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Shrubs for the remediation of contaminated Mediterranean areas: is the nurse effect mediated by increases in soil enzyme activities?

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Abstract

The use of shrubs as nurse plants to facilitate woody plant recruitment has been proved to be particularly useful for the revegetation of highly disturbed environments, such as contaminated lands. In a contaminated area from SW Spain, we compared soil fertility and microbial enzyme activity in different microhabitats associated with shrubs, along a gradient of soil contamination, 13 years after the revegetation of the area following a contamination episode. We compared soil organic matter, nutrient content, and enzyme activities in four microhabitats: underneath retama (*Retama sphaerocarpa*) shrubs nursing Holm oak (*Quercus ilex* subsp. *ballota*) seedlings, underneath separate retama and oak individual plants, and in patches of soil without any woody cover. Soil enzyme activities were influenced more by the background soil conditions (pH and organic matter content, modified by the addition of a soil amendment during soil remediation) than by the development of the vegetation. In the soils under the cover of the retama-oak association, intense acidification (pH <4) was observed at the most contaminated sites, which resulted in higher solubility of toxic trace elements and lower enzyme activities (five times lower dehydrogenase and β-glucosidase activities, in comparison to neutral soils). The results suggest that improving soil conditions before plantation through amendment application is critical to ensure the improvement of microbial activity in the long-term at such degraded sites.

Keywords: *Retama sphaerocarpa*, *Quercus ilex*; soil contamination; soil enzyme; β-glucosidase, dehydrogenase; facilitation

Highlights

- Using shrubs as nurse plants for tree recruitment is useful for restoring highly degraded sites.
- The nurse effect is often reported to be mediated by increases in microbial activity underneath shrubs.
- 13 years after revegetation of a contaminated area, soil fertility and enzyme activities were analyzed in a nurse shrub-tree association.
- Enzymes were influenced scarcely by vegetation development, but strongly by pH and organic matter.
- In this area, the nurse effect was not mediated by increases in soil microbial activity.

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1. Introduction

Shrubs are considered to be hotspots of soil fertility and biological activity in dry ecosystems (Aguilera et al., 1999; Duponnois, 2011; Goberna et al., 2007; González-Polo and Austin, 2009; Sasaki et al., 2010). They enhance belowground mutualisms that may benefit other plant species established under the shrub cover (Duponnois et al., 2011; Requena et al., 2001). These effects on soil quality, together with the amelioration of extreme temperature conditions underneath the shrubs (Callaway, 1992; Gómez-Aparicio et al., 2004; Leiva et al., 2015), result in the facilitation of the recruitment of other woody plant species, which is often referred to as the *nurse effect*. In the last decade, many studies have shown that applying this nurse effect to the restoration of degraded dry ecosystems is a promising tool for improving the success of woody plant plantations in these systems, which is usually limited by high mortality rates during the first years after planting (Blanco-García et al., 2011; Gómez-Aparicio, 2009; Torroba-Balmori et al., 2015).

Legume shrubs, such as retama (*Retama sphaerocarpa* (L.) Boiss), have a great potential as nurse plants in Mediterranean ecosystems (Gómez-Aparicio et al., 2004; Padilla and Pugnaire, 2006). The presence of retama shrubs buffers against extreme temperatures at ground level (Muñoz-Vallés et al., 2011; Padilla and Pugnaire, 2006), increases water availability in the topsoil due to the hydraulic lift of their roots (Prieto et al., 2010), and increases organic matter mineralization rates (Moro et al., 2007) and N and P concentrations in the soil (Rodriguez-Echeverria and Perez-Fernandez, 2003). The positive effects of retama shrubs on woody plant recruitment may be enhanced in particularly harsh environments, such as contaminated areas - where this species can be successfully established due to its relatively high tolerance of soil contamination (De la Fuente et al., 2014; Moreno-Jiménez et al., 2011).

In a contaminated and remediated area in SW Spain, we found that the shade provided by the retama canopy allowed nursed seedlings of Holm oak (*Quercus ilex* subsp. *ballota*) to cope with the toxicity provoked by the concurrence of low pH and high trace element concentrations in the soil (Domínguez et al., 2015). Here, we have studied whether this nurse effect is also related to increases in soil fertility and microbial extracellular enzyme activities underneath the retama shrubs. Thirteen years after the initial revegetation of the contaminated area, we compared soil fertility and enzyme activities in different microhabitats along a gradient of soil contamination, focusing on the retama-Holm oak association.

2. Materials and methods

The study was conducted in the Guadiamar River Valley (37º30–13´N, 6º13´W), which has a Mediterranean climate with mild, rainy winters and warm, dry summers (more information about the area is provided in Domínguez et al., 2008). The area was affected by a large mining accident in 1998, which left the soils severely contaminated by trace elements. To mitigate the effects of this accident, a large-scale restoration plan was launched in 2000, which included the addition of soil amendments and the revegetation of the affected lands with native woody plant species (Domínguez et al., 2008). Sugar beet lime (with P, N, and K concentrations of 5.1, 9.8, and 5.3 g kg^{-1}, respectively, Madejón et al., 2006) was applied to the soils to stabilize the pH and to improve the soil fertility. In 2005, we conducted an experimental sowing of Holm oak acorns at different microsites (under shrub cover vs. open, uncovered sites) and along a gradient of soil contamination, which was intended to test the usefulness of positive plant-plant interactions (nurse effect) in the revegetation of the area (Domínguez et al., 2015). In 2012, we analyzed the fertility and microbial enzyme activities in soils from different microhabitats: 1) underneath the canopy of *R. sphaerocarpa* individuals planted during the initial afforestation of the area (in 1999); 2) underneath Holm oak juveniles, planted also in 1999 following the traditional afforestation method, without any plant cover protection; 3) under the canopy of *R. sphaerocarpa* individuals, where Holm oak seedlings had been established experimentally in 2005; and 4) in patches without any woody vegetation, dominated by ruderal herbaceous species. Four sites were selected along the gradient of soil contamination: site 1 was not affected by the accident, but was exposed to historical trace-element contamination due to its proximity to the mine, sites 2 and 3 were severely contaminated by the mining accident, and site 4 was unaffected by the accident and was not exposed to historical contamination (Supplementary Information, Fig. S1). The soils at these sites were mainly fluvisols. The soil conditions in 2003, four years after the planting of the retama shrubs, are shown in Supplementary Material Table S1. The main differences in soil conditions among the sites were related to soil pH (ranging from 3.2 to 7.3) and the total trace element concentrations (for example, As concentrations ranged from 249 to 15.6 mg kg^{-1}, Domínguez et al., 2010). At each site four replicates per microhabitat were selected in 2012, and the soils were sampled (0-10 cm) at the end of the spring season using a cylindrical auger. Soil samples (bulk soil) were collected at a maximum distance of 5 cm from the Holm oak or *R. sphaerocarpa* trunks; thus, a high incidence of roots within the sampled area can be assumed.

A fraction of each sample was dried and sieved (< 2mm) for analysis of pH and electrical conductivity (1:2.5 soil:water suspension), organic matter (OM) (Walkley and Black, 1934), available P (Olsen et al., 1954), available K (Bower et al., 1952), and total N (Kammerer et al., 1967). To determine the soluble trace element concentrations (As, Cd, Cu, Pb, and Zn), the soils were sieved to < 1 mm, extracted with 0.01 M $CaCl_2$, and analyzed using a Varian ICP 720-ES spectrometer (simultaneous ICP-OES with axially viewed plasma). A second fraction of each sample was sieved (< 2mm) and kept at 4 ºC until the analysis of the activities of three enzymes, which was completed within one week after collection. We analyzed three extracellular enzyme activities that are highly responsive to soil heavy metal contamination (Pan and Long, 2011) and are commonly used as indicators of microbial activity recovery after remediation of soil contamination (Hinojosa et al., 2004; Pérez de Mora et al., 2005). Dehydrogenase activity was determined by incubation of 1 g of soil with 2-(p-iodophenyl)-3-5-phenyltetrazolium (INT) for 20 h at 25 ºC, in the dark (Trevors, 1984). Urease activity was determined as the rate of ammonium release after incubation of 1 g of soil with 80 mM urea at 37 ºC, for 2 hours (Kalender et al., 1999), and β-glucosidase was analyzed colorimetrically by incubation with p-nitrophenyl-linked substrate at 37 ºC for 1 hour (Tabatabai et al., 1982). For each enzyme three replicate assays per soil sample were performed. The analyses were conducted with fresh soils and the results were based on the oven-dried weights.

Mixed linear models were applied to analyze the differences in soil fertility and microbial activity, with microsite type (shrub vs. open) as a fixed factor, site as a random factor, and the microsite × site interaction as a random factor. The soil pH data did not meet normality, and a generalized linear model was applied instead, using a gamma distribution. Bivariate correlations between the enzyme activities and the other soil properties studied were explored, and the fitting of linear, quadratic, or exponential equations was carried out using Sigma Plot v.12.

3. Results

Thirteen years after the start of the remediation activities in the area, the soil OM and nutrient contents were scarcely influenced by the development of the vegetation: in general, there were no significant differences among microsite types, but all these variables differed significantly across sites (Table 1). In contrast, for soil pH a significant microsite × site interaction was found. At the more acidic sites (sites 1 and 3) the retama-Holm oak microsite soils tended to have lower pH than the soils of the other three microsites (Fig. 1). The change in pH was particularly important at the most contaminated site (site 3), where the soils under the cover of the retama-oak association were extremely acid (pH < 3.5).

The soil enzyme activities were also hardly influenced by the type of microsite (Table 1). For urease activity, there was a significant microsite × site interaction; it was similar among microsites at all sites except site 2, where it was higher in the open microsites dominated by ruderal herbs and lower under oak saplings. The three enzymes studied seemed to be controlled by slightly different environmental factors (Table 2). Dehydrogenase activity was controlled strongly by soil pH and to a lesser extent by electrical conductivity and available K. Urease activity, in contrast, was influenced more by soil fertility (OM, N, and K contents), while β-glucosidase activity was controlled by both pH and fertility (Table 2). For the two spill-affected sites, there were stronger links between soil pH and the activities of the three enzymes analyzed (see example for dehydrogenase and β-glucosidase, Fig. 2a, b): β-glucosidase increased from < 0.5 μmol PNP g^{-1} h^{-1} at pH < 4 to 2.5 μmol PNP g^{-1} h^{-1} at pH 7.5, while dehydrogenase increased five-fold (from 2 to 10 μmol INT g^{-1} h^{-1}) within this range of soil pH. At these contaminated sites, the activities were negatively affected by the soluble concentrations of Cd, Cu, and Zn (see example for Cd in Fig. 2c, d).

4. Discussion

In dry Mediterranean ecosystems pioneer shrub species (often called nurse plants) can facilitate the establishment of other late-successional species under their canopies, mainly due to the amelioration of extreme temperature conditions and the improvement of plant water status (Callaway, 1992; Castro et al., 2004; Gómez-Aparicio et al., 2005; Padilla and Pugnaire, 2009). The improvement of soil conditions under the shrubs is also one of the mechanisms mediating the nurse effect (Pugnaire et al., 1996). As this positive plant-plant interaction can be especially intense under high abiotic stress (Callaway et al., 2002; Lortie and Callaway, 2006), we expected a clear effect of the shrub cover on soil fertility and microbial activity in the area studied, 13 years after soil contamination and the subsequent remediation.

However, our results suggest a limited influence of the revegetation on the most important physico-chemical properties, as well as on some microbial enzyme activities in the soils studied. These results contrast with previous reports which show a clear improvement of soil fertility under retama shrubs in natural semi-arid areas (Pugnaire, 2004; Rodriguez-Echeverria and Perez-Fernandez, 2003), but are in agreement with other studies showing a lack of medium-term effects of retama shrubs on soil fertility after the revegetation of former agricultural lands (Cuesta et al., 2010). Our work supports previous evidence that, in the retama-Holm oak association in this contaminated area, the nurse effect is not mediated by the improvement in soil fertility and microbial activity underneath the retama shrubs, but is due mainly to the amelioration of extreme microclimatic conditions (Domínguez et al., 2015). In other contaminated areas from different regions, studies have shown that the facilitation of plant recruitment by nurse plants is mediated by increases in soil OM under the nurse canopy (Ginocchio et al., 2004) and by increased stabilization of pollutants in the nurse root system (Frèrot et al., 2006), which leads to a reduction of trace element availability. Conversely, Eränen and Kozlov (2007) showed a decrease in plant performance below the nurse canopy, due to a greater accumulation of airbone pollutants underneath shrubs.

Interestingly, the establishment of oak plants under the retama canopy resulted in an unexpected acidification of the soils at the two sites with naturally acidic soils, which was not recorded during the first years after oak emergence. This might have been caused by the intensive removal of base cations from the soils by the roots of the two species growing close together in this type of microsite on soils poor in base cations. At the most contaminated site, strong acidification of soils occurred due to the oxidation of the remnants of sulfites deposited during the mine accident (Domínguez et al., 2016). It seems that at this site the amendments applied were not enough to neutralize the acids produced by the progressive sulfide oxidation over the years. Therefore, the application of new amendments to these extremely acid soils is highly recommended, as low-pH conditions hamper the recovery of microbial activity. Because of the further acidification of the soils underneath this plant association, and given that the solubility of toxic trace elements such as Cd and Pb increases exponentially at pH values lower than 4 (Domínguez et al., 2009), the planting of Holm oak seedlings under the canopy of retama shrubs at sites with an initial soil pH of less than 5 should be avoided.

As the soil fertility and microbial activities were not influenced much by the development of vegetation at this stage of this study, but were affected considerably by the inter-site differences in soil pH and nutrient contents, it seems essential to ensure that certain soil conditions are achieved before conducting the afforestation of similar contaminated areas. In our work, the differences in soil conditions among sites resulted from different background geological factors, differing degrees of contamination, and irregular treatment during the remediation program. Previous experimental work with retama plantations in Mediterranean areas has shown that enzyme activities increase notably when the establishment of the plantation is preceded by the addition of soil amendments, and that amendments have a much greater effect on enzyme activities than mycorrhizal inoculation of retama seedlings (Caravaca et al., 2003). Soil pH and nutrient content are properties that can be managed through the addition of amendments, but, critically, the effectiveness of the added materials must be monitored over time to ensure the recovery of microbial activity in contaminated soils in the long-term.

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Figure captions

Fig. 1. Soil pH (mean ± standard error) in the studied microsites at the different sites along the gradient of soil contamination (indicated by the symbol color intensity). RET = underneath *R. sphaerocarpa* individuals planted 13 years before soil sampling; RET-OAK = underneath *R. sphaerocarpa* individuals, where Holm oak seedlings had been experimentally established seven years before soil sampling; HERB = in patches without any woody vegetation, dominated by ruderal herbaceous species; OAK = underneath Holm oak juvenile trees, planted 13 years before soil sampling.

Fig. 2. Influence of soil pH and $CaCl_2$-soluble Cd on enzyme activities at the two sites affected by the mining accident that contaminated the soils in 1999 (sites 2 and 3). The fitted line and parameters are indicated.

Tables

Table 1. Results of the general linear models applied to enzyme activities and soil fertility across sites and microhabitat types. Significant results ($p \leq 0.05$) are highlighted with bold letters.* for pH, the results correspond to the generalized linear model applied (Wald statistics).

Variable	Statistics	Site	Microsite	Site × Microsite
Dehydrogenase	*F*	**7.00**	1.05	1.07
	p	**0.001**	0.381	0.403
Urease	*F*	**8.50**	1.10	**2.26**
	p	**< 0.001**	0.360	**0.037**
β-glucosidase	*F*	**5.64**	0.64	0.73
	p	**0.002**	0.592	0.676
pH*	*Wald*	**256.9**	**29.6**	**55.4**
	p	**< 0.001**	**< 0.001**	**< 0.001**
Organic matter	*F*	**22.72**	0.81	1.13
	p	**< 0.001**	0.496	0.365
Total N	*F*	**16.01**	0.95	1.25
	p	**< 0.001**	0.427	0.294
Available P	*F*	**11.56**	2.56	1.86
	p	**< 0.001**	0.068	0.086
Available K	*F*	**18.20**	0.83	1.42
	p	**< 0.001**	0.484	0.213

Table 2. Bivariate correlations between enzyme activities and the rest of the soil factors studied. Asterisks indicate the significance level (* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns: non-significant). EC: electrical conductivity; OM: organic matter; N: total nitrogen; P: available phosphorus; K: available potassium.

Variable	Dehydrogenase	Urease	β-glucosidase
pH	0.52***	ns	0.48***
EC	-0.28*	ns	ns
OM	ns	0.41**	0.40**
N	ns	0.34*	0.42**
P	ns	0.52***	ns
K	0.46**	ns	0.29**