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Trait-based selection of nurse plants to restore ecosystem functions in mine tailings

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Abstract

1. Metal mining in drylands generates waste tailings with high toxicity, physical instability, as well as water and thermal stresses, that hamper their biological

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colonisation. This limits the restoration of ecosystem functions that are essential to re-integrate these artificial micro-deserts within the landscape matrix.

2. We assessed the functional role of local nurse plant species and their traits to restore ecosystem functions related to soil fertility, soil microbial productivity and the reduction of abiotic stress. We sampled 30 metalliferous tailings in a mining district from semiarid Spain to detect nurse plant species and quantify their ability to promote essential functions from their establishment on the barren substrate up to the adult stage.
3. We found 11 plant species acting as nurses out of 102 species able to colonise barren soils. Ten nurses further triggered a cascade of effects increasing soil fertility and microbial productivity and/or lowering soil abiotic stress.
4. Plant species with larger life forms and longer periods of establishment since tailing abandonment contributed the most to the promotion of ecosystem functions. C4 plant species developing root systems with lower intensivity and depth: laterality ratios, as well as leaves with lower carbon : nitrogen ratios (C:N) induced a faster recovery of ecosystem functions.
5. *Synthesis and applications.* We propose a protocol for selecting key species to be used in restoration programs based on their ability to restore ecosystem functions under extremely stressful conditions. We encourage combination of multiple target species with complementary traits in order to reinforce the rehabilitation of ecosystem functions.

Keywords

Abiotic stress, ecosystem services, metal concentration, nutrient cycling, organic matter decomposition, functional diversity, microbial productivity, polluted mine soil, plant facilitation, soil fertility

Introduction

Metal mining creates waste tailings that function as micro-deserts which feature severe abiotic stresses imposed by high concentrations of trace elements, high conductivity, low pH, physical instability and scant soil and water resources (Conesa *et al.* 2006). Around 700 million tons of metalliferous wastes are laid down annually (Warhurst 2000). These extreme environments, which hinder both the natural recolonization and human-driven restoration, yield very depleted ecosystem services that require innovative restoration strategies. Conventional mine-rehabilitation techniques (e.g. waste removal, dam building, on-site containment by sealing) have been usually the methods of choice (European Commission 2009), but are often expensive and either inefficient or difficult to apply due to geosstructural risks and environmental restrictions (Tordoff *et al.* 2000; Conesa & Schulin 2010). Metal uptake by phytoextraction using hyperaccumulator plants is considered the most useful phytoremediation technique (Ali *et al.* 2013). Nevertheless, phytoextraction techniques need both of long-term programs so as to significantly decrease soil metal concentrations and ex situ disposal treatments of the extracted phytomass (Conesa *et al.* 2006). These limitations have encouraged the use of plants for stabilization of mine tailings, so-called phytoestabilization, to promote the ecological integration of mining structures within their surrounding landscape matrix (Tordoff *et al.* 2000; Conesa *et al.* 2006; Mendez & Maier 2008; Ali *et al.* 2013).

For mine tailings embedded into natural areas, phytoestabilization poses a cheap and environmentally friendly tool compared to conventional rehabilitation techniques.

Plant species selection is essential for the success of rehabilitation technologies in mine tailings. Plant selection has evolved from the use of model hyperaccumulator plants (e.g. *Festuca*, *Thlaspy* or *Trifolium*) to locally adapted species in the last years (Tordoff *et al.* 2000; Mendez & Maier, 2008; Párraga-Aguado *et al.* 2013). Ideal functional traits sought for species selection focus on their ability both to establish and rapidly grow on barren substrates and to tolerate (but not accumulate) metals (Martínez-Fernández *et al.* 2011). These functional traits typically characterise pioneer plants that show intermediate characters between stress-tolerant and ruderal species (Pierce *et al.* 2017). Pioneers, though very efficient in colonizing barren areas, do not necessarily promote the establishment of other plant species or enhance soil conditions (Lei & Duan 2008; Arocena *et al.* 2009). These limitations have been counteracted by the use of organic and/or physical amendments (Mendez & Maier 2008), although contrasting results have been reported regarding the improvement of soil structure and fertility, metal immobilisation or microbial productivity (Arco-Lázaro *et al.* 2015; Zornoza *et al.* 2016). Moreover, organic amendments can enhance metal mobility through weathering and decomposition of organic residues in the long term (Tordoff *et al.* 2000; Mendez & Maier, 2008; Párraga-Aguado *et al.* 2013). Alternatively, we propose that the phytoestabilization can be improved by selecting and combining nurse plant species that not only act as pioneers but also facilitate the establishment of other plant species, ultimately promoting the plant-soil feedbacks that trigger essential ecosystem functions (*sensu* Hooper *et al.* 2005). This approach favours the facilitation over the inhibition or tolerance models of succession proposed by Connell & Slatyer (1977).

Nurse plants are stress-resistant species that colonize barren soils, develop isles of milder microclimatic conditions, and facilitate the growth beneath their canopy of less stress-tolerant plants. In natural abiotically stressful environments a wealth of nurse plants have shown to shape plant patches with increased productivity and soil fertility compared with adjacent bare sites (Aguilar & Sala 1999; Padilla & Pugnaire 2006). Nurse plants may have specific traits that confer them the ability to i) tolerate abiotic stress and establish on barren soils, ii) transform the microclimate beneath their canopy, both reducing solar radiation and increasing water availability, and locally relaxing the abiotic filter, and iii) improve soil fertility by supplying litter and root exudates available to decomposers (Navarro-Cano *et al.* 2014). Beyond promoting plant recruitment, facilitation-driven plant communities may drive a cascade of benefits on ecosystem functions, including ecosystem properties such as the accumulation of organic carbon and nutrients in soils, and ecosystem services such as nutrient cycling, reduction of metal concentration in soils and ecosystem stability (Pugnaire *et al.* 1996; Wang *et al.* 2014; Navarro-Cano *et al.* 2015). These ecosystem functions, though rarely taken into account, are fundamental for the successful phytostabilization of mine tailings (Mendez & Maier 2008). The use of nurse plants improves the success of forest afforestations on natural soils in dry areas (Gómez-Aparicio 2009). In mine tailings, the formation of islands of increased fertility that promote soil microbial activity has been recently shown (Párraga-Aguado *et al.* 2013), suggesting that the use of nurse plants can be an attractive option for the phytomanagement of these degraded environments.

The Cartagena-La Unión Mining District (SE Spain) has been a main metalliferous area in Europe during the last two millennia (Conesa & Schulin 2010). The mining activity

was totally abandoned in 1991, leaving 89 mine tailings with high concentrations of heavy metals and metalloids (hereafter metal(loids)), that threaten the environmental and human health (García-García 2004). Nowadays, mine tailings are being colonized by plants from the surrounding natural matrix shaping a patchy structure that resembles that of facilitation-driven communities. This led us to hypothesize that local plant species might not only withstand these extreme conditions but also trigger the restoration of essential ecosystem functions through ecological facilitation (Pugnaire *et al.* 1996; Navarro-Cano *et al.* 2014, 2015). We further speculated that nurse plants may have ecophysiological and morphological functional traits that allow recruiting on barren polluted substrates and promote ecosystem functions related to the amelioration of soil fertility (organic carbon, nutrients) and soil microbial productivity (organic matter decomposition, nutrient cycling), and the reduction of abiotic stress (concentration of metal pollutants, pH, salinity). We analyzed gradients of plant size for each potential nurse identified in the study area, with the aims to: i) assess the ability of each nurse to promote soil fertility and microbial productivity, and reduce the abiotic stress associated with its growth, and ii) determine the functional traits of nurse plants that underlie the rehabilitation of the ecosystem functions. As functional diversity has proven to optimize many ecosystem properties (Hooper *et al.* 2005) we discuss how the trait-based selection of nurse plants and their combination to synergistically force the rehabilitation of multiple ecosystem functions can be a practical tool in phytostabilization programs so as to achieve an effective restoration that promotes ecosystem services, beyond the survival of planted species.

Materials and methods

Study system

The Cartagena-La Unión Mining District (Murcia, Spain; 30° S 68°15' E, 41°44'33" N) ranges 5.000 ha of a low-altitude coastal mountain range. Climate is semiarid Mediterranean with 17.9°C mean annual temperature, 316.3 and 762.2 mm mean annual precipitation and evapotranspiration respectively (data for 2005-2015). Metamorphic nappes, sedimentary and igneous materials shape the area, whose ores of iron, lead and zinc among other metals have been intermittently exploited for over 2000 years. Mine tailings from the refining process show high metal concentrations and electrical conductivity, negligible organic matter and occasionally acidic pH that hamper plant recolonization and restoration (Conesa & Schulin 2010).

Vegetation sampling

Mine tailings are covered by a sparse patchy vegetation <25% plant cover (Fig. 1). We defined plant patches as clustered communities growing beneath the canopy of a perennial plant that could potentially be a nurse plant. A nurse plant should be able, as per definition, to recruit on gaps and promote seedling establishment of third species as it grows by relaxing the abiotic filter (Navarro-Cano *et al.* 2015). We defined gaps as the open space adjacent to each patch, where isolated pioneer plants (and no adults of potential nurses) are observed.

Nurse plants were identified by an *in situ* random sampling of perennial species. We performed a preliminary sampling across 30 mine tailings from the 2nd half of the XXth century, 22 of which had conspicuous vegetation beyond some scattered individuals. We detected 102 plant species able to recruit on the barren substrate, 16 of which had

other plant species growing beneath their canopy (Table S1). Out of these 16 potential nurses, we selected 13 species that were highly represented in the tailings (from 50 to >1000 individuals; Table S1). To discard that nurses were not improving soil conditions but establishing under fertile conditions, we compared main soil parameters in the gaps and beneath the seedlings of these 13 species (Table S2). Then, we performed an in-depth sampling in spring 2015 focusing on the 13 potential nurses in seven mine tailings that showed the highest plant density for the selected species (Table 1; Fig. S1). We recorded the number of individuals growing underneath each nurse and in its adjacent gap in at least 30 randomly selected patches per potential nurse except for *Lygeum spartum* (28 patches) and *Osyris lanceolata* (16 patches), which did not have a sufficient number of individuals (Table 1). Gaps were delimited with a similar area and shape to their corresponding patches, and were systematically placed West of the patch at a distance equal to its mean canopy diameter. We sampled a total of 1286 plots, including 643 plant patches and adjacent gaps.

We estimated the year at which each potential nurse species colonized each mine tailing (AGE) by analysing a time series (1948-2013) of high resolution (0.25-0.50 m) aerial digital orthophotographs (<http://sitmurcia.carm.es/>).

Soil sampling

We collected 106 soil samples (0-5 cm) on early May 2015 along the size gradients of all 13 potential nurse plants. To minimize the site effects along with size gradients, each nurse was sampled in a single mine tailing. Size gradients included soils in the gaps between plant patches, as well as soils underneath seedlings (1-2 year old, height <10 cm), juveniles (>10 cm tall with no cues of reproduction) and adults (reproductive) of

each nurse. Specifically, we took soil samples from 93 plant patches that configured size gradients for all potential nurses and 13 samples in the open space across each gradient. The size of the patch was measured as its mean canopy diameter (Navarro-Cano *et al.* 2015). Underneath each patch we took five soil subsamples, from 10×10 cm quadrats each that were located in the cardinal points and the centre of the patch, and subsequently mixed all subsamples into a ca. 1 kg sample. In small plants, where five subsamples could not be collected beneath the canopy, the total soil surface was sampled. The number of samples collected in each one of the seven mine tailings depended on the presence of studied nurses (mine tailing A=12 samples; B=10; C=10; D=18; E=8; F=8; G=42). The number of samples taken to depict the nurse size gradient varied depending on the nurse life form: trees and shrubs (10 samples per species), perennial grasses (8) and dwarf shrubs (6). This different sample size aimed at balancing the sampling effort across the size gradients of nurse plants that can differ in their lifespan up to two decades in the study area. Unbalanced sample sizes did not cause differences across nurses in the soil studied parameters, as we repeated all the statistical analyses using balanced sample sizes (n=6) for all nurses and results were consistent (data not shown). Gap samples resulted from bulking the subsamples collected in the gaps adjacent to each patch. Soil samples were transported to the laboratory on ice, sieved ≤ 2 mm and kept at 4°C during subsequent analyses. Main substrate properties in the seven tailings are given in Table S3.

Soil fertility, microbial productivity and abiotic stress variables

Soil gravimetric humidity (GH), pH, electrical conductivity (EC), total organic carbon (TOC) and nitrogen (TN) were measured using standard procedures as in Navarro-Cano *et al.* (2015). Soil total concentrations of other nutrients and heavy metals were

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determined by digestion with HNO₃ and H₂O₂, using an Ultraclave microwave digestion system (Milestone, Milan) followed by analysis by Inductively Coupled Plasma Optical Emission Spectroscopy (ICAP 6500 ICP-OES Spectrometer, Thermo Fischer Scientific, Waltham).

Basal respiration (BR), indicative of the rates of organic carbon mineralization, was measured as the average C content respired daily per kilogram soil during a 30 day aerobic incubation assay. We quantified the production of CO₂ from 30 g soil using an infrared analyser (Headspace CO₂ analyzer 6700, Illinois Instruments Inc). We also quantified colorimetrically hydrolytic activities involved in carbon (β -glucosidase, GA), phosphorus (alkaline phosphatase, PA) and nitrogen cycling (urease, UA). Further details on all protocols are given in Navarro-Cano *et al.* (2014).

Plant functional traits

The functional role of the nurse plants on the rehabilitation of ecosystem functions was assessed by measuring morphological (life form, biovolume, pedestal height, root intensivity and root depth:laterality ratio) and physiological traits (photosynthetic metabolism, halophytism, leaf C:N ratio, leaf As and leaf Pb concentration).

Information on trait values was either obtained from the literature or from five adult plants sampled in the tailings (Table S4). Life form was estimated from categories related with plant morphology and size (tree, shrub, perennial grass, dwarf shrub), assigned based on Paula *et al.* (2009) and the authors' criterion. Plant biovolume was estimated from the plant height and major and minor canopy diameter. Pedestal height was defined as the elevation of soil beneath the nurse canopy resulting from sediment trapping, which depends on the aboveground plant architecture (branching type and density), and was estimated trigonometrically from its angle and length. Root intensivity

was estimated as the ratio between the fresh root length (maximum length of stretched roots) and the dry root weight (cleaned and 65°C oven-dried roots) after digging up the whole root system. The root depth:laterality ratio was estimated by measuring the total laterality and depth of fresh roots. Both root traits have been used as indicators of soil exploration ability and competitive strength (Guerrero-Campo 1998; Cornelissen *et al.* 2003). Photosynthetic metabolism (C3, C4 or CAM) and halophytism (a syndrome related to the ability to cope with high salinity) were obtained from the literature (Table S4). Leaf C and N was quantified as for soils from ca. 10 g of fresh ground leaves per plant. Leaf As and Pb were quantified in ground and digested samples, and analysed by ICP as above. These concentrations were used as cues of the accumulation and tolerance of toxic elements (Table S5).

Statistical analyses

Facilitation of beneficiary plant species by each potential nurse plant was analysed by Chi square tests, to explore whether the observed distribution of the total number of counted plant individuals underneath the nurse canopy and in the adjacent gaps differ significantly from the random expectation. The species-specific effects of the nurse diameter (taken as a continuous variable) on each variable associated with soil fertility (TOC, N, P, K and GH), microbial productivity (BR, GA, PA and UA) and abiotic stress (As, Cd, Cu, Pb, Zn, pH and EC) were tested by means of Generalised Linear Models (GLM) using the nurse diameter as the independent variable. For each nurse species the *p*-values in the 16 GLMs were adjusted for multiple comparisons with the Benjamini-Hochberg correction to control for the false discovery rate.

We performed three principal component analyses (PCA) to reduce the main groups of ecosystem functions to orthogonal variables in order to estimate the overall effects of the size gradient of each nurse species separately on soil fertility, productivity and abiotic stress (Fig. S2). The first PC resulting from the three individual PCAs was used as a dependent variable in separate GLMs. False discovery rates were controlled, as described above.

To determine which functional traits of the nurse plants associate with the rehabilitation of soil fertility and microbial productivity, we assessed the role of the overall selected traits on the improvement of soil TOC, N, P, GA, UA and PA beneath the potential nurses. All trait values were reduced by PCA including quantitative and qualitative variables with the *PCAmixdata* R package (Chavent *et al.* 2014). We used the first and second dimensions as predictive variables in GLMs with 'Soil improvement' as the dependent variable and 'Mine tailing' as a random factor in order to account for the effects of each one of the seven sampling sites. We measured the Soil improvement as a ratio, resulting from the division of the value of each soil variable at the final (largest patch) and starting (gap) points of each nurse size gradient. Finally, to provide a time integrated measure of the recovery period of soil properties beneath each nurse species, we used the colonization age (AGE) of each nurse as a predictor of Soil improvement as above. The *p*-values of these mixed GLMs were adjusted for multiple comparisons with the B-H correction. All analyses were performed with R 3.1.3 (R Core Team 2015).

Results

We detected 13 potential nurse plants among 102 species growing on barren mine tailings (Table S1), which included trees, shrubs, perennial grasses and dwarf shrubs

(Table 2). Eleven out of these 13 species actually functioned as nurse plants facilitating the recruitment of beneficiary species, as the plant abundance beneath their canopy was significantly higher than that in the adjacent gaps (Table 1).

Effects of potential nurses on soil fertility, microbial productivity and abiotic stress

Once we confirmed that nurse seedlings are established on barren substrate (Table S2) we tested if nurses positively shift the soil properties as they grow. The species-specific effects of the nurse diameter on the fertility, productivity and abiotic stress variables are shown in Table 2. Contrasting effects were found depending on the variable and nurse species. TOC was significantly improved by plant diameter in most trees and shrubs, excepting *D. pentaphyllum*, which actually did not act as a nurse according to our results. Three of these species (*T. canariensis*, *O. lanceolata* and *A. halimus*) also improved total N, and *A. halimus* even K. Among perennial grasses and dwarf shrubs, TOC levels only increased associated with the size of *L. spartum* and *S. tenacissima*. Similar patterns were found for soil microbial productivity (Table 2). *A. halimus* had positive effects on all productivity variables. Similarly, the trees *T. canariensis* and *O. lanceolata* and the grasses *L. spartum*, *S. tenacissima* and *H. synaica* significantly increased at least GA, PA and BR, whereas no dwarf shrubs had any effect on microbial productivity variables.

Positive effects of nurses on abiotic stress variables were less frequent (Table 2). Only *S. oppositifolia* significantly increased soil pH (from 5.5 in the gaps to 7.5 underneath the largest patches) and concurrently decreased EC (2.0 to 0.5 dS·m⁻¹). In contrast, this species increased soil As (150 to 350 mg·kg⁻¹) and Pb (300 to 1000 mg·kg⁻¹) concentrations beneath its canopy as it grew. Other species, such as *O. lanceolata*, *A.*

halimus and *L. spartum* also reduced EC. Only the grasses *L. spartum* and *H. synaica* reduced respectively soil Cd and Pb concentrations.

The first principal components of three PCAs performed to reduce soil fertility, microbial productivity and abiotic stress variables (hereafter Fertility, Productivity and Abiotic stress) were used for further analyses (Fig. S2). Fertility explained 49.2% of the total variance and was positively correlated with TOC, N, P and K. Productivity (59.3%) was positively correlated with GA, UA, PA and BR. Abiotic stress (45.1%) was negatively correlated with EC and Cd, Pb and Zn concentrations. Fertility significantly increased with the nurse size only in some of the largest life forms as *T. canariensis*, *O. lanceolata* and *S. oppositifolia* (Table 2). Productivity significantly increased in the six largest nurse species (Table 2). Finally, Abiotic stress significantly decreased in *A. halimus*, *S. oppositifolia* and increased marginally in *P. miliaceum* (Table 2). Fig. 2 shows the evolution of soil fertility, microbial productivity and abiotic stress with the mean diameter of *Osyris lanceolata* for illustrative purposes. All other species can be found in Figs. S3 to S5.

Identifying nurse functional traits related to ecosystem functions

The reduction of ten functional traits of the nurse plants by PCA yielded a first dimension (TRAITS1) that explained 31% of the variance and was positively correlated with halophytism, trees and shrubs and the C4 metabolism (Fig. S6). TRAITS1 was negatively correlated with root intensivity, root depth:laterality ratio and leaf C:N ratio. Overall, TRAITS1 would be higher in larger species with adaptations to abiotic stress and an efficient metabolism and with higher root laterality and presence of secondary roots. The second dimension (TRAITS2) explained 26.2% of variance and was

positively correlated with the nurse biovolume and pedestal height, and negatively correlated with leaf As and Pb concentration. TRAITS2 would be higher in plants shaping significant canopies, that work as sediment sinks and foliar accumulator of metal(loids). TRAITS1 significantly and positively explained the improvement in soil TOC beneath the nurses (Fig. 3); Estimate \pm SE value of the linear model; 0.29 ± 0.09 ; $t_5=3.1$, $P=0.040$), marginally explained N (0.46 ± 0.21 , $t_5=2.2$, $P=0.089$) and GA (0.47 ± 0.19 ; $t_5=2.4$, $P=0.088$), and significantly PA (0.24 ± 0.06 , $t_5=3.8$, $P=0.023$). TRAITS2 explained PA improvement (0.25 ± 0.07 , $t_5=3.6$, $P=0.023$), marginally GA (0.54 ± 0.23 , $t_5=2.3$, $P=0.088$) and N (0.22 ± 0.10 , $t_5=2.2$, $P=0.089$).

We finally examined the effect of the colonization age of the mine tailings by each nurse species (AGE) on the amelioration of ecosystem functions. AGE, even though a rough time estimate, was a good predictor of TOC improvement (0.08 ± 0.02 , $t_5=3.4$, $P=0.039$; Fig. 5), N (0.08 ± 0.02 , $t_5=4.2$, $P=0.014$), PA (0.05 ± 0.02 , $t_5=3.1$, $P=0.027$), and marginally GA improvement (0.11 ± 0.05 , $t=2.1$, $P=0.088$). The shrub *A. halimus* improved TOC and N faster than the average, whereas *S. oppositifolia* similarly improved TOC faster than other species (Fig. 3).

Discussion

Our results show that nurse plants trigger the recolonization of metalliferous mine tailings under semi-arid conditions through the assembly of patchy plant communities and further promote essential ecosystem functions. Plant traits related to the type of metabolism and root system, as well as the nutrient ratios in the leaves, appear as the main determinants of the ability of a nurse plant to restore the ecosystem functions beyond the recruitment of beneficiary species in mining areas. We underline the role of

nurse plants as ecosystem engineers that promote ecosystem functions related to plant dynamics, soil properties and microbially-mediated processes in extremely stressful ecosystems. In line with previous studies (Wang *et al.* 2014; Dominguez *et al.* 2015), these results point out the use of plant-plant facilitation as an emergent topic in mine reclamation.

The use of facilitation as a strategy to restore mining areas requires, firstly, an appropriate selection of the nurse species, and thus a clear differentiation between plants that are mere pioneers and those that can further facilitate less stress-tolerant species. The fact that metal tolerant species have been traditionally defined as pioneers can be associated with the idea that, under extreme conditions, the abiotic component of the species niche is the only determinant of species establishment and growth. This view ignores the biotic interactions that can potentially readjust the species fundamental niche, thus shaping the realized niche, as the final process of both abiotic and biotic filtering. Bruno *et al.* (2003) highlight that incorporating facilitation into the niche concept alters the notion of the realized niche, which can have a broader spatial range for beneficiary species than that predicted by their fundamental niche based on the nurse-driven habitat amelioration. In agreement with this approach, we included nurses and beneficiaries to the plant functional categories that colonize mine tailings. This classification allows detecting not only plant colonizers but also community assemblers and soil promoters that improve ecosystem functions as organic matter decomposition and nutrient cycling (Padilla & Pugnaire 2006; Navarro-Cano *et al.* 2015, 2016). A suitable functional categorization can be fundamental when the results have potential applications in restoration. Arocena *et al.* (2009) defined *T. canariensis* and *P. halepensis* as pioneers based on the incipient soil formation beneath their canopy, while

according to our results in the same study area both species are indeed nurses. Pioneers are usually early-colonizers that both resist abiotic stress (high EC, metal concentration and temperature) and are efficient competitors for soil resources, and thus can lead to an arrested plant succession (Walker & del Moral 2003). This is the case of some perennial grasses that show to effectively recover in post-mining areas but impede forest establishment (Hammond 1999; Winkler *et al.* 2014). In our study, most perennial grasses seem to facilitate the establishment of dwarf shrubby and annual Mediterranean semiarid communities (Table 1), but have a limited effect in triggering forest communities and little influence on soil fertility and productivity (Table 2 and Figs. S3-S4). This type of succession follows the inhibition model described by Connell & Slatyer (1977), and we argue that other species that follow instead the facilitation model should be selected for restoration practices.

Plant nursing ability might be ultimately determined by functional traits, which are the main drivers of community assembly and providers of ecosystem functions (De Bello *et al.* 2010, 2012). Therefore, finding out the set of traits that determine the capacity to facilitate other species and promote ecosystem functions is another relevant step in the selection of nurse species. Short- to mid-term shifts in soil properties are controlled by plant traits related with the production of leaf litter and root exudates, which are the fundamental resource for microbial decomposers and nutrient cyclers (Kellman 1979; Dakora & Philips 2002). Among the main traits that influence biogeochemical cycles (revised by Cornelissen *et al.* 2003), leaf traits are the most commonly assessed drivers of soil fertility and productivity shifts (De Deyn *et al.* 2008), whereas root traits are rarely assessed likely because seem to be more recalcitrant to decomposition (Kemp *et al.* 2003; de Bello *et al.* 2010). In Mediterranean environments, higher leaf litter

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decomposition rates are positively correlated with leaf toughness and negatively with C:N ratios (Gallardo & Merino 1993). Besides root exudates and litter, root size and architecture can potentially influence soil fertility and stability (erosion control), and water regulation (De Baets *et al.* 2007; de Bello *et al.* 2010). The type of root system is also essential at the community level, since functionally complementary roots (e.g. spreading at different depths) allow a higher shared efficiency in the use of resources through reducing inter-specific competition (Blignaut & Milton 2005). A functionally dissimilar neighbourhood can potentially increase the amount of organic inputs to the soil (Spehn *et al.* 2000) and likely the abundance of soil microbes and detritivorous fauna (Milcu *et al.* 2013). Indeed, the nurse and its beneficiaries make a synergistic contribution to soil fertility and microbial productivity underneath multi-specific plant patches (Navarro-Cano *et al.* 2015). In our study system, halophytic and C4 plant species with larger life forms, with lesser root intensivity, root depth:laterality ratio and leaf C:N ratio were more effective in increasing soil TOC, N and P (Fig. 3).

Halophytism and C4 photosynthetic metabolism are attributes that allow overcoming saline and water stress (Pyankov *et al.* 2010; Párraga-Aguado *et al.* 2014). Lesser root intensivity and depth:laterality ratio in trees and shrubs are trait expressions that might reduce water competition beneath the canopy by favouring functional complementarity with the shallower root systems from tiny plant species facilitated by the nurses (Guerrero-Campo 1998; De Baets *et al.* 2007). We also found that plant species with a larger biovolume, able to create higher pedestals beneath their canopy were more effective to promote C cycling, in agreement with the idea that dust trapping ability promotes the formation of fertility islands (Wezel *et al.* 2000). All these traits depict the ideal nurse in our tailings as a long-lifespan species with an aboveground architecture prone to retain soil particles and seeds, and enrich its canopy with labile leaf litter. This

model species also has belowground traits that minimize competition with its facilitated community. Ideally, this nurse species might contribute to reduce the metal concentration beneath its canopy without a significant accumulation in plant tissues. Nevertheless, the metal concentration patterns beneath the plant canopy are not always positively correlated, as in *S. oppositifolia* that significantly alleviates soil EC and Cd, and Zn but simultaneously increases As concentrations (Table 2 and Fig. S5).

In order to give practical guidelines for restoration programmes in the studied mine tailings, we give a multifunctional scoring of nurse species based on their ability to improve ecosystem functions (Table 3). The results can be interpreted as a sequential decision making process translatable to any other ecosystem with preponderance of abiotic stress factors, e.g. mine areas in drylands, disturbed highlands, or abandoned marginal croplands prone to desertification. The decision process pursuits to detect nurse plants able to i) build multi-specific plant patches, ii) create fertility isles, ii) promote organic matter decomposition and nutrient cycling, and iv) decrease soil abiotic stress. *O. lanceolata* and *A. halimus* had the highest global scorings, and so could be seen as the best species to positively affect the assessed ecosystem functions. This functional scoring could be also used to select the best nurse for a specific purpose in combination with data on the colonization age (if available). For instance, improvement in soil organic carbon and nitrogen respectively could be achieved with *S. oppositifolia* and *A. halimus*, which show soil improvement rates over the average (Fig. 3).

Alternatively, our functional scoring could serve as a tool to choose different nurse species that might be combined to synergistically increase the community effects on global ecosystem functions. Our results illustrate how the trait-based selection of target

species can help to more efficiently design strategies to reach the aims of ecological restoration programs (in cost and time) in stressful ecosystems.

Authors' Contributions

All authors conceived and designed the study. JANC and MG collected data. JANC analysed data and wrote a draft of the manuscript to which all authors made contributions and gave final approval for publication.

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Data accessibility

Plant species traits and soil data are available from the *Dryad Digital Repository*. DOI: 10.5061/dryad.j70qf (Navarro-Cano, Goberna, & Verdú 2018)

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Table 1. Potential nurse plant species in seven mine tailings. Mean diameter \pm SD of the plant patches formed by each nurse, and number of patches and adjacent gaps sampled (N) to test nursing ability. Paired χ^2 was used to test for statistical differences in the number of juvenile and adult plants growing in each Patch and Gap. * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$).

Species	Life form	Diameter	N	Plants in patches	Plants in gaps	χ^2
<i>Pinus halepensis</i>	Tree	195.0 \pm 128.3	96	62	33	8.85**
<i>Tamarix canariensis</i>	Tree	322.6 \pm 187.2	82	76	44	8.53**
<i>Osyris lanceolata</i>	Tree	210.9 \pm 128.4	32	55	25	11.3***
<i>Atriplex halimus</i>	Shrub	115.4 \pm 82.8	60	45	13	17.66***
<i>Salsola oppositifolia</i>	Shrub	127.5 \pm 98.9	60	59	35	6.13*
<i>Dorycnium pentaphyllum</i>	Shrub	48.1 \pm 26.4	60	11	10	0.05
<i>Lygeum spartum</i>	Perennial grass	134.4 \pm 79.9	56	21	6	8.33**
<i>Stipa tenacissima</i>	Perennial grass	76.1 \pm 53.6	64	45	23	7.12**
<i>Piptatherum miliaceum</i>	Perennial grass	32.8 \pm 17.1	194	70	20	27.78***
<i>Hyparrhenia synaica</i>	Perennial grass	25.1 \pm 15.9	190	45	12	19.11***
<i>Helichrysum stoechas</i>	Dwarf shrub	28.6 \pm 15.7	240	77	54	4.04*
<i>Paronychia suffruticosa</i>	Dwarf shrub	31.5 \pm 17.7	88	36	28	1
<i>Limonium carthaginense</i>	Dwarf shrub	18.0 \pm 12.8	64	12	3	5.4*
Total			1286	614	306	

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Table 2. Significant estimates of the effects of nurse diameter on variables related to soil fertility, microbial productivity and abiotic stress. In the Species column, A-G indicate sampled mine tailing (Fig. S2). Microbial productivity variables are abbreviated as GA (glucosidase activity), UA (urease activity), PA (phosphatase activity) and BR (basal respiration). Within each group of variables, the PC1 column refers to the first PC obtained from PCA reduction of all variables. Non-significant estimates are omitted. *** ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), no asterisk ($p<0.1$).
^a log-transformed diameter to stabilize variance.

Species	Fertility						Productivity					Abiotic stress							
	TOC	N	P	K	GH	PC1	GA	UA	PA	BR	PC1	As	Cd	Cu	Pb	Zn	pH	EC	PC1
<i>Pinus halepensis</i> (n=10, G)							0.001*		^a 0.525***		0.004*	0.313**							
<i>Tamarix canariensis</i> (n=10, G)	0.003*	0.0001*			-0.004	0.004*	0.002		^a 0.675	0.005	0.005*	0.169*	0.014*	1.633		5.099			
<i>Osyris lanceolata</i> (n=10, D)	0.007*	0.0004*		0.001*	0.008*	0.014**	0.005*		0.008*	0.011*	0.01**					4.595		-2.981*	
<i>Atriplex halimus</i> (n=10, C)	0.002*	0.0002*			-0.006*		^a 0.254	0.001	0.007	0.005*	0.009*		^a -2.274					-7.986*	0.006*
<i>Salsola oppositifolia</i> (n=10, B)	0.002		0.0001	0.001**	0.002*	0.005*				0.007		0.525*		1.227**		^a 0.319**	^a -219*	0.001*	
<i>Dorycnium pentaphyllum</i> (n=6, G)																			
<i>Lygeum spartum</i> (n=8, E)	0.001*		^a -0.000*		^a -0.344*		0.002**		0.012**		0.005**	^a -0.916*				^a 0.027*	-0.001*	^a -377***	
<i>Stipa tenacissima</i> (n=8, D)	^a 0.001						^a 0.054		^a 0.247	-0.093									
<i>Piptatherum miliaceum</i> (n=8, G)					^a -0.216*		0.011*					0.259				103	^a 0.043		-0.028
<i>Hyparrhenia synaica</i> (n=8, F)			^a -0.002*				0.03*		0.0536*	^a -0.453*				^a -583*					
<i>Helichrysum stoechas</i> (n=6, A)																			
<i>Paronychia suffruticosa</i> (n=6, A)																			
<i>Limonium carthaginense</i> (n=6, G)			^a -0.005														^a -0.047		

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Table 3. Multifunctional scoring of nurse species to support decision making for phytoestabilization programmes in the studied mine tailings, based on each species ability to nurse third species, increase soil fertility and microbial productivity, and reduce abiotic stress. The standardized difference between the initial value (barren soil) and the final value (beneath larger nurse) for each variable and species is shown (values ranging from -1 to 1). Non-significant effects are omitted. The global score for each species is calculated by summing all the individual abilities to restore ecosystem functions.

Multifunctional scoring of nurses ¹	<i>P. halepensis</i>	<i>T. canariensis</i>	<i>O. lanceolata</i>	<i>A. halinus</i>	<i>S. oppositifolia</i>	<i>D. pentaphyllum</i>	<i>L. spartum</i>	<i>S. tenacissima</i>	<i>P. miliaceum</i>	<i>H. synaica</i>	<i>H. stoechas</i>	<i>P. suffruticosa</i>	<i>L. carthaginense</i>
1. Nursing ability (Relative interaction intensity index) ²	0.31	0.27	0.38	0.55	0.26		0.56	0.32	0.56	0.58	0.18		0.60
2. Nurse ability to increase soil fertility													
Total Organic Carbon		0.75	0.83	0.52	0.66		0.33	0.22					
Total Nitrogen		0.68	0.65	0.58									
Total Phosphorous					0.02		-0.01			0.00			-0.56
Total Potassium			0.17		0.30								
Soil gravimetric humidity		-0.43	0.36	-0.34	0.10		-0.28		-0.25				
3. Nurse ability to increase microbial productivity													
C cycling	1.00	0.99	0.90	0.99			0.85	0.96	0.92	0.72			
N cycling				0.75									
P cycling	0.69	0.85	0.43	0.53			0.47	0.47	0.27				
Microbial activity		0.27	0.78	0.57				-0.12		-0.45			
4. Nurse ability to decrease abiotic stress ³													
As concentration	-0.47	-0.30			-0.32								
Cd concentration		-0.20		0.22			0.08		-0.42				
Cu concentration													
Pb concentration		-0.03			-0.44					0.17			
Zn concentration		-0.14	-0.03				-0.09		-0.37				
pH					0.19		-0.02		0.02				-0.02
Electrical conductivity			0.61	0.40	0.53		0.38						
5. Global scoring of nurses to restore ecosystem functions	0.38	0.25	0.51	0.48	0.14	0.00	0.23	0.37	0.10	0.20	0.18	0.00	0.01

¹Each value was standardized as $\frac{V_f - V_i}{V_f + V_i}$ where V_f is the final value and V_i the initial value

²Nursing ability was calculated using the RII as in Navarro-Cano *et al.* (2014): $RII = \frac{Bw - Bo}{Bw + Bo}$ where Bw and Bo are the number of plants below the nurse and in the adjacent barren soil, respectively

³For metal concentrations, negative values indicate higher concentrations and therefore an increased abiotic stress; for pH, negative values indicate higher acidity and then an increased abiotic stress; for EC, positive values indicate lower salinity which is interpreted as a decreased abiotic stress

Figure 1. Overview of two of the studied mine tailings in the study area. An acidic tailing with a *S. oppositifolia* patchy community (A), with dense annual communities beneath their canopies (B). A neutral tailing (C) with dominance of *T. canariensis* (central ring) and *A. halimus* (outer belt), in which several perennial species are colonizing the oldest *A. halimus* patches (D).

Figure 2. Evolution of soil fertility, microbial productivity and abiotic stress with the mean diameter of *Osyris lanceolata*.

Figure 3. Top graphs show the effects of the functional traits of nurse plants (TRAITS1) on soil TOC and N improvement. Linear fits are depicted. Species codes: *L. carthaginense* (Lica), *P. miliaceum* (Pimi), *H. stoechas* (Hest), *P. suffruticosa* (Pasu), *D. pentaphyllum* (Dope), *H. sinaica* (Hysi), *S. tenacissima* (Stte), *L. spartum* (Lysp), *O. lanceolata* (Osla), *A. halimus* (Atha), *T. canariensis* (Taca), *S. oppositifolia* (Saop). Bottom graphs show the effects of the colonization age of the mine tailing by the nurse species (AGE) on soil TOC and N improvement. Linear fits are depicted. Plant species above the fitting lines might be considered as fast improvers of TOC or N, whereas species below the lines would be slow improvers.



