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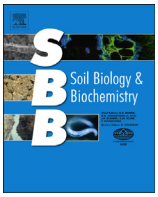

Benefactor and allelopathic shrub species have different effects on the soil microbial community along an environmental severity gradient

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ABSTRACT

Patches where shrubs have either positive or negative effects on their understory plant community are common in arid ecosystems. The intensity and balance of these effects change along environmental severity gradients but, despite the major role of soil microbes in plant interactions, little is known about the differences among soil microbial communities under these species and their possible influence on such contrasting shrub effects. We hypothesized that microbial communities associated to benefactor and allelopathic shrubs would differ among them and that differences would increase with environmental severity. To test these hypotheses we characterized soil microbial biomass, activity and community composition under a benefactor shrub species, *Retama sphaerocarpa*, an allelopathic shrub species, *Thymus hyemalis*, and in bare soil among plants (gaps) at three sites along an environmental severity gradient. Shrubs promoted an increase in soil bacterial diversity, being bacterial communities associated to benefactor shrubs, allelopathic shrubs and gaps different in composition. Microbial enzymatic activity and biomass increased under shrubs and under more mesic conditions; nonetheless, they were highest under benefactor shrubs at the most arid site and under allelopathic shrubs at the less severe site. Compared to gaps, the presence of shrubs induced changes in microbial activity and community composition that were larger at the most severe site than at the less severe site. Along the gradient, benefactor shrubs enhanced the abundance of bacterial groups involved in organic matter decomposition and N fixation as well as plant pathogens, which could contribute to *Retama*'s outstanding positive effects on understory plant biomass and diversity. Plant patches mitigate the effects of extreme conditions on associated plant and soil microbial communities and promote soil biodiversity and ecosystem functioning in arid ecosystems, with shrubs actively selecting for specific microbial groups in their understory.

1. Introduction

Shrubs in dry environments grow often in patches, which promote ecosystem heterogeneity (Goberna et al., 2007). Most shrub species have significant effects on their understory plant communities, ranging from negative to positive (Pugnaire et al., 2004). Patches where shrubs facilitate establishment and growth of other species are common in these systems (Pugnaire et al., 2011; McCluney et al., 2012). Benefactor shrubs provide shade and promote large modifications in understory soil by increasing moisture (Prieto et al., 2011), organic matter content (Pugnaire et al., 2004), microbial biomass, activity and abundance of some microbial groups (Goberna et al., 2007; Hortal et al., 2013). They therefore constitute hotspots of microbial activity, driving many ecosystem processes (Austin et al., 2004; Goberna et al., 2007; Rodriguez-Echeverria et al., 2013).

Interference processes where shrub species negatively affect the establishment and growth of other plants under their canopy have

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also been reported in these systems (Pugnaire et al., 2004; Ehlers, 2011; McCluney et al., 2012). For example, in the Mediterranean Basin, allelopathic species in the Lamiaceae produce volatile compounds in their leaves that serve as protection against high temperature and drought as well as defense against herbivores, parasites and pathogens (Linhart and Thompson, 1999; Peñuelas, 2008). When leached into the soil through leaf litter, they negatively affect seed germination and growth of neighbor plants (Inderjit, 1996; Vokou et al., 2003; Pugnaire et al., 2004) as well as soil bacteria and fungi (Kalemba and Kunicka, 2003). Volatile compounds also inhibit nitrification, increasing soil ammonium content while decreasing nitrate (Pavolainen et al., 1998; Castells et al., 2003) with consequences for both plant and soil microbial communities. However, little is known about the composition of microbial communities associated to allelopathic plants.

Despite the role of soil biota in both plant facilitation (Rodriguez-Echeverria et al., 2013) and interference (Jensen and Ehlers, 2010), what distinguishes soil microbial communities associated to benefactor and allelopathic species has not been explored yet. Moreover, differences may change with environmental conditions as does the relative importance of negative and positive plant interactions (Pugnaire and Luque, 2001; Callaway et al., 2002). It has been shown that facilitation processes are more important and frequent under extreme conditions (Bertness and Callaway, 1994; Armas et al., 2011; He et al., 2013) and also that the production of volatile compounds increases with temperature (Peñuelas, 2008). However, how soil microbial communities associated to these shrub species change with environmental conditions and how this might impact the intensity of both positive and negative plant–plant interactions is not known.

In a semiarid system we compared soil microbial communities associated to two plant species showing contrasted effects on their understory plant communities along an environmental severity gradient. We hypothesized that i) soil microbial communities under a benefactor and an allelopathic shrub species will differ in composition both between them and compared to bare soil; ii) microbial biomass and activity will be the highest in the less severe site (due to the amelioration of environmental conditions) and also higher under the benefactor shrub than under the allelopathic shrub (due to the antimicrobial properties of the later); and iii) soil microbial communities will show larger differences in composition, biomass and activity among cover types (gaps, benefactor and allelophatic shrubs) at the most arid site than at the less severe site. We expected that changes in soil microbial community composition and activity caused by shrubs will be largest at the most severe site since microhabitat conditions between shrubs and gaps will differ most. This will be particularly true for the benefactor shrub, in accordance with stronger plant facilitation effects occurring under these conditions.

To address these questions we selected three sites in which the severity of microclimatic conditions decreases with elevation, *i.e.* soil temperature decreases while soil moisture and N content increase with elevation (Armas et al., 2009). As benefactor species we selected *Retama sphaerocarpa*, a large, deep-rooted leguminous shrub showing an outstanding facilitation role (Pugnaire et al., 1996, 2004, 2011; Armas et al., 2011). As allelopathic species we selected *Thymus hyemalis*, a shrub up to 70 cm tall typically found in semi-arid environments of SE Spain (Peinado et al., 1992) that often shows an empty understory reflecting interference mechanisms (Pugnaire et al., 2004). *Thymus* species have a shallow root system (De Baets et al., 2008) and their leaves are loaded with volatile oils likely to stand hot and dry environments (Peñuelas, 2008). These oils have anti-microbial effects on fungi and bacteria development (Salmeron et al., 1995; Dorman and Deans, 2000).

2. Materials and methods

2.1. Field sites and cover types

We selected three field sites within a distance of 3 km on the northern foothills of the Sierra Alhamilla range, Almeria, Spain (37°01′N, 2°23′W). The climate is semiarid with a mean annual precipitation of 230 mm at the bottom and 347 mm at the top (Armas, 2004), with a pronounced dry season from June to September during which there is no rain in most years (Perez Pujalte et al., 1987). Mean annual temperature in Tabernas (490 m elevation, 3 km to the east) is 17.9 °C with mean maximum summer temperatures that can reach over 40 °C. Differences in soil temperature and moisture along the elevation range (Armas et al., 2009) as well as differences in bedrock cause a pronounced gradient of environmental conditions and plant communities along the slope. Plant cover increases with elevation, with the tussock grass *Stipa tenacissima* dominating the community and shrubs such as *Anthyllis cytisoides*, *Cistus clusii*, *R. sphaerocarpa*, *Rosmarinus officinalis*, and *T. hyemalis* becoming gradually more common with increasing elevation until they are replaced by an evergreen holm oak (*Quercus ilex*) woodland above 900 m (Cabello, 1997).

The three selected field sites, hereafter referred to as low, intermediate and high, were located at 340, 370 and 480 m elevation, respectively. All sites were open shrublands dominated by *R. sphaerocarpa* (L.) Boiss, hereafter *Retama*, along with smaller shrubs such as *T. hyemalis* Lange, hereafter *Thymus*, and were selected to encompass a range of soils and microclimatic conditions. Soils were orthic solochanks with inclusions of calcic regosols at the low site, calcic regosols mixed with gypsic regosols at the intermediate site and calcic regosols with weathered micaschist at the high site (Perez Pujalte et al., 1987). Mean soil pH was 8.2 with no differences among sites (data not shown). On spring and summer of an average climatic year, soil moisture increased from 15% to 25% along the same elevation gradient while soil temperature decreased from 29 °C to 25 °C (Armas et al., 2009).

At each site we selected three different cover types: understory of *Retama* shrubs, understory of *Thymus* shrubs and gaps (bare soil) among them. We randomly selected five individuals of similar size per species and five gaps in each of the three sites. Mean shrub height (±SD) and mean projected canopy area (calculated as the area of an ellipse) were, respectively, 1.96 ± 0.33 m and 4.00 ± 2.73 m^2 for *Retama* and 0.48 ± 0.08 m and 0.27 ± 0.15 m^2 for *Thymus*. We measured plant biomass and species richness in 20×20 cm^2 quadrats under each shrub and in nearby gaps and calculated the relative interaction index (RII, Armas, 2004) as Q2 $RII_{shrub} = (B_{shrub} - B_{gap})/(B_{shrub} + B_{gap})$, where B is the biomass or species richness under each shrub (B_{shrub}) and gaps (B_{gap}). RII quantifies the intensity of change in the presence of a shrub compared to gaps and ranges from −1 to 1 with negative values indicating negative effects and positive values facilitation. Mean plant biomass and richness (±SD) were 5.18 ± 4.51 g and 12.28 ± 2.99 species under *Retama*, 0.49 ± 0.46 g and 4.67 ± 1.97 species under *Thymus* and 1.37 ± 1.14 g and 7.56 ± 2.68 species in gaps. *Retama* shrubs acted as facilitator shrubs at all sites significantly promoting plant biomass (RII = 0.45 ± 0.29) and plant richness in particular at the low site (RII = 0.33 ± 0.14, 0.21 ± 0.12 and 0.14 ± 0.10 at the low, intermediate and high site respectively, see Table S1 for statistical results). On the contrary, *Thymus* shrubs had negative effects on plant biomass (RII = −0.51 ± 0.34) and plant richness in particular at the high site (RII = −0.14 ± 0.24, −0.11 ± 0.17 and −0.50 ± 0.09 at the low, intermediate and high site respectively).

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