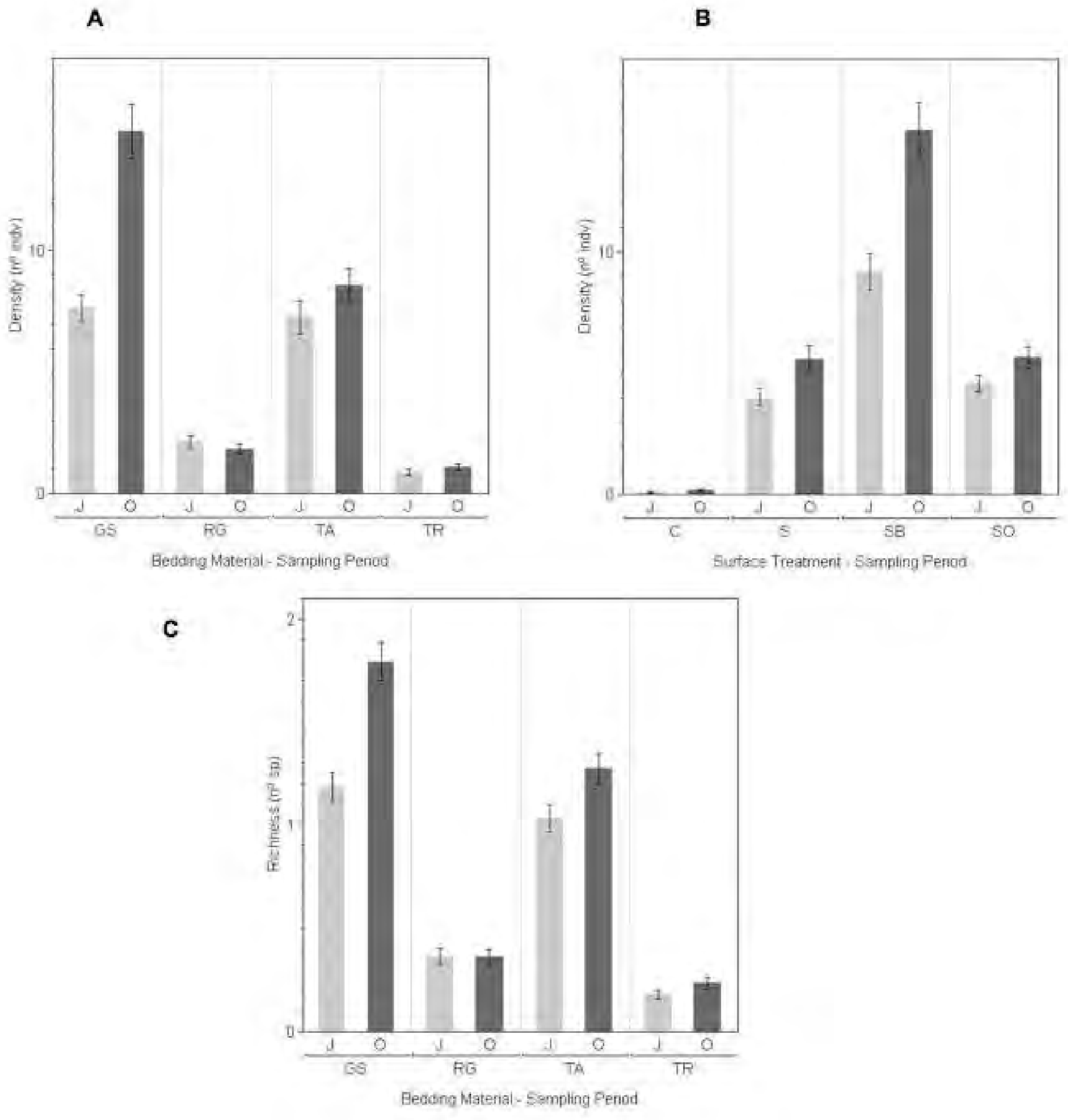


Fig. 3. Mean density (±SE) (nº individual/m^2) of target species by monitoring period and bedding material (A) or surface treatment (B), and mean richness (±SE) (nº species/0.25 m^2) by monitoring period and bedding material (C). Monitoring period: JUL (July 2010), OCT (October 2010). Soil treatments: Bedding material: GS (gypsum spoil), RG (raw gypsum), TA (topsoil addition), TR (topsoil removal); Surface Treatment: C (control), SB (sowing plus organic blanket), SO (sowing plus organic matter), S (sowing).

Herbaceous biomass

Herbaceous biomass was significantly higher (χ^2 =10175.702, P<0.0001) in the topsoil-removal (77.89 ± 5.02 gr/m^2) and topsoil-addition treatments (113.81 ± 8.06 gr/m^2) than in the gypsum spoil (1.30 ± 0.46 gr/m^2) and raw gypsum (0.01 ± 0.01 gr/m^2). There were no significant effects between surface treatments but the combination with bedding-material treatments showed significant differences (χ^2 =249.541, P<0.0001). The organic blanket favored the herbaceous biomass in the topsoil-addition treatment while limiting it in topsoil removal (Fig. 4).

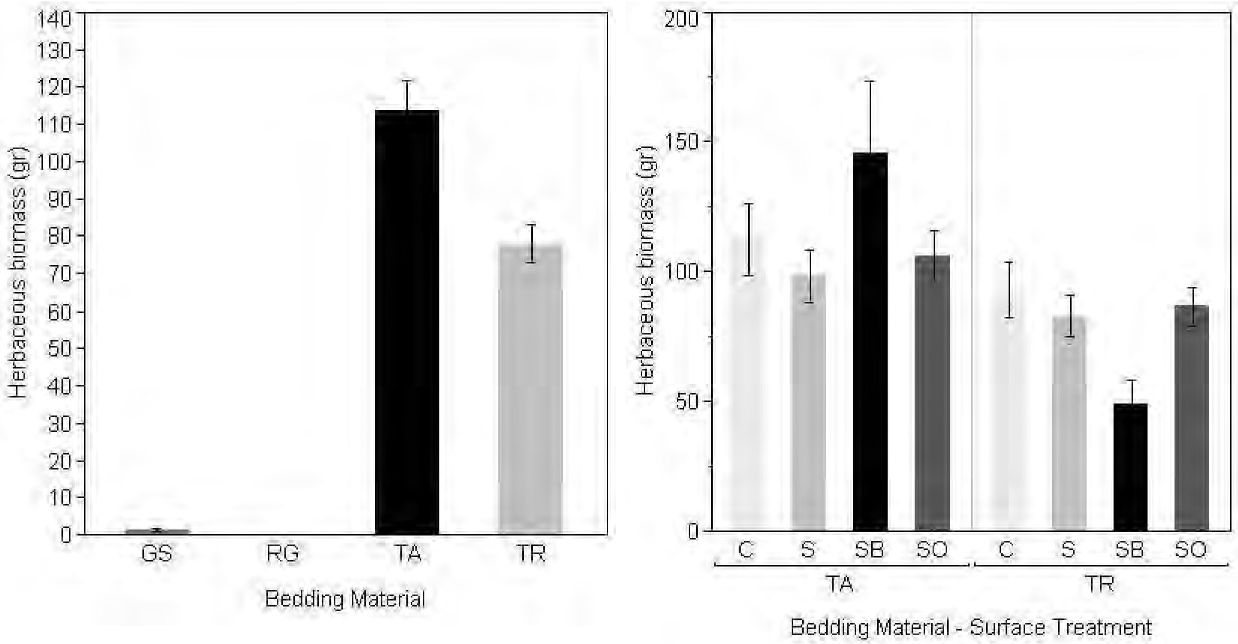


Fig. 4. Mean herbaceous biomass (gr/ m^2) (±SE) by soil treatments. Bedding material: TA (topsoil addition), TR (topsoil removal); Surface Treatment: C (control), SB (sowing plus organic blanket), SO (sowing plus organic matter), S (sowing).

Survival and growth

The survival rate of the total monitored seedlings was very high (92.7%). Despite the low mortality rate, some significant effects were found in the survival analysis. Regarding the bedding material (Wilcoxon tests: χ^2=29.7653, P<0.0001), higher mortality occurred in topsoil addition and raw gypsum treatments. In relation to surface treatments (Wilcoxon tests: χ^2=0.9858, P=0.6109) we only found significant differences in gypsum-spoil plots (Wilcoxon tests: χ^2=10.1886, P=0.0061). In this bedding material, mortality was higher in sowing plus organic blanket (see Fig. 5).

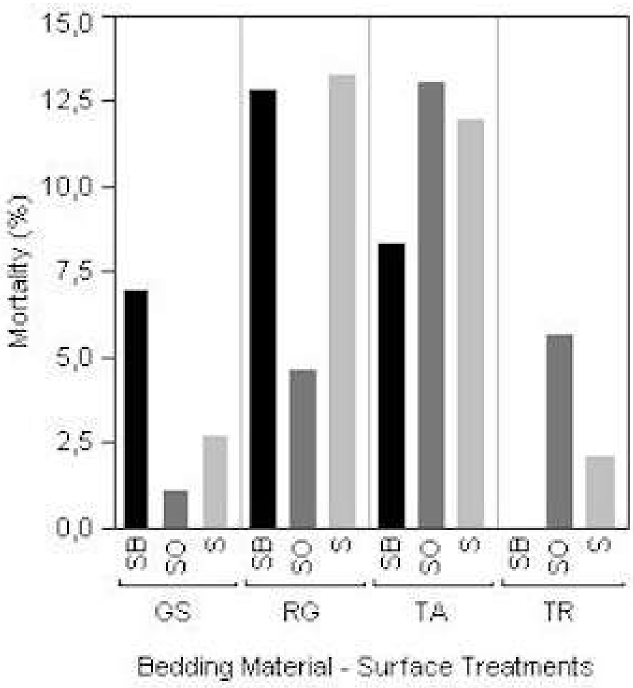


Fig. 5. Plant Mortality (%) by soil treatments combination. Bedding material: GS (gypsum spoil), RG (raw gypsum), TA (topsoil addition), TR (topsoil removal). Surface Treatment: C (control), SB (sowing plus organic blanket), SO (sowing plus organic matter), S (sowing).

The survival analysis by species showed that the bedding material influenced species survival more than did the surface treatments (Table 2). A significant effect of surface treatments on bedding materials was detected only on *T. zygis* subsp. *gracilis.* For this species, mortality was higher in the organic-blanket plots (12.5%, see Table 3). Regarding the survival rate by species, *O. tridentata* subsp. *crassifolia* registered the highest survival of all treatments.

Species	SURVIVAL						GROWTH					
	Bedding material			Surface Treatment			Bedding material			Surface Treatment		
	DF	χ2	P	DF	χ2	P	DF	χ2	P	DF	χ2	P
Hsq	1	16.0391	<.0001	2	4.3925	0.1112	1	10.3806	0.0013	2	9.2379	0.0099
Ls	1	14.1048	<.0002	2	4.2193	0.1213	1	90.0910	<0.0001	2	62.6413	<0.0001
Otc	2	0.8603	0.6504	2	3.7033	0.1570	2	11.1760	<0.0001	2	11.2217	0.0037
Ro	2	8.3197	0.0156	2	0.8677	0.6480	2	355.2281	<0.0001	2	40.0315	<0.0001
St	2	13.2074	0.0014	2	0.1474	0.9289	2	3.5631	0.1684	2	5.7693	0.0559
Tz	2	1.9941	0.3690	2	6.1973	0.0451	2	92.4410	<0.0001	2	34.1107	<0.0001

Table 2. Summary of the survival analysis (Wilcoxon test, left) and GLM (right) checking the effects of bedding materials and surface treatments on survival and growth, by species. Species: Hsq (*Helianthemum squamatum*), Hsy (*Helianthemum syriacum*), Ls (*Lepidium subulatum*), Ot (*Ononis tridentata* subsp. *crassifolia*), Ro (*Rosmarinus officinalis*), St (*Stipa tenacissima*), Tz (*Thymus zygis* subsp. *gracilis*).

For monitored seedlings the bedding materials and surface treatments, as well as their combined effect, significantly affected growth in all species except for *S. tenacissima*. The growth of the gypsophile species (*H. squamatum, L. subulatum,* and *O. tridentata* subsp. *crassifolia*) and *R. officinalis* proved higher in sown plots plus organic matter (SO) (see Tables 2 and 3 for details).

Table 3. Effects of surface treatments on each bedding material by species on survival and growth. Left, survival analysis: number of monitoring seedlings (N), percentage of mortality, and summary of the Wilcoxon test. Right, growth analysis: number of monitored seedlings alive (N), mean growth (cm) (±SE), and summary of the GLM. Species: Hsq (*Helianthemum squamatum*), Hsy (*Helianthemum syriacum*), Ls (*Lepidium subulatum*), Ot (*Ononis tridentata* subsp. *crassifolia*), Ro (*Rosmarinus officinalis*), St (*Stipa tenacissima*), Tz (*Thymus zygis* subsp. *gracilis*). Bedding material: GS (gypsum spoil), RG (raw gypsum), TA (topsoil addition), TR (topsoil removal). Surface Treatment: SB (sowing plus organic blanket), SO (sowing plus organic matter), S (sowing).

Species	Bedding material	Surface Treatment	SURVIVAL N	Mortality (%)	χ2	P	GROWTH N	Growth (cm)	χ2	P
Hsq	TA	S	26	26.92	1.4655	0.4806	19	3.09±0.29	0.1344	0.9350
		SO	12	9.09			10	3.15±0.35		
		SB	34	20.59			27	2.94±0.27		
	GS	S	34	2.94	1.8290	0.4007	33	3.23±0.23	9.4795	0.0087
		SO	36	0.00			36	4.70±0.37		
		SB	39	5.13			37	4.12±0.29		
Ls	TA	S	24	29.17	5.6456	0.0594	17	1.83±0.25	11.2422	0.0036
		SO	20	25.00			15	2.44±0.57		
		SB	40	7.50			37	3.37±0.39		
	GS	S	27	3.70	1.2401	0.5379	26	3.08±0.63	54.3852	<0.001
		SO	37	0.00			37	7.20±0.65		
		SB	39	2.56			38	6.48±0.69		
Ot	TA	S	29	0.00	1.800	0.4066	29	9.77±0.79	12.4515	0,0020
		SO	25	0.00			25	9.48±0.98		
		SB	30	3.33			29	12.29±0.98		
	GS	S	14	0.00	0.0000	0.0000	14	9.10±1.49	27.4367	<0.001
		SO	14	0.00			14	16.08±1.58		
		SB	17	0.00			17	12.58±1.48		
	RG	S	14	0.00	2.1250	0.3456	14	13.01±1.46	7.3635	0.0252
		SO	20	0.00			20	10.18±1.00		
		SB	16	6.25			15	12.71±1.72		
Ro	TA	S	40	17.50	1.8133	0.4039	33	5.24±0.38	44.7246	<0.001
		SO	40	12.50			35	4.74±0.38		
		SB	40	7.50			36	8.46±0.69		
	GS	S	41	4.88	2.1398	0.3430	39	4.77±0.65	44.6861	<0.001
		SO	40	2.50			39	8.61±0.97		
		SB	40	10.00			36	6.16±0.61		
	RG	S	40	2.50	2.1589	0.3398	39	1.79±0.24	0.2455	0.8845
		SO	32	0.00			32	1.87±0.26		
		SB	33	6.06			31	1.70±0.21		
St	TA	S	17	0.00	3.8540	0.1456	17	5.94±0.41	2.0308	0.3623
		SO	32	18.75			26	5.04±0.40		
		SB	38	10.53			34	5.79±0.35		
	GS	S	29	3.45	0.4375	0.8035	28	4.64±0.39	1.3344	0.5131
		SO	20	5.00			18	4.67±0.35		
		SB	27	7.41			25	5.29±0.29		
	RG	S	26	30.77	1.6512	0.4380	18	4.89±0.38	4.816	0.0900
		SO	10	10.00			9	4.51±0.54		
		SB	25	28.00			18	6.29±0.31		
Tz	TA	S	40	0.00	5.8139	0.0546	40	4.09±0.32	11.9569	0.0025
		SO	41	12.20			37	3.79±0.34		
		SB	46	2.17			45	5.30±0.34		
	GS	S	40	0.00	10.3478	0.0057	40	4.67±0.62	1.8611	0.3943
		SO	40	0.00			40	4.84±0.44		
		SB	40	12.50			35	5.35±0.54		
	TR	S	40	0.00	5.333	0.0695	40	6.82±0.42	13.3402	0.0013
		SO	15	6.67			14	6.01±0.59		
		SB	40	0.00			40	8.62±0.62		

Discussion

Short-term results of the experiment point the need for taking active measures to encourage rapid gypsum-habitat recovery, since natural colonization proves ineffective in the first year. The need to apply restoration measures has also been pointed out by Tormo et al. (2007) in semiarid roadfills, due to the low vegetation cover resulting in the untreated plots. This result is consistent with studies on spontaneous plant succession in abandoned gypsum quarries (Mota et al. 2003, 2004), where gypsophile species registered low establishment rates. After more than 25 years the average cover of all gypsophiles was 25%, while the cover of species characteristic of our study area, such as *L. subulatum* and *H. squamatum*, was lower than 2.5% (Mota et al. 2004). In fact, we found no plants characteristic of gypsum habitats during the first year in the control plots (no sowing, nor surface treatment).

On the contrary, native species sowing (both gypsophiles and gypsovags) showed highly satisfactory results. The advantages of using native species in revegetation have been highlighted in some studies (e.g. Harper-Lore 1996; Matesanz & Valladares 2007; Bochet et al. 2010). In our study, sowing performed better in quarry gypsum spoil (GS) and topsoil addition (TA) bedding materials, significantly increasing species richness and density with regard to raw gypsum (RG) and topsoil removal (TR). It bears noting that these two options (GS and TA) represent the most predictable situations at the end of the mining activity. On the other hand, despite the guidelines suggested the use of raw gypsum as a bedding material option in the recovery plan (technical unpublished document), we found the lowest success of the sowing, with similar results for topsoil removal, so that both bedding materials proved to be the most ineffective.

The replacement of topsoil has been widely proposed as a valuable source of seeds in restoration works (e.g. Bradshaw 1997; Tormo et al. 2007), and its preservation and storage (stockpiling) are regarded as one of the most important practices in land reclamation (Abdul-Kareem & McRae 1984; Kundu and Ghose 1994; Ghose 2004).

Topsoil provides a source of seeds, favoring the presence of target species, but also promotes the spread of possible competitors, which could diminish the performance of gypsum species (Matesanz & Valladares 2007). In our study, the presence of colonizer hemicryptophytes and herbaceous plants was significantly higher in the topsoil-addition treatment than in other bedding material. Therefore, when the topsoil contains large numbers of seeds of undesirable species, then it may be better to use the subsoil as a substrate for restoration (Biswas and Mukherjee 1989; Ghose 2004). In this sense, our results indicated that in gypsum spoil without added topsoil, the density, richness, survival, and growth were higher, suggesting a negative effect of the topsoil, which restricts the establishment and growth of target species.

Although topsoil appears not to be advantageous at an early stage in our study, over the long term it could become positive. On the one hand, herbaceous species play an important hydrological role by enhancing soil infiltration and protecting soil against erosion (Nicolau et al. 2002). Tormo et al. (2007) studying roadfill revegetation in a semiarid Mediterranean environment, found that plots with topsoil addition became less prone to erosion than did the plots without such additions, although these differences were not significant. On the other hand, apart from seeds, organic matter and plant nutrients (Ghose 2004), topsoil contains cyanobacteria, green algae, lichens, mosses and other organisms that are closely integrated with soil particles, resulting in the formation of biological soil crusts (Belnap & Lange 2003). The ecological roles and ecosystem services of these crusts have been well documented (Eldridge & Greene 1994; Bowker et al. 2005; Li et al. 2010), including the positive effects of biological soil crust on vascular plants (Su et al. 2009). Therefore, experimental plots must be monitored over time to achieve a more complete evaluation of the results.

In any case, the results were positive for sowing on both bedding materials (GS and TA), especially on gypsum spoil (see Results). These two treatments in combination with the organic blanket enhanced the presence of target species, whereas the addition of organic matter had no significant additional effects on plant density. The survival and growth analysis by species indicated that gypsum spoil was also the most effective bedding material, but jointly with sowing plus organic matter. Therefore, the surface treatments improved results in two ways. The organic blanket reduced evaporation, which could promote seed germination and consequently higher seedling density. On the other hand, organic matter improved growth and survival. This is not surprising, since organic matter benefits plant development, and some authors have even demonstrated that a paucity of organic matter could limit species establishment during primary succession on gypsum outcrops (Dana & Mota 2006). Surface treatments can raise the recovery cost but provide some technical and ecological benefits (Muzzi et al. 1997), especially in semiarid and arid environments (Bochet et al. 2010). Treatments such as the organic blanket promote a good vegetative cover, and reduce surface runoff (and consequently seed loss) as well as the erosion rate (Muzzi et al. 1997;

Benik et al. 2003). However, if the long-term goal is to reestablish native vegetation, the use of some surface treatment such as large amounts of biosolids could be negative (Paschke et al. 2005). In fact, in a experiment to rehabilitate a gypsum quarry, the use of solid waste compost as organic amendment promoted a good vegetation cover (Castillejo & Castelló 2010), although it was not provided by gypsophile or gypsovag species.

Therefore it is relevant to select optimal methods regarding bedding-material, surface treatments and seed-mixture, since we do not only need a high plant density or cover, but also an appropriate composition to recover the target habitat. Species composition is clearly a key issue in judging restoration success (Henderson 1999; Lorite et al. 2010). This fact is particularly pertinent in habitats exclusive to specific substrates such as dolomite, serpentine or gypsum, since they are composed mainly of specialist plant species, frequently rare or even endemic (Parsons 1976; Kazakou et al. 2008; Mota et al. 2008). In our experiment, all species selected for the sowing showed positive results, regarding density, survival and growth, in the most effective treatments. Plant density has been specially high for *Lepidium subulatum, Helianthemum squamatum, Rosmarinus officinalis,* and *Thymus zygis* subsp. *gracilis.* In prospect of the future recovery plan probably the seed proportion should be adjusted. Therefore, we should assess our results in the long term in order to optimize the seed mixture composition.

After the summer were recorded even higher values in plant density than before (in all sowing plots on GS and TA bedding materials), and also we obtained a high survival rate. These results are relevant for ecological restoration, since the establishment after germination is severely limited by summer drought in Mediterranean-type ecosystems (Herrera, 1992). In addition, the survival to the first summer is a key factor for the development of some gypsophiles. In fact, Escudero et al., (1999, 2000) found low survival percentages respectively for *H. squamatum* and *L. subulatum* in natural habitats, especially due to drought. However, they found that most *H. squamatum* seedlings surviving at the end of the first year were alive at the end of the second. In our sown plots most of the marked plants not just survived, but grew during this season, probably due to the characteristics of the gypsum spoil. The gypsum properties may determine a significant increase in water availability during summer drought (Meyer 1986; Meyer & García-Moya 1989), which would justify the active growth and the flowering phenology of most gypsophiles during the summer (Meyer 1986; Gómez et al, 1986; Escudero et al. 2000). In addition, besides the positive results regarding plant density, survival, and growth, after the first summer, the habitat recovery could be favored since target species have small size and fast cycle, even able to produce flowers and fruits in the first year (Author's unpublished data). Therefore, in spite of the short-term nature of our results, they point in the right direction to recover the target habitat. However, the sowing experiment should be monitored in the long term to confirm the ecological and economical viability of the restoration options planned.

In conclusion, the short-term results of this study highlight the importance of implementing measures to recover the target gypsum habitat. A proper seed mixture of gypsophiles and gypsovags sown on gypsum spoil, is adequate to guarantee a high plant-density of the key species. Some technical solutions, such as adding organic matter or laying organic blanket, can improve the effectiveness of the sowing, whereas some common practices, such as topsoil addition, may not be advantageous for the early stages of the target species.

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Chapter 6

Recovery of vegetation affected by gypsum quarrying: Sowing response to different soil treatments after five years.

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Recovery of vegetation affected by gypsum quarrying: Sowing response to different soil treatments after five years.

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Abstract

1. Mining often causes drastic environmental changes in important habitats for plant conservation. Gypsicolous vegetation requires effective restoration of the altered areas by gypsum quarrying. Alteration on the initial species pools and the soil conditions together with competitive species interactions pose a challenge to restore valuable gypsicolous plant communities.

2. We evaluated a sowing experiment under a dry Mediterranean climate in SE Spain to test the establishment of gypsicolous vegetation on four bedding materials derived from quarrying, including raw gypsum, gypsum spoil, topsoil addition and topsoil removal; and whether the application of organic matter or organic blankets as surface treatments were beneficial. We evaluated early vegetation dynamics over a 5-year period and compared the results to local plant communities in the habitat and in an abandoned quarry to evaluate restoration success.

3. Trajectories were driven by seed input and soil conditions: Divergence occurred (i) initially between sown and control treatments at all bedding materials due to seed availability, and (ii) between the topsoil removal treatment compared to the three gypsum derived substrates sharing more beneficial properties. Surface treatments had no advantageous effects over time.

4. While the recovery was limited in control plots and topsoil removal, sowing on raw gypsum, gypsum spoil and topsoil addition improved the composition, richness and cover making experimental plant communities resemble the reference habitat, except for the increasing cover of *Rosmarinus officinalis*. Control plots on these treatments had a low cover although were colonised by target species from the adjacent sown plots, specially on gypsum spoil, gaining target species missing in the abandoned quarry.

5. Synthesis and applications: Sowing on materials obtained in gypsum quarrying is a successful method to ensure rapid establishment of gypsicolous vegetation in disturbed areas. Appropriate seed mixtures must emphasize the establishment of gypsophile species and not include potential competitors able to become dominant, such as *Rosmarinus officinalis* in our study. Gypsum spoil

showed a remarkable potential for restoration achieving satisfactory establishment and effective recruitment of target species, promoting self-sustainability of restored gypsicolous communities. Raw gypsum improved remarkably the cover of the endangered species *O. tridentata* subsp. *crassifolia.* These results will allow better designed and cost-effective future restoration programs in the area and inform ecological restoration of disturbed gypsicolous habitats.

Key-words: Sowing, seed-mixture, gypsophiles, gypsum spoil, habitat restoration, vegetation dynamics, succession, topsoil, mining wastes, quarrying, plant community.

Introduction

Quarrying often damages important habitats for plant conservation due to drastic environmental changes affecting both vegetation and soil (Bradshaw, 1997). Restoration may be challenging due to limitations caused by the alteration of both topography and soil properties (Bradshaw, 2000). Common restoration practices to enhance vegetation establishment include the backfilling of the disturbed area with quarrying-wastes, amelioration of both chemical and physical soil properties, and planting or sowing (Cooke and Johnson, 2002; Cohen-Fernández and Naeth, 2013). Sowing is a common technique for quarry rehabilitation helpful to speed up succession towards target stages (Brofas and Karetsos, 2002; Novàk and Prach, 2010; Baasch *et al.*, 2012). However, the effectiveness of seed-banks to initiate vegetation recovery will be conditioned among other factors by soil management, being particularly relevant to singular substrates such as serpentine, dolomite or gypsum supporting unique plant communities (O'Dell and Claassen, 2009; Whiting *et al.*, 2010; Mota *et al.,* 2011a; Escudero *et al.*, 2015).

Gypsum habitats are considered a priority for conservation and have been listed as one of the most endangered in Europe (European Commission, 1992). These habitats support a highly diverse flora with many endemic, rare and threatened species associated to gypsum substrates. These habitats are often fragmented, degraded or destroyed due to human activities (Pueyo and Alados, 2007), quarrying being one of the most drastic causes of disturbance (Pulido *et al.*, 2004; Ballesteros *et al.*, 2013). Gypsum is an industrial mineral in global demand (Herrero *et al.*, 2013), and its extraction inevitably damages the habitat and its valuable plant species (Dana and Mota, 2006; Mota *et al.*, 2011b). Mining companies are often compelled to conduct restoration programs, but the limited understanding of the dynamics of gypsicolous communities makes difficult to envisage successful restoration strategies.

Approaches to restoring gypsum disturbed areas have considered generally two different goals: increasing the natural value of the disturbed site by recovering native plant communities (i.e. restoration), or improving ecosystem productivity or protection against erosion (i.e. rehabilitation; Marqués *et al.*, 2005; Castillejo and Castelló, 2010). Although both goals are complementary, they have rarely been addressed together in restoration programs. Despite the interest of encouraging the development of gypsicolous communities, few experiments have been designed to direct the trajectory of vegetation change and improve conservation value of disturbed sites. Spontaneous succession may be a useful, low-cost restoration tool in many situations (Bradshaw, 1997; Prach and Hobbs, 2008). However, in gypsum environments the potential of spontaneous succession is strongly conditioned by site-specific conditions, sometimes failing to recover the original species in the medium- to long-term (25-70 years; Martín *et al.*, 2003; Mota *et al.*, 2003, 2004; Dana and Mota, 2006). Active restoration methods have included common approaches, such as the introduction of native and non-native species by sowing, hydroseeding or planting, in combination with the management of the available substrates (Mota *et al.*, 2004; Matesanz *et al.*, 2007; Castillejo and Castelló, 2010; Ballesteros *et al.*, 2012, 2014, 2017). These substrates normally are spoil materials derived from quarrying involving problems such as instability, compaction or chemical imbalance that, especially under dry conditions, become restrictive factors for vegetation recovery. Studies dealing with both spontaneous succession and active restoration have directly or indirectly reported environmental filtering caused by the lack or arrival order of propagules, the soil quality and competitive species interactions strongly condition the outcomes of restoration (Mota *et al.*, 2003, 2004; Dana and Mota, 2006; Ballesteros *et al.*, 2012, 2014, 2017).

To our knowledge, few works have provided the opportunity to study the development of gypsicolous communities and experimentally manipulate the soil and test revegetation techniques to select suitable options for restoration. We conducted a field experiment seeking to develop measures to contribute to the recovery plan of a gypsum quarry in SE Spain under Mediterranean conditions (Ballesteros *et al.,* 2012). The main objective was to recover gypsicolous species in the area, especially those included in the habitat of Community interest 1520 'Iberian gypsum vegetation, *Gypsophiletalia*', (Habitat Directive 92/43/EEC). Sowing was performed on different bedding materials available in the quarry environment and treated with either organic matter or organic blankets overlays. Preliminary analysis showed the initial establishment of target species was determined by interactions between the bedding material and the surface treatment (Ballesteros *et al.,* 2012). As vegetation developed, we had an excellent opportunity to study the early successional dynamics on these systems, and judge the effectiveness of restoration treatments and against local plant communities in the target habitat and in an unrestored quarry used as references. Specifically, we aimed to answer which restoration method was more effective in enhancing species composition, richness and cover leading to the desired gypsicolous plant community. We hoped examining the patterns of vegetation establishment will help us to determine the best measures for the design of gypsum-quarry restoration plans.

2. Materials and Methods

2.1. Study area

The study area is located in Escúzar, Granada, SE Spain (37° 2' N, 3° 45' W) between 900-1000 m asl. The climate is continental Mediterranean, with relatively cold winters, hot summers, and four months of water deficit. The mean annual temperature is 15.1 °C, with an average monthly minimum temperature in January of 7.6 °C and maximum of 24.2 °C in August. Annual rainfall averages 420 mm, occurring mainly in winter. The area is in the Neogene sedimentary basin of Granada; the dominant substrates being lime and gypsum deposited in the late Miocene, the latter in combination with marls (Aldaya *et al.*, 1980). The predominant soils in the gypsum outcrops are Gypsiric Leptosols (Aguilar *et al.*, 1992; IUSS Working Group WRB, 2015). The vegetation of the area is a mosaic of fields with cereal crops and olive and almond orchards (*Olea europaea* L. and *Prunus dulcis* D.A. Webb.) and scattered patches of native plants growing over gypsum outcrops (Ballesteros *et al.*, 2012).

2.2. Target species

The target gypsicolous vegetation in the area is described in the EU Habitats Directive (European Commission, 1992) as 1520, "Iberian gypsum vegetation, *Gypsophiletalia*", and is characterized by plants exclusive to gypsum soils (gypsophiles); (see Mota *et al.*, 2011; Escudero *et al.*, 2015), such as *Helianthemum squamatum* (L.) Dum. Cours., *Lepidium subulatum* L., and *Ononis tridentata* subsp. *crassifolia* (Dufour ex Boiss.) Nyman. In addition, there are also other frequent non-exclusive species of gypsum substrates (gypsovags) such as *Rosmarinus officinalis* L., *Stipa tenacissima* L., *Helianthemum syriacum* (Jacq.) Dum. Cours., *Thymus zygis* L. subsp. *gracilis* (Boiss.) R. Morales and *Teucrium capitatum* subsp. g*racillimum* (Rouy) Valdés Berm. and Sánchez Crespo (according to Marchal *et al.*, 2008).

2.3. Experimental design

Experimental plots were built in October 2009 on a cereal old-field of 0.45 ha (37° 2' 57'' N, 3° 45' 30'' W; 950 m asl) consisting of marls, next to a gypsum active quarry, providing materials derived from the mineral extraction. These materials were chosen based on their potential applicability for restoration (see Ballesteros *et al.*, 2012, 2014). The experimental design considered two factors: (1) bedding material and (2) surface treatment. We prepared four flat plots (15 m x 60 m) on the old-field, each consisting of a bedding material (see properties in Appendix A and B; Ballesteros et al. 2014), as follows: (1) topsoil removal (TR), using the site substrate, previously removing the topsoil (c. 30 cm) and thus its seed bank (coming from the pre-existing cereal crop); (2) gypsum spoil (GS), providing a layer of gypsum mine spoil (0.5 m) derived from gypsum extraction; (3) topsoil addition (TA), placing a layer of topsoil (ca. 10 cm) retrieved from the natural habitat (after 8 months of stockpiled storage), on top of a gypsum spoil layer (0.5 m); and (4) raw gypsum (RG), consisting of a layer (0.5 m) of coarse gypsum (i.e. the same material used in the factory to be processed). Each plot was divided into 20 subplots (5 m x 5 m), where the surface treatments were randomly applied. The surface treatments were: (1) Control (no sowing or surface treatment) (C); (2) Sowing (S); (3) Sowing plus organic matter addition (SO); and (4) Sowing plus organic blanket (SB). The organic matter was added in the form of commercial substrate (organic matter = 85.4%, pH 6–7, N 260 mg·kg^{-1}, P 389 mg·kg^{-1}, K 2000 mg·kg^{-1}, Mg 678 mg·kg^{-1}, Fe 15 mg·kg^{-1}) at 160 l per subplot. The organic blanket was a natural biodegradable net made of straw and alpha grass (*S. tenacissima*). Five replicates per combination treatment were performed (four bedding material types x four surface treatments x five replicates = 80 subplots in total). The sowing was performed in November 2009. We used a mixture of native seeds (500 seeds/m^2) consisting of 60% gypsophiles and 40% gypsovags, at the following rates (seeds/m^2): Gypsophiles: *H. squamatum* (180), *L. subulatum* (120), and *O. tridentata* subsp. *crassifolia* (6); and gypsovags: *H. syriacum* (50), *R. officinalis* (50), *S. tenacissima* (50) and *T. zygis* subsp. *gracilis* (50). Seeds were collected from natural vegetation patches within the study area between June and September 2009. We included few *Ononis* seeds in the mixture due to low availability caused by disperse flowering and high seed predation.

2.4. Vegetation sampling

We sampled plant species richness, cover and composition in the experimental plots, as well as in the habitat and in an abandoned quarry within the study area to be used as references for evaluating the success of the restoration treatments. The experimental plots were sampled in June 2011, November 2011, and October 2012, 2013 and 2014. Sampling in autumn allowed to record late-emerging seedlings of target species as observed in Ballesteros *et al.* (2012). Percentage cover was estimated by placing 4 equidistant linear transects along each subplot, and assessed three contact points every 0.5 m: at the centre and 0.5 m to each side of the transect (78 points per transect). We recorded the perennial plant species occurring (i.e. chamaephytes and hemicryptophytes) plus bare soil, and calculated their cover as the proportion of points intercepted. For the reference sites, the vegetation in the habitat and in the abandoned quarry were sampled in October 2016. The habitat (NAT) was used as reference of the target community. We selected 10 separate sites (>150 m distance) in the study area where the target community was represented. At each site, we laid five (eight in one site) 25m-transects (>5m separation) and assessed three contact points every 1m; at the centre and 1m to each side of the transect (78 points per transect; 53 transects in total). Additionally, we sampled an abandoned quarry (AQ) in the study area to evaluate spontaneous succession after ~25 years since abandonment by laying 15 25m-transects as described for the habitat. We increased transect size in NAT and AQ compared to the experimental plots to represent the variability of plant communities at reference sites. The different transect size between the experimental plots and the reference sites can influence richness and cover estimation but is not likely to affect overall trends (Otýpková and Chytrý, 2006; Chytrý, 2001).

2.5. Data analyses

The species data were analysed using both univariate and multivariate methods. All statistical analyses were performed using R version 3.3.2 (R Core Team, 2016).

We analysed the effects of bedding material, surface treatment and their interaction on the richness and cover of target species, gypsophiles separately, non-target species (i.e. other than sown gypsophiles and gypsovags not included in the seed-mixture), total species, and the cover of individual species. These effects were assessed fitting linear mixed models (LMMs) using bedding material and surface treatment as fixed factors and subplot and sampling date as random factors, except for the final results for which sampling date was not included. Models were fitted applying the Laplace approximation of likelihood (Bolker *et al.*, 2009), a gaussian error distribution and identity-link function using the R "lme4" package (Bates *et al.*, 2015). Additionally, we analysed the effect of surface treatment by species for each bedding material fitting Generalized Linear Models (GLM), assuming a gaussian error distribution and identity-link function using the R "stats" package (R Core Team, 2016). Pairwise multiple comparisons with Tukey's correction were made to estimate differences between surface treatments using the R "multcomp" package (Hothorn *et al.*, 2008).

For multivariate analyses, we used the R “vegan” package (Oksanen *et al.*, 2017), and percentage cover values were log-transformed ($log_e(x+1)$). We analysed the final cover (data from October 2014) to compare species composition between bedding materials in the experimental plots, the reference habitat and the abandoned quarry (data from October 2016) using detrended correspondence analysis (DCA). Standard deviational ellipses were used to illustrate their position on the biplots (Oksanen *et al.,* 2017). We examined the variability in the composition of the plant community at the last sampling across restoration treatments, conducting a permutational multivariate analysis of variance (permanova) considering the bedding materials, surface treatments and their interaction, using the “adonis” function in R “vegan” package (Oksanen *et al.*, 2017).

We assessed the effects of bedding material and surface treatment on species composition over time using principal response curves (PRCs, Van den Brink and Ter Braak, 1999). PRCs is a special case of redundancy analysis (RDA) for multivariate responses in a design with repeated observations (Oksanen, 2017). PRCs represent changes in species composition over time for each treatment combination as deviations from a reference value established as a zero line (Alday and Marrs, 2014). In our case, we set NAT as zero line, representing the composition of the vegetation in the target community (natural gypsum habitat). Additionally, we included AQ representing the vegetation established by spontaneous succession in the unrestored quarry. The vegetation for both NAT and AQ is represented as constant because data from October 2016 were used for all sampling dates.

3. Results

A total of 28 species were recorded in the experimental plots over the 3.3-years monitoring (27 in TA, 21 in GS, 18 in TR and 17 in RG), and 19 in NAT and 11 in AQ in the one-off sampling in reference sites. The most frequent species in transects at the experimental plots were target species and the ruderals *Dittrichia viscosa* and *Picnomon acarna.* All target species except *R. officinalis* were frequent in NAT, as well as *T. capitatum*. In AQ were frequent *Retama sphaerocarpa* and the ruderal colonizers *Helichrysum stoechas, Andryala ragusina* and *D. viscosa* whereas, except for *T. zygis,* none of the target species occurred.

3.1. Effects of restoration treatments on richness and cover

LMMs revealed significant effects of bedding material (BM), surface treatment (ST) and their interaction, on the richness and cover of target species, gypsophiles, non-target species and total plant cover throughout the experiment (Table 1).

			Target species		Gypsophiles		Non-target species		Total	
		df	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Richness	Bedding material (BT)	3	1259.37	**<0.001**	889.51	**<0.001**	1431.02	**<0.001**	1531.60	**<0.001**
	Surface treatment (ST)	3	286.62	**<0.001**	63.00	**<0.001**	13.15	**0.004**	465.43	**<0.001**
	BM x ST	9	209.52	**<0.001**	211.53	**<0.001**	55.85	**<0.001**	59.79	**<0.001**
Cover	Bedding material (BT)	3	675.78	**<0.001**	545.12	**<0.001**	187.62	**<0.001**	865.13	**<0.001**
	Surface treatment (ST)	3	290.26	**<0.001**	109.66	**<0.001**	65.51	**<0.001**	203.35	**<0.001**
	BM x ST	9	222.03	**<0.001**	245.10	**<0.001**	88.31	**<0.001**	130.00	**<0.001**

Table 1. Results of linear mixed models (LMMs) testing the global effects throughout the experiment of bedding material, surface treatment and their interaction on the richness and cover of target species, gypsophiles separately, non-target species, and total plant species. The chi-square statistic (X^2) of the fixed factors and their significance are presented. All results with $p<0.05$ are in bold.

Target species showed increasingly similar richness and cover between surface treatments within bedding materials, diverging from controls throughout the 3.3-years monitoring (Fig. 1).

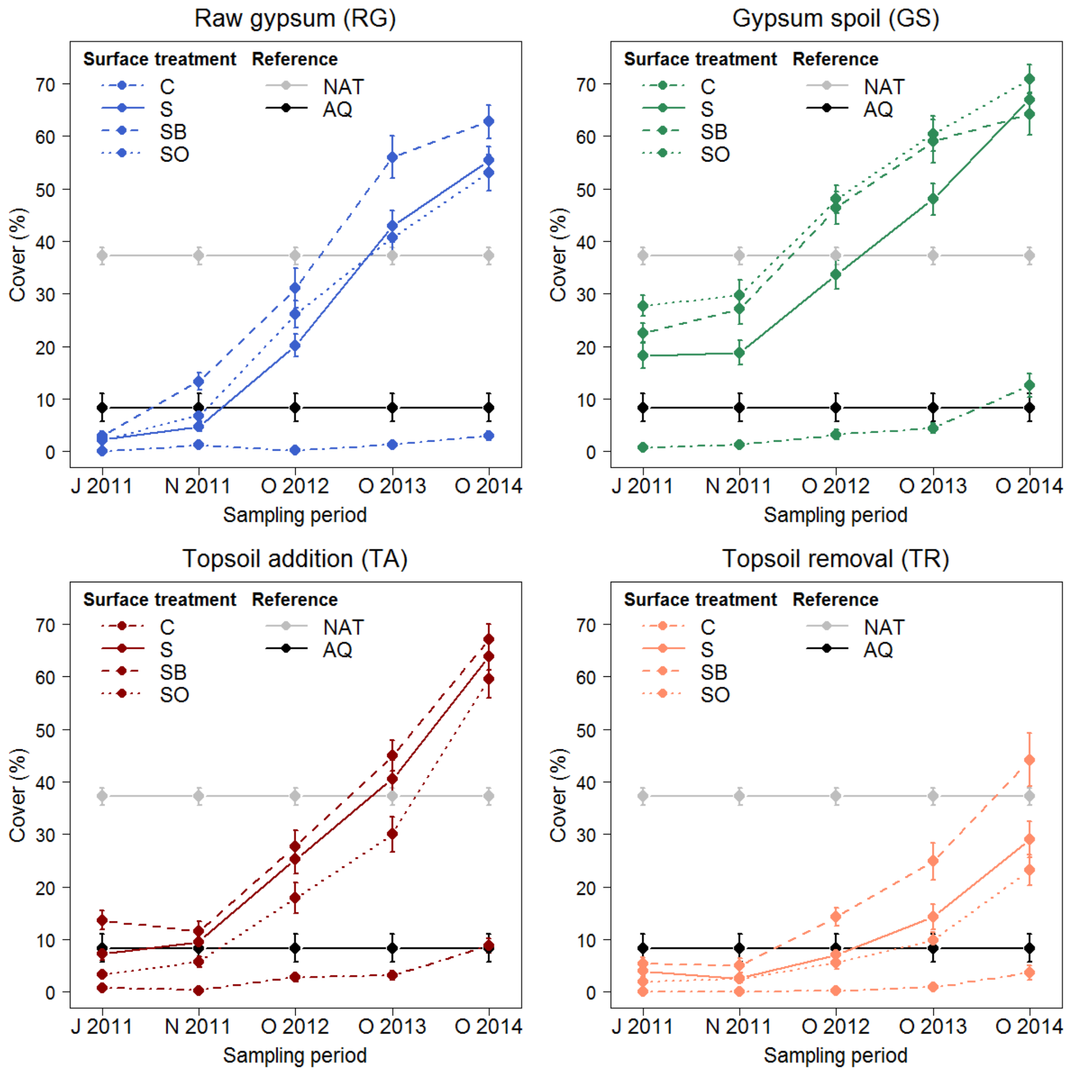

Figure 1. Changes in percentage cover (mean±SE) of target species within bedding materials between June 2011 and October 2014 compared to the habitat and the abandoned quarry used as reference (data from October 2016). Surface treatments: C: control; S: sowing; SB: sowing plus organic blanket; SO: sowing plus organic matter. Bedding materials: RG: raw gypsum; GS: gypsum spoil; TA: topsoil addition; TR: topsoil removal. Reference sites: NAT: natural habitat; AQ: abandoned quarry.

We represented the results of bedding materials pooling all three sowing treatments together to compare them to their respective controls (Fig. 2). The richness and cover of target species, including gypsophiles, were always greater in NAT than in AQ (Fig. 2 a, e, b and f). In the experimental plots, target species cover and richness of sown plots increased with time especially on GS, TA and RG, moving towards NAT, while controls were more similar to AQ throughout the monitoring (Fig. 2 a and e). Target species cover on GS was greatest at the initial sampling, reaching NAT cover first (~38%), approximately three years after sowing (October 2012). After four years (October 2013), sowings at all materials except on TR had reached similar or greater cover than NAT. At the final observation year, GS, TA, and RG had a similarly high cover (57-67%) superior to NAT, while TR had reached NAT cover slower. On the other hand, the richness and cover of target species on control treatments were low in all bedding materials throughout the study and, except for a richness increase in GS, had values similar or below AQ at the final sampling.

The richness of gypsophiles was greatest on RG, GS and TA sown plots throughout the monitoring, with the control at GS increasing in the last sampling (Fig. 2 b). Gypsophiles cover in NAT was ~15% and all treatments were below except sowings at RG due to *O. tridentata* (Fig. 2 f). The cover of gypsophiles was specially low on controls for most of the monitoring with a slight increase at the end, while in AQ it was almost non-existent.

Non-target species richness and cover were low on NAT and much higher on AQ (Fig. 2 c and g). The richness and cover on TA were initially greatest and similar to AQ, remaining high at controls and decreasing on sown plots throughout the monitoring. Non-target species richness and cover decreased in general 3-4 years (November 2011-October 2012) after the experiment started (despite fluctuations on TA), except on controls at RG.

The total cover on NAT was composed mainly of target species, and AQ of non-target species (Fig. 2 h). The total cover on experimental plots increased throughout the monitoring mainly due to the gain of target species on sown plots, and non-target species on the controls. Restoration at all bedding materials except TR showed greater total cover than reference sites, whereas all other options were similar (restoration at TR and untreated at TA) or showed lower cover.

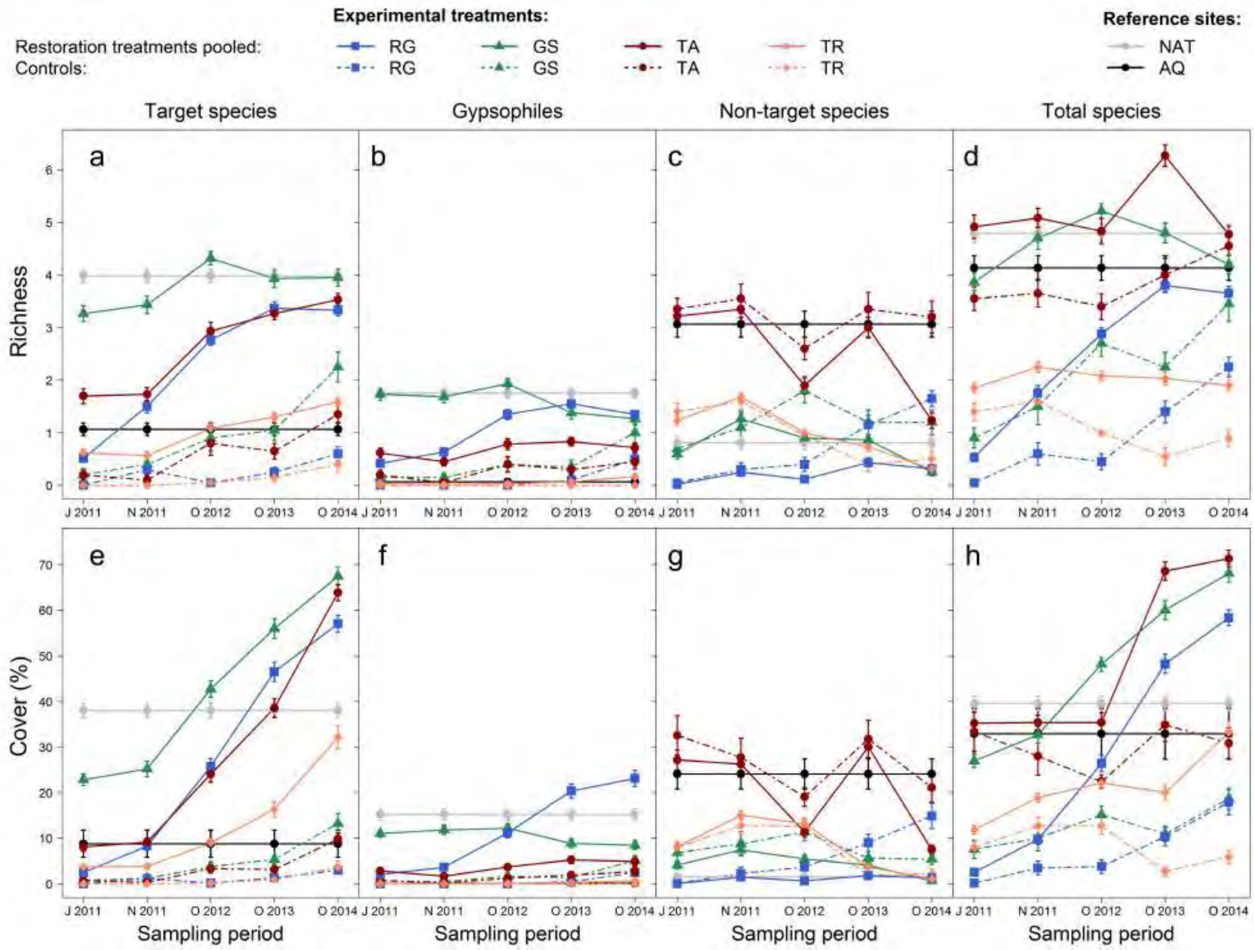

Figure 2. Changes on the richness (a-d) and percentage cover (e-h) (mean±SE) of target species, gypsophiles separately, non-target species and total species by bedding material between June 2011 and October 2014 compared to the habitat and the abandoned quarry used as reference (data from October 2016). Restoration treatments are pooled together, untreated controls are included separately. Bedding materials: RG: raw gypsum; GS: gypsum spoil; TA: topsoil addition; TR: topsoil removal. Reference sites: NAT: natural habitat; AQ: abandoned quarry.

For the final observation year, LMMs revealed significant effects of bedding material (BM), surface treatment (ST) and their combination, on the richness and cover of species (target species, gypsophiles, non-target species and total plant cover), except for the interaction between BM and ST that had no effect on the richness of gypsophiles (Table 2).

			Target species		Gypsophiles		Non-target species		Total	
		df	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Richness	Bedding material (BT)	3	288.07	**<0.001**	157.29	**<0.001**	155.22	**<0.001**	338.93	**<0.001**
	Surface treatment (ST)	3	109.67	**<0.001**	9.30	**0.03**	62.45	**<0.001**	23.48	**<0.001**
	BM x ST	9	43.13	**<0.001**	16.52	0.06	66.36	**<0.001**	24.07	**<0.001**
Cover	Bedding material (BT)	3	217.32	**<0.001**	249.22	**<0.001**	126.09	**<0.001**	288.11	**<0.001**
	Surface treatment (ST)	3	350.35	**<0.001**	46.23	**<0.001**	136.77	**<0.001**	238.25	**<0.001**
	BM x ST	9	59.08	**<0.001**	99.94	**<0.001**	79.35	**<0.001**	44.83	**<0.001**

Table 2. Results of linear mixed models (LMMs) testing the effects at the last sampling date (October 2014) of bedding material, surface treatment and their interaction on the richness and cover of target species, gypsophiles separately, non-target species, and total plant species. The chi-square statistic (X^2) of the fixed factors and their significance are presented. All results with p<0.05 are in bold.

Permanova analysis showed part of the variance on the species cover in the final observation year was mainly explained by the bedding material (BM) and to a lower extent by surface treatment (ST). R^2 for BM and ST respectively by species group were as follow: Target species (BM and ST had respectively R^2 = 0.29 and 0.16, both with p <0.001), gypsophiles (R^2 = 0.25, p = 0.001 and R^2 = 0.03, p = 0.014 respectively), gypsovags (R^2 = 0.29 and R^2 = 0.18, both with p = 0.001), non-target species (R^2 = 0.26 and R^2 = 0.04, both with p = 0.001) and total species cover (R^2 = 0.23 and R^2 = 0.21, both with p = 0.001). The interaction was significant for target species (R^2 = 0.07, p = 0.001), gypsovags (R^2 = 0.10, p = 0.001) and the total cover (R^2 = 0.08, p = 0.001) whereas had small explanatory power for gypsophiles and non-target species.

Individual response of gypsophiles throughout the monitoring showed the cover of *O. tridentata* increased, specially at RG; was always highest at GS for *H. squamatum,* and decreased *for L. subulatum* specially at GS, where the highest initial cover was recorded (Fig. 3). All gypsovags increased their cover on sowings throughout the monitoring on most bedding materials (Fig. 3).

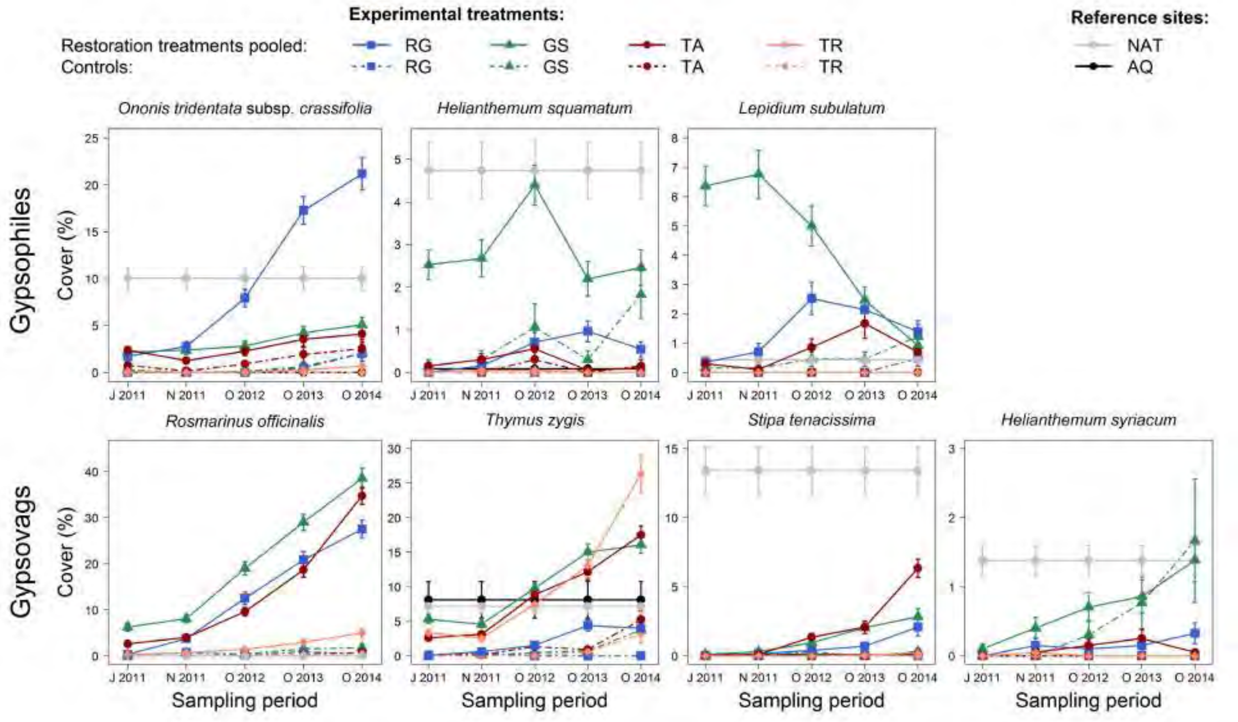

Figure 3. Evolution of the percentage cover (mean±SE) of target species, by bedding material between June 2011 and October 2014 compared to the habitat and the abandoned quarry used as reference (data from October 2016). Restoration treatments are pooled together, untreated controls are included separately. Bedding materials: RG: raw gypsum; GS: gypsum spoil; TA: topsoil addition; TR: topsoil removal. Reference sites: NAT: natural habitat; AQ: abandoned quarry.

LMMs for individual species indicated significant effects of bedding material (BM), surface treatment (ST) and their combination for all species except for *H. squamatum* and *H. syriacum* for which only bedding material had significant effects (Table 3).

		Target species													
		Gypsophiles						Gypsovags							
		O. tridentata		*H. squamatum*		*L. subulatum*		*R. officinalis*		*T. zygis*		*S. tenacissima*		*H. syriacum*	
	df	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Bedding material (BT)	3	249.93	**<0.001**	109.10	**<0.001**	21.89	**<0.001**	252.14	**<0.001**	161.30	**<0.001**	71.55	**<0.001**	49.01	**<0.001**
Surface treatment (ST)	3	42.86	**<0.001**	2.07	0.559	8.38	**0.039**	131.72	**<0.001**	59.36	**<0.001**	25.55	**<0.001**	3.43	0.329
BM x ST	9	105.83	**<0.001**	7.01	0.636	17.40	**0.043**	102.96	**<0.001**	89.11	**<0.001**	32.17	**<0.001**	7.16	0.621

Table 3. Results of linear mixed models (LMMs) testing the effects of bedding material, surface treatment and their interaction on the cover of target species (gypsophiles and gypsovags) at the last sampling date. Reference sites are not included in the analysis. The chi-square statistic (χ^2) of the fixed factors and their significance are presented. All results with p<0.05 are in bold.

Comparing reference sites to the final observation year (Fig. 4), we observed the total cover on AQ was lower than on sown plots and based mainly on non-target species and *T. zygis* as the only target species. The total cover on NAT was higher than AQ and lower than on sown plots, but target species were represented more evenly. In turn, sown plots in all bedding materials characterized by high cover of target species with large proportion based on few dominant species (i.e. *R. officinalis* and *O. tridentata* in RG*, R. officinalis* and *T. zygis* in GS and TA*,* and *T. zygis* in TR). The cover of target species, as group or individually, was generally lowest in controls in all bedding materials. Post-hoc comparisons for individual species within each bedding material at the last observation year are showed in Appendix C. *O. tridentata* performed best on RG, *L. subulatum* on RG and GS*, H. squamatum* and *H. syriacum* on GS, *S. tenacissima* on TA, *T. zygis* specially on TR, and *R. officinalis* well on all materials except TR. Within each bedding material, the surface treatments did not differ greatly in cover, showing blankets or the addition of organic matter had little effect over time. Exceptions occurred as follows: on RG, *O. tridentata* (significantly better on S and SO)*, H. squamatum* (better on S) and *H. syriacum* (better on SB); on RG and M, *R. officinalis* (better on SB and SO respectively) and *S. tenacissima* (better on SB and SO respectively)*;* and *T. zygis* on GS (better on S) and on RG, TA, and TR (better on SB). The cover of non-target species was greatest on AQ. The controls had the greatest cover of non-target species in all bedding materials, being greatest on TA and RG, whereas in sowing treatments it was low except on TA (on SB and SO), (Fig. 4).

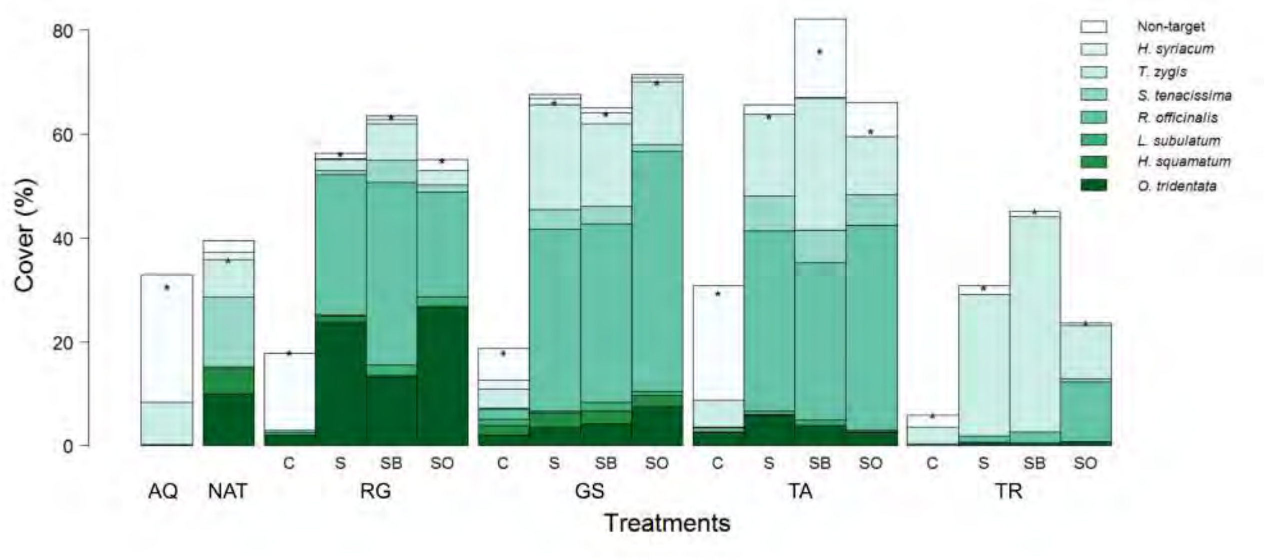

Figure 4. Mean cumulative cover (%) of species by soil treatment in the last sampling date (reference sites: October 2016, experimental plots: October 2014). Stars indicate the mean total cover. Reference sites: AQ: abandoned quarry; NAT: natural habitat. Experimental plots: Bedding material: RG: raw gypsum; GS: gypsum spoil; TA: topsoil addition; TR: topsoil removal. Surface treatment: C: control; S: sowing; SB: sowing plus organic blanket; SO: sowing plus organic matter.

3.2. Effects of restoration treatments on species composition over time

PRC curves represent compositional changes over time of each bedding material and surface treatment combination in our experimental plots, and need to be interpreted in relation to the zero line which represents the reference community (NAT). The community established in the abandoned quarry (AQ) is also represented for comparison (Fig. 5). The more strongly correlated species with the temporal trajectory, either positively or negatively, are displayed as species scores on the one-dimensional diagram (Fig. 5b).

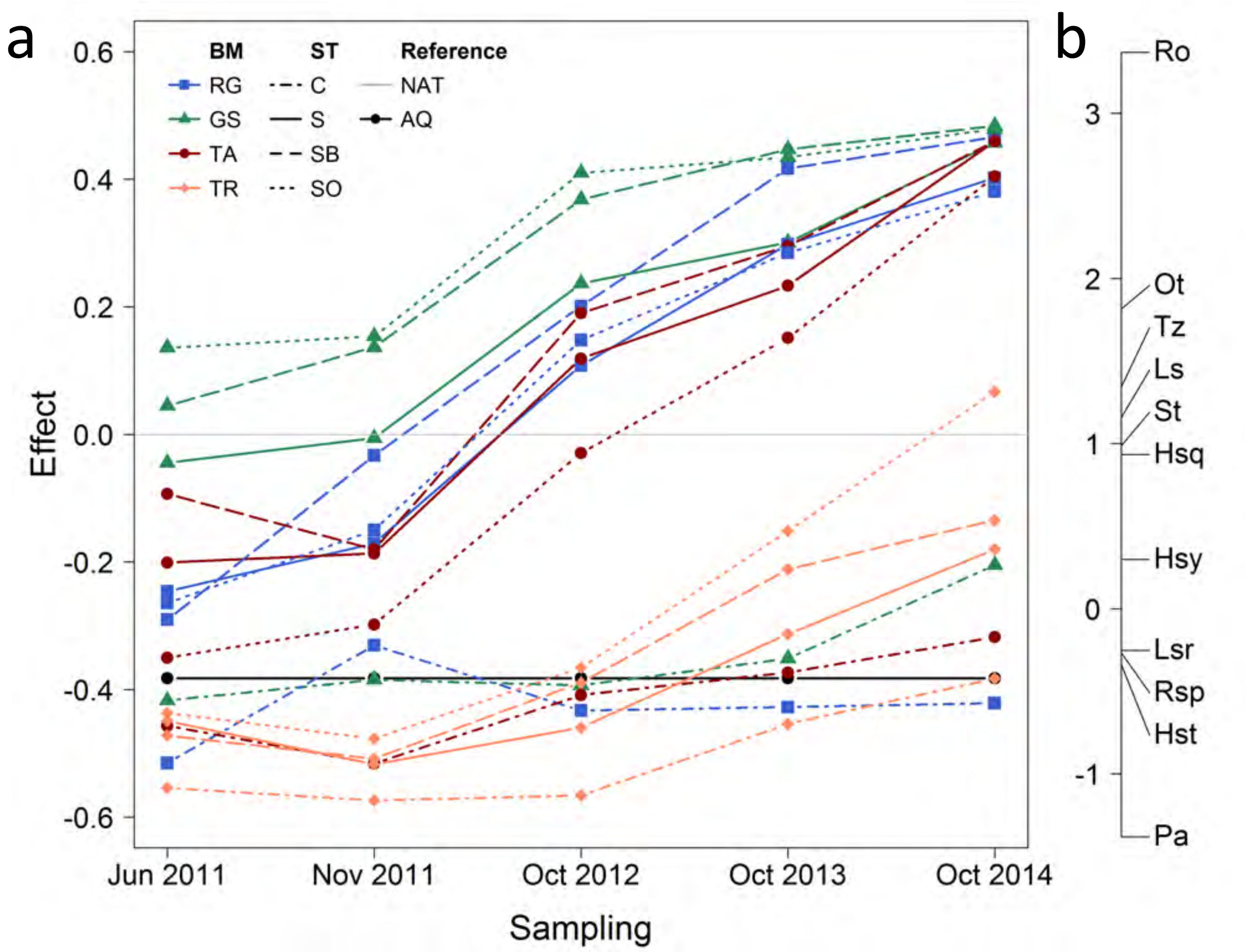

Figure 5. Principal response curves illustrating the changes in community composition for the combination of bedding materials x surface treatment 5 years after application relative to reference sites in the study area. The change in species is relative to the first axis of a RDA, and the species position on this axis is represented on the vertical axis. **Species:** Hst = *Helichrysum stoechas,* Hsq = *Helianthemum squamatum*, Hsy = *Helianthemum syriacum*, Ls = *Lepidium subulatum*, Lsr = *Lactuca serriola,* Ot = *Ononis tridentata* subsp. *crassifolia*, Pa = *Picnomon acarna*, Rsp = *Retama sphaerocarpa*, Ro = *Rosmarinus officinalis*, St = *Stipa tenacissima*, Tz = *Thymus zygis* subsp. *gracilis*. Experimental plots: Bedding material (BM): RG: raw gypsum; GS: gypsum spoil; TA: topsoil addition; TR: topsoil removal. Surface treatment (ST): C: control; S: sowing; SB: sowing plus organic blanket; SO: sowing plus organic matter. Reference sites: AQ: abandoned quarry; NAT: natural habitat.

PRC ordination explained 48% of the variation, accounted for by treatment and the treatment x time interaction (5.75% explained by time). Axis 1 accounted for a high proportion of the variation (31.43%), explaining more than higher axes (22, 12, 9.9, 6.8, 4.1, 3.5%). The PRC confirmed the restoration treatments induced significant change in species composition. The model was statistically significant (p=<0.001). The PRC showed composition differed between treatments from the start of the monitoring onward (p=<0.01 at all sampling dates). All treatments generally moved in a positive direction over time. Two main responses were observed: (i) a large positive effect of restoration treatments on GS, RG and TA, initially differing between them but converging at the end, early attaining greater cover of target species than NAT, and (ii) moderate effect of restoration on TR or very limited on the controls at all bedding materials respectively. Restoration at TR moved towards NAT more slowly than the other restoration treatments, with only TR.SO reaching the target community nearly two years after the slowest treatment in the other bedding materials (TA.SO). The composition on untreated controls changed little and remained very similar to the abandoned quarry (AQ), except for the control at GS that moved slightly towards NAT in the last sampling. Figure 5 showed target species increased their cover on the restoration treatments (from initial negative weights to positive), becoming similar or even greater than in the reference habitat on GS in Nov 2011 and RG, TA, and TR.SO in Oct 2012. The strongest changes over time occurred in *R. officinalis* that became dominant, and *O. tridentata, T. zygis, L. subulatum, S. tenacissima, H. squamatum* and *H. syriacum* in descending order in treatments with positive PRC scores. These compositional differences make evident restoration treatments promoted target species while not restoring benefited early successional species inespecific to the reference habitat. Non-target species such as *L. serriola, R. sphaerocarpa,* and *P. acarna* had lower cover (negative weights) on the habitat and treatment combinations with positive effect.

3.3. Effects of restoration treatments on species composition in the final sampling

The DCA of species composition produced eigenvalues of 0.42, 0.36, 0.25 and 0.27 and gradient lengths of 5.30, 3.99, 4.15 and 3.97 for the first four axes (Fig. 6). Experimental plots distributed around the centre of the ordination space with NAT being near in axis 2 indicating compositional similarities, whereas AQ showed markedly different occupying the right side of axis 1. The left-hand side of the plot showed target species whereas the right hand showed mainly non-target colonizer species characteristic of disturbed areas. The species plot showed target species on the left side of the ordination space associated to the reference site NAT and the experimental plots. *H. squamatum, H. syriacum* and *S. tenacissima* were nearer to NAT, *O. tridentata* occupied an intermediate position between NAT and the experimental plots, and *L. subulatum* and *R. officinalis* were more associated to the experimental plots, occurring more frequently. *T. zygis* was characteristic of TR and occupied a transitional position between the experimental plots and AQ. The right-hand side of the plot showed early successional non-target species related to disturbed sites and inespecific to the reference habitat such as *Picnomon acarna, Andryala ragusina,* *Dittrichia viscosa*, *Helichrysum stoechas* and *Retama sphaerocarpa.* Compositional differences in the final year confirmed restoration treatments promoted target species while not restoring benefited only early successional species inespecific to the target habitat.

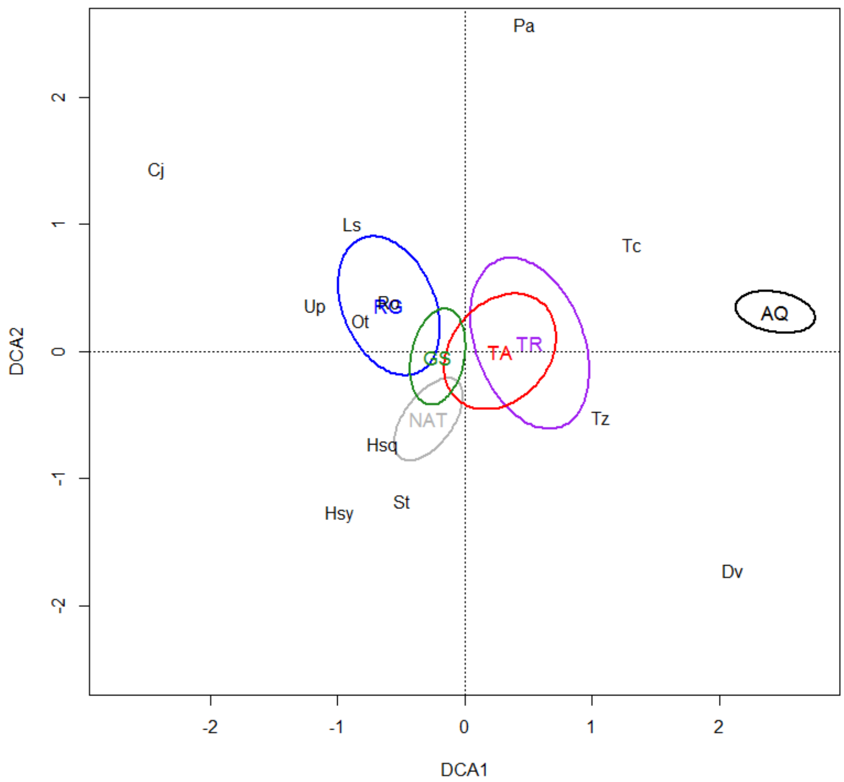

Figure 6. DCA ordination plot of treatments of the species cover ~5 years after sowing at the experimental area in Escúzar, Granada, SE Spain. The SD ellipses (95% confidence limits) of transects position by treatment are shown. **Species:** Cj = *Chondrilla juncea*, Dv = *Dittrichia viscosa*, Hsq = *Helianthemum squamatum*, Hsy = *Helianthemum syriacum*, Ls = *Lepidium subulatum*, Ot = *Ononis tridentata* subsp. *crassifolia*, Pa = *Picnomon acarna*, Ro = *Rosmarinus officinalis*, St = *Stipa tenacissima*, Tc = *Teucrium capitatum* subsp. *gracillimum*, Tz = *Thymus zygis* subsp. *gracilis,* Up= *Ulex parviflorus*. Bedding materials: RG: raw gypsum; GS: gypsum spoil; TA: topsoil addition; TR: topsoil removal. Reference sites: NAT: natural habitat; AQ: abandoned quarry.

4. Discussion

Our results confirmed that gypsicolous vegetation of high conservation value can be restored in the short term by sowing in common materials derived from gypsum extraction. Here, we found the vegetation dynamics were driven by sowing and bedding material choice. Sowing on materials derived from gypsum quarrying (i.e. GS, RG and TA) improved the composition, richness and cover of target species after 5 years, becoming increasingly similar between them, whereas surface treatments did not particularly improve the performance of vegetation within materials. Control plots had low cover, but target species colonised from adjacent sown plots, specially at GS where their cover increased significantly, indicating a remarkable recruitment potential of target species which can be exploited in restoration programs. Restoration treatments initiated vegetation trajectories towards the desired plant community in the gypsum habitat, albeit the dominant *R. officinalis* caused notable differences. All restoration treatments achieved more valuable plant communities (i.e. higher richness and cover of target species) than those in the untreated abandoned quarry. Thus, our results demonstrate that sowing on appropriate substrates ensures establishment of target vegetation in the short term, helping to jump-start succession and moving it towards the desired community.

Initial management effects reported earlier for samplings conducted in 2010 (Ballesteros *et al.,* 2012) did not persist. Despite higher richness and seedling density of target species were observed initially at GS and TA (Ballesteros *et al.*, 2012), richness and cover converged with RG over time. The initial high cover of non-target species in TA and TR reduced over time (Ballesteros *et al.,* 2012), and contrary to expected, TA had an increasingly similar trajectory to RG and GS. We also found surface treatments converged within bedding materials providing only little benefit over time. Initially, SB had improved species richness and seedling density, specially at GS and TA (Ballesteros *et al.,* 2012), but except for *T. zygis* did not traduce into the greatest cover of target species over time. Non-target species did not benefit either, except on TA where the cover kept high, but lower than in controls. In the case of SO, initially favouring the survival and growth of seedlings (Ballesteros *et al.,* 2012), did not improve the cover of target species over time. Thus, we conclude the application of these surface treatments would not be justified, and restoration of gypsicolous vegetation should be approached basing on the appropriate selection of seed mixture, seed density and bedding material.

Convergence to a similar plant community is more likely when the initial site conditions (e.g. species pools, soil properties) are more similar, specially when conditions are stressful and diversity is low as in our study (Walker and del Moral, 2003). In our experiment, all the treatments started with a similar species pool provided by sowing, so potential differences due to the order of species arrival were minimised, except on TA that contained the habitat seed bank including target and non-target species. However, despite competition by non-target species reduced the establishment of target species on TA compared to RG and GS, (Ballesteros *et al.*, 2012), the differences in cover and richness decreased with time. Convergence and better performance on RG, GS and TA in contrast to TR were more likely due to a more similar and favourable chemical composition (e.g. higher gypsum content) and water availability (Appendix A and B; Ballesteros et al. 2014). Better performance on the same substrates has also been reported for plantings of gypsophiles, where gypsum and summer water-content were higher (Ballesteros *et al.,* 2014). Gypsum or sulphur content have been claimed to improve the performance of gypsophiles (Meyer, 1986; Ruiz *et al.*, 2003). Water-availability in summer has been related to the gypsum content of substrates, and used to explain active growth and phenology of gypsophiles (Meyer, 1986; Escudero *et al.*, 1999, 2000). Crystallization water on gypsum soils represents a significant water source for shallow-rooted plants, especially during summer, accounting for 70-90% of the water used (Palacio *et al.*, 2014). Thus, gypsovags in our study could have also benefited from these properties. However, the concentration of other elements or physical properties such as soil texture and structure may have caused vegetation respond differently according to the material and require further study. Although the reasons why these materials were better for gypsicolous species are not conclusive, they proved to greatly benefit target species, so that their use in restoration would be very recommendable.

Reference ecosystems considering local heterogeneity are crucial to validate the success of the restoration (Mota *et al.*, 2003; SER, 2004; Dana and Mota, 2006; Castillejo *et al.*, 2011). In our study, the most satisfactory results in comparison to NAT were obtained in sowings at RG, GS and TA which, regardless of the surface treatment, had similar richness and superior cover. The main difference with NAT was due to the high cover of *R. officinalis* in sowings (contributing approximately to half of the total cover). Despite *R. officinalis* is frequent in gypsicolous communities (Mota *et al.,* 2011a), this species was outside well-preserved patches in our study area (Marchal *et al.,* 2008), explaining why it was not recorded in the reference habitat. Without this species, the cover of target species in sowings at RG, GS and TA would be not far from NAT and agree with Marchal *et al.* (2008). Gypsophiles in RG and GS had similar richness, and the most satisfactory cover compared to NAT; in the case of RG, it was due to the high cover of *O. tridentata.* Control plots and TR had a much slower successional response, showing low species establishment and cover, more in line with the poor results obtained in AQ. In this sense, abandoned quarries may take considerable time to recover the characteristics of unaltered communities. Mota *et al.* (2003) and Dana and Mota (2006) studied abandoned quarries in SE Spain with chronosequences spanning 70 years, reporting significant soil and vegetation changes through time, but limitations for the recovery of the original species. Thus, the introduction of key species combined with the management of substrates, as our study indicates, can help to overcome some of these limitations in the short-term, improve the establishment of gypsicolous vegetation and jump-start succession towards the desired community.

In our study, it was possible to compare the effects of management against reference plant communities in the target habitat and the abandoned quarry at one-time point, but further monitoring would allow to include ongoing changes due to environmental fluctuation (e.g. temperature and precipitation), (Alday and Marrs, 2014). In addition, there was only one available abandoned quarry in the area, and thus our results are contingent to site-specific conditions and should be interpreted with caution. This is a common problem in restoration studies, where is not always possible to have enough restored and reference sites to avoid site-specific idiosyncrasies (Dana and Mota, 2006; Stuble *et al.*, 2017).

The choice for restoration must be based on the capacity of RG, GS, and TA to ensure the regeneration of target species. Recruitment of gypsophiles likely relies on the formation of seed-banks in the vicinity of mother plants and nucleation (Martinez-Duro *et al.*, 2010). In our experiment, control plots helped us to understand the recruitment process on the tested materials. We obtained the best results on GS, where target species were more evenly represented and achieved the highest richness and cover compared to RG and TA, which in turn had a high proportion of non-target species. This means recruitment was more effective on GS, revealing particularly convenient for restoration. Plantings of gypsophiles on the same substrates tested here developed bigger plants, able to produce more seed in RG (Ballesteros *et al.*, 2014). However, the higher recruitment in GS suggests its seed-bank can be as or more effective than RG in establishing target species. Similarly happened with TA which, even with the seed contribution to the controls from the seed-bank or plants on the adjacent sown plots, was less effective at establishing target species, probably due to the abundance of non-target species. This is consistent with the high cover of ruderals (~47%) nearly 10-years after the application of topsoil on abandoned quarries in SE Spain (Mota *et al.,* 2004). Topsoil yielded moderate cover of gypsophiles in our study and in Mota *et al.* (2004); (>5 and ~7 %, after 5 and 10 years respectively). Thus, if non-target species persist over time, hindering the establishment of the gypsicolous community, its use would be preferred for different goals such as improving the productivity or control erosion.

Seed input has proved to be key in the recovery of gypsicolous vegetation and to overcome critical dispersal limitations evident in the area. The replacement of substrates may not be enough to recover gypsicolous vegetation in the long term without an early input of target species seed. Materials may remain barren or dominated by ruderals hindering succession towards a desirable community. The chances of arrival of target species seeds from the vicinity are low given well-conserved habitat patches are normally fragmented and isolated , and gypsophiles do not have efficient dispersal mechanisms (Escudero *et al.,* 1999, 2000). Sowing or other restoration methods such as hydroseeding or planting (Ballesteros *et al.,* 2014, 2017), can be useful in overcoming these limitations and provide the starting species pool to jump-start succession. However, these methods can not recreate the exact unaltered community. Thus, keeping well-conserved gypsicolous vegetation patches adjacent to the quarry could facilitate colonisation of target species and other organisms in the long-term (e.g. missing vascular plants, cryptogamic crusts, microbial communities). The assembly process needs to be followed to evaluate success in the long-term. Facilitation-competition trade-offs structuring gypsicolous communities under site-specific conditions require further study (Pueyo *et al.*, 2007; Saiz *et al.*, 2014). Particularly, *R. officinalis* can become very competitive under moderate environmental stress, and because its allelopathic potential, able to displace desirable species (Pueyo *et al.*, 2007; Chen *et al.*, 2013; Saiz *et al.*, 2014, García-Robles *et al.*, unpublished results). Additional measures may be required to divert succession from less-valuable plant communities and emphasize gypsophiles cover, either adjusting seed proportions initially, sowing at later stages or controlling competitive species. In our case, satisfactory results were obtained with the initial seed proportion at the most effective treatments, with GS showing general good results for most species. *L. subulatum* was above the cover of the habitat, but its strong decrease throughout the experiment suggests it may require further management. Because *R. officinalis* is absent in the target community in the area, its dominance and high competitive power, would not be recommendable to include it in the seed mixture in the future restoration program.

The availability and cost-effectiveness of each approach must be borne in mind in extrapolating experimental results when planning large-scale restoration programmes. The use of gypsum spoil is of particular interest given it is an inexpensive quarrying by-product produced in large quantities, used to fill quarry pits and reshape the landscape after quarrying (Ballesteros *et al.,* 2014). Raw gypsum has a higher cost, but its remarkable benefits to *O. tridentata* subsp. *crassifolia*, also observed in Ballesteros *et al.* (2014), must be considered when designing the restoration plan or specific conservation measures, given this subspecies is endemic to the area and particularly affected by quarrying (Ballesteros *et al.*, 2013). Spreading small amounts of topsoil may also be helpful to introduce other desirable components not included on the sowing such as missing species or biological soil crusts (e.g. cyanobacteria, green algae, lichens, mosses) important on gypsum habitats (Belnap and Lange 2003, Ballesteros *et al.,* 2017).

We have demonstrated that sowing on the most favourable substrates can help the establishment of a local set of target species, but its necessary to validate this measures through regular long-term monitoring over time, and in other places with different species pools and site conditions to inform and design better strategies for the ecological restoration of gypsicolous habitats.

5. Conclusions

Our experiment demonstrates sowing on appropriate substrates can ensure the establishment of gypsicolous species in the short-term. Sowing on gypsum spoil, raw gypsum and topsoil made composition, richness and cover approach that of the reference habitat, generating more valuable plant communities than the spontaneously generated in the abandoned quarry. Early seed input on appropriate substrates can overcome dispersal limitations of target species and priority effects caused by competition with non-target species. The seed mixture must emphasize gypsophiles species and not include species such as *R. officinalis,* that may hinder the establishment of the other target species. Additionally, we found that the application of surface treatments (i.e. organic matter addition or organic blanket overlays) does not improve the performance of vegetation enough to justify the cost increase in restoration programs. Gypsum spoil confirmed as the most satisfactory restoration option due to the good establishment and recruitment of target species, which may promote more self-sustainable gypsicolous communities. Although satisfactory results were obtained longer time frames are required to judge the success of restoration. Thus, this experiment should be monitored over the long term and evaluate the ecological, technical and economic viability of management methods and confirm their applicability to achieve effective large-scale restoration of disturbed gypsum environments. The knowledge derived from this study will help to develop future programs for the management of gypsum habitats.

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Appendices

Appendix A. Mean values (±SE) of the edaphic variables of the bedding materials studied. Different letters indicate statistically significant differences in the *post hoc* test after Bonferroni correction at $p<0.05$. Five samples were randomly collected on each bedding material (20 in total) at 0-30 cm depth. Analyses were conducted following the methodology in Mañares et al. (1998) and MAPA (1994). Table reproduced from Ballesteros et al. 2014.

Variable	Raw gypsum	Gypsum spoil	Topsoil addition	Topsoil removal
Gravel (>2mm) (%)	55.38 ± 1.42 a	31.72 ± 2.44 b	7.74 ± 2.60 c	2.37 ± 0.30 c
Sand (2-0.05 mm) (%)	31.26 ± 2.21 a	32.55 ± 3.33 a	39.89 ± 3.07 a	5.01 ± 0.48 b
Coarse silt (0.05-0.02 mm) (%)	5.76 ± 0.73 b	11.89 ± 1.60 ab	16.43 ± 4.84 a	5.45 ± 0.68 b
Fine silt (0.02 mm) (%)	62.98 ± 1.57 b	55.56 ± 4.29 b	43.68 ± 2.73 c	89.54 ± 0.91 a
pH	7.91 ± 0.02 a	8.04 ± 0.01 a	7.90 ± 0.06 a	7.99 ± 0.05 a
CEC (meq/100g)	8.23 ± 0.32 b	7.40 ± 0.42 b	9.24 ± 0.21 b	20.11 ± 1.30 a
Ca^{2+} (mg/l)	34.68 ± 0.97 b	39.73 ± 1.33 a	39.68 ± 0.54 a	35.00 ± 0.84 b
Mg^{2+} (mg/l)	9.81 ± 0.41 a	4.40 ± 0.61 b	3.24 ± 0.30 b	0.83 ± 0.03 c
Na^{+} (mg/l)	1.95 ± 0.34 a	1.97 ± 0.26 a	1.90 ± 0.21 a	1.62 ± 0.36 a
K^{+} (mg/l)	3.74 ± 0.45 a	1.18 ± 0.13 b	1.44 ± 0.10 b	0.65 ± 0.40 b
S^{2-} (mg/l)	2664.62 ± 61.98 a	2352.00 ± 73.33 b	2358.86 ± 98.65 b	1999.20 ± 43.13 c
Organic carbon (%)	0.33 ± 0.07 b	0.42 ± 0.12 b	1.03 ± 0.07 a	0.92 ± 0.17 a
Organic matter (%)	0.56 ± 0.13 b	0.73 ± 0.20 b	1.78 ± 0.12 a	1.59 ± 0.29 a
N (%)	0.03 ± 0.00 c	0.03 ± 0.00 c	0.06 ± 0.01 b	0.10 ± 0.01 a
$CaCO_3$ (%)	30.45 ± 1.13 b	23.97 ± 0.36 c	23.37 ± 0.74 c	52.52 ± 1.32 a
Gypsum (%)	87.18 ± 1.12 a	88.22 ± 1.92 a	93.56 ± 1.39 a	27.30 ± 11.73 b
Quartz (%)	0.58 ± 0.12 b	0.52 ± 0.11 b	0.82 ± 0.36 b	5.06 ± 1.08 a
Calcite (%)	0.48 ± 0.16 b	3.82 ± 0.78 b	3.44 ± 0.44 b	46.56 ± 7.43 a
Dolomite (%)	8.18 ± 0.29 a	3.92 ± 0.68 b	0.38 ± 0.38 c	0.00 ± 0.00 c
Phyllosilicates (%)	2.30 ± 1.02 b	1.78 ± 1.09 b	0.66 ± 0.50 b	18.54 ± 3.90 a

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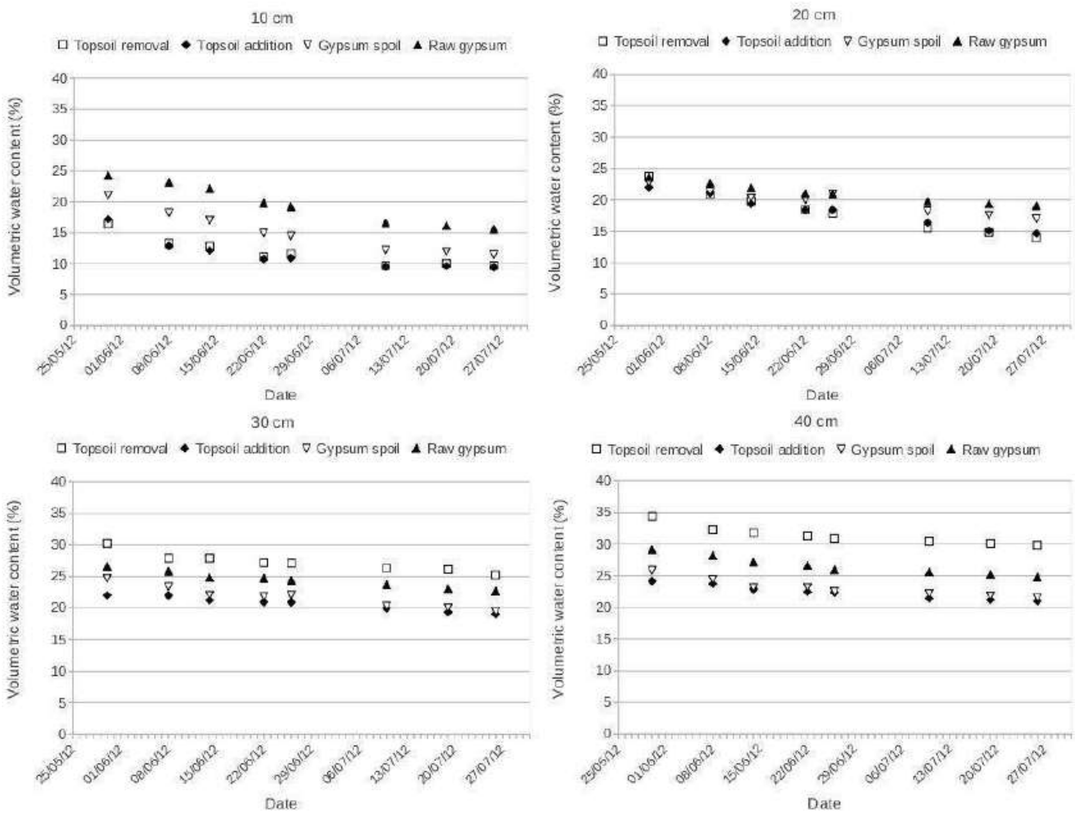
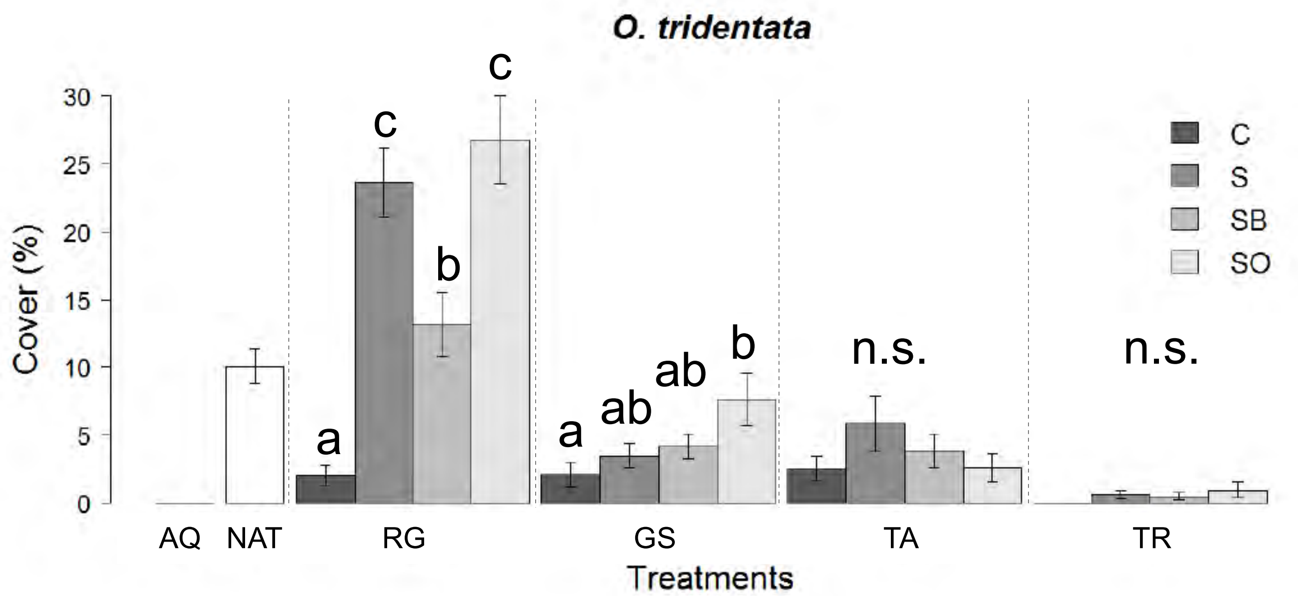
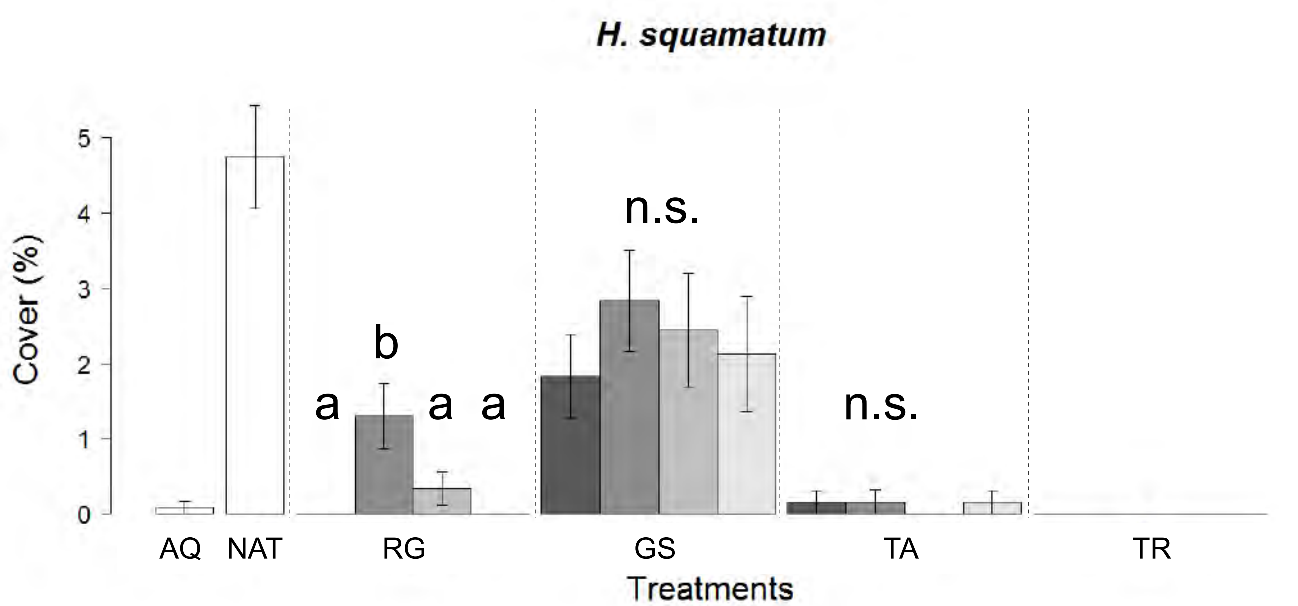
Appendix B. Volumetric soil-water content (%) on the bedding materials from May to July 2012, monitored in Ballesteros et al. 2014 at 10, 20, 30, and 40 cm depth in four access tubes using a Profile probe PR2 and a HH2 moisture meter (Delta-T Devices). Bedding materials: RG, raw gypsum; GS, gypsum spoil; TA, topsoil addition; TR, topsoil removal. Figure reproduced from Ballesteros et al. 2014.

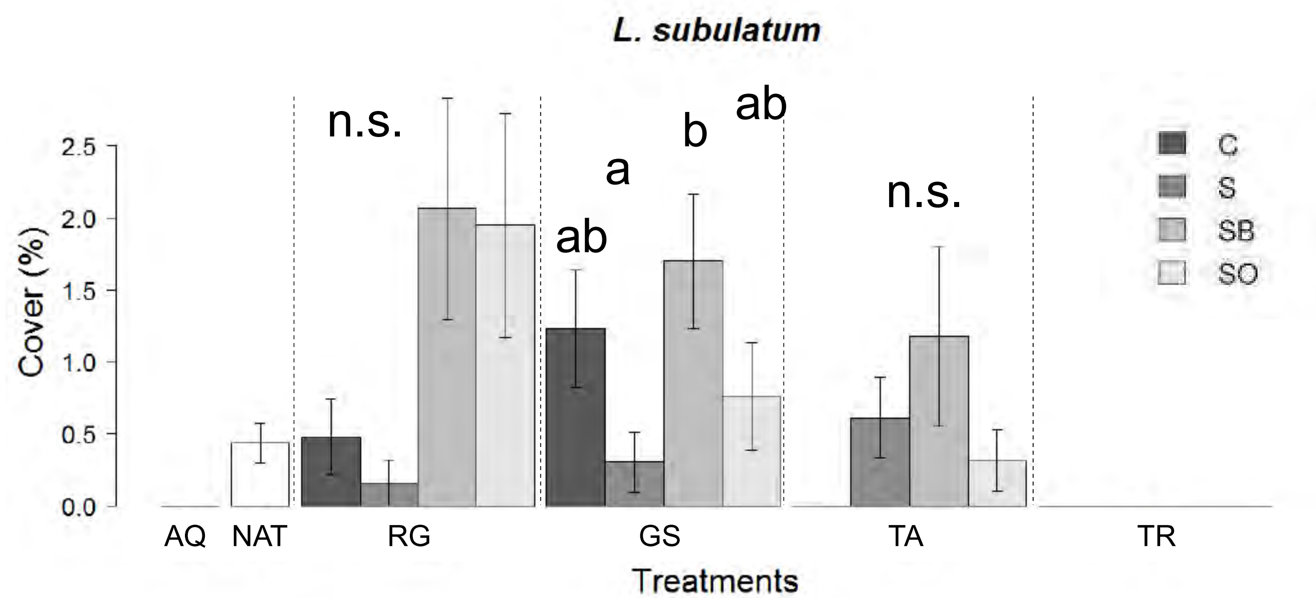


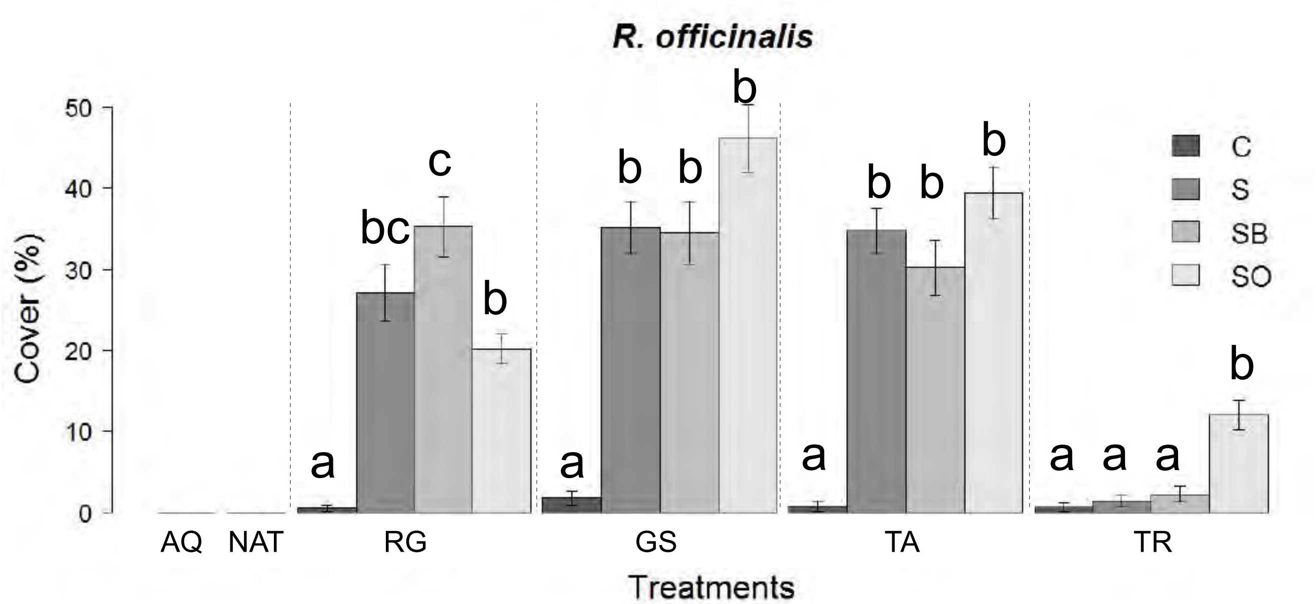
Ballesteros M, Cañadas EM, Foronda A, Peñas J, Valle F, Lorite J. 2014. Central role of bedding materials for gypsum-quarry restoration: An experimental planting of gypsophile species. *Ecological Engineering* **70**: 470–476.

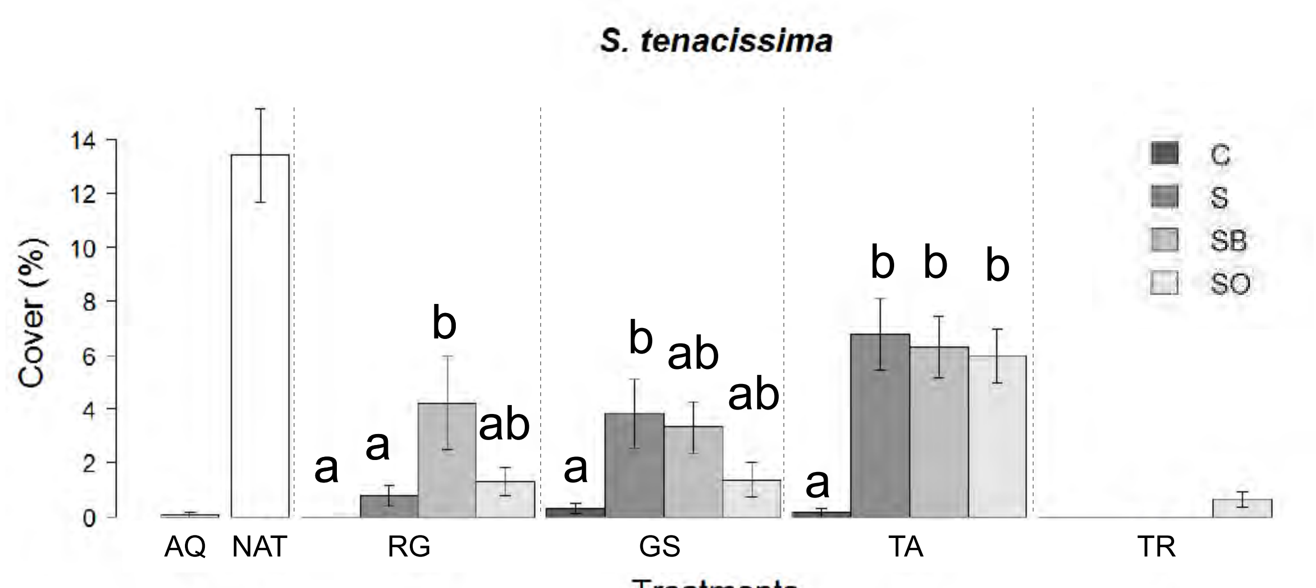
Appendix C. Mean percentage cover (± SE) of species by surface treatment in each soil treatment in the last sampling date (reference sites: October 2016, experimental plots: October 2014). Different letters above the bars represent significant differences between surface treatments at p<0.05 in the post hoc Tukey tests after the GLMs; n.s. indicates not significant differences. Reference sites: AQ: abandoned quarry; NAT: natural habitat. Experimental plots: Bedding material: RG: raw gypsum; GS: gypsum spoil; TA: topsoil addition; TR: topsoil removal. Surface treatment: C: control; S: sowing; SB: sowing plus organic blanket; SO: sowing plus organic matter.

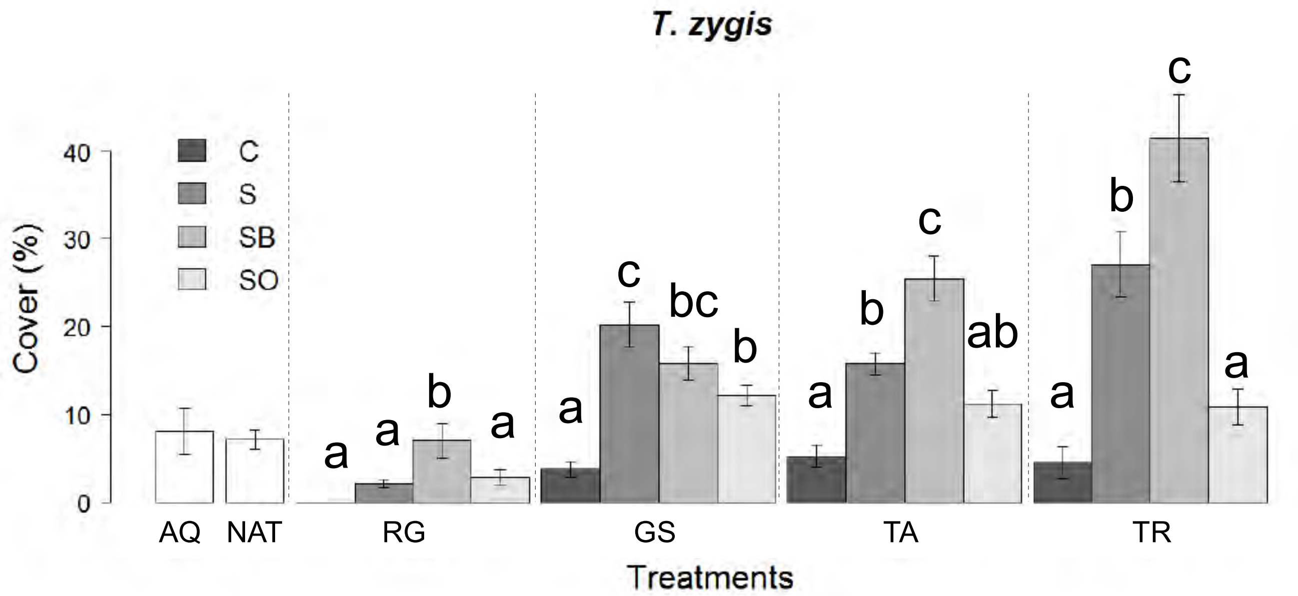


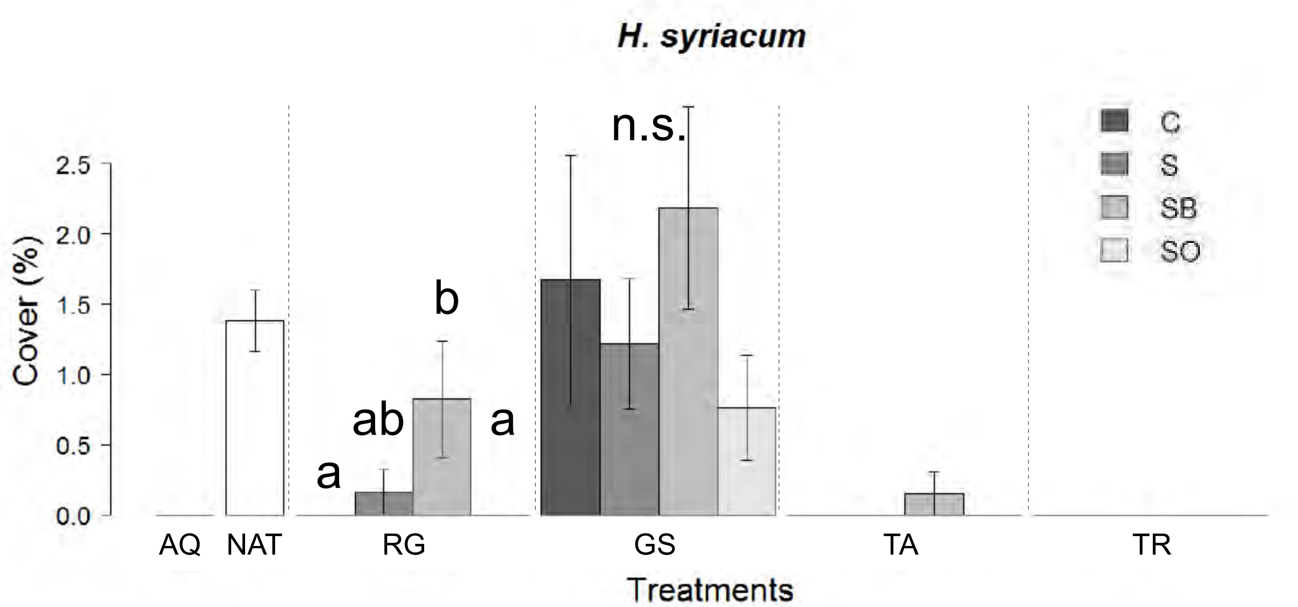



L. subulatum
Cover (%)
0.0
0.5
1.0
1.5
2.0
2.5
n.s.
ab
a
b
ab
n.s.
C
S
SB
SO
AQ
NAT
RG
GS
TA
TR
Treatments


R. officinalis
Cover (%)
0
10
20
30
40
50
a
bc
c
b
a
b
b
b
a
b
b
b
a
a
a
b
C
S
SB
SO
AQ
NAT
RG
GS
TA
TR
Treatments


S. tenacissima
Cover (%)
0
2
4
6
8
10
12
14
a
a
b
ab
a
b
ab
ab
a
b
b
b
C
S
SB
SO
AQ
NAT
RG
GS
TA
TR
Treatments


T. zygis
Cover (%)
Treatments
C
S
SB
SO
AQ
NAT
RG
GS
TA
TR
a
a
b
a
a
c
bc
b
a
b
c
ab
a
b
c
a
0
10
20
30
40


H. syriacum
Cover (%)
Treatments
C
S
SB
SO
AQ
NAT
RG
GS
TA
TR
a
ab
b
a
n.s.
0.0
0.5
1.0
1.5
2.0
2.5

Sowing on gypsum spoil showing most target species (June, 2017).

Sowing on raw gypsum showing *Ononis tridentata* subsp. *crassifolia, Rosmarinus officinalis* and *Stipa tenacissima* in the foreground (June, 2017).

Sowing on topsoil addition treatment showing mainly colonizer species (non-target species); (June, 2013).

Sowing on topsoil removal (called marls in Chapter 4) dominated by *Thymus zygis* (June, 2017).

Chapter 7

Restoration of gypsicolous vegetation on quarry slopes: Guidance for hydroseeding under contrasting inclination and aspect.

Ballesteros M, Cañadas EM, Marrs RH, Foronda A, Martín-Peinado FJ, Lorite J.

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Restoration of gypsicolous vegetation on quarry slopes: Guidance for hydroseeding under contrasting inclination and aspect

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Abstract

The establishment of gypsicolous vegetation of high conservation value on land impacted by quarrying requires restoration measures to overcome constraints imposed by the new landforms created in the process. The aim of this study was to assess the suitability of three standard hydroseeding methods to restore gypsicolous vegetation on quarry spoil slopes under a dry Mediterranean climate. The treatments were: paper cellulose mulch; paper cellulose mulch + organic blanket; and wood fibre mulch; compared against a control. These treatments were tested on two slopes (10-15% vs 60-65%) and two contrasting aspects (north vs south). We evaluated the cover of all plant species 2.8 years after treatment, assessing both target gypsicolous species and non-target species. Our results showed strong compositional and cover differences between hydroseeded and control plots. Control plots had a low cover of target species with a vegetation composed of early-successional species that had the potential to hinder target species establishment over time. All hydroseeding treatments improved target vegetation cover, with wood fibre performing best in most situations studied here, alternatives being the cheaper but less effective paper mulch on shallow slopes; or the more expensive paper mulch + blanket on steep slopes in case of high erosion risk. Shallow and southern-steep slopes were more suitable for the recovery of gypsum vegetation by hydroseeding, compared to northern-steep slopes where non-target species developed more readily outcompeting target species. These results will help to guide management decisions to restore gypsicolous vegetation by hydroseeding in disturbed gypsum habitats.

Keywords: Aspect, hydroseeding, gypsum habitat, restoration techniques, slope.

1. Introduction

The restoration of native vegetation affected by quarrying poses challenges due to limitations caused by the alteration of both topography and soil properties (Bradshaw, 2000). Quarrying usually produces low-quality spoil materials with inherent stability problems, both causing severe difficulties for the re-establishment of former vegetation (Martín-Duque et al., 2010; Espigares et al., 2011; Cohen-Fernández et al., 2013). Common practices to enhance vegetation establishment and stabilising slopes include the spreading of topsoil, use of geotextiles, and the planting or sowing of plants (Theisen, 1992; Singh et al., 2002; Ghose, 2004; Matesanz et al., 2006; Gilardelli et al. 2013). Hydroseeding is a common sowing technique for quarry and road-side rehabilitation that is increasingly used in ecological restoration; this approach often requires the use of various mulches, stabilisers, fertilizers as well as mixtures of commercial and native species seeds (Matesanz et al., 2006; Brofas et al., 2007; García-Palacios et al., 2010). The inclusion of native species is increasingly being used in restoration projects especially under adverse climatic and soil conditions (Matesanz & Valladares, 2007; Bochet et al., 2010; Oliveira et al., 2012), and is particularly relevant when the recovery of specific vegetation targets associated with singular substrates is the restoration goal (O'Dell & Claassen, 2009; Whiting et al., 2010; Ballesteros et al., 2014).

Gypsum substrates in arid and semi-arid regions are often important habitats for plant conservation that must be preserved (European Commission, 1992). These habitats support a highly-specialized flora with many rare and endemic species which have a range of strategies to cope with the physical and chemical limitations imposed by gypsum substrates (see Mota et al., 2011; Escudero et al., 2014). However, gypsum is a mineral in global demand (Herrero et al., 2013), and its extraction by mining inevitably damages the valuable gypsicolous vegetation and the habitat (Mota et al., 2011). Thus, mining companies are compelled to conduct restoration programs despite the lack of information on the most appropriate ecological restoration techniques and procedures. The restoration of gypsicolous flora affected by quarrying has been the focus of previous studies (Mota et al., 2004; Dana & Mota, 2006; Ballesteros et al., 2012, 2014). Spontaneous succession may take considerable time due to site-specific environmental conditions, as unstable and unsuitable substrates, lack of propagules or competition with non-target species (Mota et al., 2004; Dana & Mota, 2006; Prach & Řehounková, 2006; Gilardelli et al., 2015). Active measures such as planting (Ballesteros et al., 2014) and sowing (Ballesteros et al., 2012) have been shown to provide good restoration of gypsicolous plant communities, but they have mainly been implemented on relatively flat landforms. Techniques for successful restoration of steeper landforms have only been partially addressed (e.g. Pastor & Hernández, 2002; Martín et al., 2003; Matesanz & Valladares, 2007). However, gypsum quarry waste areas are often remodelled and usually have relatively steep slopes, which depending on orientation may differ greatly on surface temperature and water availability in Mediterranean climates (Kutiel, 1992; Pueyo & Alados, 2007; Alday et al., 2010). One way to tackle steep slopes is through hydroseeding; although hydroseeding is widely used in restoration, to our knowledge, there is limited technical or scientific literature resulting in specific guidelines that can be used to design restoration programs for disturbed gypsum habitats.

The aim of our study is to assess the suitability of three hydroseeding methods to restore gypsicolous vegetation affected by quarrying on spoil slopes under a dry Mediterranean climate. Our underlying hypothesis was that early vegetation response would be determined by interactions between the hydroseeding method, slope and site aspect. We hoped the results would inform future ecological restoration of spoil materials left after gypsum quarrying, allowing better designed and cost-effective future restoration programs.

2. Materials and Methods

2.1. Site description

The study was performed in an experimental area next to an active quarry in Escúzar, Granada, SE Spain (37° 2' 57'' N, 3° 45' 30'' W) at 950 m asl. The climate is continental Mediterranean, with relatively cold winters, hot summers, and four months of water deficit. The mean annual temperature is 15.1 °C, with an average monthly minimum temperature in January of 7.6 °C and maximum of 24.2 °C in August. Annual rainfall averages 420 mm, occurring mainly in winter. The area is in the Neogene sedimentary basin of Granada; the dominant substrates being lime and gypsum deposited in the late Miocene, the latter in combination with marls (Aldaya et al., 1980). The predominant soils in the gypsum outcrops are Gypsiric Leptosols (Aguilar et al., 1992; IUSS Working Group WRB, 2015). The vegetation of the area is a mosaic of fields with cereal crops and olive and almond orchards (*Olea europaea* L. and *Prunus dulcis* D.A. Webb.) and scattered patches of native plants growing over gypsum outcrops (Ballesteros et al. 212).

2.2. Target species

The target gypsicolous vegetation in the area is described in the EU Habitats Directive (European Commission, 1992) as 1520, “Iberian gypsum vegetation, *Gypsophiletalia*”, and is characterized by plants exclusive to gypsum soils (gypsophiles); (see Mota et al. 2011, Escudero et al. 2014), such as *Helianthemum squamatum* (L.) Dum. Cours., *Lepidium subulatum* L., and *Ononis tridentata* subsp. *crassifolia* (Dufour ex Boiss.) Nyman. In addition, there are also other frequent non-exclusive species of gypsum substrates (gypsovags) such as *Stipa tenacissima* L., *Rosmarinus officinalis* L., *Helianthemum syriacum* (Jacq.) Dum. Cours., *Thymus zygis* L. subsp. *gracilis* (Boiss.) R. Morales and *Teucrium capitatum* subsp. g*racillimum* (Rouy) Valdés Berm. & Sánchez Crespo (according to Marchal et al. 2008). Total plant cover in the habitat is approximately 42%, 30% for target species and 22% for gypsophiles (transforming Braun-Blanquet scale data in Marchal et al., 2008, following Van der Maarel 1979).

2.3. Experimental design

Experimental slopes were built in October 2011 on an area of 0.7 ha using spoil (see properties in Table S1), derived from gypsum extraction. This material was chosen on the basis of pilot experiments (Ballesteros et al., 2012, 2014). The design of the experimental slopes (Figure 1) included three factors: slope (1), aspect (2) and treatment (3). We considered: (1) two slopes: steep slopes (60-65 %, limited by the angle of rest of the spoil material) and shallow slopes (10-15 %, typical slopes left after quarrying, according to the quarry management plan) in combination with (2) two aspects: north- and south-oriented. In each of these 4 slope x aspect combinations we set up eight experimental plots (steep slopes = 5 x 10 m; shallow slopes = 5 x 20 m) and applied (3) three hydroseeding treatments and one control randomly to each of two replicate plots. The hydroseeding treatments were: (a) Paper cellulose (PC), consisting of water, seeds, paper cellulose mulch (200 $g·m^{-2}$), soil stabiliser (0.5-0.8 % by mulch weight) and fertilizer (30 $g·m^{-2}$ NPK 15-10-10 + 3MgO + 6S); (b) Paper cellulose + blanket (PCB), equal to PC but also covered with a straw and coir fibre blanket; (c) Wood fibre (WF), equal to PC, but mulch consisted of wood fibre (220 $g·m^{-2}$), and (d) control (C), where no hydroseeding was applied. This provided 2 slopes angles x 2 aspects x 4 treatments x 2 replicates = 32 plots.

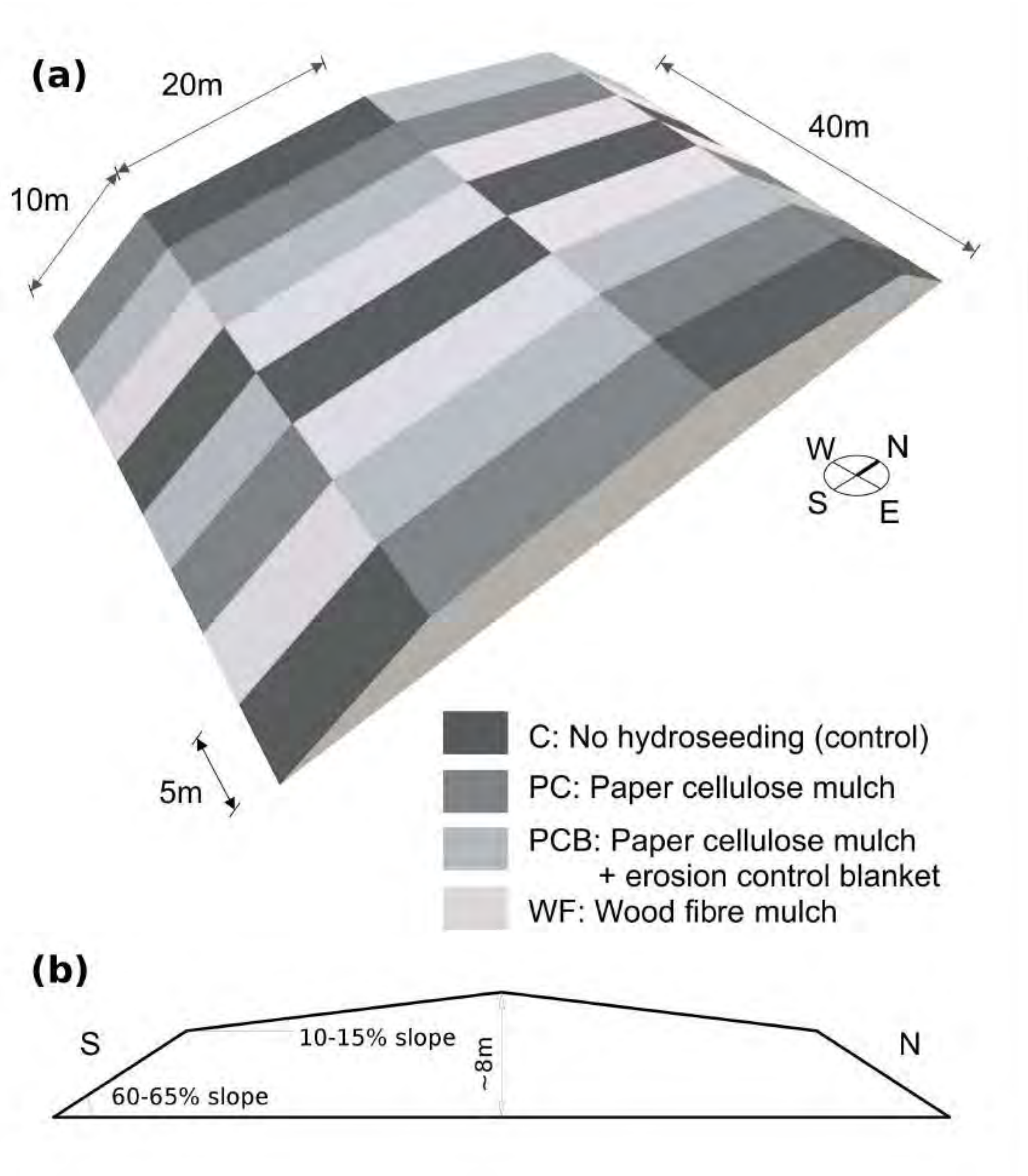


Figure 1. (a) Schematic of the experimental slopes showing the hydroseeding treatments distributed on two contrasting slopes: shallow (10-15%) and steep (60-65%); and two aspects: north (N) and south (S). (b) Cross section of the experimental slopes. The space between the plots is not represented.

Hydroseeding was conducted in December 2011. The substrate was previously tilled to 10 cm depth to aid seed establishment. We used a mixture of native seeds (655 seeds/m^2) consisting of 47% gypsophiles and 53% gypsovags. Based on pilot experiments (Ballesteros *et al*. 2012), seeds of all taxa were added at the following rates (seed m^{-2}): Gypsophiles included *H. squamatum* (180), *L. subulatum* (120), and *O. tridentata* subsp. *crassifolia* (10); and gypsovags, *S. tenacissima* (100), *R. officinalis*

(45), *H. syriacum* (100), *T. zygis* subsp. *gracilis* (50) and *T. capitatum* subsp. *gracillimum* (50). Seeds were collected from natural vegetation patches within the study area between June and September 2011.

2.4. Vegetation sampling

We sampled plant species composition and cover 2.8 years later (October 2014). Sampling in this season ensures to record late-emerging seedlings of target species (as observed in Ballesteros et al. 2012). We placed 4 equidistant linear transects along each of the 32 plots, and assessed three contact points every 0.5 m: at the centre and 0.5 m to each side of the transect (123 and 63 points per transect for shallow and steep slopes respectively). We recorded the perennial plant species occurring (i.e. chamaephytes and hemicryptophytes) plus bare soil, and calculated their cover as the proportion of points intercepted.

2.5. Data analyses

Canonical correspondence analysis (CCA) was performed to relate species composition to the explanatory variables (following Oksanen, 2015) using the “vegan” package (Oksanen et al., 2016). The species dataset was reduced by omitting the less frequent species (<5% of transects). Vegetation cover data were arcsin transformed before analyses (Crawley, 2007). Constraining variables (hydroseeding treatment, slope and aspect) were included in the model using forward selection based on the use of the AIC statistic as the selection criterion (Oksanen, 2015), with significance assessed using 200 permutations. Standard deviational ellipses (95% confidence limits) were used to illustrate the area covered by the hydroseeding treatments in the biplot. We also tested the relative influence of the explanatory variables (treatment, slope and aspect) on plant composition using the “adonis” function in the “vegan” package (Oksanen et al., 2016).

We analysed the effects of hydroseeding treatment, slope (shallow versus steep), aspect (north versus south) and their interaction on the cover of target species, gypsophiles separately, non-target species (i.e. other than sown gypsophiles and gypsovags), and total species. These effects were assessed fitting generalised linear mixed models (GLMMs) using treatment, slope and aspect as fixed factors and plot as random factor. Models were fitted applying the Laplace approximation of likelihood (Bolker et al., 2009), a Poisson error distribution and log-link function using the R “lme4” package (Bates et al., 2015). Similarly, we assessed the effects of treatment and its interaction with slope or aspect as fixed factors using aspect and slope respectively as random factors, and performing multiple comparisons with the R "multcomp" package (Hothorn et al., 2008). All statistical analyses were performed using R version 3.2.3 (R Core Team, 2015).

3. Results

3.1. Species composition

We recorded 28 perennial species in the plots. The mean number of species was greater in all hydroseeded treatments; mean values (±SE) were: PCB: 10.8±0.6, WF: 10.2±0.6, PC: 9.7±0.6, and C: 6.6±0.6. The most frequent species on the hydroseeded plots were *Lolium perenne* (94.8% of transects), *T. zygis* (89.6%), *R. officinalis* (81.3%), *Moricandia arvensis* (79.2%), *Medicago sativa* (76%), *Picnomon acarna* (74%), *H. squamatum* (70.8%), *O. tridentata* (70.8%), *L. subulatum* (67.7%), *H. syriacum* (62.5%), *T. capitatum* (50%) and *S. tenacissima* (37.5%). Frequency for all these species was always lower in control plots except for *M. arvensis* (93.8%) *and P. acarna* (75%) and other non-target species (e.g. *Lactuca serriola*, 53.1%, *P. miliaceum*, 28.1%, *Dittrichia viscosa*, 25%, *Ulex parviflorus*, 18.8%).

In the multivariate analysis, all explanatory variables were included in the model after forward selection in CCA reducing the AIC of the null model from 179.96 to 154.57; the resultant model was significant ($p < 0.001$). The constrained inertia within this CCA was 0.70 (37% of explained variance) and eigenvalues for the first five axis $\lambda_1 = 0.20$, $\lambda_2 = 0.14$, $\lambda_3 = 0.09$, $\lambda_4 = 0.08$ and $\lambda_5 = 0.05$. Hydroseeding treatment was the main factor in explaining species composition ($R^2 = 0.24$, $F = 18.96$, p-value = 0.001) followed by slope ($R^2 = 0.08$, $F = 19.22$, p-value = 0.001) and aspect ($R^2 = 0.07$, $F = 16.43$, p-value = 0.001). There were marked compositional differences between hydroseeded and control plots. The species plot (Figure 2) showed target species on the right of the ordination next to *L. perenne* and *M. sativa*. The hydroseeding treatments occupied a similar region on the right hand side of the ordination space overlapping near the origin because of the presence of the target species. The hydroseeding treatments shared all target species, except for paper cellulose that did not contain *L. subulatum* and *S. tenacissima.* Early-successional non-target species like *P. acarna* and *D. viscosa* were characteristic of PCB and PC, and *P. miliaceum* was only characteristic of PC. The control treatment was separated on the left hand side of the ordination and was related to *L. serriola*. Species such as *M. arvensis* and *Reseda stricta* occupied an intermediate position between the hydroseeding treatments and the control. Target species were associated to the southern aspects and shallow slopes (except *O. tridentata*).

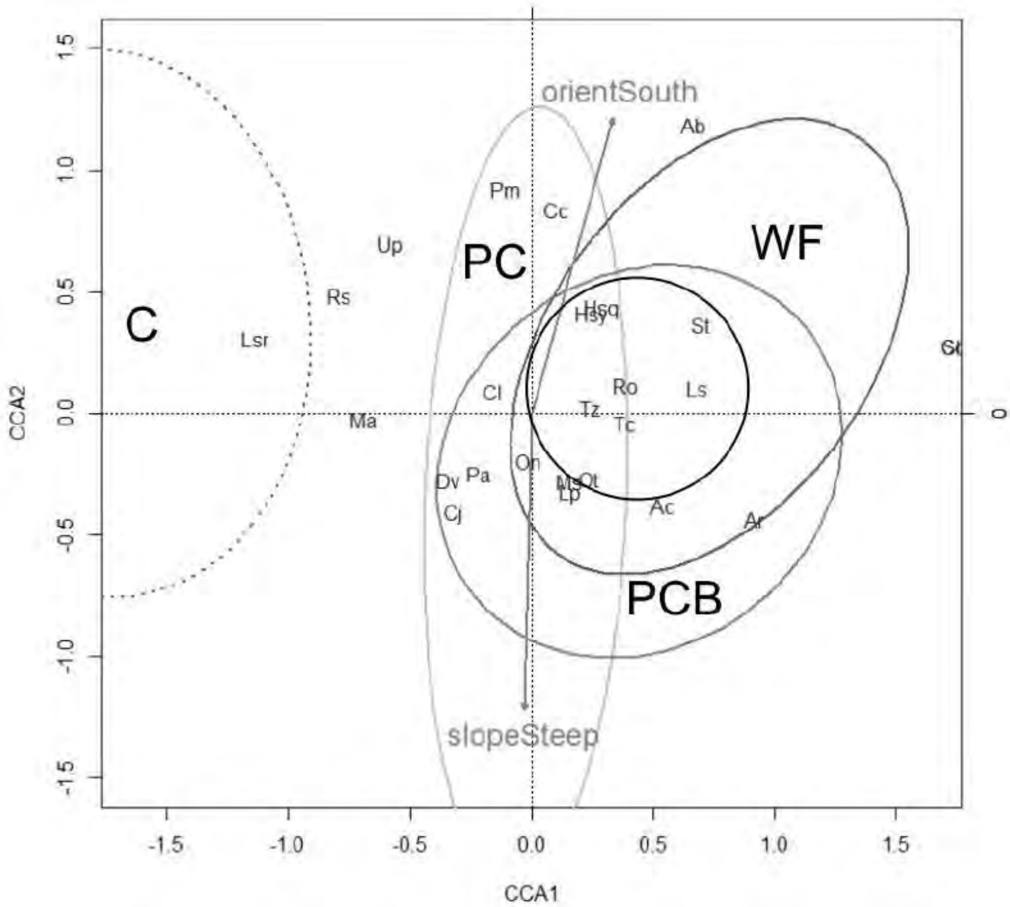


Figure 2. Constrained CCA ordination plot of treatments of the species cover 2.8 years after hydroseeding at the experimental area in Escúzar, Granada, SE Spain; the ordination was constrained on hydroseeding treatment. The SD ellipses (95% confidence limits) of transects position by treatment are shown. **Treatments:** C: control; PC: hydroseeding with paper cellulose mulch; PCB: hydroseeding with cellulose mulch plus erosion control blanket; WF: hydroseeding with wood fibre mulch. Treatments were tested on two contrasting slopes**:** shallow (10-15%) and steep (60-65%); and two aspects: north and south. **Species:** Target species are highlighted with a black circle. Ab = *Artemisia barrelieri*, Ac = *Anthyllis cytisoides*, Ar = *Andryala ragusina*, Cc = *Centaurea calcitrapa*, Cj = *Chondrilla juncea*, Cl = *Carthamus lanatus*, Col = *Colutea arborescens*, Dv = *Dittrichia viscosa*, Hsq = *Helianthemum squamatum*, Hsy = *Helianthemum syriacum*, Lp = *Lolium perenne*, Ls = *Lepidium subulatum*, Lsr = *Lactuca serriola*, Ma = *Moricandia arvensis*, Ms = *Medicago sativa*, On = *Onopodum nervosum*, Ot = *Ononis*

tridentata subsp. c*rassifolia*, Pa = *Picnomon acarna*, Pm = *Piptatherum miliaceum*, Ro = *Rosmarinus officinalis*, Rs = *Reseda stricta*, Sh = *Scolymus hispanicus*, St = *Stipa tenacissima*, Tc = *Teucrium capitatum* subsp. *gracillimum*, Tz = *Thymus zygis* subsp. g*racilis,* Up= *Ulex parviflorus*.

3.2. Species cover

The dominant species according to their cover on the hydroseeded plots were the non-target species *L. perenne* (12.5%)*, M. arvensis* (9.6%)*, M. sativa* (6.3%)*,* followed by the target species *T. zygis* (4.8%)*, R. officinalis* (4.6%)*, H. squamatum* (4.3%) in this order. Values for the remaining target species were *L. subulatum* (2.4%), *O. tridentata* (2.1%), *T. capitatum* (0.9%) and *S. tenacissima* (0.8%). The cover of non-target species was greater on control plots, where *M. arvensis* was the dominant species (34.7%).

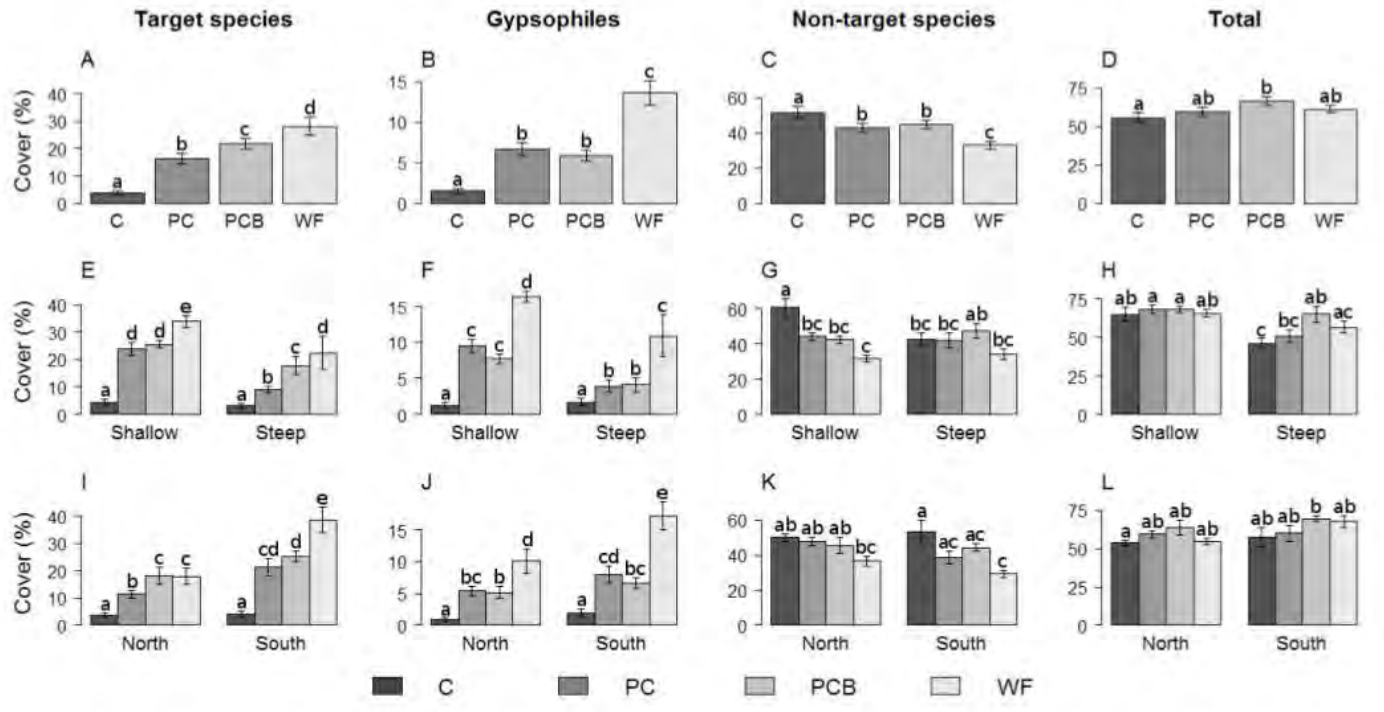
The cover of target species and gypsophiles showed a significant response to hydroseeding treatment, slope and aspect, but not their interaction (Table 1). The target species cover was greatest on WF (28.2 out of a total cover of 61.1%), followed by PCB (21.7 out of 66.6%), PC (16.4 out of 59.5%) and C (3.8 out of 55.5%), with all treatments differing significantly (Figure 3A and D). In the case of gypsophiles, there were significant differences between treatments except for PC and PCB (Figure 3B). Target species cover was always greater on the shallow slopes (Figure 3E) and southern aspect (Figure 3I) when comparing hydroseeding treatments to their counterpart. The same was true for gypsophiles (Figure 3F and J). Target species showed no differences in the control treatment either among slopes or aspects (Figure 3E and F), as also occurred for gypsophiles (Figure 3I and J). The results were supported by the individual response of target species, except for *O. tridentata* that performed better on the northern slope. The best treatment for most target species was WF, excepting *R. officinalis* and *T. capitatum* that performed better in PCB (Figure S1; Figure S2).

Table 1. Results of generalised linear mixed models (GLMMs) testing the effects of hydroseeding treatment, slope, aspect and their interaction on the cover of target species, gypsophiles separately, non-target species, and total plant species. The chi-square statistic (χ^2) of the fixed factors and their significance are presented. All results with p<0.05 are in bold.

		Species cover							
		Target species		Gypsophiles		Non-target species		Total	
	df	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Treatment (T)	3	30.29	**<0.001**	36.60	**<0.001**	10.97	**0.012**	2.88	0.410
Slope (S)	1	7.38	**0.007**	6.01	**0.014**	0.61	0.434	6.54	**0.011**
Aspect (A)	1	8.45	**0.004**	4.45	**0.035**	0.79	0.374	1.55	0.213
T × S	3	2.45	0.484	2.88	0.410	4.74	0.192	1.86	0.602
T × A	3	4.91	0.178	2.78	0.427	1.51	0.681	0.92	0.820
S × A	1	1.79	0.180	2.40	0.121	12.13	**<0.001**	4.33	**0.037**
T × S × A	3	4.69	0.196	7.69	0.053	0.27	0.965	2.85	0.415

The cover of non-target species (mainly early-successional colonizers) was affected by hydroseeding treatment and the interaction of slope with aspect (Table 1). Cover was greatest on control plots and the lowest on WF (Figure 3C). The C treatment had its maximum on shallow slopes and southern aspects, where WF reached its minimum (Figure 3G and K).

Total plant cover showed a significant response to slope and slope by aspect interaction (Table 1). There were significant differences between treatments, with the greatest total plant cover in PCB, followed by WF, PC and C (Figure 3D). These differences were due to different performance of treatments on the steep slopes, as they all had similar total cover on the shallow one (Figure 3H). Total cover was greater on the shallow slopes in C and PC compared to their counterparts on the steep slopes, whereas PCB and WF performed similarly on both inclinations (Figure 3H). Total cover was similar for all treatments in the two aspects, with greatest cover achieved in PCB on the southern slope (Figure 3L).



We observed marked differences in the cover and proportion of species between northern steep slopes and all other aspect and slope combinations, with the first showing a particular increase of non-target species cover at the expense of target species (Figure 4).

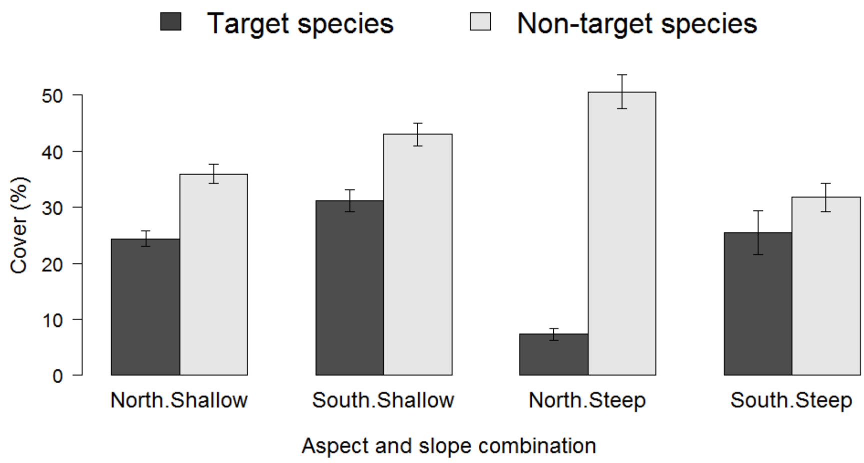


Figure 4. Cover (%) of target and non-target species (mean±SE) by aspect and slope combination showing idiosyncratic effects on north steep slopes (hydroseeding treatments are pooled together, control treatment is not included).

4. Discussion

Our findings show gypsicolous vegetation of high conservation value can be restored in quarry spoil slopes in the short term using standard hydroseeding methods. Natural succession has previously proved to have a limited potential for the restoration of gypsicolous vegetation in the short- to medium-term (<25 years; Martín et al. 2003; Mota et al., 2003, 2004; Dana & Mota, 2006; Ballesteros et al., 2012). Here, our results demonstrate hydroseeding ensures the establishment of target vegetation in the short-term, helping to jump-start succession and moving it towards the desired community.

The vegetation response was conditioned by the hydroseeding method, slope and site aspect. Hydroseeding method had the greatest effect on target vegetation. Target vegetation established better using wood fibre, paper mulch + blanket, or paper mulch than in the control treatment (in descending order). Restored and control plots differed remarkably. Hydroseeded plots had a more desirable species composition with greater target vegetation cover than control plots, which were almost completely occupied by non-target species typical of early-successional stages (i.e. colonizers). This was true despite other non-target species such as *L. perenne* and *M. sativa* being similarly abundant in the hydroseeded plots, probably because of seed remaining unintentionally in tanks from previous hydroseedings, thus explaining why these species were more frequent than in control plots.

Wood fibre was the most effective treatment for the establishment of target vegetation, specifically for gypsophile species. This treatment achieved the most similar cover to undisturbed gypsophilous vegetation for target species (28 vs ~30% respectively) and gypsophiles (16 vs ~22%); (calculated from Marchal et al. 2008), especially on shallow slopes, and south orientation. The improved results with wood fibre could be attributed to its capability of creating a thicker mat, holding seeds in place, resisting erosion more effectively than paper cellulose, or retaining more soil moisture thus creating a more favourable environment for target species seeds (Gruda, 2008; Profile, 2011). In addition, wood fibre not only produced the greatest target species cover but also the lowest cover of undesirable species, minimising the chances of potential competitors becoming dominant, and overall producing the greatest chance of favouring the recovery of the gypsicolous plant community.

The establishment of target species on paper mulch and paper mulch + blanket was less effective overall. On shallow slopes, both treatments produced similar results, but on steep ones paper mulch + blanket was better. This result was expected, given organic blankets are widely used to improve hydroseeding outcomes by retaining seeds and controlling erosion and run-off on steep slopes (e.g. Muzzi et al., 1997; Katritzidakis et al., 2007; Cohen-Fernández & Naeth, 2013).

The target vegetation performed better on shallow slopes in all hydroseeding treatments. Steep slopes are more prone to erosion and run-off (Kapolka & Dollhopf, 2001), and gravity allows seeds to be dragged downwards causing substantial seed losses (Cerdà & García-Fayos, 1997) hence limiting plant establishment (Matesanz et al., 2006; García-Fayos et al., 2010). These results are commonly found in other areas with variable slopes (e.g. roadsides and other mine wastes); in most cases steeper slopes perform worse than shallower ones (García-Fayos et al., 2010, Bochet et al., 2011).

Target vegetation produced a satisfactory response on shallow slopes in the two orientations and on steep northern slopes. The xerophytic and stress-tolerant nature of the target species allowed them to perform well in most situations, except steep slopes on northern aspects. This latter combination produced the worst results due to idiosyncratic effects that reduced considerably target vegetation cover in favour of non-target species. The lower insolation on steep, northern slopes appears to reduce water and physical limitations of gypsum substrates, allowing generalist vegetation (i.e. non-target species) to develop more readily (Pueyo & Alados, 2007) competing with the desired species of the target habitat (Pueyo et al., 2007). At this latitude, north-facing aspects receive less solar radiation, soil moisture is higher, and surface temperatures are generally more favourable for vegetation (Kutiel, 1992; Pueyo & Alados, 2007; Alday et al., 2010). This pattern has also been found on the vegetation on gypsum quarry landfills in SE Spain (Martín et al., 2003), where northern aspects had a much greater plant cover than southern aspects, although it did not seem to affect negatively the cover of gypsicolous species or gypsophiles as in our study. However, the identity of species and slope angles were not reported and hence direct comparisons are difficult. In turn, our results for the three gypsophile species agree those of Pueyo et al. (2007) with *H. squamatum* performing better in south oriented and shallow slopes, *L. subulatum* in both orientations, and *O. tridentata* in northern slopes. These results must be taken into account when designing the restoration plan, specifically for *O. tridentata* subp. *crassifolia*, endemic to the area and particularly affected by quarrying (Ballesteros et al. 2013). All the other target species generally performed better on southern aspects (exceptions being *T. zygis* and *T. capitatum* in wood fibre). On steep, southern slopes, target species as a whole performed similarly well as on shallow slopes, proving this harsher situation can also be restored by means of hydroseeding. Therefore, except on steep northern slopes, where non-target species become more competitive, our results showed target species can be established satisfactorily by hydroseeding.

The present study helps to guide decisions for the restoration of disturbed gypsum vegetation affected by quarrying. Figure 5 summarises an approach to treatment selection based on our results. The “no restoration” option led to the occurrence of early-successional species, slow succession and uncertain long term recovery. By contrast, this study demonstrates the short-term benefits of conducting hydroseeding early after disturbance. The effectiveness of measures was greater on shallow slopes where wood fibre produced the best results. Alternatively, paper mulch obtained reasonably good results, so the choice between the two methods can be based on the cost-benefit trade-off. On the other hand, the effectiveness of hydroseeding methods was affected strongly by steep slopes, and thus minimising them wherever possible would generally improve the restoration outcome. When this is not possible, designing stable slopes must be a priority, taking into account geomorphological principles and adequate drainage. If the erosion risk cannot be mitigated, application of organic blankets should help control erosion and run-off until a vegetation cover develops (Lorite et al., 2015). Conversely, in the absence of prominent erosion risks, wood fibre was the most effective and hence recommended measure. Our results showed gypsicolous target vegetation established reasonably well on steep southern slopes whereas non-target vegetation established better on steep northern slopes, at the expense of target species. The reduced environmental suitability of these areas combined with increasing competitive interactions suggest simple approaches such as increasing the seed supply would not be cost-effective in very steep, northern slopes. In this case, the extension of northern steep slopes in the global project must be taken into account to assess whether they could be managed with less ambitious goals (e.g. slope stabilisation with non-specific target species) or, if the recovery of gypsicolous vegetation was imperative, additional and costly site-specific actions will probably be required such as planting schemes (Ballesteros et al., 2014).

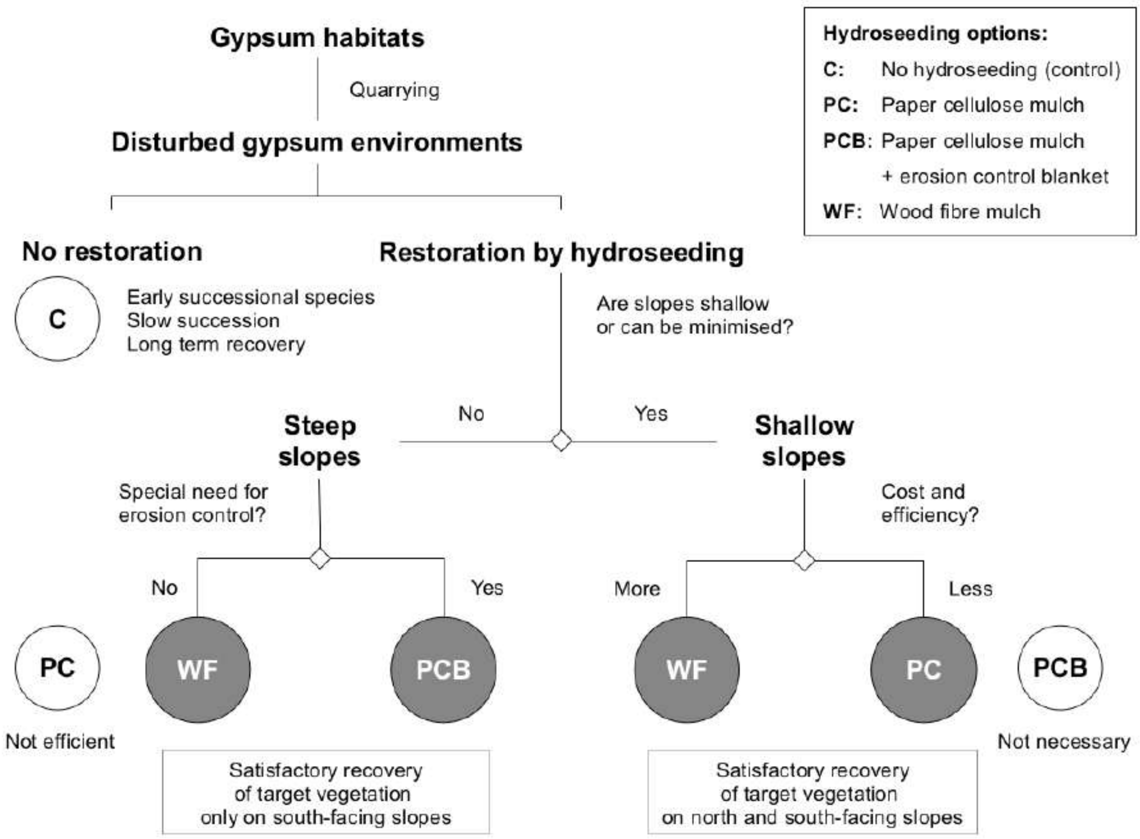


Figure 5. Decision pathways for the selection of hydroseeding methods to restore gypsum vegetation on quarry slopes according to our results.

The cost-effectiveness of each approach must be borne in mind when planning restoration programs. Contouring of the slopes should be carefully planned in advance to minimise overall costs. The treatments tested differ strongly in economic terms. The least costly was paper mulch (0.56 €/m^2), followed by wood fibre (0.72 €/m^2) and paper mulch + blanket (3.17 €/m^2). Although paper mulch showed limited results in steep slopes, this option could be considered for shallow slopes, given it can be very helpful to restore target vegetation at large scale at a low price provided favourable topography. The most effective option was wood fibre, with the best performance in both shallow and steep slopes for only a narrow cost increase compared to that of paper mulch hydroseeding. Being more expensive, paper mulch + blanket could be considered with an additional focus in increasing slope stability in very steep slopes. In this sense, the treatment application should be site-specific to minimise costs and optimise their performance.

5. Conclusions

Our results prove the establishment of gypsicolous vegetation can be achieved in disturbed quarry slopes by conventional hydroseeding methods in the short-term. All hydroseeding treatments were useful for ecological restoration of the target vegetation. However, the success of the intervention was strongly conditioned by the slope, with more limited results achieved in steep slopes. The most satisfactory results were obtained using wood fibre mulch with the greatest establishment of gypsicolous vegetation on shallow slopes. Comparable results were only attained by the paper cellulose + blanket treatment on the steep slopes. In spite of being more expensive, the wood fibre mulch treatment could be considered for its additional applicability to prevent erosion problems and improve slope stability. However, wood fibre or paper cellulose mulches should be preferred in moderate slopes given the lower-cost, easy application and greater ecological benefits of these options. This experiment should be monitored over the long term to evaluate the ecological, technical and economic viability of the tested hydroseeding methods and confirm their applicability to achieve effective large scale restoration of gypsum disturbed environments. The knowledge derived from this study will help to develop future programs for the management of gypsum habitats.

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Supporting Information

Table S1. Mean values (± SD) of the physicochemical characterization of the gypsum spoil used.

Figure S1. Cover (%) of target species (mean±SE) by treatment and slope.

Figure S2. Cover (%) of target species (mean±SE) by treatment and aspect.

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Table S1. Mean values (± SD) of the physicochemical characterization of the gypsum spoil used. Twelve gypsum spoil samples were randomly collected in the study site at 0-30 cm depth in order to evaluate the main properties that might have influenced species response in our study. N is the number of samples used for analyses. Analyses were conducted following the methodology in Mañares et al. (1998) and MAPA (1994). [a] Exchangeable cations.

Variable	N	Gypsum spoil
Gravel (>2mm) (%)	4	33.48 ± 3.78
Sand (2-0.05 mm) (%)	12	8.99 ± 2.17
Coarse silt (0.05-0.02 mm) (%)	12	9.60 ± 6.22
Fine silt (0.02 mm) (%)	12	41.44 ± 7.60
Clay (<0.02 mm) (%)	12	39.97 ± 8.22
pH	12	7.79 ± 0.04
Cation exchange capacity ($cmol_+$/kg)	12	8.15 ± 1.77
Ca^{2+} ($cmol_+$/kg)[a]	12	7.68 ± 1.83
Mg^{2+} ($cmol_+$/kg)[a]	12	0.22 ± 0.06
Na^{+} ($cmol_+$/kg)[a]	12	0.04 ± 0.01
K^{+} ($cmol_+$/kg)[a]	12	0.20 ± 0.07
Total carbon (%)	12	3.26 ± 0.45
Inorganic carbon (%)	12	3.27 ± 0.42
Organic carbon (%)	7	0.04 ± 0.03
Total N (%)	12	0.029 ± 0.005
$CaCO_3$ (%)	12	27.25 ± 3.52
Gypsum (%)	12	47.96 ± 28.22
Electrical conductivity (dS/·m)	12	2.27 ± 0.01
Water retention at field capacity (%)	8	31.09 ± 1.18
Water retention at wilting point (%)	8	20.98 ± 0.96
Available-water content (%)	8	10.11 ± 1.01

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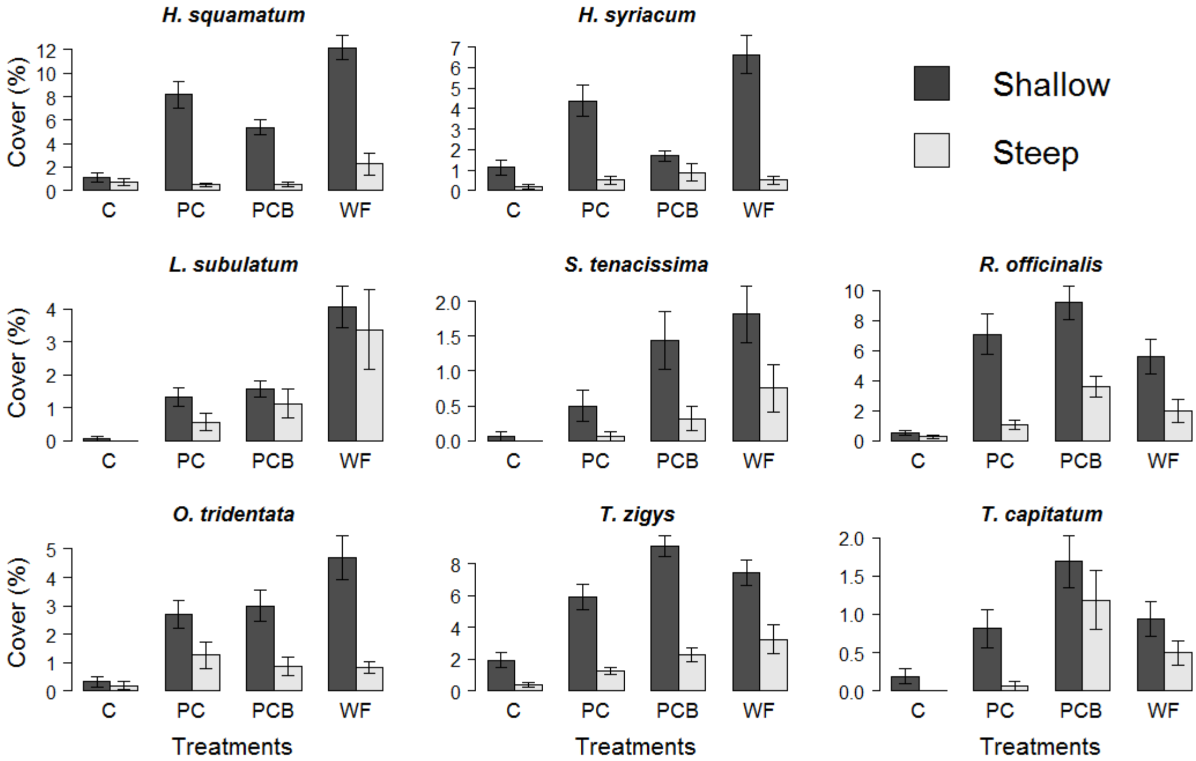

Figure S1. Cover (%) of target species (mean±SE) by treatment and slope (north- and south-facing slopes are pooled together). Hydroseeding treatments: C: No restoration (no hydroseeding); PC: paper cellulose mulch; PCB: paper cellulose mulch plus an erosion control blanket; and WF: wood fibre mulch. Slope inclination: shallow (10-15%) and steep (60-65%).

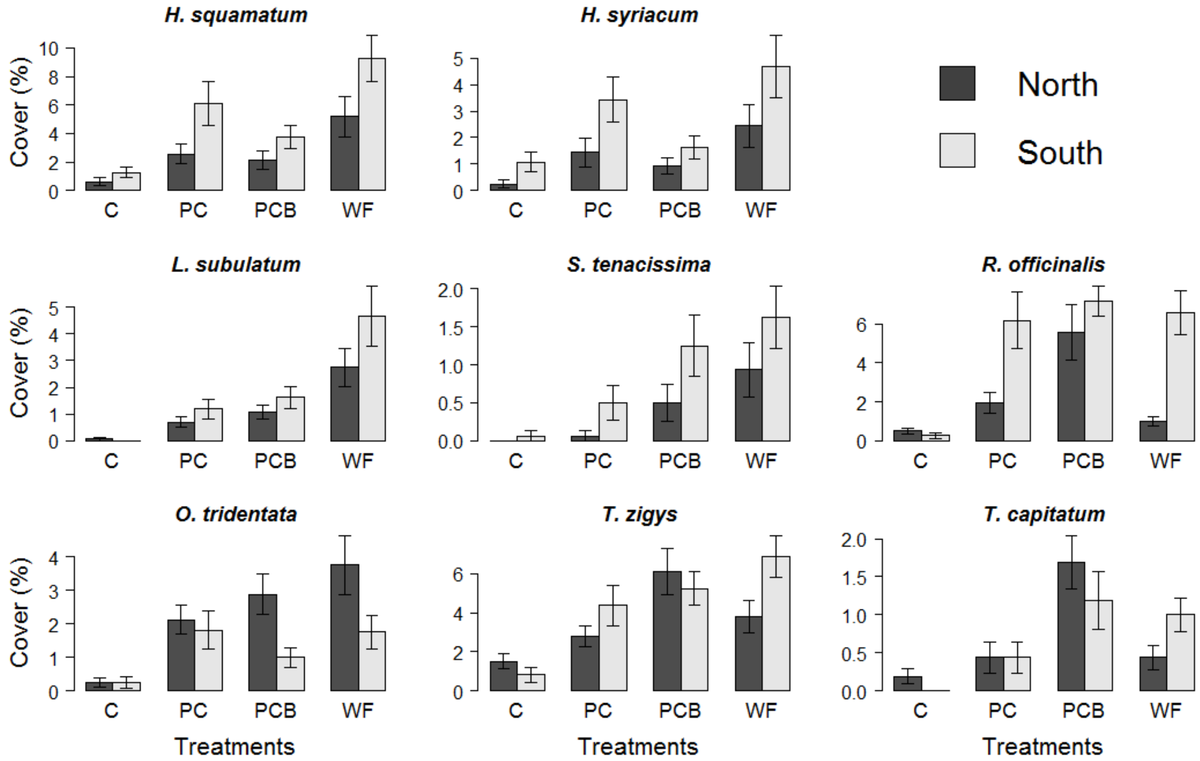

Figure S2. Cover (%) of target species (mean±SE) by treatment and aspect (shallow and steep slopes are pooled together). Hydroseeding treatments: C: No restoration (no hydroseeding); PC: paper cellulose mulch; PCB: paper cellulose mulch plus an erosion control blanket; WF: wood fibre mulch.

View of the.experimental slopes with hydroseeding treatments distributed on two contrasting slopes: shallow (10–15%) and steep (60–65%); and two aspects: north (N) and south (S). North aspect is showed here.

North aspect of shallow slopes in November 2011.

North aspect of shallow slopes in May 2015.

Chapter 8

Successful lichen translocation on disturbed gypsum areas: A test with adhesives to promote the recovery of biological soil crusts.

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Successful lichen translocation on disturbed gypsum areas: A test with adhesives to promote the recovery of biological soil crusts

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Abstract

The loss of biological soil crusts represents a challenge for the restoration of disturbed environments, specifically in particular substrates hosting unique lichen communities. However, the recovery of lichen species affected by mining is rarely addressed in restoration projects. Here, we evaluate the translocation of *Diploschistes diacapsis,* a representative species of gypsum lichen communities affected by quarrying. We tested how a selection of adhesives could improve thallus attachment to the substrate and affect lichen vitality (as CO_2 exchange and fluorescence) in rainfall-simulation and field experiments. Treatments included: white glue, water, hydroseeding stabiliser, gum arabic, synthetic resin, and a control with no adhesive. Attachment differed only in the field, where white glue and water performed best. Adhesives altered CO_2 exchange and fluorescence yield. Notably, wet spoils allowed thalli to bind to the substrate after drying, revealing as the most suitable option for translocation. The satisfactory results applying water on gypsum spoils are encouraging to test this methodology with other lichen species. Implementing these measures in restoration projects would be relatively easy and cost-effective. It would help not only to recover lichen species in the disturbed areas but also to take advantage of an extremely valuable biological material that otherwise would be lost.

Introduction

Biological soil crusts (BSCs), consisting of complex associations of lichens, mosses, algae, cyanobacteria, and other organisms are remarkable components of dryland ecosystems worldwide [1]. They play a critical role in their structure and function, acting on soil stability, biogeochemical cycling, and plant establishment [1–3]. These crusts are particularly notable in gypsum ecosystems, and are normally dominated by lichens [1,4,5]. They have a high conservation value due to their potential to form covers (sometimes >80%), their function, and their diversity [6–8]. However, large extensions covered by BSCs are disturbed by human activities [9,10], quarrying inflicting particularly severe damage [6,11]. Gypsum is a mineral in global demand [12] and its quarrying inevitably damages BSCs and their habitat. Despite that mining companies are obligated to conduct environmental restoration (e.g. geomorphology and plant cover), BSCs are normally ignored. Limitations such as the slow growth of their components, low reproduction rates, the destruction of propagules and habitat together with the lack of a clear restoration methodology make the recovery of BSCs especially challenging [3,10,13,14].

Few studies have addressed the assisted recovery of BSCs. Practice to enhance BSC establishment include soil stabilisation, resource augmentation, and inoculation-based techniques [10]. Inoculation is the best-studied and consists of adding cultured or salvaged BSC components to increase propagule availability in the disturbed area [14–17]. The recovery of cyanobacteria and algae has been achieved in gypsum BSCs over the short term [14–16]. However, the recovery of later successional components such as lichens can be extremely slow unless these are translocated [9,14,15]. Lichen translocation can be particularly advisable to accelerate species recolonisation in the disturbed area, but the loss of thalli due to environmental factors (e.g. wind, rain-storms) can arrest or greatly delay establishment [9,18].

The attachment of propagules to the substrate is key for full lichen development (e.g. nutrient and moisture intake, hyphae formation, and favourable reproduction) [19,20] and acts as one of the main ecological filters for colonisation and recovery [21]. The first step for propagules is to attach to the substrate in an entirely physical process [20]. Thereafter, propagules start to grow hyphae and actively attach to the substrate [20]. Otherwise, dispersed propagules remain erratic at risk of being removed off by wind [22] or water [23] and lost. Thus, keeping thalli in place would be useful in reducing their loss, favouring establishment, and eventually augmenting the source of propagules over time to hasten BSC recovery. Accordingly, a range of adhesives has been used previously for lichen translocation on different substrates (see Smith [24] for a review), terricolous habitats being especially challenging. The aim of our study is to assess thallus translocation for restoration using a representative species of gypsum lichen communities [*Diploschistes diacapsis* (Ach.) Lumbsch]. We tested whether the application of various adhesives could improve thallus attachment onto gypsum mine spoil without compromising their vitality. The results may be helpful for future restoration programs to recover BSCs in disturbed gypsum habitats.

Material and methods

Site description: The field work was conducted in an experimental area next to an active quarry in Escúzar, Granada, SE Spain (37°2'N, 3°45'W) at 950 m asl. The climate is continental Mediterranean, with relatively cold winters, hot summers, and four months of water deficit. The mean annual temperature is 15.1°C, with an average monthly minimum temperature in January of 7.6°C and maximum of 24.2°C in August. The annual rainfall, occurring mainly in winter, averages 420 mm. The area is in the Neogene sedimentary basin of Granada, where the dominant substrates are lime and gypsum deposited in the late Miocene, the latter in combination with marls [25]. The predominant soils where gypsum crops out are Gypsiric Leptosols [26,27]. The vegetation of the area is a mosaic of fields with cereal crops and olive and almond orchards (*Olea europaea* L. and *Prunus dulcis* D.A. Webb.), and scattered patches of native scrub described as 1520, "Iberian gypsum vegetation, *Gypsophiletalia*" [28]. Open areas between native plants are often colonised by a well-developed biocrust community characterised by lichens such as *D. diacapsis, Acarospora placodiiformis*, *A. nodulosa* var. *reagens*, *Buellia zoharyi*, *Squamarina lentigera, S. cartilaginea*, *F. desertorum*, *F. fulgens, F. poeltii*, *F. subbracteata*, *Psora decipiens,* and *P. saviczii* (Fig. 1a, Supplementary Table S1).

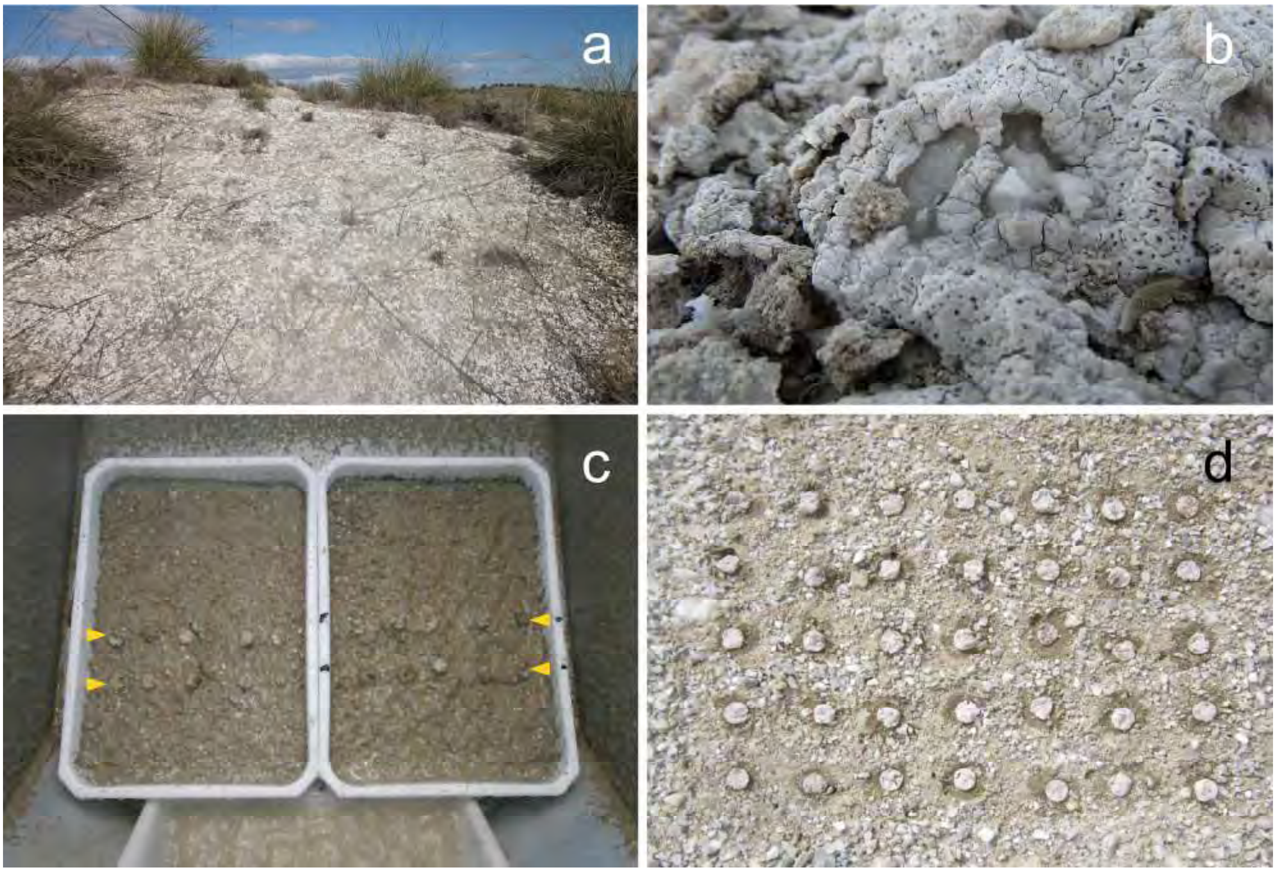

Figure 1. (a) Soil crust community on gypsum soil dominated by the lichen *Diploschistes diacapsis* in a Mediterranean gypsum shrub community in Granada, Spain. (b) *D. diacapsis* growing on gypsum soil. (c and d) Thalli of *D. diacapsis* used in our experiments to test for the effect of various adhesives on thalli attachment to gypsum spoil; (c) in trays during the rainfall-simulation (see arrows), and (d) in one of the plots when the field experiment was set up in March 2014.

Study species

D. diacapsis (Fig. 1b), is a sub-cosmopolitan terricolous crustose lichen species occurring mainly on calcareous and gypsaceous soils in open xeric habitats in Mediterranean and arid climates [1,29,30]. This lichen species has a thick thallus (1-3 mm) of variable aspect, verrucose-areolate with urceolate apothecia and white pruina [31]. It is one of the most frequent lichens in gypsum BSCs in the Iberian Peninsula, normally reaching the greatest cover and biomass (~40% cover [4]; ~55% cover and >80% biomass in the study area according Ibarz [32]). Having a central role in gypsum communities, it acts as host to numerous fungi and lichen species [33]. *D. diacapsis* was selected as a model species for its dominance at the study site and for its similar ecology and morphology with respect to other crust-forming lichens in gypsum habitats (e.g. *Acarospora, Buellia, Squamarina*, *Lecidea)* [34].

Common set up for rainfall-simulation and field experiments

Thallus collection: In March 2014, thalli were detached at the study site from the parent gypsum substrate and collected from shrub interspaces in the area where quarrying was scheduled. The samples were taken immediately to the laboratory in paper bags. Thalli were soaked in tap water (for 5-10 min), cleaned and die cut into disks of 15 mm to obtain homogeneous experimental units. Thalli were transferred to be used, respectively, in rainfall-simulation and field experiments within the next 24 hours.

Substrate used: Gypsum spoil was assayed as the recipient substrate for translocation (properties in Supplementary Table S2). It is a by-product of gypsum quarrying normally used to fill quarry pits and reshape the landscape after quarrying. Pilot studies have confirmed its suitability to conduct gypsum vegetation recovery [35,36]. This material, often remaining in the quarried areas, is a suitable substrate to test lichen and adhesive performance.

Adhesive treatments: Several commercial natural or synthetic adhesives were applied to thalli in rainfall-simulation and field experiments. These included: (1) white glue (G): wood and craft white glue consisting of polyvinyl acetate (Wolfpack, A Forged Tool S.A.); (2) hydroseeding stabiliser (HS): synthetic polyacrylamide polymer (Bonterra Ibérica S.L.) as powder dissolved in water at 5 g/L; (3) gum arabic (GA): a complex of glycoproteins and polysaccharides derived from *Acacia* tree exudates (Prager, Orita S.A.); (4) synthetic resin (SR): contact adhesive polymeric glue (Kollant, Impex Europa S.L.); and (5) water (W): wetting spoil with water. A control treatment (C) where no adhesive was applied to thalli was used in both experiments. The adhesives were used at their commercial concentrations.

Rainfall-simulation experiment: We tested the effect of adhesives on thallus attachment under controlled rainfall and slope conditions in a rainfall-simulation experiment recreating the effect of two separate disturbing rain events. In March 2014, 30 plastic trays (35 x 25 x 5 cm) were perforated to allow drainage, filled with gypsum spoil (sieved at 0.5 cm; approx. 2.5 Kg), watered, and left to dry in order for the spoil to gain cohesion. Ten thalli were transferred to each of five replicate trays after applying one of the six adhesive treatments (2 ml with a 200-ml syringe) to the lower surface of the thallus (i.e. closest side to substrate), except for the water treatment, which was applied by spraying tap water (~100 ml) to moisten the tray substrate (10 thalli x 5 replicates x 6 adhesive treatments = 300 thalli). Thalli were placed in two lengthwise parallel rows (5 each) in the middle of the tray (Fig. 1c), using a quadrat with a 5 x 5 cm grid, which also served to monitor detachment after rainfall simulations. Simulations were conducted in a greenhouse, using a rainfall simulator (modified from Fernández-

Gálvez et al.[37]), consisting of a drop-forming chamber (controlled by a pump connected to a water reservoir) on top of a structure (2 m high) that housed two random trays at the same time tilted for a 25° slope (near the angle of rest of the spoil material to simulate an extreme situation). We conducted two 15-min simulations at 50 $l \cdot m^2 \cdot h$ intensity (selected according to rainfall record in the study area from 2000 to 2013) in consecutive weeks, allowing the substrate to dry in between. We visually checked thallus detachment after each simulation, removing detached thalli to avoid interference on the following check. Once the substrate dried after the second simulation, we assessed thallus attachment by manual inspection applying gentle sideways pressure on the thalli to verify whether the thalli were attached or moved without resistance. We measured CO_2 exchange as an indicator of thallus vitality after adhesive application [38,39]. The thalli used in the rainfall simulation were left in their trays outdoors for two months (May-June) under ambient conditions. The CO_2 exchange was measured with an infrared gas analyser (EGM-4 Environmental Gas Monitor and a SRC-1 Soil Respiration Chamber, PP systems, Hitchin, U.K.). Six thalli per tray (30 per treatment) were moistened and placed together inside the chamber (to improve CO_2 detection) on a sterile plastic surface (to avoid substrate interference in the measurements). Five measurements per treatment were performed by alternating treatments. Measurements were taken between 10:00 and 13:00 h (local time, GMT+1). This period is considered representative of daily averages of CO_2 exchange for these lichens [40,41].

Field experiment: The experiment was set up in March 2014 on a conditioned flat area consisting of bare gypsum spoil generated from gypsum quarrying. A total of 30 permanent plots of 0.5 x 0.5 m were established with a randomised design, including 5 replicate plots per each of the 6 adhesive treatments. In the centre of each plot (Fig. 1d), 35 thalli were positioned using a 50 x 50 cm quadrat with a 5 x 5 cm grid (5 replicate plots x 6 treatments x 35 thalli = 1050 thalli). Each thallus was transferred and fixed to the centre of a grid square with the corresponding plot adhesive applying gentle pressure to attach it, and its position was identified in order to record thallus detachment over time. Thalli were fixed by applying an adhesive treatment (2ml with a 200-ml syringe) to the lower surface of the thallus, except for the water treatment, which was applied by spraying tap water (~200ml) to moisten the plot substrate. We monitored lichens visually for thallus detachment over a 15-month period (March 2014 to May 2015), weekly during the first three months and monthly afterwards. Detached thalli were removed to avoid interference on the following sampling dates. Additionally, we measured chlorophyll fluorescence using the ratio F_v/F_m as an indicator of lichen vitality 16 months (June, 2015) after the application of the adhesives. Chlorophyll fluorescence is a sensitive indicator of photosystem II (PSII) efficiency, used to detect stress and to assess lichen vitality [39,42,43]. Fluorescence was measured on 10 thalli (when available) per replicate plot with a chlorophyll fluorimeter (Handy PEA, Hansatech Instruments, Norfolk, U.K.) on soaked (with a spray of water) and dark-adapted (measured by night) samples, applying a saturating flash of light of 3580 μmol s^{-1} m^{-2} for 1 s. As a reference of vital lichens, we included 50 15-mm thalli prepared with fresh material collected from the undisturbed habitat (Hab) two hours before the measurements were taken following the same procedure, and we evaluated the efficiency of PSII by measuring the F_v/F_m ratio [44].

Data analyses: We assessed the effect of adhesives on thallus attachment over time using the Kaplan-Meier log-rank survival analysis (R "survival" package [45]) and fitting mixed-effects Cox proportional hazard models (R "coxme" package [46]) using trays or quadrats as random factors, respectively, in rain-simulation or field experiments. The effect of adhesives on CO_2 exchange in rainfall simulations was evaluated by fitting generalised linear models (GLMs) with a Poisson error distribution and log link function (R "stats" package [47]). We tested for the effect of adhesives (i.e. fixed factor) on fluorescence yield in the field experiment, fitting generalised linear mixed models (GLMMs) with a binomial error distribution and logit link function, using quadrats as random factors (R "lme4" package [48]). Model parameters were estimated using the Laplace approximation of likelihood. Pairwise multiple comparisons with Tukey's correction (R "multcomp" package [49] were made to estimate differences between adhesive treatments. All statistical analyses were performed using R version 3.2.2 [47].

Results

Rainfall-simulation experiment: Rain simulation showed 100% attachment in G, W, and GA and very high for the rest of treatments at the end of the experiment (SR, 98%; HS, 94% and C, 82%; Table 1; Supplementary Fig. S1). As expected, treatment C recorded the lowest attachment, although the post hoc analysis indicated no differences with respect to the other treatments. All treatments had lower detachment risk than did C (Table 1, Cox regression). Thalli detached during the first simulation only in treatments C (12% detachment) and HS (2%), and no detachment was found during the second one. Manual inspection after the substrate dried showed additional detachment in treatments C (6% detachment), HS (4%), and SR (2%). Remarkably, the remaining thalli on the C treatment were attached at the end of this experiment. The analysis of CO_2-exchange measurements taken on thalli detached for this purpose at the end of the experiment showed significant differences between adhesives, with higher respiration rates in C, HS, W, GA, G, and SR in descending order (Fig. 2, Supplementary Table S3).

Table 1. Thallus attachment (%) and results of the Cox proportional-hazard regression for adhesive treatments in the rainfall-simulation experiment (control treatment established as reference). Treatments with 100% attachment (white glue, water, gum arabic) are not included in the analysis. HR: Hazard ratio <1 means thalli using this adhesive treatment had a lower risk of detachment than did control. Results with $p < 0.05$ are in bold.

		Cox proportional-hazard regression				
Treatment	Attachment (%)	HR	Estimate	SE	z	p
Synthetic resin	98	0.10	-2.292	1.096	-2.09	**0.037**
Hydroseeding	94	0.31	-1178	0.731	-1.61	0.110
Control	82	1	-	-	-	-
		Random effects: Variance: 0.222; SD: 0.472				

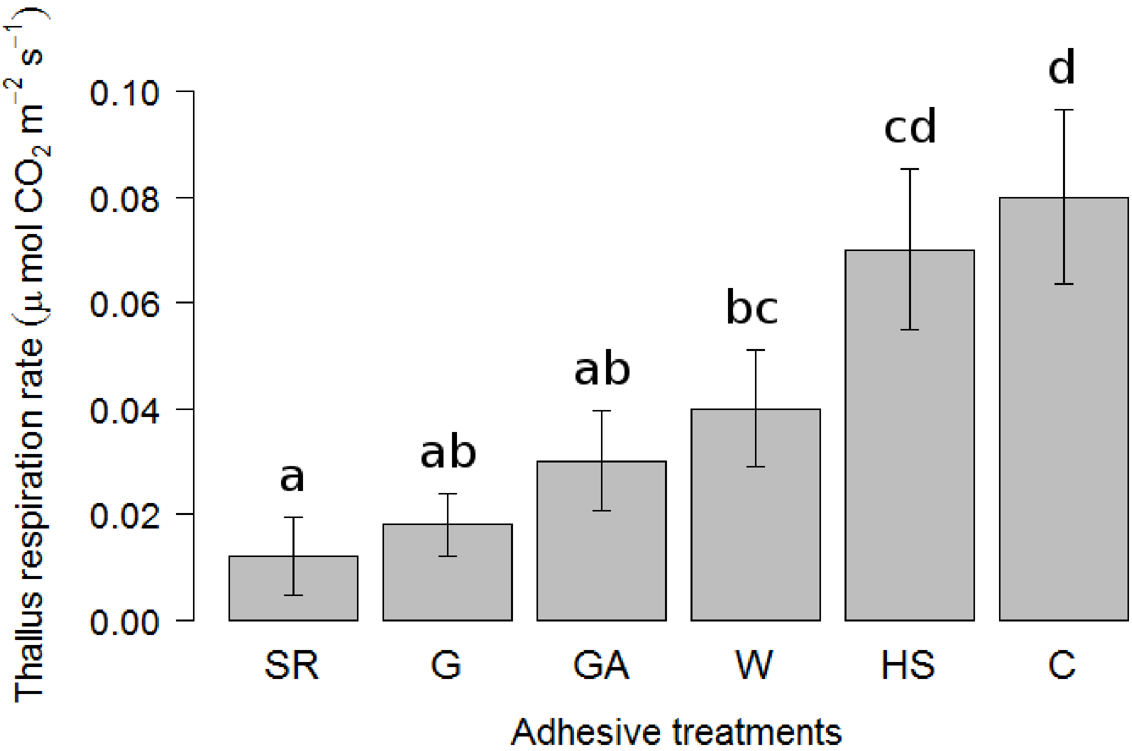

Figure 2. Thallus respiration rate (mean values ± SE) for each adhesive treatment in rain simulations. Treatments: SR, synthetic resin; G, white glue; GA, gum arabic; W, water; HS, hydroseeding; C, control. Different letters represent statistically significant differences between adhesives ($p < 0.05$).

Field-experiment results: After a 15-month follow-up of the field experiment, our results showed that the adhesive treatment significantly affected thallus attachment (Fig. 3; Table 2). Responses varied depending on the adhesive, with better results in G (87.4% attachment), W (80%), HS (59.9%), GA (54.3%), C (45.7%), and SR (36.6%) in this order, with significant differences between G and W compared to the rest (Table 2). Kaplan-Meier curves showed only moderate detachment in G and W and significantly greater in the rest of the treatments (Fig. 3). Treatment C plummeted in the first month, followed by steady detachment until declining almost completely towards the eighth month. Detachment was more gradual in HS, GA, and SR, with the first two listed treatments with little detachment from the tenth month onwards, and the latter falling steadily until the end of the follow-up, even underperforming the C treatment (Fig. 3). The Cox proportional hazard analysis also showed significant differences between treatments. All treatments reduced the risk of detachment compared to the control, except SR, which presented a similar risk (Table 2, Cox regression). The photosynthetic activity as F_v/F_m for the treatments was C (0.16), SR (0.18), W (0.22), HS (0.24), GA (0.25), Hab (0.28) and G (0.31), with significant differences only between C, and G, and Hab (Fig. 4, Supplementary Table S4).

Table 2. Thallus attachment (%) and results of Cox proportional-hazard regression for adhesive treatments in the field experiment (control treatment established as reference). HR: Hazard ratio <1 means thalli using this adhesive treatment had a lower risk of detachment than did control. Results with $p < 0.05$ are in bold.

		Cox proportional-hazard regression				
Treatment	Attachment (%)	HR	Estimate	SE	z	p
White glue	87.4	0.13	-2.024	0.365	-5.55	**<0.001**
Water	80	0.22	-1.528	0.341	-4.48	**<0.001**
Hydroseeding	59.9	0.52	-0.669	0.320	-2.09	**0.04**
Gum arabic	54.3	0.62	-0.482	0.315	-1.53	0.13
Control	45.7	1	-	-	-	-
Synthetic resin	36.6	0.93	-0.073	0.310	-0.24	0.81
		Random effects: Variance: 0.190; SD: 0.436				

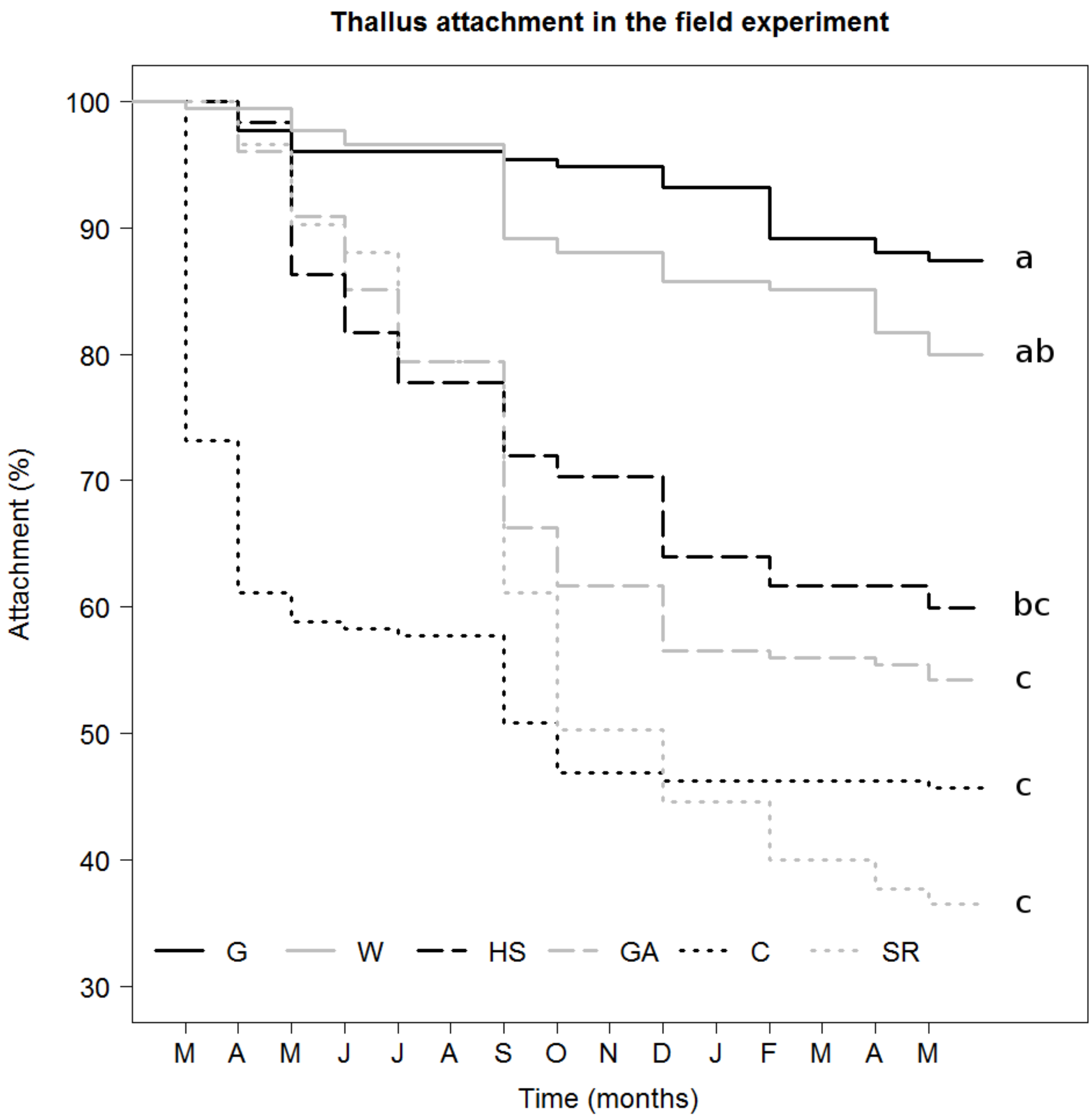

Figure 3. Kaplan-Meier survival curves representing thallus attachment for each adhesive treatment from March 2014 to May 2015 in the field experiment. Treatments: G, white glue; W, water; HS, hydroseeding; GA, gum arabic; C, control; SR, synthetic resin. Different letters at the end of curves represent statistically significant differences between adhesives ($p < 0.05$).

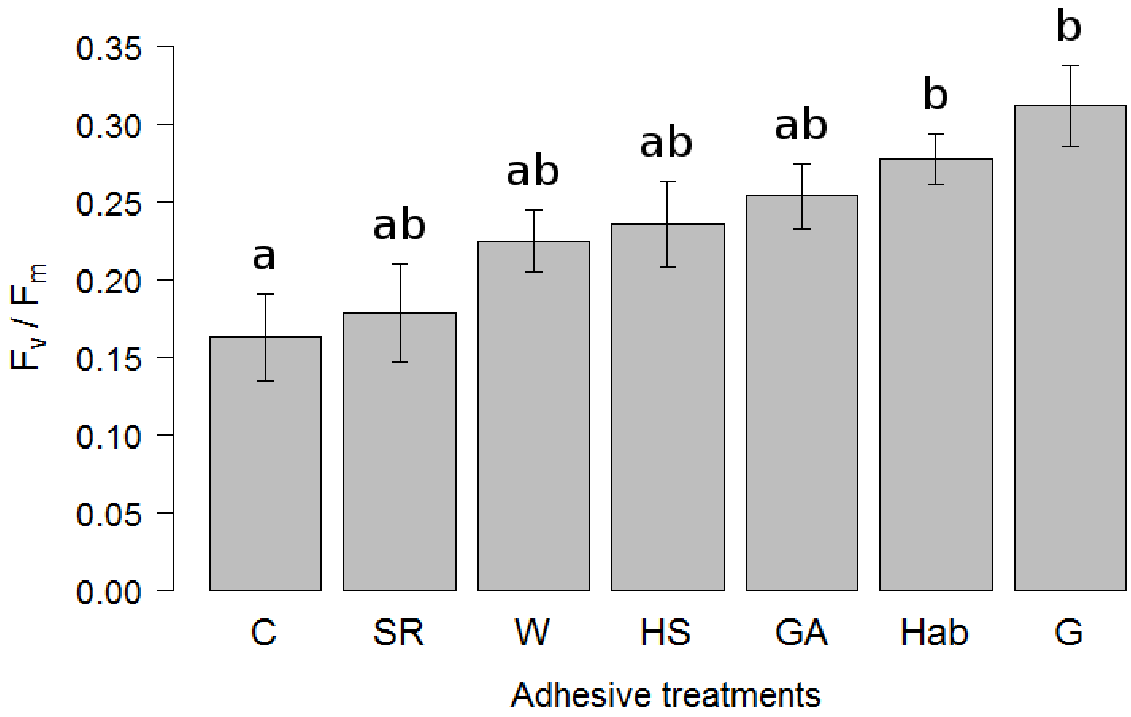

Figure 4. Maximum quantum yield (mean values ± SE) of PSII photochemistry (Fv/Fm) in thalli of the lichen D. diacapsis 16 months after translocation to gypsum spoils using adhesive treatments: G, white glue; W, water; HS, hydroseeding; GA, gum arabic; C, control; SR, synthetic resin. Thalli from the undisturbed habitat (Hab) were transferred to the same substrate before measurements as a reference. Different letters represent statistically significant differences between adhesives ($p < 0.05$).

Discussion

Our findings indicated that white glue, water, hydroseeding stabiliser and gum arabic improved the attachment of *D. diacapsis* thalli, although they also altered lichen vitality. Remarkably, the water treatment (i.e. wetting the spoils) significantly improved thallus attachment without compromising their vitality, which would be especially advantageous to optimise crust material in translocation actions.

Thallus attachment to the substrate was similar for all adhesives in rainfall simulations. Although the thalli were exposed to considerable rain intensity and inclination in this experiment, the level, angle or time exposed to these factors were not sufficient to show differences between treatments. By contrast, thallus attachment differed depending on the adhesive when exposed to field conditions over a longer period (differences were noticeable from the first month onwards). The difference between experiments appears to be explained by the greater exposure time in the field to rain and wind. These factors can encourage lichen dispersal in open areas [4,21], but for the same reason they can also make it difficult for thalli to remain at the same specific site, hindering establishment. In our field experiment, we found thallus attachment using white glue and water was greatest and remarkably better than for the other treatments throughout the follow-up. By contrast, the performance of hydroseeding stabiliser and gum arabic was inferior and similar to the control towards the end of the experiment. Although the attachment achieved with the synthetic resin was similar to that using the hydroseeding stabiliser and gum arabic for nearly 8 months, it lost its adhesive properties and eventually registered the lowest results.

Our results suggest that water followed by substrate drying played a central role in thallus attachment. Although we assumed *a priori* that making the spoils wet would have an antagonistic effect on thallus attachment, the opposite happened in both the rainfall-simulation and field experiments. In rainfall simulations, most of the thalli in all treatments were attached to the substrate after it dried, this being especially remarkable in the control treatment, where no adhesive had been applied, but where water acted as such after the first simulation. This pattern was observed in the field also. Most thalli in the control treatment detached in the first two months but stabilised afterwards despite heavy rain in the following months. In fact, all treatments seemed to stabilise except the synthetic resin. A possible explanation is that the synthetic resin is a hydrophobic adhesive and remained on the thalli, preventing them from reattaching. While water may weaken the relationship between thalli and the substrate during a rain event, it also helps to create some bonds with the substrate surface [18,50]. The surface becomes sticky due to the high cohesiveness of silt and clay particles when mixed with moderate amount of water. Then the drying process makes these bonds more stable, helping lichens to attach. This pattern has been reported in nature (e.g. with seeds [51] or soil particles [52]) and, as our results reveal, it is one of the ways lichen propagules create bonds and attach to certain substrates.

Another key issue is how the different treatments affect thallus vitality. A given adhesive could aid thallus attachment, but if significantly harmed the lichen’s physiology, then use of the product would be unacceptable. In this regard, vitality measurements determined that some of the treatments tested reduced thallus activity, although no one was so aggressive as to completely inhibit respiration or photosynthetic processes. The results of CO_2 exchange showed thallus activity in rainfall simulations to be reduced by the adhesive applied. The highest respiration rate was registered in the control treatment, followed by the hydroseeding stabiliser, water, and the rest of treatments.

Contrary to expectations, the water treatment differed from control despite that thalli in both treatments were treated only with water (prior or during the rainfall simulation). Although the CO_2 exchange was not as high as in the control treatment, thalli treated with water were active and there is no apparent reason why water would reduce their vitality, but rather the opposite. In the field experiment, the efficiency of PSII (F_v/F_m) used as stress indicator [53] showed no differences between the thalli translocated using adhesives and those freshly collected in the undisturbed habitat. Although no significant differences were found between treatments, with the use of the synthetic resin, thalli showed reduced activity and some signs of necrosis, and thus this adhesive is not recommendable. The control treatment recorded the lowest value, probably due to the lowest attachment to the substrate. The activity for all the other adhesives was between 0.22 and 0.31, and the activity for thalli in the habitat (Hab) used as reference was within this range (0.28). These values are similar to PSII efficiency on undisturbed north and south-facing populations of *D. diacapsis* studied in Pintado et al. [54], estimated, respectively, as 0.25 and 0.28 by Maestre et al. [3]. Thus, we can infer that translocation onto the gypsum spoil using most of the adhesives studied here was not so stressful as to alter the photosynthetic process and make the F_v/F_m differ from nature.

Therefore, our results suggest that translocation can help some components of the BSCs to recover. We have demonstrated that *D. diacapsis* is amenable to translocation and can be moved from its habitat onto gypsum spoils, but this methodology could be extended to other gypsum-crust components in substrates having similar properties (i.e. disturbed original habitat or topsoil used for restoration). More than 50% of the lichen species in gypsum habitats have similar morphology and ecology [4] and the use of this methodology with them is especially appealing. In addition, other terricolous lichen communities on similarly performing substrates in other drylands could benefit from this approach. The crust material can be salvaged from donor habitats prior quarrying. Complete thalli or fragments can be scalped from the parent material along with other BSCs species [14,15]. This material could be applied directly or stored for relatively long periods providing some flexibility for translocation actions [14,55]. The recipient substrate can be sprayed with water to create a sticky surface to receive the crust material, and then let the substrate dry until the thalli bind, as our results reveal.

Areas meeting the environmental requirements that define the occurrence of these crusts should be selected for translocation [24]. Their occurrence has been related to variables such as substrate stability [4,56], orientation and water availability [54], soil respiration [56], and complex relations with the surrounding vegetation [3,56–58].

Accordingly, Martínez et al. [56] reported that high bare-soil cover and low plant-litter cover were positively related to the presence of gypsum crustose lichens. While this scenario is frequent right away after quarrying, invasive early successional plant species (e.g. *Dittrichia viscosa, Pitptatherum milliaceum, Moricandia arvensis,* in our area) could strongly reduce open areas available for lichens over the short term. Thus, where possible, it would be advisable to translocate lichens to plant interspaces in areas where gypsum vegetation had already developed (naturally or restored) aiding it to escape early successional competitors. Additionally, these areas would provide a gradient of conditions for translocation, depending on crust species-specific requirements related to plant proximity (shade, moisture, organic matter, etc.[56–59]. Other components of gypsum BSCs (i.e. cyanobacteria and algae) have been reported to develop comparatively fast through colonisation [15] or inoculation [14–16]. Thus, a combination of translocation with additional inoculation measures [16] or the use of already colonised substrates (i.e. restored gypsum spoils, topsoil, disturbed original habitat) could help facilitate certain limiting processes (e.g. contact between mycobiont spores and photobionts [60]) and promote a more complete recovery of gypsum BSCs.

In conclusion, here we have shown that the use of certain adhesives can significantly increase lichen attachment to the substrate without compromising their vitality. Particularly, our study shows the central role that water and the subsequent drying of the substrate play for thallus attachment, and offers a methodology with implications for restoration of disturbed gypsum BSCs. This study represents one of the first studies to evaluate the translocation of lichens in gypsum drylands and the first to test adhesives for lichen attachment onto gypsum quarry spoils. This methodology needs to be tested over time using other species and considering disturbances in gypsum habitats. Further protocols should be designed, assessing the ecological, technical, and economic viability of translocation, in order to confirm their applicability to large-scale restoration of gypsum BSCs. The results in the present study are encouraging and open an opportunity to accelerate the recovery of BSCs in disturbed gypsum habitats.

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Author Contributions

J.L., M.B., and M.C. conceived and designed the study. M.B., J.A., and E.M.C. undertook the experiments. M.B. conducted the analysis and wrote the manuscript. All authors reviewed the manuscript.

Additional Information

Supplementary information accompanies this paper.

Supplementary Table S1

Supplementary Table S2

Supplementary Table S3

Supplementary Table S4

Supplementary Figure S1

Competing financial interests: The authors declare no competing financial interests.

Supplementary Table S1. Cover (%) of lichens and number of species (mean ± SE) determined on 18 soil cores of 25 x 25 cm and 10 cm depth collected in the study area (Ibarz, 2012).

	Mean ± SE
Total lichen cover per soil core (%)	76.8 ± 4.3
Number of species	9.1 ± 0.6
Individual species cover (%)	
Acarospora nodulosa var. *reagens* (Zahlbr.) Clauzade & Cl. Roux	2.1 ± 0.7
Acarospora placodiiformis H. Magn.	7.3 ± 1.8
Buellia zoharyi Galun	1.0 ± 1.0
Cladonia foliacea (Huds.) Willd	0.1 ± 0.1
Collema sp.	4.3 ± 1.2
Diploschistes diacapsis (Ach.) Lumbsch	54.7 ± 5.4
Fulgensia desertorum (Tomin) Poelt	0.9 ± 0.5
Fulgensia fulgens (Sw.) Elenkin	6.3 ± 1.8
Fulgensia poeltii Llimona	0.8 ± 0.4
Fulgensia subbracteata (Nyl.) Poelt	7.8 ± 2.1
Psora albilabra (Dufour) Körber	0.6 ± 0.3
Psora decipiens (Hedw.) Hoffm.	7.4 ± 2.8
Psora saviczii (Tomin) Follmann & Crespo	1.0 ± 0.3
Squamarina cartilaginea (With.) P. James	1.9 ± 0.6
Squamarina lentigera (Weber) Poelt	6.8 ± 2.6
Toninia sedifolia (Scop.) Timdal	3.8 ± 1.5

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Supplementary Table S2. Mean values (± standard deviation) of the physicochemical characterization of the gypsum spoil used (Aguilera, 2012). Twelve gypsum spoil samples were randomly collected in the study site at 0-30 cm depth to determine the substrate properties. N is the number of samples used for the analyses. The analyses were conducted following the methodology in Mañares et al. (1998) and MAPA (1994). [a] Exchangeable cations.

Variable	N	Gypsum spoil
Gravel (>2mm) (%)	4	33.48 ± 3.78
Sand (2-0.05 mm) (%)	12	8.99 ± 2.17
Coarse silt (0.05-0.02 mm) (%)	12	9.60 ± 6.22
Fine silt (0.02 mm) (%)	12	41.44 ± 7.60
Clay (<0.02 mm) (%)	12	39.97 ± 8.22
pH	12	7.79 ± 0.04
Cation exchange capacity ($cmol_+$/kg)	12	8.15 ± 1.77
Ca^{2+} ($cmol_+$/kg)[a]	12	7.68 ± 1.83
Mg^{2+} ($cmol_+$/kg)[a]	12	0.22 ± 0.06
Na^+ ($cmol_+$/kg)[a]	12	0.04 ± 0.01
K^+ ($cmol_+$/kg)[a]	12	0.20 ± 0.07
Total carbon (%)	12	3.26 ± 0.45
Inorganic carbon (%)	12	3.27 ± 0.42
Organic carbon (%)	7	0.04 ± 0.03
Total N (%)	12	0.029 ± 0.005
$CaCO_3$ (%)	12	27.25 ± 3.52
Gypsum (%)	12	47.96 ± 28.22
Electrical conductivity (dS/·m)	12	2.27 ± 0.01
Water retention at field capacity (%)	8	31.09 ± 1.18
Water retention at wilting point (%)	8	20.98 ± 0.96
Available-water content (%)	8	10.11 ± 1.01

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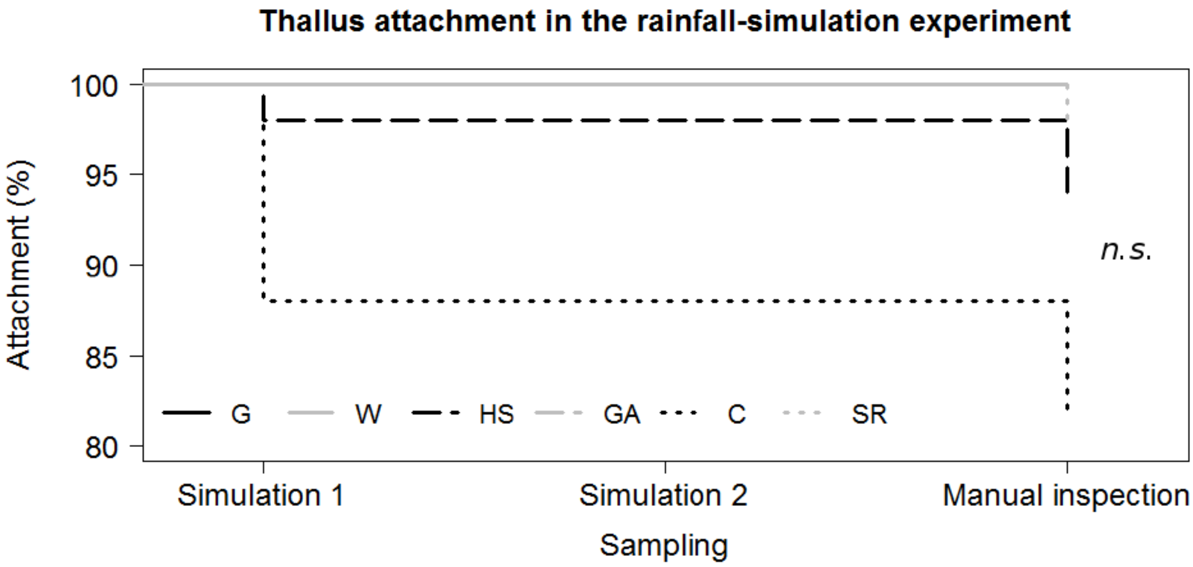
Supplementary Table S3. Effect of adhesive treatment on thallus respiration rate (CO_2 exchange) in the rainfall-simulation experiment, evaluated fitting a generalised linear model (GLM). Results with $p < 0.05$ are in bold.

Treatment	Estimate	SE	z	p
Intercept	-2.369	0.131	-18.044	**<0.001**
White glue	-1.496	0.307	-4.874	**<0.001**
Water	-0.659	0.225	-2.931	**0.003**
Hydroseeding	-0.148	0.193	-0.769	0.442
Arabic gum	-1.016	0.255	-3.989	**<0.001**
Synthetic resin	-1.663	0.329	-5.055	**<0.001**

Supplementary Table S4. Effect of adhesive treatment on maximum quantum yield of PSII photochemistry (F_v/F_m), evaluated fitting a generalised linear mixed model (GLMM). Adhesive treatment as fixed factor and quadrat as random factor. Results with $p < 0.05$ are in bold.

Treatment	Estimate	SE	z	p
Intercept	-1.75	0.143	-12.203	**<0.001**
Synthetic resin	0.111	0.215	0.514	0.607
Water	0.341	0.163	2.094	0.036
Hydroseeding	0.400	0.171	2.344	0.019
Arabic gum	0.350	0.168	2.079	0.038
Habitat	0.564	0.152	3.722	**<0.001**
White glue	0.596	0.159	3.758	**<0.001**
Random effects: Variance: 0.004; SE: 0.064				



Supplementary Figure S1. Kaplan-Meier survival curves representing thallus attachment for each adhesive treatment after two simulated rainfall events and manual inspection once the substrate dried. Note treatments with glue, water, and gum arabic had 100% attachment. Treatments: G, white glue; W, water; HS, hydroseeding; GA, gum arabic; C, control; SR, synthetic resin. There were no significant differences between adhesives at $p < 0.05$.

Discussion

Discussion

Synthesis

Throughout this thesis we have analysed several aspects to assist in the recovery of gypsicolous vegetation affected by quarrying, including: a) the assessment of the local native plant communities to establish appropriate references for restoration, b) the effect of gypsum at the initial stages of plant development, c) the suitability for restoration of various substrate management and revegetation methods, and d) the potential of lichen translocation to recover gypsum lichenic crusts. Herein, we briefly summarise the main findings, and discuss the conclusions and implications of Chapters 1 to 8. Limitations of the study and suggestions for further research are considered.

In this thesis, we determined the habitat of Community interest 1520 'Iberian gypsum vegetation, *Gypsophiletalia*', (European Commission, 1992) in CW Granada had been destroyed, fragmented or degraded due to several disturbances. Quarrying, ploughing, overgrazing and afforestation affected the gypsum habitat, and particularly the narrow endemic *O. tridentata* subsp. *crassifolia* (Chapter 1). Habitat depletion from quarrying, assuming the projected exploitation plan, would involve a decline of the area of occupancy, extent of occurrence and quality of the habitat, together with the drastic population reduction of *O. tridentata* subsp. *crassifolia,* suggesting it should be considered as Vulnerable, with criteria A3cd, D2 according the IUCN categories (IUCN, 2001, 2011). Therefore, its recovery and the ecological restoration of altered areas are required to mitigate negative effects of quarrying and improve the overall conservation of the gypsum habitat. Throughout this thesis, we could identify appropriate local references of the habitat in the area to guide future restoration efforts (Chapters 1, 6 and 8).

A further step in this thesis was to determine the effect of gypsum at different stages of plant development, with the final aim of gaining insight into the propagation of a selection of native species for habitat-restoration purposes in and beyond our study area. The presence of gypsum in the seed and seedling environment, rather than posing a constraint (Duvigneaud and Denaeyer-De Smet, 1966, Ruiz *et al*., 2003), improved the germination, emergence, survival and growth of certain species (Chapter 2 and 3). The dissolved gypsum in aqueous solutions had no adverse effect at the germination stage for many species regardless of their substrate preference in nature (Chapter 2). This is in agreement with Herrero *et al.* (2009) who claimed gypsum has a negligible effect on the osmotic potential or ion-specific toxicity for plants. On the contrary, chemical features of gypsum could be advantageous for some gypsophiles, particularly for *Lepidium subulatum* and *Gypsophila struthium,* and at specific concentrations to *Helianthemum squamatum* or gypsovags such as *Lygeum spartum* and *Pinus halepensis*. Moreover, adding gypsum to a standard nursery growing medium (peat) benefited the emergence of seven species, survival of three species, and growth of two gypsophiles out of nine species tested (Chapter 3). Thus, both chapters suggest applying gypsum treatments has a potential to improve efficiency in the propagation of some gypsicolous species, having special interest for threatened species such as *Ononis tridentata* subsp. *crassifolia*, which require a particularly efficient use of its seeds, very scarce in the habitat. These measures can help to optimize production of native species for gypsum habitat restoration implying better use of the available seeds and seedlings and a reduction in costs associated with seed harvesting, watering or nursery space. Finally, both experiments highlighted the importance of using appropriate growing media to propagate plants characteristic of special substrates when planning restoration measures.

Throughout this thesis we confirmed using adequate substrates together with the introduction of plant propagules (i.e. seed or seedlings) through various revegetation methods can effectively establish gypsicolous vegetation affected by quarrying (Chapters 4, 5, 6 and 7). In Chapters 6 and 7, spontaneous succession led to limited vegetation cover and undesired plant communities in the short-middle term, consistent with other studies reporting middle-long term results (Dana and Mota, 2006; Mota *et al.*, 2003, 2004). As expected, our observations of the initial vegetation establishment showed recovery was much faster on restored plots than on control plots and the reference abandoned-quarry after ~25 years, indicating assistance is necessary and that restoration is strongly recommended. The use of appropriate substrates may not be enough to recover gypsicolous vegetation in the long term without the early introduction of target species. Plant material introduction has proved to be key in the recovery of gypsicolous vegetation by overcoming dispersal limitations (typical of gypsophile plants) and the lack of propagules of target-species due to low connectivity between habitat patches and disturbed sites evident in the study area (Chapters 4, 5, 6 and 7).

Plantings on raw gypsum and gypsum spoil (and topsoil to a lesser extent) proved effective to achieve good survival, growth and seed production of the gypsophiles *H. squamatum, L. subulatum* and *O. tridentata* subsp. *crassifolia* (Chapter 4). The sowing experiment confirmed raw gypsum, gypsum spoil and topsoil can make composition, richness and cover approach satisfactorily that of the reference habitat, generating remarkably more valuable plant communities in 5 years than spontaneous succession on the local abandoned quarry over ~25 years (Chapter 6). Raw gypsum was the best treatment to increase plant size and seed production of planted or sown gypsophiles (Chapter 4), improving remarkably the cover of *O. tridentata* subsp. *crassifolia* (Chapter 6). In turn, gypsum spoil supported good growth and production of gypsophiles, better recruitment of target species and lower non-target species cover, leading to a more self-sustainable gypsicolous community. In contrast, topsoil produced smaller gypsophiles with less seed, and was less efficient to establish and recruit the gypsophiles *H. squamatum* and *L. subulatum* through planting and sowing. Topsoil was less beneficial for gypsophiles than raw gypsum and gypsum spoil even removing non-target species twice in the planting experiment, suggesting substrate properties and not only higher competition were the cause of the more limited plant performance (Chapter 4). Additionally, despite initial positive effects (Chapter 5), we concluded surface treatments (i.e. organic matter addition and blanket overlays) had no advantageous effects over time on flat landforms (Chapter 6).

Based on the previous chapters confirming the effectiveness of gypsum spoil for restoration, we conducted an experiment on gypsum spoil slopes to test the effect of three hydroseeding methods, slope (10-15% vs 60-65%) and orientation (north vs south) in vegetation recovery (Chapter 7). All hydroseeding treatments tested were useful for ecological restoration of the target vegetation (i.e. paper cellulose mulch, paper cellulose mulch + organic blanket, and wood fibre mulch, compared against a control). However, the success of the intervention was strongly conditioned by the slope, with more limited results achieved in steep slopes. The most satisfactory results were obtained using wood fibre mulch with the greatest establishment of gypsicolous vegetation on shallow slopes. Comparable results were only attained by the paper cellulose + blanket treatment on the steep slopes. In spite of being more expensive, the wood fibre mulch treatment could be considered for its additional applicability to prevent erosion problems and improve slope stability. However, wood fibre or paper cellulose mulches should be preferred in moderate slopes given the lower-cost, easy application and greater ecological benefits of these options.

A crucial factor in restoration implementation is to select appropriate species in proper combinations, densities and patterns (Walker and del Moral, 2003). Species performance are sometimes unpredictable without references in literature or from pilot studies under similar circumstances (Walker and del Moral, 2003). The use of non-native broad-purpose species has already been discouraged in gypsum habitats to avoid potential competition and preserve an appropriate species composition (Matesanz and Valladares, 2007). Planting, sowing and hydroseeding of native target-species allowed us to better understand vegetation performance in response to our experimental conditions (Chapters 4, 5, 6, 7). We based on cover development, recruitment, and how results compared to the reference habitat to assess the suitability of species and seeding rates used in our experiments (Chapters 6 and 7). Covers were already reasonably good, although some species were still below the cover in the habitat suggesting more time is needed to achieve the values in the habitat. Although *O. tridentata* increased cover and recruitment slowly, is likely to be sustained over time given the high survival and growth observed on gypsum spoils. If a higher cover is required, the seed rate could be increased. Additional measures such as plantings of *O. tridentata* subsp. *crassifolia* on raw gypsum patches within the large-scale restoration on gypsum spoils could be used as nuclei to help this species to colonise the restoration site and to provide seed for revegetation purposes. The species *H. squamatum* and *H. syriacum* established and recruited successfully despite some fluctuations, suggesting restoration could be achieved over time with the current seeding rate. *L. subulatum* was above the low cover in the habitat, but a strong decrease throughout the experiment suggests further assessment of the suitability of conditions and management may be required. *S. tenacissima* was more abundant in the habitat and needs to be reinforced with more seed or through planting. In turn, *Thymus zygis* and particularly *Rosmarinus officinalis* were overrepresented compared to the habitat. Our results, suggest the number of seeds of *T. zygis* can be lower than in our experiments. It also would be recommendable to exclude *R. officinalis* in the seed mixture, because it was absent in the local target community and became dominant in the sowing experiment, having potentially adverse effects in the future restoration program (Chapter 6).

The loss of biological soil crusts represents a challenge for the restoration of gypsum disturbed environments. In Chapter 8 we addressed the translocation of *Diploschistes diacapsis,* a representative species of gypsum lichen communities affected by quarrying. We tested how a selection of adhesives could improve thallus attachment to the substrate and affect lichen vitality (measured as CO_2 exchange and fluorescence) in rainfall-simulation and field experiments. Notably, making spoils wet allowed thalli to bind to the substrate after drying without compromising vitality, revealing as the most suitable option tested for translocation. The satisfactory results applying water on gypsum spoils are encouraging to test this methodology with other lichen species. Implementing these measures in restoration projects would be relatively easy and cost-effective. It would help not only to recover lichen species in the disturbed areas but also to take advantage of an extremely valuable biological material that otherwise would be lost.

The results obtained throughout this thesis allow a greater understanding of the ecology of the gypsum species and the applicability of various restoration methods that, filtered through economic constraints, will help to develop better restoration plans. In the next section, we suggest some guidelines based on the results obtained in previous chapters.

Guidelines to recover gypsicolous vegetation affected by quarrying

Through this thesis, we gathered site-specific information useful to guide decisions for the restoration of disturbed gypsum vegetation affected by quarrying. Figure 1 summarises an approach to selection of substrate management, revegetation method and plant material based on our results.

Spontaneous succession or the “no restoration” option makes vegetation recovery more sensitive to site-specific conditions, leading to slower succession and uncertain long-term recovery. Restoration sped up succession compared to the reference abandoned-quarry and control plots, indicating some of the measures tested here are strongly recommendable.

The first step in the restoration must be the physical stabilisation of the site. Available wastes in the area such as gypsum spoils and overburden are the most common and feasible options to contour the landscape to a desirable state. When necessary, structural and erosion problems must be reduced through geotechnical solutions. Careful planning will reduce labour and costs of stabilisation and remodelling of gypsum quarry waste areas prior to revegetation.

Our study determined gypsum spoil, raw gypsum and topsoil had applicability to restore gypsicolous vegetation. All materials tested to cover gypsum quarry waste areas prior revegetation need active introduction of target species. Gypsum spoil supports good growth and production of gypsophiles, better recruitment of target species and lower non-target species cover, leading to a more self-sustainable gypsicolous community. Gypsum spoil is available in large quantities and has no commercial value, adding to the ecological benefits technical and economic advantages. Thus, we recommend using gypsum spoil for the general restoration of gypsicolous vegetation. Raw gypsum should be considered occasionally to raise bigger *O. tridentata* plants and make seed available for restoration, controlling plant provenance to preserve genetic integrity. Topsoil can be used to increase productivity or reduce erosion, but should not be routinely recommended to restore gypsum habitats.

Two paths can be considered for the restoration of gypsum spoils depending on the site topography. On flat and shallow slopes revegetation can be conducted successfully through planting, sowing and hydroseeding with wood fibre or paper mulches, so the choice between these methods must be based on the cost-benefit trade-off. If planting is considered, we recommend using growth substrates with gypsum to propagate gypsophiles (e.g. standard peat mixed with 25-50% of gypsum by weight), while for gypsovags is not necessary (e.g. standard peat or 0-10%). Our results showed the application of organic matter or organic blankets overlays are not effective enough to justify their use in flat and shallow slopes. A preliminary assessment conducted at large-scale on flat and shallow slopes (1.5 ha in total) indicated hydroseeding is the most practical option (not presented in this thesis). On steep slopes hydroseeding is technically the most suitable option. Designing stable slopes must be a priority, taking into account geomorphological principles and adequate drainage. The success of hydroseeding is strongly affected by steep slopes, and thus minimising them wherever possible would generally improve the restoration outcome. If the erosion risk cannot be mitigated, application of organic blankets should help control erosion and run-off until a vegetation cover develops (Lorite *et al.*, 2015). All hydroseeding treatments improve target vegetation cover, with wood fibre performing best in most situations studied, alternatives being the cheaper but less effective paper mulch on shallow slopes; or the more expensive paper mulch + blanket on steep slopes in case of high erosion risk. Shallow and southern-steep slopes are more suitable for the recovery of gypsum vegetation by hydroseeding, compared to northern-steep slopes where non-target species can develop more readily outcompeting target species (Chapter 7).

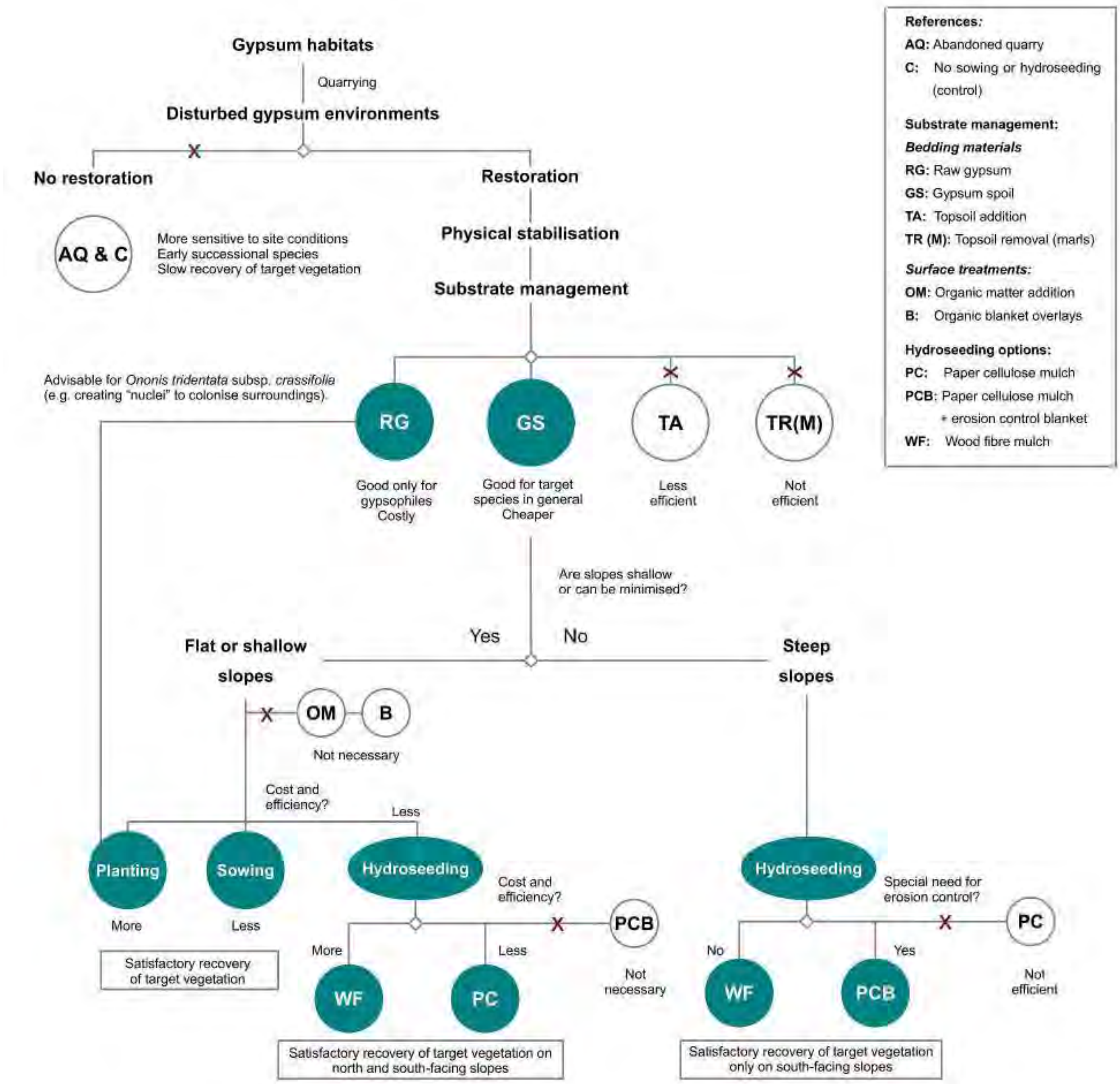


Figure 1. Decision pathways for the selection of restoration methods to restore gypsum vegetation on quarry slopes according to our results. The most satisfactory and recommendable options are in green and in white the options ruled out in our experiments.

Limitations and future research directions

Restoration *sensu stricto* seeking to fully recreate pre-disturbed ecosystems is difficult and unlikely (Walker and del Moral, 2003). Restoration methods can improve the environmental conditions of the disturbed site and provide the starting species pool to make plant communities resemble those in the reference habitat. However, no restoration method can recreate the exact unaltered community (Walker and del Moral, 2003; Hobbs *et al.*, 2007). Thus, further research must identify how other important biotic components in the habitat respond to both degradation and restoration, considering missing vascular plant species, cryptobiotic crusts, microbial communities and fauna. For example, it is necessary to determine whether gypsum ephemeral communities including species such as *Campanula fastigiata* and *Chaenorhinum grandiflorum* subsp. *carthaginense* in the area can establish under the conditions recommended in this thesis. Additionally, more research is needed to improve the recovery of biological soil crusts, and to further assess the suitability of translocation and inoculation methods to recover important components (e.g. cyanobacteria, algae, lichens, mosses, microbial communities); (Maestre *et al.*, 2006; Bowker, 2007; Chiquoine *et al.*, 2016). Restoration projects can be completed with lower biodiversity than the reference unaltered areas. If the re-established habitat is stable and allows colonisation of other desirable species it will diversify (Walker and del Moral, 2003). Therefore, it is also necessary to identify ways to improve the ecological connectivity between disturbed/restored sites and nearby habitats (McDonald *et al.*, 2016). If other relevant native species are not able to colonize from the vicinity over time, further assistance may be required to improve biodiversity.

Long-term monitoring is needed to re-evaluate the effectiveness of management actions and identify potential problems. The results of this thesis are associated to the duration of two restoration projects and limited by the time of the PhD program but long term follow up will allow further assessment. Early successional studies in restored mining areas have critical importance to understand the initial vegetation establishment and how community structure develops (Alday *et al.*, 2011, Prach *et al.*, 2014). However, other processes come into play once the initial community establishes due to environmental changes or species interactions, making predictions uncertain following restoration measures (Tischew and Kirmer, 2007). It is necessary to determine how edaphic variables control plant performance in the tested bedding materials and to determine whether changes over time will affect target vegetation (e.g. water, nutrients, organic matter content). Facilitation-competition trade-offs are important in gypsum habitats (Pueyo *et al.*, 2007; Saiz *et al.*, 2014) and require further study under our experimental conditions. Additional measures may be required to divert succession from less-valuable plant communities and emphasize gypsophiles cover, either adjusting seed proportions, sowing at different stages or controlling competitive species. Applying the knowledge learned through assessment of previous practices and adaptive management will improve future restoration programs (Murray and Marmorek, 2003; Hilderbrand *et al.*, 2005).

Site and year effects are potential constraints in comparing the outcomes of different restoration actions almost impossible to avoid in practice (Dana and Mota, 2006; Stuble *et al.*, 2017). Revegetation methods in our study were tested on independent experiments and may be influenced by interannual variability (e.g. rainfall and temperature). However, our experiments were conducted next to each other using gypsum spoils with the same provenance, reducing site variability. Thus, comparison of the outcomes and cost-analysis of the different revegetation methods is particularly appropriate on this substrate, which in turn is the most recommendable for restoration. In addition, although site and year effects make difficult to generalise successional patterns in restoration, our conclusions can be extrapolated beyond our study area and methods improved and adapted to achieve specific goals according to local targets in other disturbed sites with similar characteristics.

Successful restoration of gypsicolous vegetation is possible but does not justify the destruction of the natural resources, and the preservation of gypsum habitats must be encouraged. Impacts must be reduced by identifying areas with good quality habitat and designing reserves composed of patches of habitat (Mota *et al.*, 2011). The negative impacts of quarrying can be reduced through restoration programs and the recovery of gypsicolous vegetation ensured through the methods recommended in this thesis. The transfer of this knowledge into practice is crucial and needs the cooperation with stakeholders and local authorities (Tischew and Kirmer, 2007). Our results will be put at the disposal of the regional government and taken into account in the design of future plans to restore the vegetation of gypsum habitats in our area.

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Conclusions

Conclusions

The habitat of Community interest 1520 'Iberian gypsum vegetation, *Gypsophiletalia*' in CW Granada is affected by several disturbances. Gypsum quarrying, assuming the projected exploitation plan would cause a decline of the area and quality of the habitat, together with the drastic population reduction of the narrow endemic *Ononis tridentata* subsp. *crassifolia*. Therefore, this subspecies must be categorized as Vulnerable (A3cd, D2; IUCN 2001) and both improving its conservation and the ecological restoration of gypsum habitats affected by quarrying are required.

Enhancing propagation of native characteristic species can benefit the restoration of gypsum habitats. Our germination and growth-chamber experiments showed gypsum increased the germination, emergence, survival and growth of certain species. Adding gypsum to the commercial substrate can improve the propagation of native species, implying better use of the available seeds and a reduction in costs associated with seed harvesting, watering or nursery space.

Our study showed null or slow spontaneous recovery of gypsicolous vegetation in our reference abandoned-quarry in the mid-term (~25 years) as well as in our experimental control plots, being consistent with the slow recovery described in other abandoned quarries in SE Spain. Quarrying leaves altered areas with unsuitable substrates or dominated by generalist species, which leads to a slow succession and uncertain recovery of gypsicolous vegetation, therefore requiring active restoration.

Active restoration through revegetation methods with characteristic species on adequate substrates ensures the recovery of gypsicolous vegetation in the short-term. The introduction of plant material (seeds or seedlings) has proved to be key in the recovery of target-species by overcoming dispersal limitations and the lack of propagules due to low connectivity between habitat patches and disturbed sites. The three methods tested (planting, sowing and hydroseeding) are useful to overcome these limitations and establish the characteristic species of the habitat.

The efficacy of revegetation methods is conditioned by the choice of substrate for restoration. Gypsum spoil supported good growth and seed-production of gypsophiles, recruited target species better and generated lower non-target species cover, leading to a more self-sustainable gypsicolous community. Together with these ecological advantages, the wide availability and low commercial value of gypsum spoil make it the most recommendable option for the general restoration of gypsicolous vegetation.

Raw gypsum has a higher cost, but its remarkable benefits for gypsophiles, particularly for *O. tridentata* subsp. *crassifolia* should be considered when designing the restoration plan or specific conservation measures. Topsoil can be used to increase productivity or reduce erosion, but should not be routinely recommended to restore gypsum habitats.

The application of organic matter or organic blanket overlays do not improve the performance of gypsicolous vegetation on flat surfaces enough to be justified in restoration plans.

In gypsum spoil slopes hydroseeding with wood fibre is recommendable in most situations, alternatives being the cheaper but less effective paper mulch on shallow slopes, or the more expensive paper mulch + blanket on steep slopes in case of high erosion risk. Shallow and southern-steep slopes are more suitable for the recovery of gypsum vegetation by hydroseeding, compared to northern-steep slopes where target species are outcompeted by generalist species.

The translocation onto gypsum spoils of *Diploschistes diacapsis,* a representative species of gypsum lichen communities affected by quarrying is feasible without compromising its vitality. We confirmed making spoils wet can make thalli remain longer in place after translocation than with other methods. Translocation using this simple but effective methodology could help to recover key biological material in gypsum habitats and must be considered in the design of restoration plans.

Successful restoration of gypsum habitats is possible but does not justify its destruction, and the conservation of these environments must be encouraged. This thesis improves the understanding of the recovery of gypsicolous vegetation affected by quarrying and will contribute to develop better restoration programs and management of gypsum habitats.

Conclusiones

Conclusiones

El hábitat de interés comunitario 1520 “Vegetación gipsícola mediterránea, *Gypsophiletalia”* en CW Granada se ve afectado por varios tipos de perturbación. En concreto, la explotación de canteras de yeso, de acuerdo con los proyectos existentes causaría una disminución del área y calidad del hábitat así como la drástica disminución poblacional del endemismo local *Ononis tridentata* subsp. *crassifolia.* Por ello, esta subespecie debe catalogarse como Vulnerable (IUCN A3cd, D2; IUCN 2001) siendo necesaria la mejora de su estado de conservación y la restauración ecológica del hábitat de yesos en las áreas alteradas por la minería.

La propagación eficiente de especies propias de yesos puede beneficiar la restauración de este hábitat. Así, nuestros experimentos de germinación y cámara de cultivo mostraron que el yeso aumentaba la germinación, emergencia, supervivencia y crecimiento de ciertas especies. La adición de yeso al sustrato comercial mejoró la propagación de ciertas especies gipsícolas, implicando un mejor uso de las semillas disponibles y una reducción en costes de recolección, riego o espacio de vivero.

Nuestro estudio mostró una nula recuperación espontánea de la vegetación gipsícola en nuestra cantera a medio plazo (~25 años) y lenta en las parcelas control, de acuerdo con resultados descritos en otros estudios de canteras abandonadas en el SE de España. Las canteras dejan áreas alteradas con sustratos inadecuados o dominados por especies generalistas, lo cual conlleva una lenta sucesión y la recuperación incierta de la vegetación gipsícola, requiriéndose por tanto su restauración activa.

La restauración activa mediante métodos de revegetación con especies características y la selección de sustratos adecuados asegura la recuperación de la vegetación gipsícola a corto plazo. La introducción de planta o semillas ha demostrado ser clave en la recuperación del hábitat objetivo, permitiendo superar limitaciones en la dispersión y falta de propágulos debidas a la baja conectividad entre los parches de hábitat natural y las zonas alteradas. Los tres métodos testados (plantación, siembra e hidrosiembra) son útiles para superar estas limitaciones y establecer las especies características del hábitat.

La eficacia de los métodos de revegetación está condicionada por el sustrato empleado para la restauración. El rechazo de yeso favoreció el crecimiento y la producción de semillas de las especies gipsófitas, reclutó mejor las especies objetivo y logró baja cobertura de especies generalistas, generando una comunidad más autosostenible. Además de las ventajas ecológicas, su disponibilidad y bajo valor comercial convierten al rechazo de yeso en el sustrato más recomendable para la restauración general de la vegetación gipsícola.

El yeso bruto tiene un coste superior, pero dado sus notables beneficios para los gipsófitos, particularmente para *O. tridentata* subsp. *crassifolia,* puede considerarse en el diseño de medidas de restauración y conservación puntuales. Por otro lado, la capa de rescate puede usarse para aumentar la cobertura vegetal y reducir la erosión para casos concretos, pero no se recomienda de forma generalizada para restaurar el hábitat de yesos.

La aplicación de materia orgánica o el uso de manta orgánica en superficies planas no mejoran la respuesta de la vegetación gipsícola lo suficiente como para ser justificados en los planes de restauración.

En terraplenes de cantera, la hidrosiembra con fibra de madera es recomendable en la mayoría de las situaciones, siendo el mulch de pasta de papel una alternativa más económica pero menos eficiente en pendientes suaves, o el mulch de pasta de papel + manta orgánica más caro pero útil en pendientes fuertes con riesgo de erosión. Las pendientes suaves en general y las fuertes con orientación sur son más favorables para establecer la vegetación gipsícola mediante hidrosiembra, en comparación con las pendientes fuertes orientadas al norte, donde las especies objetivo son desplazadas por especies generalistas.

La traslocación sobre rechazo de yeso del liquen *Diploschistes diacapsis* representativo de las costras biológicas del hábitat, es factible sin comprometer su vitalidad. Comprobamos que humedecer el rechazo permite que los talos permanezcan más tiempo que con otros métodos en el lugar de traslocación sin comprometer su vitalidad. Esta metodología simple pero efectiva permite recuperar material biológico clave en hábitats de yesos y debe ser considerada en el diseño de planes de restauración.

La restauración de los hábitats de yesos se puede llevar a cabo con éxito, aunque esto no justifica su destrucción, y se recomienda que sean preservados sin alterar. Esta tesis aumenta el conocimiento sobre la recuperación de la vegetación gipsícola afectada por la minería y contribuirá a desarrollar mejores programas de restauración y manejo de los hábitats de yeso.