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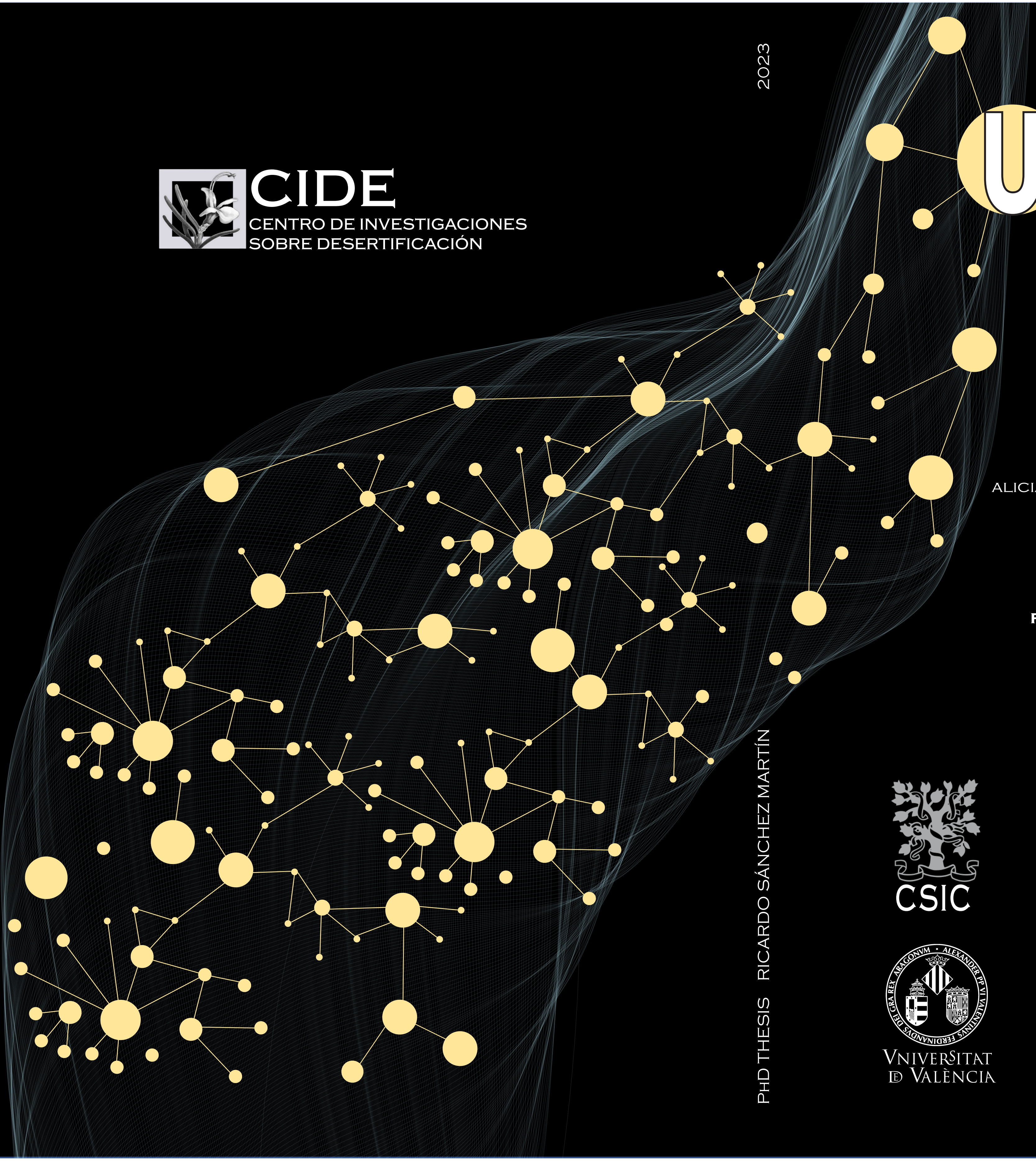

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PhD THESIS   RICARDO SÁNCHEZ MARTÍN

UNVEILING SPECIES ASSEMBLAGE RULES IN GYPSUM PLANT COMMUNITIES

RICARDO SÁNCHEZ MARTÍN

PHD THESIS

SUPERVISED BY:

ALICIA MONTESINOS NAVARRO AND MIGUEL VERDÚ DEL CAMPO

ACADEMIC ADVISOR:

MANUEL SERRA GALINDO

PROGRAMA DE DOCTORADO EN BIODIVERSIDAD Y BIOLOGÍA EVOLUTIVA

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Unveiling species assemblage rules in gypsum plant communities

TESIS DOCTORAL

Ricardo Sánchez Martín

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Dirigida por: Dra. Alicia Montesinos Navarro y Dr. Miguel Verdú del Campo

Tutor: Dr. Manuel Serra Galindo

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Los doctores Alicia Montesinos Navarro y Miguel Verdú del Campo del Departamento de Ecología y Cambio Global del Centro de Investigaciones sobre Desertificación (CIDE-CSIC-UV-GV)

CERTIFICAN:

Que los trabajos de investigación desarrollados en la memoria de la tesis doctoral “Unveiling species assemblage rules in gypsum plant communities”, han sido realizados bajo su supervisión y son aptos para ser presentados por Ricardo Sánchez Martín ante el tribunal que en su día se consigne, para aspirar al grado de doctor en el programa de doctorado en Biodiversidad y Biología Evolutiva de la Universidad de Valencia.

En Valencia, a 20 de febrero de 2023

Fdo. Directora de tesis
Alicia Montesinos Navarro

Fdo. Director de tesis
Miguel Verdú del Campo

Fdo. Tutor
Manuel Serra Galindo

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Coauthors' affiliations

Alicia Montesinos Navarro, Miguel Verdú del Campo: Centro de Investigaciones Sobre Desertificación (CIDE, CSIC-UV-GV), Moncada, Valencia, Spain.

José I Querejeta, Iván Prieto: Cetro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), Murcia, Spain.

Jordi Voltas: Departamento de Producción Vegetal y Ciencia Forestal (Universitat de Lleida), Lleida, Spain.

Juan Pedro Ferrio: Aragon Agency for Research and Development (ARAID). Zaragoza, Spain.

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SUMMARY

Plant communities are not merely snapshots of species located in the same area. Instead, they are dynamic entities connected through complex biotic interactions. Understanding the interplay of processes that bind species within a community is vital for comprehending species maintenance and may assist us in predicting how communities respond to the perturbations of a changing world. This Ph.D. aims to unveil how abiotic conditions, plant strategies, and biotic interactions shape plant communities growing in stressful environments.

Plant species can thrive in stressful environments through contrasted strategies, either showing species-specific adaptive strategies or reliance on facilitative interactions with neighboring species. While some species rely on adaptive traits for overcoming specific stressful conditions, although it may imply unaffordable costs in other environments, others stress-sensitive species may show generalist strategies to deal with stressful environments and take advantage of facilitative interactions with neighboring species. Facilitation occurs when the presence of one species enhances the establishment of another, and as with any interaction, its outcomes vary over time due to changes in the environment, the ontogenetic state of the involved plants, or the intervention of third species through indirect interactions.

Therefore, the maintenance of stress-sensitive species, or the co-occurrence of adult plants in general, should provide long-term benefits for both of them or a more equitable performance when they grow associated. For plant species that rely on facilitation, their resilience to perturbations may be affected by their dependence on specific nurses (i.e., species from which they benefit). Here, the more alternative nurses they have, the more likely they will switch nurses and thus survive (i.e., facilitation rewiring). Here, we assess different processes shaping plant communities in stressful environments by exploring (a) contrasted species-specific strategies to thrive in these environments and the potential trade-offs among them, (b) potential mechanisms of long-term co-occurrence among adult plant species in these communities, and (c) the limitations to switch among nurse species in environments with contrasted stresses.

To do so, we explore plant communities in two semi-arid environments in Southeast Spain varying in stress levels. Specifically, we studied plant communities growing in stressful gypsum outcrops and their immediate surrounding of limestones, which constitute a milder environment. To address our aims, we approached the exploration of these communities from a broad perspective. We quantify species' affinity to the stressful substrate as their percentage of cover in the stressful environment relative to the milder environment. Then, we relate this affinity to several species traits and test whether species dependence on facilitation

is related to this affinity. To do so, in both communities, we quantify the tendency of each species to recruit under nurse plants (i.e., species reliance on facilitation) and whether it depends on the species' affinity for the substrate. To assess weather facilitative effects remain throughout the lifespan of the interacting species, we focused on adult individuals. We explored whether plants of two different species show a more equitable performance (i.e., size asymmetry) when they are co-occurring in multispecific patches compared to their conspecifics living in isolation. We propose this as a mechanism of long-term co-occurrence, as it may reduce the likelihood of species competitively outperforming each other. Finally, we assessed whether facilitation rewiring is constrained and which nurse traits produce such constraints in contrasted environments.

Our findings show that species living in stressful soils employ various coping strategies to face such conditions. On the one hand, species with high gypsum affinity showed traits that allowed them to overcome stressful gypsum constraints. However, we did not find collateral costs to their success in other environments as a result of these adaptations. On the other hand, rare and stress-sensitive species rely on facilitation for establishing, resulting in multispecific patches where species balance their performance, enhancing their co-occurrence in the long term. Finally, we explore general patterns in facilitation rewiring. Nurses’ traits can shape facilitative interactions, limiting the rewiring capacity to nurse species harboring similar

traits. Nurses' species selection varied between environments. Specifically, we found that in gypsum environments, facilitation rewiring was constrained to nurse species with gypsum-specialized traits, while in milder environments, rewiring occurs with nurse species that are close relatives of the preferred nurses. Altogether, these results show that functional or phylogenetic species redundancy is key to ensuring the survival of species requiring facilitation, thus contributing to the resilience of the entire community.

Keywords: Contrasting stress, facilitation, gypsum, gypsum affinity, limestone, neighborhood, nurses, phylogeny, Plant communities, rewiring.

GENERAL INTRODUCTION AND AIMS

Background

In nature, all species face environmental pressures that determine how they struggle to survive (Darwin, 1859). The survival capacity of each species would be strongly determined by how they perceive and respond to their abiotic environments (Lewis et al., 2017). Moreover, species are not solitary players since no single species exists in isolation (Vizentin-Bugoni, Debastiani, Bastazini, Maruyama, & Sperry, 2020). Instead, they are bound in a web of interactions with other species upon which they rely to varying degrees (Vizentin-Bugoni et al., 2020). These interspecific interactions, which act synergistically with abiotic factors (Lewis et al., 2017), shape the diversity of communities through negative, positive, or complex higher-order interactions (Bairey, Kelsic, & Kishony, 2016; Bruno, Stachowicz, & Bertness, 2003; Bulleri, Bruno, Silliman, & Stachowicz, 2016; Chesson, 2000; Hirn et al., 2022). Understanding the underlying processes entangling species in manifold interactions can be vital to assessing the factors favoring diversity maintenance and how they could respond to environmental changes (Valladares, Bastias, Godoy, Granda, & Escudero, 2015), a critical aspect considering the current diversity loss crisis (Ceballos et al., 2015; Cowie, Bouchet, & Fontaine, 2022; Kolbert, 2014).

Plant communities usually harbor species that face environmental constraints differently (Corlett & Tomlinson, 2020; Valiente-Banuet, Rumebe, Verdu, & Callaway, 2006). While some species have specific adaptations to deal with environmental stressors (Corlett & Tomlinson, 2020), others have not, but may still survive in the system by taking advantage of biotic interactions with neighboring species (Baraza et al., 2006; Bertness & Callaway, 1994; Callaway, 2007; Schlau, Huxman, Mooney, & Pratt, 2021). Therefore, the success of each species will depend on the species' traits and their relationship with the abiotic and biotic conditions. In this scenario, the co-occurrence of stress-adapted and stress-sensitive species can result from functional segregation (Navarro-Cano, Goberna, Valiente-Banuet, & Verdú, 2021; Schöb, Macek, Pistón, Kikvidze, & Pugnaire, 2017; Verdú, Gómez, Valiente-Banuet, & Schöb, 2021). The segregation of functional traits can reduce interspecific competition, thus contributing to community diversity.

However, both showing an adaptive-strategy, or relying on other species to survive, can lead to different costs. On the one hand, the adaptations that allow species to thrive under stressful circumstances can undermine their success outside their optimum habitat (Corlett & Tomlinson, 2020; Futuyma & Moreno, 1988; Palacio et al., 2007; Rajakaruna, 2018). For instance, many adapted species growing in stressful soils are restricted to these environments and cannot migrate to other surrounding environments (Escudero, Palacio, Maestre, & Luzuriaga, 2015; Sianta & Kay, 2019). This may be critical for maintaining diversity if these species get locally extinct, as their recolonization from neighboring unsuitable habitats is strongly undermined (Corlett, 2020; Corlett & Tomlinson, 2020). On the other hand, poorly adapted species follow a more opportunistic strategy as they can quickly recolonize from adjacent sources (Büchi & Vuilleumier, 2014). However, they thrive in a stressful ecological context, so they will likely rely on others to survive (Graff & Aguiar, 2017; Liancourt, Le Bagousse-Pinguet, Rixen, & Dolezal, 2017). This reliance is based on an intricate and frequently unpredictable interplay between abiotic and biotic factors (Poisot, Stouffer, & Gravel, 2015), which could jeopardize species' persistence.

The traditional view of species as resource consumers has resulted in the perception that competition is the predominant force in plant species’ interactions (Brooker et al., 2008; McIntire & Fajardo, 2014). Although this view is valid, it neglects the fact that some species are also habitat modifiers (hereafter nurses) that can ameliorate the surrounding conditions in ways that increase the success of other species less adapted to the original conditions (hereafter facilitation; Bronstein, 2009; McIntire & Fajardo, 2014). Facilitation may help biodiversity maintenance by generating a myriad of different microhabitats that can relax the environmental filters for multiple species that find a window of opportunity for survival in unsuitable environments (Brooker et al., 2008; Bruno et al., 2003; Soliveres, Maestre, Berdugo, & Allan, 2015). Indeed, there is evidence of many ecosystems in which positive interactions operate across different biomes, from desert to polar zones (Brooker et al., 2008; Callaway, 2007; Callaway & Walker, 1997; He, Bertness, & Altieri, 2013; Holmgren & Scheffer, 2010; McIntire & Fajardo, 2014; Soliveres, Smit, & Maestre, 2015).

Facilitative interactions between two species depend on the balance between the interacting species' traits and the environment (Navarro-Cano et al., 2021; Poisot et al., 2015). Facilitation occurs when the favorable environmental conditions provided by nurse species overcome the adverse effects of interspecific competition (Schlau et al., 2021). This reduction of interspecific competition is favored when interacting species segregate their functional traits to acquire resources (Navarro-Cano, Goberna, & Verdú, 2019; Valiente-Banuet & Verdú, 2013). Furthermore, according to the stress gradient hypothesis, facilitation will predominate as environmental stress increases (Bertness & Callaway, 1994; Liancourt et al., 2017; Maestre, Callaway, Valladares, & Lortie, 2009). However, under extremely stressful conditions, facilitative interactions tend to disappear (Maestre & Cortina, 2004; Michalet et al., 2006; Tielbörger & Kadmon, 2000).

Traditionally, the role of facilitation in structuring diversity has been viewed through the lens of environmental improvements that promote the establishment of stress-sensitive species (Liancourt, Callaway, & Michalet, 2005), and later on, the co-occurrence of adult plants of functionally different species due to low competition among them (Butterfield & Briggs, 2011; Cavieres & Badano, 2009). However, other less-studied mechanisms beyond competition avoidance can also enhance the long-term co-occurrence of species as adult plants, increasing the potential of facilitation in preserving biodiversity. In this line, there is empirical evidence of resource sharing among plants, suggesting that positive effects among plants can persist beyond the establishment. For example, facilitative interactions can be mediated by nitrogen or water transfer between adult plants of different species (Montesinos-Navarro, Verdú, Querejeta, Sortibrán, & Valiente-Banuet, 2016; Montesinos-Navarro, Verdú, Querejeta, & Valiente-Banuet, 2017, 2019), or the sharing of beneficial mycorrhizae (Sortibrán, Verdú, & Valiente-Banuet, 2019). The aforementioned mechanisms may result in a more equitable use of resources among plant species, favoring their coexistence in natural communities; This can be a potential role of facilitation that has been unnoticed so far.

Interspecific interactions of different signs and intensities link species within communities, giving rise to structures with ecological implications (i.e., networks; Bascompte, 2007, 2010; CaraDonna et al., 2017). Interspecific interactions are not static but dynamic components within ecological networks that vary in time and space (CaraDonna et al., 2021; Poisot et al., 2015; Trøjelsgaard & Olesen, 2016; Tylianakis & Morris, 2017). These changes can be especially critical in facilitation-driven communities, since the disappearance of positive interactions can be critical for facilitated species' survival (Jordano & Vespucio, 2016).

The lability of interactions provides facilitated species the advantage of rewiring with different nurse species, increasing the robustness of facilitation networks. However, the rewiring of facilitative interactions can be limited since certain species interactions are unlikely, and also since there is some evidence of general patterns in these reconnections, such as a tendency to reconnect with species closely related to the preferred interacting species (Montesinos-Navarro, Díaz, Torres, Caravaca, & Roldán, 2019; Raimundo, Guimarães, & Evans, 2018). The magnitude of this rewiring can be critical for the maintenance of plant species highly dependent on facilitation. Despite its importance in predicting how communities may respond to environmental changes, rewiring patterns and how they vary in different environments are not fully understood.

Thesis justification and general aims

Understanding the principles governing plant communities' assembly is essential to predict how environmental changes could impact species diversity. The diversity of a community depends on abiotic variables, how species respond to them, and the biotic interactions occurring within the community (Lewis et al., 2017). Biological interactions are crucial to maintaining diversity, as their loss can have perverse effects on the diversity of the entire community, resulting in the unleashing of co-extinction cascades (Janzen, 1974; Valiente-Banuet et al., 2015). Despite the advances in this area, the puzzle remains incomplete, limiting our ability to assess how multiple species survive in natural communities, particularly in stressful environments with many stress-sensitive species (Rajakaruna, 2018).

This thesis project explores the assembly of plant species in two environments with contrasted stress levels to unveil how the interplay between abiotic conditions, plant traits, and biotic interactions shapes the communities of these systems. Specifically, we aim to explore (a) the distinct strategies for thriving in these environments and possible trade-offs of investing in each of them, (b) the potential mechanisms for long-term co-occurrence among adult plant species in these communities, and (c) the constraints on reconnecting to distinct nurse species in these two environments. We expect that species with specific traits for overcoming adverse abiotic factors will be able to thrive in these stressful environments, whereas stress-sensitive species will depend on other species to thrive. We further explore the mechanisms by which these species associations may persist over the long term (i.e., into the adulthood of both species). One of them is that a more equitable distribution of resources among the interacting species may result in more similar performances among plant species when they grow together than when they grow in isolation. Finally, we look for general patterns in the rewiring of facilitation networks, detecting constraints to reconnect to different nurse species in each environment.

Specific aims

Chapter 1 assesses whether co-occurring species display different morphophysiological strategies to survive in gypsum soils. Gypsum-specialized traits may give the advantage of avoiding abiotic stress. However, harboring these traits may come at a cost for survival in other environments if it implies the lack of critical traits for species' survival in other stressful conditions not related to gypsum soils. Conversely, stress-sensitive species, those without gypsum-specialized traits, may be subjected to all the rigors of these stressful conditions, severely limiting their chances of survival unless they can benefit from growing associated with species with gypsum-specialized traits.

In this regard, in Chapter 2, we investigated how biotic interactions with neighboring species can enhance the presence of stress-sensitive species. Specifically, we assessed whether stress-sensitive species, which also tend to be rare species, benefit more from facilitation than species possessing gypsum-specialized traits, which tend to be more abundant. Then, we checked if individuals growing together in interspecific patches showed a more equitable performance than individuals growing isolated, a potential mechanism of species coexistence that could support diversity in these environments.

Finally, in chapter 3, we explored potential functional and phylogenetic constraints in the selection of nurse species. Biotic interactions are dynamic and can vary in space and time due to alterations in the environments (both abiotic and biotic). Therefore, we assessed how the environment determines the flexibility of facilitative interactions by analyzing the ability of recruit species to switch nurses. At the same time, we identified the patterns limiting this rewiring capacity and their relation with the environmental context. An approach that may help us identify which species traits are critical for realizing facilitative interactions and may have profound implications for the stability of the whole community under changing scenarios.

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GENERAL METHODS

Although each chapter describes the method section fully, we provide insight into common elements emphasizing the study site selection and the main variables and methods used across chapters.

Study sites

This thesis has been conducted in plant communities developed over gypsum outcrops and their immediate surrounding limestone environments. Here, plant communities are characterized by sparse vegetation, generally grouped in multispecific patches, mainly composed of camephytes and small shrubs where facilitative interactions domain (Delalandre & Montesinos-Navarro, 2018; Montesinos-Navarro, Storer, & Perez-Barrales, 2019). Our system constitutes an environmental mosaic with contrasting diversity and stress levels. Specifically, the three chapters were developed on four sites of 2 ha each, located along the Vinalopó valley in the semi-arid southeast of the Iberian Peninsula. We chose two contiguous 1 ha subsites within each site, one within a gypsum outcrop (stressful subsite hereafter) and one in the surrounding non-gypsum lithology (limestones in our case; mild subsite hereafter). The four sites have their stressful and mild subsites separated by a few meters to minimize changes in climatic conditions in each pair.

The reasons why the selected environments constitute an ideal scenario for assessing our aims are three-folded: Firstly, we studied plant communities growing on two contrasting stress levels (mild and stressful subsites). Both subsites face similar climatic stress derived from a semi-arid climate. In all subsites, the species are subjected to a severe drought, accentuated in the summer months (June- September). The rainy periods are scarce and irregular during the rest of the year (365.9±97.7mm). This regional stress is maximized within the gypsum outcrops (i.e., stressful subsite) since plants must face additional stress derived from the gypsum structure and chemical composition ($CaSO_4 \cdot 2H_2O$). On the one hand, gypsum physical structure with hard and even crystallized layers displaying a low porosity and high aggregation and instability represents a challenge for the establishment and root development of the plants growing under this environmental context (Figure 1; Bridges & Burnham, 1980; Escudero, Palacio, Maestre, & Luzuriaga, 2015; Guerrero Campo, Alberto, Hodgson, García Ruiz, & Montserrat Martí, 1999). On the other hand, Ca and S excess have been proved to interfere with other macronutrient acquisition or become toxic for plants (Duvigneaud & Denaeyer-de Smet, 1966; Guerrero-Campo, Alberto, Maestro, Hodgson, & Montserrat-Martí, 1999; Romão & Escudero, 2005; Ruiz, López-Cantarero, Rivero, & Romero, 2003).

Figure 1. *Helianthemum syriacum* plant growing surrounded by gypsum crystals.

Secondly, the harshness of the abiotic conditions in gypsum favors the cohabitation of species possessing traits to cope with the particular stress (i.e., gypsum-adapted species) and other species with no specific strategies to deal with gypsum stress (i.e., stress-sensitive species). Gypsum-adapted species present traits related to avoiding chemical stress (Palacio et al., 2014) and overcoming soil physical resistance (Bridges & Burnham, 1980; Palacio, Azorín, Montserrat-Martí, & Ferrio, 2014; Romão & Escudero, 2005). These adaptations may constrain the ecological niche of these species, limiting them to a gypsum-exclusive existence (i.e., gypsum-specialists species) (Damschen, Harrison, Ackerly, Fernandez-Going, & Anacker, 2012; Escudero et al., 2015; Mota, Sánchez-Gómez, & Guirado, 2011).

Thirdly, gypsum habitats occur as isolated outcrops separated by a matrix of different lithology, mainly limestone. These archipelagoes of habitat islands may favor that lineages with adaptative traits show low dispersal capacity (Escudero et al., 2015; Isnard, L'huillier, Rigault, & Jaffré, 2016; Silveira et al., 2019; Spasojevic, Damschen, & Harrison, 2014) since plants investing in long-distance dispersal could reduce their performance if seeds go out of the boundaries of their optimum habitat (Schenk, 2013). Due to the difficulty of dispersal throughout the non-gypsum surrounding environments, the colonization of new sites or recolonization of sites where populations have been locally extinct is unlikely, resulting in local extinctions (Corlett & Tomlinson, 2020). This scenario would be critical for those species that rely on a low number of nurse species, which can become locally extinct unless they can rewire with other nurse species, mitigating co-extinction cascades.

Sampling design

Our sampling design comprised 80 (150x150 cm) plots in each subsite, except for one site where the mild subsite had 79 plots. We distributed the plots randomly to occupy the 1 ha entirely, avoiding places presenting difficult access. We sampled the four localities between April 2019 and February 2020.

Firstly, we identified all adult plants inside each plot and measured their cover. We also identified whether each plant grew alone (i.e., non-associated) or in a vegetation patch with other individuals (i.e., associated). We also estimated the cover of each patch. Then, we calculated the bare ground cover by subtracting the surface occupied by patches and non-associated plants from the sampled area. Secondly, we identified all recruits within the plots and identified whether they were recruiting on bare ground or beneath an adult plant, identifying the species of the adult plant. We considered as recruits all plants ranging from seedlings (with at least the first two leaves) to tiny plants in comparison with adults (<15% in height compared to adults), showing neither signs of reproductive structures nor lignification at the stem base.

Defining community species properties

To characterize the species in our communities, we calculated the following metrics.

The gypsum affinity index

We calculated the "gypsum affinity index" that determines the degree of affinity of each species for this substrate. Gypsum affinity (g) is the proportion of species' cover found in the gypsum subsite divided by the sum of cover in that site (gypsum-subsite and non-gypsum subsite), only considering the cover of plants growing alone in both subsites to reduce the effect of plant-plant interactions on the metric:

$$g = \frac{Cs}{Cs + Cm}$$

Where Cs is the species' cover in the stressful gypsum subsite and Cm in the mild non-gypsum subsite, usually with a limestone lithology. Gypsum affinity is a continuous variable with a range [0, 1] measuring the species' cover in the gypsum subsite compared with the surrounding non-gypsum environments. Therefore, g= 0.5 represent an equal species' cover within and outside gypsum. Lower values ($g<0.5$) will be associated with species with a reduced cover in the gypsum subsite compared to the non-gypsum subsite. In contrast, species with higher values ($g>0.5$) show a higher cover within the gypsum subsite, being values of 1, the gypsum specialists restricted to the gypsum soil.

Facilitation: The relative interaction intensity index (RII)

We evaluated the intensity of the interactions between species in the studied communities. To do so, we used slight variations of the relative interaction intensity index (RII hereafter) defined in Armas, Ordiales, & Pugnaire (2004):

$$RII = \frac{k_a - k_{na}}{k_a + k_{na}}$$

The signs and intensity of the interaction between two plants can be deduced by comparing the survival, growth or reproduction of plants growing associated with others (k_a) vs. non-associated (K_{na}), thus growing isolated. RII gives symmetric values around zero and with defined limits [-1,1], with positive values suggesting that growing associated with another plant increases the plant performance (i.e., facilitation) and negative values indicating that it reduces it (i.e., competition; Armas et al., 2004). In this Ph.D. thesis, recruitment density (i.e., number of recruits per cm^2 of nurse species or bare soil) was used as a proxy for performance (i.e., k in the formula). The RII index can be used to assess interactions at different levels. For instance, In Chapter 2, we calculate RII by comparing recruits' density below vegetation (adding up the cover of all species) vs. bare ground, while in chapter 3, we focused on each interspecific interaction, calculating the RII for each pairwise interaction of recruit and nurse species, i.e., we compared the recruit density of a species k growing beneath a specific nurse j vs. recruit density of k in the bare ground.

Exploring facilitation rewiring

We address the factors limiting facilitation rewiring by assessing how beneficiary species shift their nurse species depending on the environment and the species traits, using a space-for-time substitution design. Gypsum outcrops provide the perfect arena to assess facilitative rewiring since they harbor plant communities with similar species composition. Each system may have its own environmental idiosyncrasy that would affect the realization of facilitative interactions. In these systems, we identified those facilitative interactions that tend to occur when the two interactants are present (i.e., preferred interactions) and those which are not usually realized, even when the two species are present (i.e., non-preferred interactions). As a result, we obtained the pool of preferred and non-preferred nurses for each facilitated species. Then, we compare the functional traits of no-preferred and preferred nurses, or their phylogenetic distances, with null models that re-define non-preferred nurses by allowing a random rewiring with all adult species in the community. That assists us in identifying non-random patterns in the facilitative reconnections and whether they vary in different environments.

Phylogenetic relationships

Species share an evolutionary history to a greater or lesser degree (Revell, 2010), so related species tend to resemble each other and share similar traits or responses to coping with their environmental context (Pausas & Verdú, 2010). To consider phylogenetic relationships between species in the patterns of species' responses to environmental stress, we built the phylogenetic tree of the species found in the study sites by pruning our target species from the mega tree "GBOTB.extended.tre". This mega tree includes 74,533 species, resulting from combining two previously published mega trees (Smith & Brown, 2018; Zanne et al., 2014), whose branch lengths indicate the chronological time (millions of years). Species not matched with the mega tree were inserted following the criteria established in the phylomaker package (Jin & Qian, 2019; Qian & Jin, 2016). Then, we employed the phylogenetic distances between species to inform the analyses carried out in Chapters 1 and 2 to discern whether more closely related species tend to exhibit similar strategies of response to gypsum-imposed stress. Finally, in chapter 3, we used this information to test whether facilitated species tend to reconnect with nurse species that are close relatives to their preferred nurses.

Statistical analyses

In Chapter 1, we used a multivariate approach to identify sets of traits that may be related to overcoming gypsum-specific stressors, while other traits may be related to responses to more general stresses. Then, using phylogenetically informed mixed models, we examined whether the strategies defined by the multivariate analyses relate to species' gypsum affinity. In chapter 2, we used a Bayesian phylogenetically informed mixed model to examine the relationship between recruits' gypsum affinity, species' relative abundance, and their reliance on facilitation. We also test whether size asymmetries among species in the community are reduced when they are associated with neighbors by comparing the observed size differences with the random expectation generated by a null model. Finally, in Chapter 3, we built facilitation networks for the studied communities and quantified their dissimilarity between locations with different stress levels. Furthermore, we investigated the patterns limiting facilitative reconnections using null models based on simulations to unveil whether phylogeny or gypsum affinity explains reconnection patterns with non-preferred nurse species.

Table 1 lists the tools used by chapter; all the details and other additional analyses can be found in each chapter. All the statistical analyses were conducted with R studio (R Core Team, 2019).

Table.1

Chapter	Analysis	Function	R Package
1	Phylogenetically informed principal component analysis	phyl.pca	phytools /0.7.47 (Revell, 2012)
	Phylogenetic generalized least square models (PGLS)	gls	nlme /3.1.147 (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2019)
2	Markov Chain Monte Carlo Generalised Linear Mixed Model	MCMCglmm	MCMCglmm/ 2.29 (Hadfield, 2010)
	Null models based on simulations	sample	Stats/3.6.2 (R Core Team, 2019)
3	Analysis of partition of interaction dissimilarity (species turnover, rewiring)	Network-betadiversity	betalink/2.2.1 (Poisot, 2016)
	Generalized linear models	glm	Stats/3.6.2 (R Core Team, 2019)
	Null models based on simulations	sample	Stats/3.6.2 (R Core Team, 2019)

Table 1. Summary of the primary statistical analyses used by chapter. This table only includes the statistical analyses used to obtain the main results. All analyses are detailed in the corresponding chapter.

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CHAPTER 1

Plant's gypsum affinity shapes responses to specific edaphic constraints without limiting responses to other general constraints

"Though the organisms may claim our primary interest, when we are trying to think fundamentally, we cannot separate them from their special environments, with which they form one physical system."

A. G. Tansley (1935)

Plant's gypsum affinity shapes responses to specific edaphic constraints without limiting responses to other general constraints

Ricardo Sánchez-Martín*[1], José I. Querejeta[2], Jordi Voltas[3,4], Juan Pedro Ferrio[5,6], Iván Prieto[2], Miguel Verdú[1] and Alicia Montesinos-Navarro[1].

Authors affiliation:

[1] Centro de Investigaciones Sobre Desertificación (CIDE, CSIC-UV-GV), Carretera de Moncada-Náquera Km 4.5, 46113, Moncada, Valencia, Spain.

[2]Departamento de Conservación de Suelos y Agua, Centro de Edafología y Biología Aplicada del Segura – Consejo Superior de Investigaciones Científicas (CEBAS-CSIC), Murcia (Spain).

[3]Joint Research Unit CTFC - AGROTECNIO, Av. Alcalde Rovira Roure 191, E-25198 Lleida, Spain.

[4]Departamento de Producción Vegetal y Ciencia Forestal, Universitat de Lleida, Av. Alcalde Rovira Roure 191, E-25198 Lleida, Spain.

[5] Aragon Agency for research and development (ARAID), E-50018 Zaragoza, Spain.

[6] Department of Forest Resources, Agrifood Research and Technology Centre of Aragon (CITA). Avda. Montañana 930, 50059, Zaragoza, Spain.

Abstract

Harsh edaphic environments harbor species with different soil affinities. Plants' responses to specific edaphic constraints may be compromised against responses to prevalent stresses shared with other semiarid environments. We expect that species with high edaphic affinity may show traits to overcome harsh soil properties, while species with low affinity may respond to environmental constraints shared with arid environments.

To do so, we quantified the edaphic affinity of 12 plant species co-occurring in gypsum outcrops and measured traits related to plant responses to specific gypsum constraints (rooting and water uptake depth, foliar accumulation of Ca, S and Mg), and traits related to common constraints of arid environments (water use efficiency, macronutrients foliar content).

Our results showed that species in gypsum outcrops differed in their strategies to face edaphic limitations. A phylogenetic-informed PCA segregated species based on their foliar Ca and S accumulation and greater water uptake depths associated with plant responses to specific gypsum limitations. Species' gypsum affinity explained this segregation, but traits related to water or nutrient use efficiency did not contribute substantially to this axis.

In conclusion, plants' specializations to respond to specific edaphic constraints of gypsum soils do not limit their ability to deal with other non-specific environmental constraints.

Keywords: Gypsum affinity, Niche segregation, Nutrients, Stable isotopes, Trade-off, Water source

CHAPTER 1

Introduction

Harsh edaphic environments can be limiting for many organisms. As a result, the plant communities inhabiting these soils are characterized by sparse coverage and low biomass compared to those growing on more fertile soils in neighboring areas (Damschen, Harrison, Ackerly, Fernandez-Going, & Anacker, 2012; Escudero, Palacio, Maestre, & Luzuriaga, 2015). Some plants living on stressful soils often have mechanisms to tolerate the toxicity imposed by certain elements (Moore, Mota, Douglas, Olvera, & Ochoterena, 2014), but other less stress-tolerant species can also colonize these environments without such specific mechanisms. This might result in differentiated strategies to deal with the harsh edaphic constraints for plant life found in these environments, potentially enhancing species coexistence and richness (Escudero et al., 2015; Palacio et al., 2007).

Plants adapted to harsh soils can be classified as edaphic endemics (hereafter specialists) or non-endemics (hereafter generalists). Specialists tend to show narrow edaphic tolerances, which restrict their ecological niche, while generalists have broader edaphic tolerances that allow them to survive in a wider array of soil types (Büchi & Vuilleumier, 2014). It is commonly assumed that specialists have adapted to, and perform better, in environments with particularly stressful characteristics for plant growth than in other habitats (Futuyma & Moreno, 1988; Jasmin & Kassen, 2007; Levins, 1968). However, some generalists can also thrive in these harsh habitats following an opportunistic strategy favored by environmental heterogeneity in space and time (Büchi & Vuilleumier, 2014; Futuyma & Moreno, 1988). Indeed, the coexistence of edaphic specialists and generalists is widely observed in harsh edaphic environments such as those derived from gypsum (Escudero et al., 2015; Moore et al., 2014), serpentine (Sianta & Kay, 2019), granite (Murdy, 1968) or dolomite (Mota et al., 2008).

Gypsum soils occupy over 100 million hectares worldwide (Verheye & Boyadgiev, 1997). Gypsum ecosystems are mostly found in arid and semi-arid regions (Parsons, 1976), limiting the establishment and survival of many plant species. Besides, gypsum also imposes other more specific edaphic stresses on plants, arising from its physicochemical properties. On the one hand, the low soil water and macronutrient (N, P, K) availability can be considered a common limitation that gypsum soils share with many other dryland environments. On the other hand, some of the particularly adverse physical limitations imposed by gypsum soils are the presence of a hard physical crust that limits plant establishment (Escudero et al., 2015) and its mechanical instability, high aggregation and low porosity (Bridges & Burnham, 1980; Guerrero Campo, Alberto, Hodgson, García Ruiz, & Montserrat Martí, 1999). These properties make gypsum a limiting substrate for vertical root penetration and development (Guerrero-Campo, Alberto, Maestro, Hodgson, & Montserrat-Martí, 1999; Moore et al., 2014). Another adverse property of gypsum derives from its chemical composition ($CaSO_4 \cdot 2H_2O$), which generates an excess of Ca and S in the soil solution that can be detrimental for plant growth (Escudero et al., 2015; Romão & Escudero, 2005). An excess of Ca in soil interferes with the uptake of other essential nutrients by plants due to Ca exchange with other soil ions (Guerrero-Campo et al., 1999), whereas S excess can be toxic for plants (Duvigneaud & Denaeyer-de Smet, 1966; Ruiz, López-Cantarero, Rivero, & Romero, 2003).

In gypsum ecosystems, species with different degrees of gypsum affinity or specialization co-occur within the same plant community. These range from specialists only found on gypsum (gypsophytes) to a wide variety of generalists that can thrive on gypsum but also on other lithologies (gypsovags). Plants living on gypsum exhibit different survival strategies that may respond to some of the harshest constraints of gypsum (e.g., high Ca and S concentrations or a hard-physical crust, high aggregation, presence of pure gypsum crystals and low porosity), or to other more general constraints shared with many arid ecosystems (e.g., low fertility and water availability). On the one hand, plant responses to deal with specific gypsum limitations could be related to facing chemical toxicity and soil physical resistance against root penetration and growth. An avoidance strategy to prevent chemical toxicity is the accumulation of Ca and S in plant tissues in response to their high concentrations (Palacio, Aitkenhead, et al., 2014; Ruiz et al., 2003). On the other hand, plants capable of overcoming rooting difficulties gain access to deeper soil layers with usually greater water storage during drought periods and lower inter-plant competition (Ryel, Ivans, Peek, & Leffler, 2008). Plants living on gypsum can also show strategies to respond to other more common limitations, which could also be beneficial in other nutrient-poor and dry environments, such as an efficient nutrient acquisition or efficient water use.

Trade-offs among plant traits may emerge due to physiological constraints that limit the functional diversity of plant species. Trade-offs have been reported, for instance, between rooting depth, transpiration and water use efficiency (Brooks, Flanagan, Buchmann, & Ehleringer, 1997; Moreno-Gutiérrez, Dawson, Nicolás, & Querejeta, 2012). Plants living on gypsum may develop contrasting but equally successful strategies to cope with the stressful conditions imposed by the soils’ physicochemical properties. Therefore, plants that safely accumulate excess ions (Ca and S), avoiding toxicity, might show a reduced ability to assimilate other essential nutrients such as N, P or K (Marschner, 2012).

This study assesses whether there are trade-offs among traits so that a plant’s investment to face specific edaphic constraints is compromised against dealing with more prevalent stresses shared with other semi-arid environments. We hypothesize that a functional specialization to deal with specific gypsum constraints (e.g., deeper rooting and water uptake depth, Ca-S-Mg accumulation) may prevent water use efficiency and nutrient acquisition (e.g., higher transpiration and lower water use efficiency, lower N-P-K and C contents) due to the expected trade-off between the plant’s investment in strategies to face specific and general constraints in semi-arid gypsum ecosystems.

Materials and methods

Study area

We performed the study in a semi-arid Mediterranean ecosystem on gypsum soils located in the Vinalopó valley in southeastern Spain (Alicante, 38º 29' 39'' N; 0º 47' 00'' W). We selected flat areas to avoid topographical heterogeneity, demarcated within a radius of 13 km between 412 and 490 m.a.s.l. The dominant soil type was Keuper gypsum appearing abruptly in the form of intrusive outcrops, surrounded by other lithologies consisting mainly of limestone but also clay and marl. The climate is semi-arid, with an average temperature of 16ºC and a mean annual precipitation of 395 mm. Precipitation is strongly seasonal and falls mainly in spring (March-May) and autumn (September-November), with very low, or absent, precipitation in summer (June-August).

Evaluation of gypsum affinity and experimental design

We focused on 12 plant species commonly found on gypsum outcrops with a varying degree of gypsum affinity, including a wide phylogenetic diversity (Families in Table 1). For measuring gypsum affinity (i.e., gypsophily), we selected four localities in the same region where the boundary between the gypsum soil and the surrounding lithology (hereafter non-gypsum) was clearly demarcated. In each locality, we selected two contiguous subareas of approximately 1 ha, one within gypsum soil and another in non-gypsum soil (mainly limestone). Both types of substrates were closely located (< 100 m) in the four localities, sharing similar climatic conditions. We selected gypsum and non-gypsum areas to be as similar as possible in topography, avoiding areas with steep slopes. Sampling comprised 80 plots (150 × 150 cm) in each locality, except in one non-gypsum locality with 79 plots. The plots were semi-randomly distributed to occupy the 1 ha extension. The localities were sampled in four days periods twice per month between April 2019 and February 2020. Inside each plot, we identified all adult plants of the 12 target species (11,453 individuals) and measured each individual´s coverage employing the ellipse equation:

$$\text{coverage} = \pi ab$$

Being (a) the semi-major diameter and (b) the semi-minor diameter. Then, separately for each location, we calculated each species' gypsum affinity (g) as the proportion of plant coverage found in gypsum as follows:

$$g = \frac{Cg}{Cg + Cn}$$

Being Cg the coverage in gypsum areas, and Cn the coverage in non-gypsum areas. The plants' coverage was estimated considering only the plants living alone, thus avoiding possible effects derived from the interactions between co-occurring plants not related to soil affinity. Gypsum affinity (g) values range from 0 to 1, where 0 indicates species found in the non-gypsum areas that never occur on gypsum, and 1 indicates gypsophytes that only occur on gypsum. Species´ gypsum affinity was determined as the mean g value for the target species in the four localities. This index gives a reliable measure of the degree of gypsum affinity for our studied community since it was estimated from in situ data. Finally, we measured traits in a total of 57 plant individuals of 12 species encompassing a wide gypsum affinity gradient (Table 1).

Species	Family	g	Ng	Nt	Height (mean±SE)
*Helianthemum squamatum**	Cistaceae	1	1954	3	17.67±4.67
*Teucrium libanitis**	Lamiaceae	1	1834	4	17.00±2.97
*Herniaria fruticosa**	Caryophyllaceae	1	345	4	6.25±0.75
*Ononis tridentata**	Fabaceae	1	8	8	44.00±8.20
Dorycnium pentaphyllum	Fabaceae	0.79	88	2	40.00±0.00
Helianthemum syriacum	Cistaceae	0.70	2473	8	10.50±0.96
Anthyllis cystisoides	Fabaceae	0.68	185	6	58.83±7.14
Thymus moroderi	Lamiaceae	0.62	510	2	4.00±0.00
Thymus vulgaris	Lamiaceae	0.25	290	4	16.00±1.08
Stipa tenacissima	Poaceae	0.22	1448	2	90.00±10.00
Fumana ericoides	Cistaceae	0.17	1684	10	25.80±3.014
Rosmarinus officinalis	Lamiaceae	0.06	634	4	38.75±13.98

Table 1. Description of studied shrub species, including gypsum affinity index (g), number of individuals of each species used to calculate g (Ng), number of individuals of each species used for traits measurement (Nt), and individual plant height (cm, mean ± SE).

Plant responses to specific and general constraints in semi-arid gypsum ecosystems

Plant water sources

Assessing rooting depth in the field can be challenging, but the analysis of xylem water's isotopic composition allows an indirect assessment of water uptake depth in woody plants (Dawson, Mambelli, Plamboeck, Templer, & Tu, 2002). In seasonally dry areas like the Mediterranean region, the intense evaporation of water from the soil surface during the hot, dry summer produces isotopic fractionation resulting in an enrichment of the heavier oxygen (^{18}O) and hydrogen (^{2}H) isotopes in topsoil water. This evaporative isotopic enrichment generates a steep gradient in soil water isotopic composition with depth, with more enriched water in shallow soil layers and progressively less enriched water with depth (Allison, Barnes, & Hughes, 1983; Smith, Jarvis, & Odongo, 1997; Teixeira, Sinclair, Schroth, & Schroth, 2003). Little isotopic fractionation occurs during plant water uptake, especially for oxygen (Brunel, Walker, & Kennett-Smith, 1995; Dawson et al., 2002; Ehleringer & Dawson, 1992), but see: (Barbeta et al., 2019; Ellsworth & Williams, 2007) so the xylem water isotopic composition matches the mean isotopic composition of the different water sources taken up by active roots along the soil profile (Teixeira et al., 2003).

We analyzed the xylem water oxygen ($\delta^{18}O$) and deuterium isotopic composition ($\delta^{2}H$) of each plant in peak summer. We harvested lignified stem samples on August 14, 2017, early in the morning (7–9 am, solar time), once the plant is photosynthetically active, but evaporative demand is low, to minimize stem water evaporation. The bark and phloem were scraped off the stems with a knife to avoid xylem water contamination with phloem water and organic compounds present in living cells and/or the bark (Ehleringer & Dawson, 1992). After cutting, samples were immediately stored in individual airtight capped crystal vials and kept refrigerated in the field in a cooler until transportation to the lab, where they were kept frozen at -80 ºC until extraction. Both xylem water extraction and stable isotope analysis of water were conducted at the Serveis Científico-Tècnics of the University of Lleida (Spain). Xylem water was extracted by cryogenic vacuum distillation (Ehleringer & Osmond, 1989; Martín-Gómez et al., 2015). Sample vials were placed in a heated silicone oil bath (110–120 °C) and connected with Ultra-Torr unions (Swagelok Co., Solon, OH, USA) to a vacuum system (ca. 10^{-2} mbar) including U-shaped water traps in series that were cooled with liquid N2. The extraction time was 90 min. Captured water was then transferred into cap-crimp 2-ml vials and stored at 4 °C until analysis. The hydrogen and oxygen isotopic composition of the extracted xylem water samples were analyzed by isotope ratio infrared spectroscopy (IRIS) on a wavelength-scanned cavity ring-down spectrometer (WS-CRDS) model L2120-i coupled to an A0211 high-precision vaporizer (Picarro Inc., Sunnyvale, CA, USA). Residual organic contaminants in distilled water can interfere with the analysis of plant samples conducted with IRIS (Martín-Gómez et al., 2015). The presence of contaminants was checked using Picarro's ChemCorrect™ post-processing software and corrected, when necessary, following Martín-Gómez et al. (2015). We expressed isotope values in δ-notation (per thousand [‰]) as follows :

$$(\delta^{2}H \text{ or } \delta^{18}O) = [(Rsample)/(Rstandard)-1] \times 1000$$

Where Rsample is the ratio ($^2H/^1H$ or $^{18}O/^{16}O$) of the less abundant (heavy) to the more abundant (light) isotope in the water sample, and R standard is the same ratio ($^2H/^1H$ or $^{18}O/^{16}O$) in standard reference water (VSMOW).

Finally, we calculated the deuterium-excess (d-excess) for each xylem water sample using the relationship proposed by Dansgaard (1964).

$$d\text{-}excess = \delta^2H - 8 \times \delta^{18}O$$

Given that d-excess is derived from the relationship between δ^2H and $\delta^{18}O$, it provides a precise measure to detect evaporative isotopic fractionation and hence, differences in soil water uptake depth among plants. Here, we assumed that low (more negative) values of d-excess imply enrichment in heavy isotopes and thus plant utilization of intensely evaporated water from shallow soil layers (Allison et al. 1983).

Plant water use efficiency

We measured foliar $\delta^{13}C$ and $\delta^{18}O$ to infer the time-integrated water use efficiency and stomatal conductance over the growing season in the studied plants. The carbon isotopic composition ($\delta^{13}C$) of the leaf is used as a time-integrated proxy for intrinsic water use efficiency. The ratio between carbon uptake and stomatal conductance, i.e., the intrinsic water use efficiency (WUEi = A/g_s), can be estimated by the carbon isotopic fractionation occurring during CO_2 diffusion between the atmosphere and the sites of carboxylation, and during carboxylation itself (Farquhar & Richards, 1984). The oxygen isotopic composition ($\delta^{18}O$) of foliar tissues provides a time-integrated measure of stomatal conductance and, thus, cumulative transpiration (Barbour, 2007; Barbour, Fischer, Sayre, & Farquhar, 2000), being the foliar $\delta^{18}O$ negatively correlated with transpiration (Farquhar, Cernusak, & Barnes, 2007). Foliar $\delta^{18}O$ is unaffected by changes in photosynthetic rates (Ramírez, Querejeta, & Bellot, 2009; Scheidegger, Saurer, Bahn, & Siegwolf, 2000), but it is affected and includes the water source isotopic signal (Barbeta & Peñuelas, 2017; Sarris, Siegwolf, & Körner, 2013). When both carbon and oxygen isotopes are considered together, it is possible to separate the independent effects of carbon fixation and stomatal conductance on water use efficiency. Finally, it is important to remark that the transpiration rate is positively correlated with water uptake (Aston & Lawlor, 1979; Cienciala, Lindroth, Čermák, Hällgren, & Kučera, 1994).

In the summer of 2015, we collected 5 g of fully developed leaves from each plant individual, which were dried at 50ºC for 3 days and ground to a fine powder. We encapsulated 4 mg of ground leaf material into tin capsules for carbon isotope analysis ($\delta^{13}C$) and 0.2 mg into silver capsules for oxygen isotope analyses ($\delta^{18}O$). Samples were analyzed at the Centre for Stable Isotope Biogeochemistry, University of California, Berkeley (USA). Leaf $\delta^{13}C$ was analyzed using an elemental analyzer (Carlo-Erba NS-

1500, Milan, Italy) coupled with an isotope ratio mass spectrometer (Isoprime100, Elementar, UK). Leaf $\delta^{13}C$ is expressed in delta notation (‰) relative to the Vienna Pee Dee Belemnite standard (V- PDB). Leaf $\delta^{18}O$ was determined using an isotope ratio mass spectrometer (IRMS, ANCA/SL elemental analyzer) coupled with a Finnigan MAT Delta PlusXL IRMS Elemental Analyzer (Finnigan MAT, Bremen, Germany). Leaf $\delta^{18}O$ is expressed in delta notation (‰) relative to the Vienna Standard Mean Ocean Water for $\delta^{18}O$. Long-term (3 + years) external precisions for $\delta^{13}C$ and $\delta^{18}O$ measurements of leaf material are 0.10 and 0.20 ‰, respectively.

Nutrient concentration in leaves

We measured the concentrations of all macronutrients, including those found in excess in gypsum (Ca, S and Mg) and those that can be limiting in gypsum and other semi-arid environments worldwide (N, P and K). We also measured the C concentration to assess differences in foliar stoichiometry due to the accumulation of certain ions. Leaves were dried at 50 °C, milled, and P, K, Ca, Mg and S concentrations were measured using inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Elemental Iris Intrepid II XDL, Franklin, MA, USA) after microwave-assisted digestion with HNO_2:H_2O_2 (4:1, v:v). Foliar C and N concentrations were measured in an ANCA/SL elemental analyzer. Nutrient concentrations were measured at the Ionomic Service of CEBAS-CSIC (Murcia, Spain).

Analyses

Phylogenetic relationships

All the statistical analyses considered the phylogenetic relationships among the studied plant species, as closely related species will tend to present similar traits and, therefore, should not be considered independent observations (Revell, 2010). We assembled the phylogenetic relationships among the studied plant species with the R function "S.PhyloMaker" (Qian & Jin, 2016), which matches a given species list (our plant community) with an expanded version of the time-calibrated angiosperm species-level mega-tree that includes more than 31,000 species with branch length representing chronological time (millions of years) (Zanne et al., 2014). Species not present in the mega-tree were randomly added to our phylogeny within their corresponding genera (scenario 3, described in Qian & Jin (2016)). Finally, taxa not present in our community were pruned from our tree.

Statistical analyses

We used a multivariate approach to assess whether different plant strategies emerged using the measured variables. For this, we carried out a phylogenetically informed principal component analysis (herein, pPCA), using all the measured variables (foliar Ca, Mg, S, N, P, K, C concentrations, d-excess of xylem water,

$\delta^{18}O_{leaf}$, and $\delta^{13}C_{leaf}$), including plant height as a variable in the p-PCA to account for possible effects derived from plant size. All variables were scaled previously to run the p-PCA with the “scale” R base function. The p-PCA was run using the R function “phyl.pca” in the R package “phytools 0.7.47” (Revell, 2012). Finally, we conducted two phylogenetic generalized least square models (PGLS) using the first-axis (PC1) and second-axis (PC2) scores from the p-PCA as the response variable and gypsum affinity (g) as the predictor. PGLS is a comparative phylogenetic method that allows testing for the relationship between gypsum affinity and species strategy (defined by the p-PCA axis), considering the expected covariance structure of residuals for a given phylogeny (our phylogenetic tree). The correlation structure was derived from a maximum likelihood estimate of Pagel’s λ (Pagel, 1997), using the “corPagel” function of the R package “ape 5.3” (Paradis, Claude, & Strimmer, 2004). The PGLS was run using the “gls” function in the R package “nlme 3.1.147” (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2019). All the analyses were performed using the statistical software R 4.0 (R Core Team, 2019).

Results

Species differed widely in traits related to water uptake depth and foliar nutrients (Table 2; phylogenetic relationships between the studied species are presented in Figure 1).

Species	Xylem			Leaf								
	δ^2H	$\delta^{18}O$	*d-excess*	$\delta^{13}C$	$\delta^{18}O$	C	N	P	K	Ca	S	Mg
*Helianthemum squamatum**	-46.12±2.45	-3.05±1.10	-21.70±6.58	-28.04±0.13	27.91±0.87	38.39±1.82	0.95±0.07	0.04±0.01	0.45±0.03	1.75±0.42	1.42±0.27	0.66±0.17
*Teucrium libanitis**	-43.80±-3.69	-3.88±0.35	-12.74±1.22	-27.72±0.20	27.56±0.98	49.59±0.56	1.11±0.06	0.03±0.01	0.63±0.02	1.03±0.11	0.27±0.05	0.21±0.05
*Herniaria fruticosa**	-35.34±5.37	-0.87±1.57	-28.39±7.34	-27.69±0.57	28.61±0.72	41.59±0.84	1.19±0.15	0.03±0.01	0.87±0.04	2.30±0.33	0.81±0.08	0.54±0.01
*Ononis tridentata**	-44.39±1.94	-5.37±0.63	-1.38±3.34	-27.41±0.45	21.99±0.31	25.52±1.05	1.03±0.11	0.04±0.01	0.37±0.14	4.52±0.64	5.06±0.34	3.00±0.33
Dorycnium pentaphyllum	-37.11±1.30	-2.02±1.20	-20.97±8.32	-28.65±0.43	28.87±1.36	44.89±0.63	1.57±0.32	0.01±0.01	0.14±0.11	1.06±0.41	0.05±0.02	0.09±0.06
Helianthemum syriacum	-34.31±1.72	0.15±0.56	-35.51±2.96	-28.92±0.21	29.08±0.41	40.53±0.28	1.00±0.07	0.03±0.01	0.41±0.07	2.43±0.06	0.66±0.04	0.29±0.03
Anthyllis cystisoides	-50.25±2.53	-4.61±0.39	-13.37±1.38	-27.87±0.46	20.42±0.46	40.77±0.78	0.89±0.08	0.04±0.01	0.87±0.22	2.32±0.33	0.37±0.07	0.52±0.13
Thymus moroderi	-40.17±1.93	-1.29±0.63	-29.81±3.10	-30.00±0.25	26.76±0.18	44.99±0.07	1.19±0.04	0.03±0.01	0.80±0.17	2.03±0.02	0.45±0.08	0.21±0.04
Thymus vulgaris	-33.72±4.53	0.12±1.28	-34.67±5.88	-27.87±0.59	30.13±0.66	46.46±0.41	1.33±0.16	0.03±0.01	0.53±0.17	1.19±0.29	0.27±0.07	0.20±0.06
Stipa tenacissima	-44.87±0.56	-2.18±0.14	-27.41±0.57	-25.74±0.97	30.68±0.77	44.95±0.89	0.85±0.06	0.02±0.01	0.25±0.01	0.30±0.03	0.08±0.01	0.06±0.01
Fumana ericoides	-40.06±2.21	-1.94±0.69	-24.70±6.58	-26.50±0.18	31.60±0.83	44.49±0.26	1.10±0.06	0.04±0.01	4.84±1.88	1.60±0.39	0.55±0.21	0.91±0.24
Rosmarinus officinalis	-39.58±4.38	-1.65±1.56	-26.36±8.11	-25.75±0.27	26.97±0.63	60.97±10.41	1.36±0.20	0.03±0.01	1.27±0.11	0.65±0.04	0.21±0.05	0.31±0.07

Table 2. Measured traits. Isotopic data include δ^2H, $\delta^{18}O$ and *d-excess* (mean ±SE) measured in xylem water, and $\delta^{13}C$ and $\delta^{18}O$ measured in leaves (mean ± SE; units in ‰). Nutrient concentrations (mean ± SE) measured in leaves are also presented (g $100g^{-1}$). * Species considered as gypsophytes.

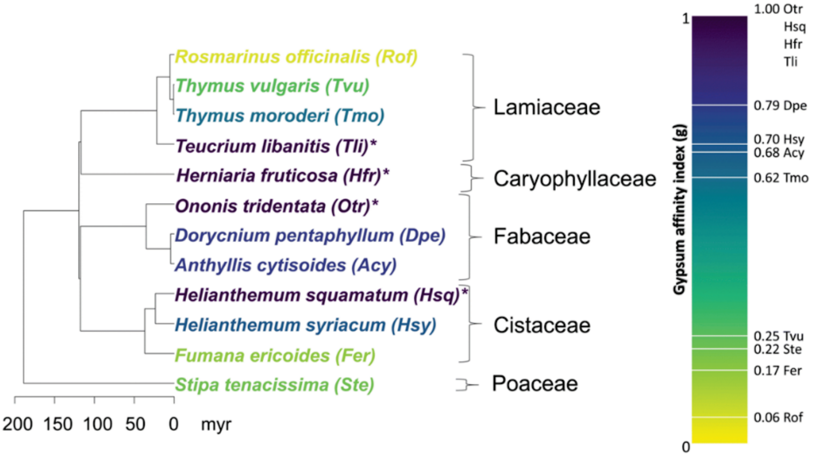

Figure 1. Phylogenetic relationships among the studied species. Species colors range from light yellow (species with low gypsum affinity) to dark purple (species with high gypsum affinity) along a gypsum affinity gradient (*g*). Species marked with an asterisk (*) are strict gypsophytes (*g*=1). Plant families appear in the figure. The units of the axis scale are millions of years (myr).

The first (PC1) and second (PC2) principal components of the p-PCA explained 43% and 21% of the total variance, respectively. Variables contributing the most to PC1 were foliar S, Mg, Ca concentrations and *d-excess* in xylem water (i.e., those specifically related to physical and chemical gypsum constraints), which showed highly negative loadings, and $\delta^{18}O_{leaf}$, foliar C and, to a lesser extent, N concentration, which exhibited highly positive loadings (Figure 2, Table 3). Other variables, such as foliar P and K concentration and $\delta^{13}C_{leaf}$ showed low absolute PC1 loadings (Figure 2, Table 3).

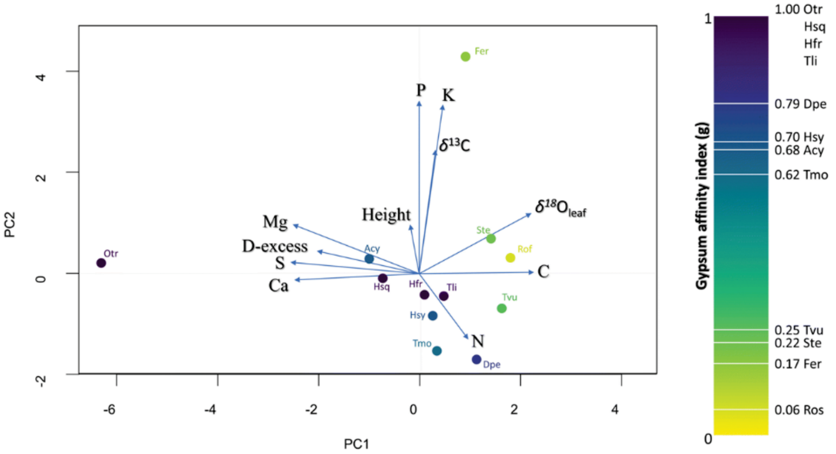

Figure 2. Biplot for the Phylogenetic principal components analysis. The first principal component (PC1) is inversely correlated with species gypsum affinity according to the PGLS analysis. Each dot represents the score value of a species. Codes: *Rosmarinus officinalis* (Rof), *Thymus vulgaris* (Tvu), *Thymus moroderi* (Tmo), *Teucrium libanitis* (Tli), *Herniaria fruticosa* (Hfr), *Ononis tridentata* (Otr), *Dorycnium pentaphyllum* (Dpe), *Anthyllis cytisoides* (Acy), *Helianthemum squamatum* (Hsq), *Helianthemum syriacum* (Hsy), *Fumana ericoides* (Fer), *Stipa tenacissima* (Ste). Dot colors range from dark purple (species with high gypsum affinity, g=1) to light yellow (species with low gypsum affinity, g=0). Arrows represent the loadings of each variable in the pPCA.

The p-PCA also showed highly positive PC2 loadings for plant height, P and K concentration, and $\delta^{13}C_{leaf}$, and a negative PC2 loading for N concentration.

Variable	PC1 (43%)	PC2 (21%)
S	-0.95	0.06
Mg	-0.93	0.26
Ca	-0.91	-0.03
d-excess	-0.76	0.12
Height	-0.07	0.24
P	0.00	0.90
$\delta^{13}C$	0.12	0.64
K	0.17	0.88
N	0.36	-0.34
$\delta^{18}O$	0.82	0.32
C	0.84	0.01

Table 3. PC1 and PC2 loadings of each measured plant variable.

The PGLS analysis showed that the species scores along the PC1 of p-PCA were significantly and negatively correlated with gypsum affinity (standardized coefficient = -2.54 ± 0.64, *F*-value = 15.80, *P*-value = 0.003) (Figure 3). Similar results were observed for individual relationships, with foliar Ca, S, Mg concentrations and *d-excess* of xylem water being positively correlated with *g*, whereas leaf $\delta^{18}O$ and foliar C concentration were negatively correlated with *g* (check Appendix 1, Table S1 for univariate responses). Results did not change substantially after excluding *O. tridentata* from the analysis (standardized coefficient = -1.90 ± 0.61, *F*-value = 10.00, *P*-value = 0.012), which indicates that the observed patterns were not exclusively driven by the extremely negative score of *O. tridentata*.

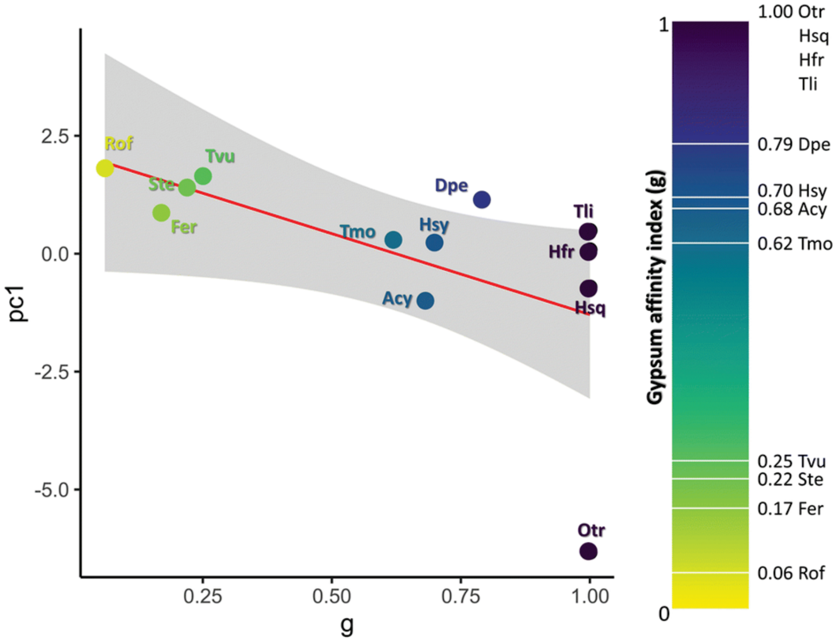

Figure 3. Regression between species scores along PC1 and gypsum affinity index (*g*). Each dot represents the mean PC1 score value and *g* of a particular species and the grey area represents the 95% CI for predictions. Codes: *Rosmarinus officinalis* (Rof), *Thymus vulgaris* (Tvu), *Thymus moroderi* (Tmo), *Teucrium libanitis* (Tli), *Herniaria fruticosa* (Hfr), *Ononis tridentata* (Otr), *Dorycnium pentaphyllum* (Dpe), *Anthyllis cytisoides* (Acy), *Helianthemum squamatum* (Hsq), *Helianthemum syriacum* (Hsy), *Fumana ericoides* (Fer), *Stipa tenacissima* (Ste). Dot colors range from dark purple (species with high gypsum affinity, *g*=1) to light yellow (species with low gypsum affinity, *g*=0).

Species with high gypsum affinity (low PC1 scores) exhibited strategies associated with traits having negative loadings, mainly related to high accumulation of Ca, Mg and S in leaves and acquisition of water from deeper soil layers. In contrast, species with low gypsum affinity (high PC1 scores) showed strategies mainly defined by low cumulative transpiration (high $\delta^{18}O_{leaf}$), high foliar C and, to a lesser extent, high N concentration. This indicates that gypsum affinity (*g* values) explained, at least in part, some of the variability along this PC1. On the contrary, we did not find a significant correlation between gypsum affinity and species scores along PC2 (standardized coefficient = -2.12 ± 1.18, *F*-value = 3.25, *P*-value 0.102), although foliar $\delta^{13}C$ and, to a lesser extent, K concentration were negatively correlated to *g* when considering those variables individually (Appendix 1, Table S1).

Discussion

Main findings

Our results show that different strategies emerge to deal with the harsh edaphic environment imposed by gypsum. In this regard, the variation defined by the PC1 was mainly explained by the contrasting degrees of gypsum affinity of the target species. In one extreme of the PC1, the observed species strategy consists of responding to the edaphic constraints imposed by gypsum through deeper roots, hence overcoming the soil hardness, along with enhanced foliar Ca and S accumulation to deal with the soil chemical toxicity. The other extreme of this axis is defined by a combination of lower time-integrated transpiration and higher foliar C concentration and, to a lesser extent, a slightly higher N concentration. In agreement with our expectations, the lower scores of species with higher gypsum affinity on the PC1 indicate that their resource use strategy specifically responds to the edaphic constraints imposed by gypsum. However, contrary to our expectations, our results do not show traits related to plant responses to non-specific constraints (i.e., shared with other arid ecosystems) at the other extreme of the PC1 axis, such as high efficiency in water and nutrient use, although univariate analyses show that species with low gypsum affinity present high water use efficiency (foliar $\delta^{13}C$) and, to a lesser extent, K concentration (Appendix 1, Table S1). Therefore, we conclude that species with high level of specialization respond specifically to the edaphic constraints imposed by gypsum, without hampering their response to prevalent constraints shared with other arid ecosystems.

Contrasting plant strategies depending on gypsum affinity

Species with higher gypsum affinity may accumulate ions found in excess (S, Ca and Mg) as a mechanism to tolerate the high concentrations of these elements in gypsum soils or to adjust their osmotic potential to take up water from ionically extreme soils (Chen & Jiang, 2010). This pattern is stronger for Ca and S but less consistent for Mg, as Mg accumulation ability is more species-dependent (Moore et al., 2014). Indeed, gypsophytes' ability to accumulate Ca, S and Mg ions have been previously demonstrated in Iberian gypsophytes (Cera, Montserrat-Martí, Ferrio, Drenovsky, & Palacio, 2020; Duvigneaud & Denaeyer-de

Smet, 1966; Palacio et al., 2007), where this accumulation can occur in cell vacuoles directly in the form of gypsum crystals ($CaSO_4{\cdot}2H_2O$) (Palacio et al., 2014). Our results suggest that the accumulation of inorganic S, Ca, and Mg may influence other physiological responses in plants living in this environment. On the one hand, the accumulation of inorganic elements can affect the foliar stoichiometry due to the high content of inorganic ions that may reduce, in turn, the foliar carbon concentration (Palacio et al., 2007). On the other hand, the accumulation of inorganic ions might help reduce the plant water potential, thereby improving soil water uptake (Ajmal Khan, Ungar, & Showalter, 2000; Flowers, Troke, & Yeo, 1977). Moreover, deep soil layers usually remain wetter during long drought periods than shallow layers due to lower evapotranspiration. The greater access to water stored in deeper soil layers can also be associated with somewhat higher cumulative transpiration (lower $\delta^{18}O_{leaf}$) and Ca accumulation, although greater utilization of deep, non-enriched water may have also contributed to lower $\delta^{18}O_{leaf}$ values in species with higher gypsum affinity (Sarris et al., 2013). Contrary to our expectations, species without responses to gypsum-specific limitations do not show either a high nutrient or water use efficiency, despite being traits favorable to deal with common limitations in stressful dry environments. Instead, they seem to tolerate gypsum limitations without any specific strategies, showing a combination of low transpiration rate, potentially resulting from a low water availability derived from their limitations to access water in deep soil layers, and high foliar concentrations of C and, to a lesser extent N, potentially due to the reduced accumulation of excess elements such as S, Ca, and Mg.

Water source segregation based on gypsum affinity

A far less explored topic is the potential vertical niche segregation regarding root scavenging for water at different depths in the soil profile, depending on the degree of species' gypsum affinity. It has been demonstrated that root systems typical of gypsovags face difficulties in penetrating gypsum soils (Bridges & Burnham, 1980), while those of gypsophytes are better adapted to overcome gypsum structural difficulties, both at seedlings (Romão & Escudero, 2005) and adult stage (Palacio, Azorín, Montserrat-Martí, & Ferrio, 2014). However, the traits or mechanisms that make specialists' roots better adapted to overcome gypsum physical constraints are still unknown. Our results suggest that species with different gypsum affinities have access to different water sources after considering their dimensions (height). Differential access to water pools can be considered a proxy for rooting depth by accounting for variation in species size (Schenk & Jackson, 2002). These functional differences might segregate the water pool niches exploited by coexisting species depending on their gypsum affinity, thereby promoting the coexistence of individuals of species with different edaphic affinities on gypsum soils. Niche partitioning and complimentary use of limiting resources reduce competition among coexisting plants and favor their coexistence (Chesson, 2000), which may explain the final composition of the plant community on gypsum outcrops. Indeed, specialists and generalists coexistence is widely observed not only in gypsum ecosystems, but also in many other harsh edaphic environments such as serpentine (Sianta & Kay, 2019), granite (Murdy, 1968) or dolomite soils (Mota et al., 2008). Niche partitioning occurs in some of these systems, thereby stabilizing their high diversity, as observed in serpentines (Levine & HilleRisLambers, 2009; Sianta & Kay, 2019). However, the extent to which the coexistence of plants with contrasting degrees of edaphic affinity is due to niche partitioning must be further examined, not only in gypsum soils but also in other harsh edaphic environments.

Conclusions

Our study shows that individuals of species living on gypsum rely on different responses and strategies to deal with gypsum edaphic constraints based on their particular gypsum affinity. Species with high gypsum affinity rely on functional responses to deal with specific gypsum edaphic constraints (i.e., soil structural hardness and Ca and S excess). They respond to these edaphic limitations by accumulating Ca, S, and Mg, highly abundant in gypsum soils and accessing water from deeper soil layers despite gypsum's strong physical constraints limiting root penetration and development. However, whether species with lower gypsum affinity rely on more generalist strategies such as higher water and nutrient use efficiency strategies useful in other non-gypsum arid ecosystems remain uncertain.

Further research

Further research on edaphic generalists' physiological performance on gypsum soils will help understand the ecological filters that harsh edaphic environments impose on plants. However, our results do not show any compromise derived from edaphic specialization in terms of efficiency in water and nutrient acquisition and use. So, the riddle of why specialists do not spread beyond their narrow edaphic optimum warrants further research by considering, for example, the importance of gypsum affinity on different fitness components, ranging from reproductive effort (traits related to flowering, fruit and seed production) to plant growth and survival. Reciprocal transplant experiments or greenhouse studies using gypsum and non-gypsum soils would be valuable for assessing specialists' performance in and off gypsum lithologies (Cera et al., 2020). It might also be interesting to explore whether the segregation of strategies observed between specialists and generalists to face the specific edaphic limitations imposed by gypsum can be generalized to other harsh edaphic environments, which may be fundamental to advancing our understanding of plant species coexistence in these habitats.

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CHAPTER 2

Interspecific facilitation favors rare species establishment and reduces performance disparities among adults

"Natural selection cannot possibly produce any modification in a species exclusively for the good of another species; though throughout nature one species incessantly takes advantage of, and profits by, the structures of others…."

Darwin, Charles. 1859. On the origin of species by means of natural selection, or preservation of favoured races in the struggle for life.

Interspecific facilitation favors rare species establishment and reduces performance disparities among adults

Ricardo Sánchez-Martín[1], Miguel Verdú[1], Alicia Montesinos-Navarro[1]

Authors affiliation:

[1]Centro de Investigaciones Sobre Desertificación (CIDE, CSIC-UV-GV), Carretera de Moncada- Náquera, Km 4.5, 46113 Moncada, Valencia, Spain.

Abstract

A variety of mechanisms sustain diversity in natural communities as a result of ecological interactions between organisms. Competition has been studied extensively in the context of species maintenance, but facilitation is often conceptualized as simply reducing competition between functionally different species, which tends to decline throughout the plants' life span. Here we explore how interspecific facilitation may sustain diversity throughout the species' life by avoiding the extinction of locally rare species at juvenile stages and reducing performance disparities between neighbors of differing species at mature stages.

To do so, we measured whether rarer species relied more on facilitation than abundant ones in semiarid shrubland in Southeast Spain. A mechanistic explanation of this relationship was subsequently tested by correlating rarity with the species' affinity to a particularly edaphic stressful environment. Finally, we assessed whether growing associated with neighbors in vegetation patches shaped by facilitation could balance performance disparities between species when they become adults.

We show that facilitation (i) favors the rare species, which in addition, tend to be those with low affinity to the stressful environment, and (ii) reduces the performance dissimilarities among plants growing associated within multispecific vegetation patches compared to plants growing alone.

These facilitative effects, beyond the reduction of competition between functionally similar species, might ensure positive and long-lasting effects of biotic interactions, implying a more critical role for facilitation in preserving biodiversity than previously thought.

Keywords: Diversity maintenance, facilitation, neighborhood, gypsum affinity, performance differences, rarity, stress sensitivity, stress tolerance

CHAPTER 2

Introduction

Interactions between organisms play a fundamental role in structuring the diversity of natural communities. Competitive interactions have largely been regarded as essential drivers of community diversity (Grime, 1973; Huston, 1979; MacArthur & Levins, 1964). However, the role of facilitation was not widely recognized until the turn of the last century, despite its potential importance in maintaining species diversity across many different biomes (Brooker et al., 2008; Bruno, Stachowicz, & Bertness, 2003; Callaway, 2007; Callaway & Walker, 1997; Cavieres et al., 2014; Hacker & Gaines, 1997; He, Bertness, & Altieri, 2013; Holmgren & Scheffer, 2010; Le Bagousse-Pinguet, Maalouf, et al., 2014; Mcintire & Fajardo, 2014; Michalet et al., 2006; Soliveres, Smit, & Maestre, 2015). Traditionally, facilitation has been viewed mainly through the lens of microenvironmental improvements that promote the establishment of sensitive species (Liancourt, Callaway, & Michalet, 2005) and the subsequent adult co-occurrence of functionally distinct species that are likely to experience little interspecific competition (Butterfield & Briggs, 2011; Cavieres & Badano, 2009).

It is commonly considered that as beneficiary plants grow, the role of facilitation weakens due to competitive pressures with neighboring species (Miriti, 2006; Valiente-Banuet & Verdú, 2008). However, it is also common, especially in arid environments, that plant species co-occur as adults in vegetation patches. Therefore, in these cases, other mechanisms besides shading or water redistribution, through which nurse plants benefit the establishment of facilitated seedlings, may be operating to enhance the co-occurrence between adult plants. Some of the long-term mechanisms enhancing the co-occurrence of adult plants in vegetation patches can be related to an increase in resource availability or a more equitative use of resources, allowing the maintenance of their coexistence over time. For instance, an increase in nutrient availability due to a greater contribution of leaf litter or an enhancement of symbiotic biotic interactions, such as pollinators or mycorrhizal fungi in the shared micro-environment (Callaway, 2007; Losapio & Schöb, 2020; Navarro-Cano, Horner, Goberna, Verdú, & Address, 2019), can result in persistent co-occurrence among adult plants (Montesinos-Navarro, Segarra-Moragues, Valiente-Banuet, & Verdú, 2012). Moreover, there is also evidence of some mechanisms that can result in a more equitable use of resources, such as nutrient transfer between adult plants cohabiting vegetation patches (Montesinos-Navarro, Verdú, Querejeta, & Valiente-Banuet, 2017). Although these mechanisms could equalize the interacting species' performance, these interactions among adult plants have received much less attention. Considering the effects of facilitation, not only on interactions between nurses and facilitated seedlings but also among adult plants, will contribute to a better understanding of the impact of facilitation at the community scale.

Facilitation favors the establishment of rare species

Facilitation may act as a niche generator, providing opportunities to establish new species that otherwise would be excluded. This process is mediated by nurse plants, which are mature plants that ameliorate their surrounding environment, favoring the establishment and survival of other plants under their canopy (Padilla & Pugnaire, 2006). This positive effect is more prominent when facilitated and nurse species differ in their ecological requirements (i.e., broad niche differences; Navarro-Cano, Goberna, & Verdú, 2019; Valiente-Banuet & Verdú, 2013), resulting in the well-documented pattern that stress-sensitive species rely more on facilitation than stress-tolerant species. (Gross et al., 2009; Le Bagousse-Pinguet, Xiao, et al., 2014; Liancourt et al., 2005; Qi et al., 2018; Valiente-Banuet, Rumebe, Verdu, & Callaway, 2006). Species that deeply depend on facilitation can find their abundance limited by the availability of the habitat provided by their nurse plants. That might be an important factor explaining why the species that rely more on facilitation are more locally rare than those that rely less on facilitation, a worldwide pattern linking facilitation and plant species coexistence (Soliveres, Maestre, Berdugo, & Allan, 2015), but see Lortie et al. (2021); Lucero, Callaway, Faist, & Lortie (2021) or Vega-Álvarez, García-Rodríguez, & Cayuela (2019) for particular cases in which facilitation favors abundant species.

Facilitation balances species performances among adult species

Positive interactions can change their signs and intensities over time depending on the ontogenetic states of the interacting species (Valiente-Banuet & Verdú, 2008) and indirect effects among neighboring species (Castillo, Verdú, & Valiente-Banuet, 2010; Schöb, Armas, & Pugnaire, 2013). When facilitated plants become adults, they face a new ecological context where they must deal with the cohabiting neighbors of the patch. Under this context, species may endure when the net balance of competition and facilitation remains positive or neutral (Valiente-Banuet & Verdú, 2013). Plant interactions might remain positive over time by attenuating species' performance disparities. Such attenuation could be produced by reciprocal benefits between the interacting species beyond establishment. For example, some facilitative interactions are mediated by nitrogen or water transfer between interacting species (Montesinos-Navarro, Verdú, Querejeta, Sortibrán, & Valiente-Banuet, 2016; Montesinos-Navarro et al., 2017; Montesinos-Navarro, Verdú, Querejeta, & Valiente-Banuet, 2019) or the sharing of beneficial mycorrhizae (Sortibrán, Verdú, & Valiente-Banuet, 2019). Mechanisms that could palliate the differences in performance between co-occurring species, an equalizing force that, if maintained across time and generations, can guarantee the species' survival in a mature stage.

Aims

The impact of facilitation on natural communities will be determined by how the two processes mentioned above interact throughout plants' lifespans. We aim to show that, under natural conditions, facilitative effects can occur at all stages of plant life, implying that facilitation plays a more significant role in species maintenance than previously thought. To that end, we examined these effects in plant communities growing in gypsum outcrops where the preponderance of positive interactions has been documented (Montesinos-Navarro, Storer, & Perez-Barrales, 2019; Sánchez-Martín, Verdú, & Montesinos-Navarro, 2022), favored by intense physical and chemical stresses that gypsum imposes on plant life (Escudero, Palacio, Maestre, & Luzuriaga, 2015). Here, we propose that facilitation may (i) favor the establishment of rare species, which we hypothesize can also be those with low affinity to stressful edaphic soils and (ii) balance the performance disparities of the species in the community when species grow in patches promoted by facilitation.

Methods

Study site and experimental design

We explore the role of facilitation in buffering species loss by characterizing plant communities growing on gypsum outcrops. Here, plant communities must not only deal with a lack of water and other essential macronutrients due to growing in a semi-arid climate but also with chemical and physical stresses associated with gypsum accumulations, such as Ca and S excess, and the presence of hard-even crystallized soil layers. (Escudero et al., 2015). Under such stressful conditions, these communities are characterized by sparse vegetation with multispecific patches derived from the facilitative effects of nurse species (Delalandre & Montesinos-Navarro, 2018; Montesinos-Navarro, Storer, et al., 2019). These properties make the system a suitable model for studying facilitation. Specifically, we conducted the study in four localities with gypsum outcrops in the Vinalopó valley, Alicante, southeast Spain (Figure 1:38º 29' 28.5" N, 0º 44' 18.1" W).

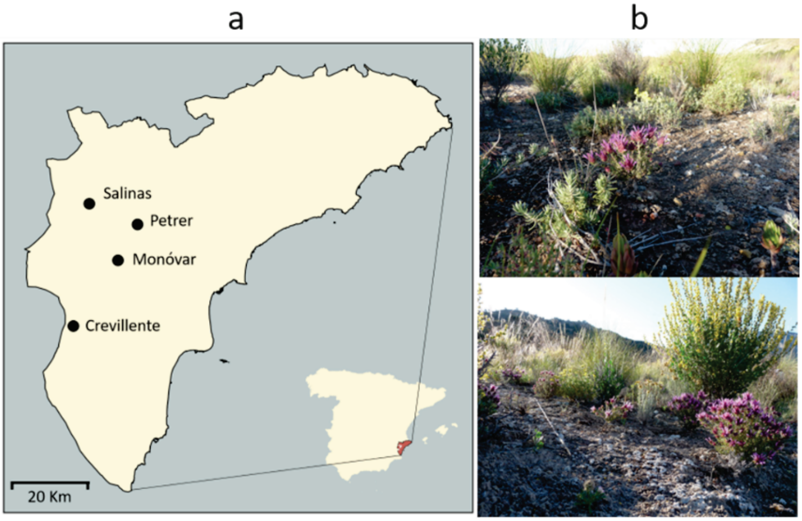


Figure 1. (a) Map of the study sites located throughout the the Vinalopó Valley (Alicante), southeast Spain. **(b)** Photographs showing the type of vegetation growing on the gypsum outcrops.

The climate is semi-arid Mediterranean, with an average annual rainfall of 360 mm, most of which falls during seasonal pulses in spring (March-June) and autumn (September-December). We selected two contiguous (<10 m apart) sub-localities of 1 ha in each locality, one within the gypsum outcrop (stressful sub-locality hereafter) and another in the surrounding less stressful limestones area (non-stressful sub-locality hereafter). The four localities were no more than 20 km apart to minimize changes in climatic conditions among them. Within each locality, the non-stressful sub-localities were always contiguously located uphill from the stressful sub-localities, ensuring the potential seed dispersal from plants in the non-stressful sub-locality to the stressful sub-locality (Bochet, 2015). Our experimental design comprised 80 plots (150x150cm) in each sub-locality, except in one non-stressful sub-locality with 79 plots. We distributed the plots randomly to wholly occupy the 1 ha sub-localities, avoiding places presenting difficult access.

We sampled the four localities between April 2019 and February 2020. Firstly, inside each plot, we quantified the number of individuals per species and estimated the species' cover by adding up the cover of all the plants per species recorded, considering the cover of recruits as negligible. Each plant cover was estimated by measuring the elliptical area of the canopy as follows:

$$(1)\quad plant\ cover = (\pi ab)$$

Where (a) is half of the widest length of the plant canopy measured through its center, and (b) is half the width of the canopy perpendicular to (a). We also registered whether plants lived alone (i.e., non-associated) or in a vegetation patch with other individuals of the same or other species (i.e., associated). Each patch's cover was estimated following the same procedure described above, and the bare ground cover was obtained as the subtraction between the sampled area and the surface occupied by patches plus non-associated plants. Secondly, we identified all recruits within the plots, both in the bare ground (non-associated) and beneath other plants (associated). We considered recruits all plants ranging from seedlings (with at least the first two leaves) to tiny plants in comparison with adults (<15% in height compared to adults), showing neither signs of reproductive structures nor lignification at the stem base. Overall, we sampled 639 plots in 4 localities, identified 85 different species, and measured 28,566 adult plants and 20,081 recruits.

Facilitation favors the establishment of rare species

We first tested whether the species abundance was directly related to its degree of affinity to the edaphic stressful soil (i.e., gypsum affinity). Then, we assessed whether species with low gypsum affinity tend to rely more on facilitation. To do so, we used species' relative abundance in each gypsum stressful sub-locality as a proxy for abundance and gypsum affinity index (g) as a proxy for edaphic affinity. Gypsum affinity was measured as in Sánchez-Martín et al. (2021) (equation 2):

$$(2)\quad g = \frac{Cs}{Cs + Cns}$$

Where Cs is the cover (%) of a specific species in the stressful sub-locality and Cns is the cover (%) of the same species in the contiguous non-stressful sub-locality. This metric measures which proportion of the total species' cover is found in the stressful sub-locality. This index was calculated with plants growing alone (i.e., excluding those growing in vegetation patches), thus avoiding the potential effects of plant-plant interactions within patches. It has been previously shown that the species' affinity in this community is positively related to harboring specific strategies to cope with harsh gypsum constraints, such as the ability of roots to penetrate hard gypsum layers and the accumulation of elements found in excess in those soils such as Ca, Mg and S (Sánchez-Martín et al., 2021). Species with g values close to 1 (high gypsum affinity) are those with the strategies mentioned above, while species with g values close to 0 (low gypsum affinity) do not show any specific response to survive in those soils (Sánchez-Martín et al., 2021).To ensure that g values were reliably estimated from a sufficient sample size, we only retained those species with at least 45 adult plants in each locality (i.e., considering both stressful and non-stressful sub-localities). Quantification of gypsum affinity was carried out for 20 different species and was based on the plant cover of 16,132 adult individuals (8,504 in the stressful and 7,628 in the non-stressful sub localities).

To assess whether species with low gypsum affinity rely more on facilitation than species with high affinity, we tested a correlation between species' gypsum affinity and species' tendency to associate with other plants (i.e., facilitation or competition). We measured this tendency for those species that account for at least 0.5% of relative abundance in each stressful sub-locality. Then, to ensure reliable estimations of the association tendencies, only species with at least 15 recruits in each stressful sub-locality were considered. Each species' association tendency was measured according to the relative interaction intensity index (RIIj) described in Armas, Ordiales, & Pugnaire. (2004) (equation 3):

$$(3)\ RIIj = \frac{RDa - RDna}{RDa + RDna}$$

Where RDa is the recruits´ density of species j growing associated beneath plants of any species (i.e., number of recruits per m^2 of vegetation cover) and $RDna$ is the recruit's density of the same species growing non-associated in the bare ground (i.e., number of recruits per m^2 of the bare ground). RII values range from -1 to 1, with positive values suggesting facilitation (i.e., recruits grow preferentially associated with other plants) and negative competition (i.e., recruits grow preferentially in the bare ground). RII was quantified for 20 species by analyzing the recruiting patterns of 6,986 recruits growing in stressful sub-localities.

Facilitation balances species' performances

We used relative plant cover as a performance proxy to assess whether facilitation reduces performance disparities of interacting species. Non-associated plants are less likely to remain isolated as they grow, resulting in a smaller cover than associated plants. In order to make the plant's cover range comparable between associated and non-associated individuals, in each stressful sub-locality, we selected only those associated plants whose cover fell within the range defined by the non-associated plants. Moreover, we eliminated individual plants below the percentile 1% for each species cover distribution to verify that all selected plants are unquestionably adults and that cover reflects adult performance rather than differences in the relative abundance of developmental stages (Appendix 2, Table S1). Then, to estimate differences in plant performance between species (i.e., differences in plant relative cover), we followed the following procedure: Firstly, we relativized plant cover for each individual by the maximum cover of a conspecific in each sub-locality to make species cover comparable among species. This relativization preserves intraspecific cover variation, but reduces interspecific variation due to the comparison of species with different life forms. Secondly, we calculated separately for each species the median performance (i.e., median relative cover; hereafter P) of plants associated (hereafter P_a) and non-associated (hereafter P_{na}), including only those species with at least 10 individuals (i.e., 5 non-associated and 5 associated; Figure 2). We used the median since it is a good population descriptor for non-normal distributed data with extreme right or left tails (Sainani 2012). Thirdly, within each sub-locality, we extracted all the possible combinations of species pairs and calculated the performance differences (PD) for each combination of species (PD (ij)), separately for non-associated ($PD(ij)_{na}$: equation 4) and associated plants ($PD(ij)_{a}$: equation 5), being i and j any specific possible pair of species (Figure 2).

$$(4)\ PD(ij)_{na} = \frac{Max\,[P(i_{na}), P(j_{na})]}{Min\,[P(i_{na}), P(j_{na})]}$$

$$(5)\ PD(ij)_{a} = \frac{Max\,[P(i_{a}), P(j_{a})]}{Min\,[P(i_{a}), P(j_{a})]}$$

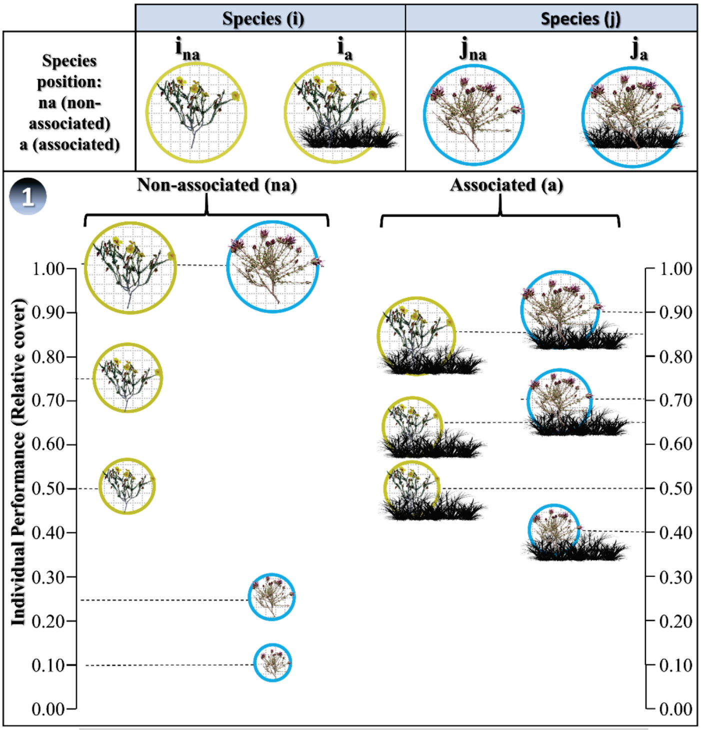
PD can only produce values ranging from 1 (i.e., no differences in P between the two species) to any positive value (the greater the value, the greater the P differences between species).

We calculated the effect of the neighborhood on performance disparities (NPD) for each species' pair through the differences between PD obtained with associated individuals (PD_{a}) and those that were found living non-associated (PD_{na}) (equation 6; Figure 2):

$$(6)\ NPD\ (ij) =\ PD(ij)a - PD(ij)na$$

Finally, we calculated the overall NPD as the mean of all the paired NPD (ij). If the overall NPD is negative, living associated will attenuate performance differences between species at the community level, a process driven by facilitation that could favor diversity. The NPD assessment was based on the cover of 7,761 adult individuals (4,616 associated and 3,145 non-associated) of 23 species.

Assesment of the effect of the neighborhood on performance disparities (NPD)



Median Performance (P)

$P(i_{na})=0.75$	$P(j_{na})=0.25$	$P(i_a)=0.65$	$P(j_a)=0.70$

2

$$PD(ij)_{na} = \frac{\text{Max }[P(i_{na}), P(j_{na})]}{\text{Min }[P(i_{na}), P(j_{na})]} = \frac{0.75}{0.25} = 3.00$$

$$PD(ij)_{a} = \frac{\text{Max }[P(i_{a}), P(j_{a})]}{\text{Min }[P(i_{a}), P(j_{a})]} = \frac{0.70}{0.65} = 1.08$$

3

$$NPD(ij) = PD(ij)_a - PD(ij)_{na} = 1.08 - 3.00 = -1.92$$

Figure 2. Visual scheme of NPD calculation for a specific pair of species (NPDij). First (**1**), we select only those individuals in the range of cover defined by the plants living non-associated individually for each species (i and j) in each stressful sub-locality. Then, we relativized each plant cover by the maximum cover of a conspecific in each stressful sub-locality to make species with different life form comparable, which provides the values observed in the figure ranging from 1 to ca. 0. Second (**2**), we calculated the Performance disparities between the interacting species separately for the individuals growing alone PD(ij)na and the individuals growing associated to other plants PD(ij)a. PD is calculated as a ratio, with the species with the higher median performance in the numerator and the other species in the denominator. Finally (**3**), the NPD is calculated as the differences in PD between the pair when plants grow associated (PD(ij)a) minus the same pair when plants grow non-associated (PD(ij)na). Negatives values in NPD (ij) suppose a reduction in performance disparities when species grow associated. We then calculated the overall NPD as the mean of all the possible interacting pairs.

Statistical analyses

First, we evaluated the relationship between the reliance on facilitation and species' gypsum affinity and abundance. Second, we assessed if facilitation reduces performance dissimilarities at the community level, testing whether performance differences between species were reduced when plants are associated.

In order to test whether facilitation promotes the survival of rare species with low gypsum affinity, we first checked whether species with low gypsum affinity are less abundant. Considering that closely related species could display more similar traits than distant relatives (Revell 2010), we applied a Bayesian phylogenetic-informed model with plant abundance in the stressful environment as the response variable, gypsum affinity (g) as the predictor, and locality as a random effect. Then, we tested whether facilitation (RII) was stronger in species with low gypsum affinity with another phylogenetic-informed Bayesian model. In this case, RII was the response variable, g was the predictor (fixed effect), and locality was a random effect.

We used Markov Chain Monte Carlo Generalised Linear Mixed Model (MCMCglmm; Hadfield, 2010) for both models. This approach allows phylogenetic corrections while using multiple replicates per species (i.e., the same species in different locations). We implemented both models with the default prior (i.e., assuming normal posterior distribution for fixed effects and inverse-Wishart prior for the random effects) and ran 600,000 MCMC iterations with a burn-in period of 100,000 iterations and sampled every 100 iterations, finally yielding 5,000 iterations for each model. We ran both models using the "MCMCglmm" function of the R package "MCMCglmm 2.29" (Hadfield, 2010), and we ensured model convergence through plot inspections. We also checked that autocorrelation, both in mean and variance, remained below 0.1 using the "autocorr" function of the "coda" R package (Plummer, Best, Cowles, & Vines, 2006). Moreover, we extracted the conditional R^2 to assess how the models fit our data, including the variance explained by the whole model (fixed and random effects), using the method proposed by Nakagawa & Schielzeth (2013).

To inform both MCMC models phylogenetically, we built the phylogenetic relationship among the studied plant species with the "V.phylomaker 0.1.0" package for R (Jin & Qian, 2019). V.phylomaker matches our given species list with the mega tree "GBOTB.extended.tre" which includes 74,533 species, a product of the combination of two previous mega trees (Smith & Brown, 2018; Zanne et al., 2014) with branch lengths indicating the chronological time (millions of years). Species that were not matched with the mega tree were added to the resulting tree following the criteria of Scenario.2, where new tips are inserted to randomly selected nodes below the genus or family level basal nodes (Jin & Qian, 2019; Qian & Jin, 2016).

Finally, to test whether facilitation balances performance disparities among associated plants in the studied communities, we compared the overall NPD against a null model, in which the position of the plants (associated vs. non-associated) was randomly reshuffled 10,000 times. The reshuffling process kept our data structure regarding replicates per sub-locality and species unaltered. Then we checked if our observed overall NPD was below the 95% credible interval extracted for the null model. We calculated the 95% credible interval using the method “HDI” with the function “ci” hosted in the “bayestestR” R package (Makowski, Ben-Shachar, & Lüdecke, 2019). All analyses were carried out with the analytical software R (R Core Team, 2019).

Results

Our results indicate that plant communities on gypsum outcrops were strongly shaped by facilitation since all the studied species benefited from the facilitation (i.e., for all species RII>0). These species represent the target community well since they include 93.18% of the adult plants and 91.23% of the community recruits. Besides, these species vary in their relative abundance (from 1% to 20%) and are gradually distributed along the gypsum affinity gradient (Figure 3).

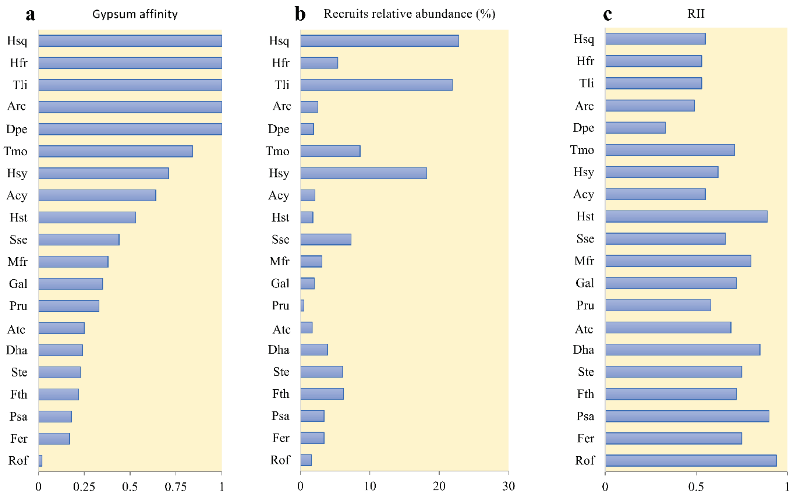


Figure 3. Characteristics of the examined species: **(a)** gypsum affinity (g), **(b)** relative abundance of recruits, **(c)** Relative Interaction Index (RII) showing the degree of facilitation, as all RII values are greater than 0. Check out Appendix 2, table S2 for detailed numbers and variation across localities. Species codes: *Anthyllis cytisoides* (Acy), *Artemisia campestris* (Arc), *Atractylis cancellata* (Atc), *Diplotaxis harra* (Dha), *Dorycnium pentaphyllum* (Dpe), *Fumana ericoides* (Fer), *Fumana thymifolia* (Fth), *Globularia alypum* (Gal), *Herniaria fruticosa* (Hfr), *Helianthemum squamatum* (Hsq), *Helichrysum stoechas* (Hst), *Helianthemum syriacum* (Hsy), *Matthiola fruticulosa* (Mfr), *Phagnalon rupestre* (Pru), *Phagnalon saxatile* (Psa), *Rosmarinus Officinalis* (Rof), *Sedum sediforme* (Sse), *Stipa tenacissima* (Ste), *Teucrium libanitis* (Tli), *Thymus moroderi* (Tmo).

The evaluation of the effect of neighborhood effect on performance differences (NPD) is based on 23 species that represent well the target community, as their coverages account for 88.60% of total cover in the stressful sub localities. The plants growing associated were grouped in 2513 patches with an average of 2.96± 1.62 species per patch and a maximum of 13 species in a unique patch. In addition, there was considerable variation in the difference between plant size associated vs. non-associated across species, with some species showing up to 67% reduction of plant size when growing associated, while others increased their size up to 77% (mean ± SD =17.03±35.67). These differences across species regarding the influence of the neighborhood on their size provide the arena to observe compensatory effects potentially resulting in a reduction of species' dissimilarities in size (i.e., performance dissimilarities).

Facilitation favors the establishment of rare species

Our results show a significant relationship between species abundance and gypsum affinity (g), with abundant species showing a greater gypsum affinity (model (a), g post mean effect= 0.142, CI 95%= (0.075, 0.215), R^2=0.2001). At the same time, species with low gypsum affinity, and therefore rare species, show higher facilitation strength (model (b), *g* post mean effect=-0.257, CI 95%= (-0.386, -0.146), R^2=0.479; Figure 4). These relationships were significant after accounting for the species' phylogenetic relationships (Appendix 2, Figure S1) and locality effects in both models: (a) phylogeny post mean effect=0.002, CI 95% (0, 0.006), locality post mean effect<0.001, CI 95% (0, <0.001); (b) phylogeny post mean effect<0.001, CI 95% (0, <0.001), locality post mean effect= 0.043, CI 95% (0, 0.115).

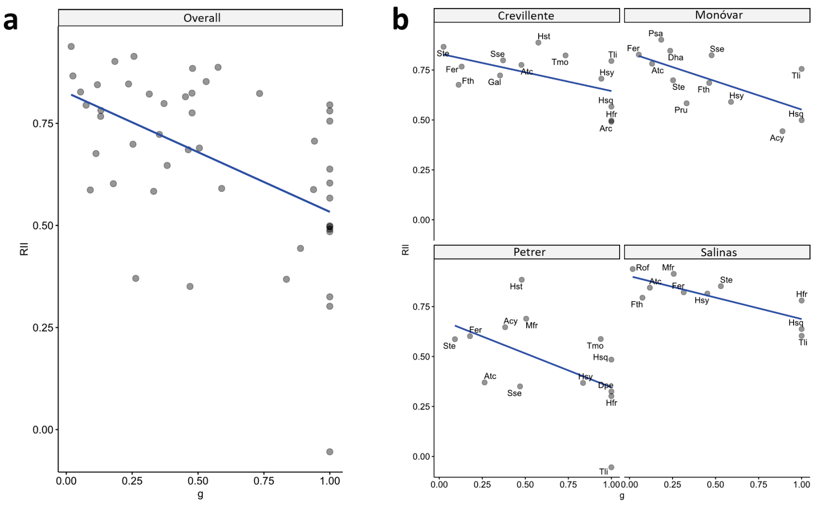


Figure 4. (a) Overall correlation between plant-plant interaction strength (RII) and gypsum affinity index (g), showing a negative correlation between facilitation and species gypsum affinity, pooling data from all the localities together. **(b)** Correlation between RII and g for each locality showing that the overall pattern found in (a) is consistent across localities. Species codes: *Anthyllis cytisoides* (Acy), *Artemisia campestris* (Arc), *Atractylis cancellata* (Atc), *Diplotaxis harra* (Dha), *Dorycnium pentaphyllum* (Dpe), *Fumana ericoides* (Fer), *Fumana thymifolia* (Fth), *Globularia alypum* (Gal), *Herniaria fruticosa* (Hfr), *Helianthemum squamatum* (Hsq), *Helichrysum stoechas* (Hst), *Helianthemum syriacum* (Hsy), *Matthiola fruticulosa* (Mfr), *Phagnalon rupestre* (Pru), *Phagnalon saxatile* (Psa), *Rosmarinus Officinalis* (Rof), *Sedum sediforme* (Sse), *Stipa tenacissima* (Ste), *Teucrium libanitis* (Tli), *Thymus moroderi* (Tmo).

Facilitation balances species' performances

Performance disparities were lower between plants growing associated than those growing non-associated with other plants, as indicated by the negative effect of the neighborhood in performance disparities (NPD= -1.012). The observed overall NPD was lower than 99.21% of the overall NPDs obtained with randomized data under the null model (Appendix 2, Figure S2). In addition, the negative NPD was consistent for the majority of the species in the community (Figure 5). Thus, when we independently considered the averaged NPDs across all the pair-wise interactions in which each species is involved, 17 of the 23 species presented overall negative values, representing 97,46% of total relative abundance in the community.

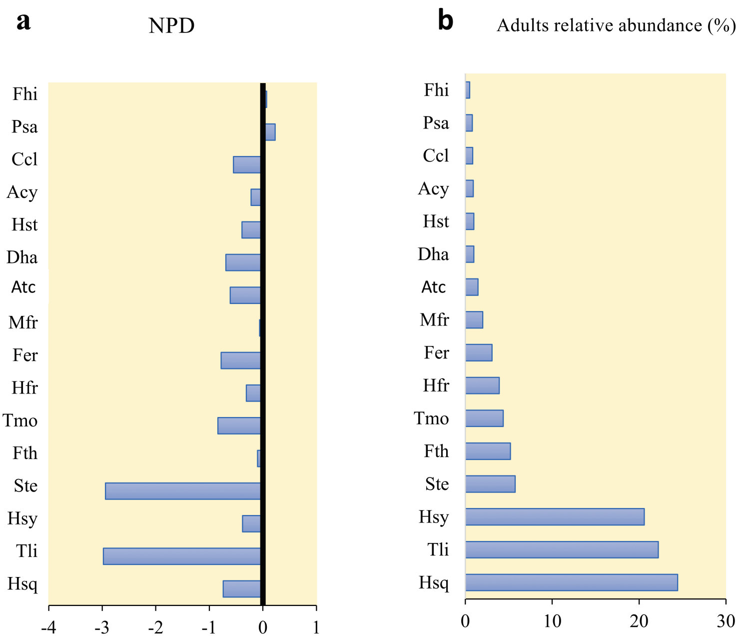


Figure 5. (a) Average effect of the neighbourhood on performance disparities (NPD) for each species and **(b)** their relative abundance (%). The results show consistency regarding the effect of the neighbourhood on reducing performance disparities. Only species with a relative abundance greater than 0.5% are displayed. *Species codes: Anthyllis cytisoides* (Acy), *Atractylis cancellata* (Atc), *Cistus clussi* (Ccl), *Diplotaxis harra* (Dha), *Fumana ericoides* (Fer), *Fumana hispidula* (Fhi), *Fumana thymifolia* (Fth), *Herniaria fruticosa* (Hfr), *Helianthemum squamatum* (Hsq), *Helichrysum stoeachas* (Hst), *Helianthemum syriacum* (Hsy), *Matthiola fruticulosa* (Mfr), *Phagnalon saxatile* (Psa), *Stipa tenacissima* (Ste), *Teucrium libanitis* (Tli), *Thymus moroderi* (Tmo).

Discussion

Our findings show two critical aspects of facilitation than can buffer species loss. First, we show that facilitation favors the establishment of rare species with low affinity for the substrate. Second, when plants become adults, we show that facilitation reduces performance dissimilarities between the community species. These observations could have implications for species maintenance.

Facilitation favors the establishment of rare species

Our results indicate that rare species tend to show low gypsum affinity, and facilitation is stronger for these species. These findings suggest that facilitation could maintain higher species richness than expected on the bare ground by preventing the local extinction of these low-abundant species. Some studies have shown that facilitation favors rare species (Calatayud et al., 2020; Soliveres, Maestre, et al., 2015), while others have provided empirical evidence that facilitation is more determinant for those species living out of their ecological optimum (Gross et al., 2009; Le Bagousse-Pinguet, Xiao, et al., 2014; Liancourt et al., 2005; Qi et al., 2018). Our results help bring together these two visions, showing that rarity and environmental fitting can be related. Species with low edaphic affinity may show limited abundance as they live out of their ecological optimum. These species differ from those with high gypsum affinity in the functional traits determining their responses to overcome environmental constraints (Sánchez-Martín et al., 2021).

Facilitation balances species' performances among adult species

We show that co-occurring adult species growing in interspecific vegetation patches reduce performance disparities compared to their isolated adult conspecifics. Although our approach does not allow us to identify the precise mechanisms that balance species sizes, the observed effect must be directly or indirectly mediated by facilitative effects between the neighboring plants. Among the potential underlying mechanisms at work could be those related to nutrient and water transfer (Montesinos-Navarro, Verdú, Querejeta, Sortibrán, & Valiente-Banuet, 2016; Montesinos-Navarro et al., 2017; Montesinos-Navarro, Verdú, Querejeta, & Valiente-Banuet, 2019), or the sharing of beneficial mycorrhizae (Sortibrán, Verdú, & Valiente-Banuet, 2019), among others. Moreover, the observed reduction in NPD is consistent when we independently considered the averaged NPDs across all the pair-wise interactions, which implies that the facilitation role in reducing performance disparities is not derived from the effect of particular species but something general to the community. Our findings highlight that facilitative effects may endure throughout the species' life. Despite the potential implications of our findings, further research is needed to understand what specific processes or mechanisms underpin our observational data to comprehend how facilitation shapes the diversity of natural communities

We show that facilitation not only plays an essential role in allowing the establishment of rare species but also balances species' performances at the adult stage. That balance in performance can guarantee the interactant's survival by complex mechanisms beyond abiotic amelioration that reduces the likelihood of the species' exclusion. Indeed, both processes may have additive effects, boosting the role of facilitation in preserving diversity. It is tempting to make an analogy between these two processes and the two major forces for species coexistence (Chesson, 2000): on the one hand, the promotion of rare, stress-sensitive species could be assimilated with stabilizing niche differences that limit species dominance when species become common while avoiding the extinction of rare ones; on the other hand, the reduction of disparities in performance could be viewed as equalizing mechanisms reducing fitness differences between species. Our findings suggest that the role of facilitation in species coexistence is more intricate and substantial than previously thought. Combining multiple facilitation mechanisms may result in emerging effects that favor diversity maintenance in communities driven by facilitative effects through the whole lifespan of the species. These findings have implications for species conservation. The demonstrated influence of facilitation in shaping the assembly of natural communities prompts a greater understanding of the interplay of processes that determine the realization of these interactions and how these may be affected by environmental changes.

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CHAPTER 3

Phylogenetic and functional constraints of plant facilitation rewiring

“There is a much more insidious kind of extinction: the extinction of ecological interactions.”

Janzen, D.H. 1974. The deflowering of Central America. Natural History. 83:48–53.

Phylogenetic and functional constraints of plant facilitation rewiring

Ricardo Sánchez-Martín[1], Miguel Verdú[1], Alicia Montesinos-Navarro[1]

Authors affiliation:

[1]Centro de Investigaciones Sobre Desertificación (CIDE, CSIC-UV-GV), Carretera de Moncada- Náquera, Km 4.5, 46113 Moncada, Valencia, Spain.

Abstract

Facilitative interactions bind community species in intricate ecological networks, preserving species that would otherwise be lost. The traditional understanding of ecological networks as static components of biological communities overlooks that species interactions in a network can fluctuate. Analyzing the patterns that cause those shifts can reveal the principles that govern the identity of pair-wise interactions and whether they are predictable based on the traits of the interacting species and the local environmental contexts in which they occur. Here we explore how abiotic stress and phylogenetic and functional affinities constrain those shifts. Specifically, we hypothesize that the rewiring of facilitative interactions is more limited in stressful than in mild environments. We present evidence of a distinct pattern in the rewiring of facilitation-driven communities at different stress levels. In highly stressful environments with a firm reliance on facilitation, rewiring is limited to growing beneath nurse species with traits to overcome harsh stressful conditions. However, when environments are milder, rewiring is more flexible, though it is still constrained to nurses that are close relatives. Understanding the ability of species to rewire their interactions is crucial to predict how communities may respond to the unprecedented rate of perturbations on earth.

Keywords: Facilitation, rewiring, species turnover, networks, phylogenetic constraints, functional constraints, gypsum affinity, traits.

Introduction

The traditional view of ecological networks as static elements of biological communities neglects the fact that interactions between species in a network may shift in time and space (Poisot et al., 2015; Trøjelsgaard & Olesen, 2016; Tylianakis & Morris, 2017; CaraDonna et al., 2021). The realization of interactions is determined by the community's composition and a complex combination of environmental conditions and interacting species traits (Poisot et al., 2015). Shifts in species interactions may avoid coextinction cascades due to the loss of particular species, potentially increasing communities' robustness to face perturbation (Vizentin-Bugoni et al., 2020; Sheykhali et al., 2020). However, partner switches (i.e., hereafter interaction turnover) are not unlimited (Raimundo et al., 2018; Montesinos-Navarro et al., 2019) since certain species combinations may be unlikely. Therefore, it is essential to assess whether there are general patterns in the topological shifts of networks' interactions to predict how different communities may respond to perturbations.

These shifts in interactions can be assessed through β-diversity changes between networks (i.e., interactions turnover; Poisot, Canard, Mouillot, Mouquet, & Gravel, 2012). Interactions turnover between two networks can come from variation in species composition and thus their associated interactions (shifts due to species turnover) or changes in the interactivity patterns among the pool of species shared in the two networks (changes due to rewiring; Poisot et al., 2012). Assessing interactions turnover and whether it follows predictable patterns can be especially important in fragmented landscapes, where the inhospitable matrix may limit the recolonization of a locally extinct species (Corlett & Tomlinson, 2020). This scenario would be critical for those species that depend on disappeared species unless they can rewire with other community species to compensate for the interaction loss, thus alleviating extinction cascades caused by the extinction of key species.

A framework to approach the β-diversity of interactions has been assessed across ecological networks in different spatial and temporal contexts, unveiling patterns in structuring natural communities that cannot be inferred directly from changes in species composition (Poisot et al., 2012; Carstensen et al., 2014; CaraDonna et al., 2021). For instance, Carstensen, Sabatino, Trøjelsgaard, & Morellato (2014) demonstrated that species involved in frequent pair-wise interactions tend to rewire less often than species involved in rare interactions. Montesinos-Navarro et al. (2019) showed that rewiring between mycorrhizal fungi and plants is not random but phylogenetically constrained. Analyzing the patterns driving rewiring interactions can shed light on the environmental factors and the species' evolutionary history behind the establishment of pair-wise interactions. Understanding these community dynamics is essential to correctly predict shifts in the structure of communities facing an unprecedented rate of environmental changes.

The role of rewiring can be critical for the survival of many species in communities governed by facilitation. Facilitation is defined as a biological interaction in which one (nurse) species alters the environment in a way that enhances the performance of a second (facilitated) species (Bronstein, 2009; Mcintire and Fajardo, 2014). These interactions can prevent the loss of species that require facilitation to survive (Bulleri et al., 2018). Mutualistic networks have traditionally been considered sensitive to the extinction of generalist species (i.e., species supporting multiple species), while they seem robust to the extinction of highly specialized species (i.e., species supporting few other species; Bascompte & Jordano, 2007), a pattern also observed for facilitation networks (Verdú & Valiente-Banuet, 2008). However, this static vision of facilitative interactions does not consider the possibility of facilitated plants to establish beneath a new nurse when the positive association effect compensates for interspecific competition, a balance dependent on the environmental context and the interacting species traits (Qi et al., 2018). For example, it has been shown that some competitive interactions can turn facilitative under an increase in the severity of the stressful conditions (Bertness & Callaway 1994; Qi et al., 2018), allowing the establishment of new facilitative interactions. However, this rewiring of partners is not unlimited since rewiring with highly competitive species could be very unlikely at any stress level. In contrast, facilitation rewiring would be easier with new species showing facilitative traits similar to those harbored by the original nurses.

Traits driving facilitative interactions depend on the environmental context (Butterfield and Callaway, 2013; Navarro-Cano et al., 2021). If particular traits are essential for the assembly of positive interactions, these traits could coerce the availability of nurses, so the stricter the requirements under specific stressful conditions, the more constrained the rewiring would be. There are several examples in restorations of stressful ecosystems where stress-tolerant nurses harbor more facilitated species than stress-sensitive nurses suggesting a better amelioration derived from harboring specific traits to overcome harsh stressful conditions (Saiz et al., 2014; Foronda et al., 2019, 2020). In contrast, in milder environments, facilitation could occur with a broader range of nurse species because the traits that limit facilitation in stressful contexts may be meaningless in milder conditions where other less specific traits can shape facilitative interactions (Chen et al., 2015; Catorci et al., 2016), allowing for more alternative facilitative interaction configurations. Phylogeny can provide clues about the relevant traits for the establishment of interactions when they are phylogenetically conserved, and therefore closely-related species are expected to show similar patterns of responses to environmental pressures (Webb et al., 2002; Ackerly, 2003; Gómez et al., 2010). However, some selection pressures can lead to adaptive convergence in distantly related species that respond similarly to the selection pressure (Webb et al., 2002; Freeman et al., 2014). Convergent evolution has been revealed, for instance, in harsh edaphic environments where specialized traits appear in distantly related taxa across phylogeny (Moore et al., 2014).

Understanding how abiotic stress and phylogenetic constraints modulate rewiring will help us predict ecological communities' responses to different disturbance scenarios. Here, we hypothesize that the rewiring of facilitative interactions is more limited in stressful than in mild environments. Specifically, we propose that facilitated species rewire with nurses that are closely related to their preferred nurses in mild environments, while in harsh environments, facilitated species recruit preferentially beneath species with traits to overcome stressful conditions, which reduces the number of available nurses, and thus rewiring.

Methods

Study site and sampling design

Gypsum outcrops in southeastern Spain are scattered in the landscape, surrounded by a different lithology. This generates a fragmented system for the edaphic specialist plants living on them that constitutes an excellent system to answer questions related to facilitation rewiring. The plant communities in these outcrops include well-adapted specialist plants and stress-sensitive edaphic generalist species, and all of them face, besides water scarcity, critical edaphic limitations (Palacio et al., 2007; Escudero et al., 2015; Sánchez-Martín et al., 2021). Moreover, these outcrops are surrounded by other communities developed in less stressful substrates, avoiding harsh edaphic conditions but not semi-arid conditions. These two soil types generate contrasting stress levels while maintaining plant communities under similar climatic conditions (See Appendix 3, Figure S1 to check basic community information differing between environments).

Position of study sites within Alicante province, Spain

Table of distances between study sites

Site x	Site y	Distance km
1	2	32.85
1	3	21.61
1	4	33.32
2	3	11.15
2	4	15.13
3	4	17.00

Figure 1. Location of the study sites in southeast Spain. Each point represents one site, including both a stressful and mild subsite. The table on the right represents the distance between each pair (xy) of sites.

We developed the study in four sites in the Vinalopó valley, southeast Spain (38°29' 39"N, 0°47' 00" W; Figure 1). Here, plant communities grow under a semi-arid Mediterranean climate, with an average annual rainfall of around 360 mm, occurring in intense seasonal pulses, mainly in spring (March- June) and autumn

(September-December). Plant communities are characterized by sparse vegetation, generally grouped in multispecific patches, mainly composed of camephytes and tiny shrubs. Within each site, we selected two contiguous subsites of 1ha, one within a gypsum outcrop (stressful subsite hereafter) and another in the surrounding non-gypsum lithology (limestones in our case; mild subsite hereafter). Stressful and mild subsites are in the four sites separated by a few meters to minimize changes in climatic conditions in each pair.

Our sampling design comprised 80 (150x150cm) plots in each subsite, except for one site where the mild subsite had 79 plots. We randomly distributed the plots to occupy 1 ha entirely, avoiding places with difficult access. We sampled the four sites between April 2019 and February 2020. Firstly, inside each plot, we identified all adult plants, independently whether they live isolated or in clumps, and measured the maximum and minimum diameter to approximate each plant cover using the ellipse equation:

$$cover = (\pi ab)$$

Where (a) is the semi-major diameter and (b) is the semi-minor diameter. We also identified whether each plant grew alone (i.e., non-associated) or in a patch with other individuals (i.e., associated). Moreover, the cover of each patch was estimated following the same method described for plants. Then, we calculated the bare ground cover by subtracting the surface occupied by patches and non-associated plants from the sampled area. Secondly, we identified all recruits within the plots and identified whether they were recruiting on the bare ground or under an adult plant, identifying the species of the adult plant. When recruits inhabit patches with multiple species, we assign the recruit the closest adult plant whose canopy covers the recruit (Alcántara et al., 2019). We considered recruits all plants ranging from seedlings (with at least the first two leaves) to tiny plants compared to adults (<15% in height compared to adults), showing neither signs of reproductive structures nor lignification at the stem base. All the species found in the adult stage are considered potential nurses independently whether they harbored recruits beneath their canopy or not, as far as they account for at least 0.5% of relative cover within each subsite. To assess whether they are actually acting as nurses or not, for each species of recruits, we tested for a significantly positive association among them, as explained in the "Analyses" section.

Analyses

The assemblage of facilitation networks

We constructed bipartite facilitation networks for the eight subsites (4 sites x 2 edaphic environments), where species are classified as adults or recruits based on the size of individuals in the sampling. Most species appear in both roles, as we observed individuals in both ontogenetic stages (Alcántara et al., 2019). To reduce stochasticity associated with low sample size, we only included as adults species (i.e., potential nurses), those with more than 0.5% of the relative coverage. Species absolute cover is available in “Adult species data” at Sánchez Martín et al. (2022). As for recruit species, we only considered those with at least 20 recruits per subsite. Then, we defined the edges of the networks as the significant facilitative associations between recruit and nurse species, calculated as follows:

Firstly, each interspecific interaction effect was calculated through the RII index (Relative interaction index: Armas, Ordiales, & Pugnaire, 2004). We measured this metric for each interaction (i.e., RII of each interaction; recruit-nurse, hereafter RIIi) which compared for each combination of recruit and nurse species in a subsite, the recruits density growing beneath the specific nurse species and the recruits density growing in the bare ground:

$$RIIi = \frac{\frac{RiNj}{CNj} - \frac{RiB}{CB}}{\frac{RiNj}{CNj} + \frac{RiB}{CB}}$$

Being *RiNj* the number of recruits of a specific species (Ri) growing beneath a specific nurse (Nj), *RiB*, the number of recruits of the same species (Ri) growing in bare ground (B), *CNj* is the cover occupied by the specific Nurse (Nj), and *CB* the bare ground cover. RIIi values range from -1 to 1, being positive when the density or recruits of a species under a given canopy is higher than in the bare ground, and negative when the opposite is true.

Secondly, we tested for the significance of each observed RIIi by comparing it to a null model that randomly assigned, 1000 times, the number of recruits of each species to the available canopies in a given subsite based on their relative cover. This redistribution considered that the nurse species are not equal in terms of cover, so we used the relative cover of each nurse species as a weighted element to be considered to obtain the weighted recruits' random distribution. Finally, all specific interactions whose observed RIIs were above 97.5% of the RIIs null model distribution were deemed significant and assigned a value of 1 in a matrix combining all possible pair-wise interactions of recruits and nurse species (edges of the networks). Non-facilitative interactions, including both non-significant (i.e., those between 2.5% and 97.5% of the RIIs null model distribution) and significant but negative interactions (i.e., those below 2.5% of the RIIs null model distribution), were set to zero as we are only interested in the facilitative interaction, that constitutes the core of the significant interactions found in the interaction networks. Specifically, of 347 significant interactions, we found 325 positives (93.66%) and 22 negative interactions (6.34%). We also found 606 pairwise interactions that were not significant, which means that their observed RIIs result from co-occurrence patterns that cannot be separated from the random expectation given the recruits' abundance and nurses' cover in the study community. All the interactions and significance tests can be checked in “Interactions data” at Sánchez Martín et al. (2022).

Rewiring and abiotic conditions

To assess whether rewiring is more limited in stressful environments than in mild environments, we explored the dissimilarity of the networks by assessing the different components of the dissimilarity in each possible pair of sites segregated by edaphic environments (i.e., stressful and mild environments) ($N = 6$ pairs of sub-localities per edaphic setting). According to Poisot et al. (2012), the dissimilarity of interactions (i.e., interactions turnover; β_{WN}) can be decoupled into two components: (1) the dissimilarity due to species turnover (β_{ST}) and (2) the dissimilarity of interactions established between species common to both networks, also called the rewiring component (β_{OS}). That means that overall interaction dissimilarity (β_{WN}) equals the sum of the dissimilarity explained by species turnover and rewiring ($\beta_{ST}+ \beta_{OS}$). Moreover, we calculated the dissimilarity in species composition (β_{S}) to assess how taxonomic changes in our communities could influence interaction dissimilarity. All these metrics were obtained by using the "network_betadiversity" function hosted in the "betalink/2.2.1" package of R (Poisot, 2016). Then, we assessed the differences between these metrics in the two contrasting edaphic environments. To do so, we conducted a generalized linear model with β_{WN}, β_{ST}, β_{OS}, or β_{S} as response variables and the edaphic setting (stressful/mild) as the predictor. We also included the identity of the pair sites as a random effect, but as it was not significant, it was not considered in further analyses. The information on the differences in species composition between stressful and mild subsites within each site and also the amount of positive interactions unique and shared between each pair of subsites is available in Appendix 3, Figure S2.

Fründ (2021) proposed an alternative normalization of dissimilarities to the Poisot's partition that avoids overestimating rewiring. However, in a recent article, Poisot claims that the Fründ approach is inappropriate for measuring network diversity because rewiring is relative to the number of interactions in the global network (since considered unique interactions) and thus dependent on both rewiring probability and species turnover (Poisot, 2022). To ensure that our main conclusions were not the result of different methodological choices, we reanalyzed as mentioned above but following Fründ's approach.

Phylogenetic constraint to rewiring

We estimated the phylogenetic constraints to rewiring following the method described in Montesinos-Navarro, Díaz, Torres, Caravaca, & Roldán (2019). Using the information from the eight subsites, separately for stressful and mild soils, we calculated the number of potential interactions between nurse and recruit species by counting the number of times each interaction could potentially occur across sites (up to 4 if both recruit and nurse are available at the four subsites of each soil). We only considered cases where there were two or more potential interactions, avoiding extremely high or low stochastic percentages due to a small number of potential interactions. Then, for each recruit, we defined the preferred nurse species as the species that facilitated it in more than 50% of their potential interactions (i.e., times that both species are connected across facilitation networks). The rest of the nurse species that also facilitated the recruits in any of the subsites were considered non-preferred nurse species (>0 % and ≤50% of their potential interactions. We also tested our analyses using other preferred/ non-preferred thresholds and checked the consistency of the results. The sensitivity test can be found in Appendix 3, Table S1.To calculate the phylogenetic distances between preferred and non-preferred nurse species, we generated the phylogenetic relationships among all of the plant species found in the sampling using the "V.phylomaker" function from the "V.phylomaker/0.1.0" R package (Jin and Qian, 2019). This function matches a given species input with the" GBOTB.extended.tre" phylogeny that includes 74,533 species as a result of the combination of two previous mega-phylogenies (Zanne et al., 2014; Smith and Brown, 2018). The species from our input that did not appear in the mega-phylogeny were inserted following scenario.2 (S2 adds unknown species randomly to nodes below the genus or family basal nodes; Jin & Qian, 2019; Qian & Jin, 2016). The branch length of the resulting phylogeny is displayed in chronological time units, specifically in millions of years (myr). Finally, to calculate the phylogenetic distances between non-preferred and preferred canopies, we used the "cophenetic" function hosted in the "ape/5.5" R package (Paradis and Schliep, 2019).

For each recruit species in each subsite, we calculated the minimum phylogenetic distance between the non-preferred nurse that was actually facilitating it and its closest relative among the preferred nurses of the recruit species. Then we obtained the average distances per recruit species in each subsite.

Then, we developed a null model to compare the obtained average with a random expectation. The null model aims to randomly assign a nurse from the adult plants present in a subsite. For this, for each recruit species at each site, the number of adult plants under which this species was found was counted. Then, that same number of plants was randomly selected from the total number of adult plants present at the site (excluding those belonging to nurse species preferred by the recruit species). Then, we used the nurses random selection to calculate the same phylogenetic distance described above. Finally, we assessed whether, for all recruit species at all subsites within each environment, the differences between the observed and expected values were statistically different from 0 by using the “t.test” function hosted in R base (R Core Team, 2019). This comparison was performed separately for networks sampled in stressful environments and for networks in mild environments.

Functional constraint to rewiring

To identify whether recruit species tend to recruit beneath specialized species, we first measured the degree of gypsum affinity (g) as a functional proxy of the species' ability to respond to gypsum constraints (Sánchez-Martín et al., 2021). The metric g for each species is strictly the proportion of its cover found in the stressful subsite divided by its total cover in the site (sum of cover in the stressful and mild subsite) but only considering the cover of plants growing alone to reduce biotic interference:

$$g = \frac{Cs}{Cs + Cm}$$

Where Cs is the species' cover in the stressful soil (i.e., gypsum) and Cm in the mild soil (i.e., limestones). Gypsum affinity (g) values range from 0 to 1, where 0 are species found in the limestone that never enter the gypsum and 1 represents strict gypsophytes restricted to gypsum. This metric is a good functional metric since it has been previously shown that the species' gypsum affinity in this community is positively related to harboring specific strategies to cope with harsh gypsum constraints, such as the ability of roots to penetrate hard gypsum layers and the accumulation of elements found in excess in those soils such as Ca, Mg and S (Sánchez-Martín et al., 2021). Species with g values close to 1 (high gypsum affinity) are those with the strategies mentioned above, while species with g values close to

0 (low gypsum affinity) do not show any specific response to survive in those soils (Sánchez-Martín et al., 2021). The g values calculated with few individuals may generate poor estimates of gypsum affinity due to stochasticity; therefore, we only calculated g for those species with a sufficient number of adults (20 in both stressful and mild environments). Finally, we averaged the values of g for each species across sites.

Then, we compared the average g of the non-preferred nurse species with the predicted by a null model that chose a set of non-preferred nurses at random, which could have facilitated each recruit in each subsite. To do so, from the pool of nurses presents in each subsite (excluding the preferred nurses for each recruit), we randomly selected the same number of observed non-preferred nurses facilitating the recruits and calculated their average gypsum affinity. Finally, we assessed whether the differences between the actual and predicted values, based on the null model, were statistically different from 0 by using the "t.test" function hosted in R base (R Core Team 2019). This comparison was performed separately for networks sampled in stressful and mild environments.

Results

Facilitation networks capture the most prevalent interactions in the studied communities in both edaphic environments, being the reliance on facilitation more prominent in the stressful subsites, as shown by the higher average RIIi in these soils (Appendix 3, Figure S3). These networks contain 325 interactions in the four sites resulting from the analysis of the recruitment patterns of 11,929 recruits (4,645 growing beneath a nurse species and 7,284 on the bare ground). These interactions can be grouped in two meta-webs that combine the facilitation patterns across communities in each edaphic environment (Figure 2a). Bringing together the networks of all the sites allows us to discern between interactions exclusive to one network from those that could potentially occur across networks (Figure 2b). Of the latter, we can differentiate between those that occur with high frequency (i.e., preferred) and those that can potentially occur in many places but are only realized in a few (i.e., non-preferred; Figure 2c). This distinction serves as the foundation for assessing limitations in the rewiring of interactions. For detailed facilitation networks of each subsite, see Appendix 3, Figure S4.

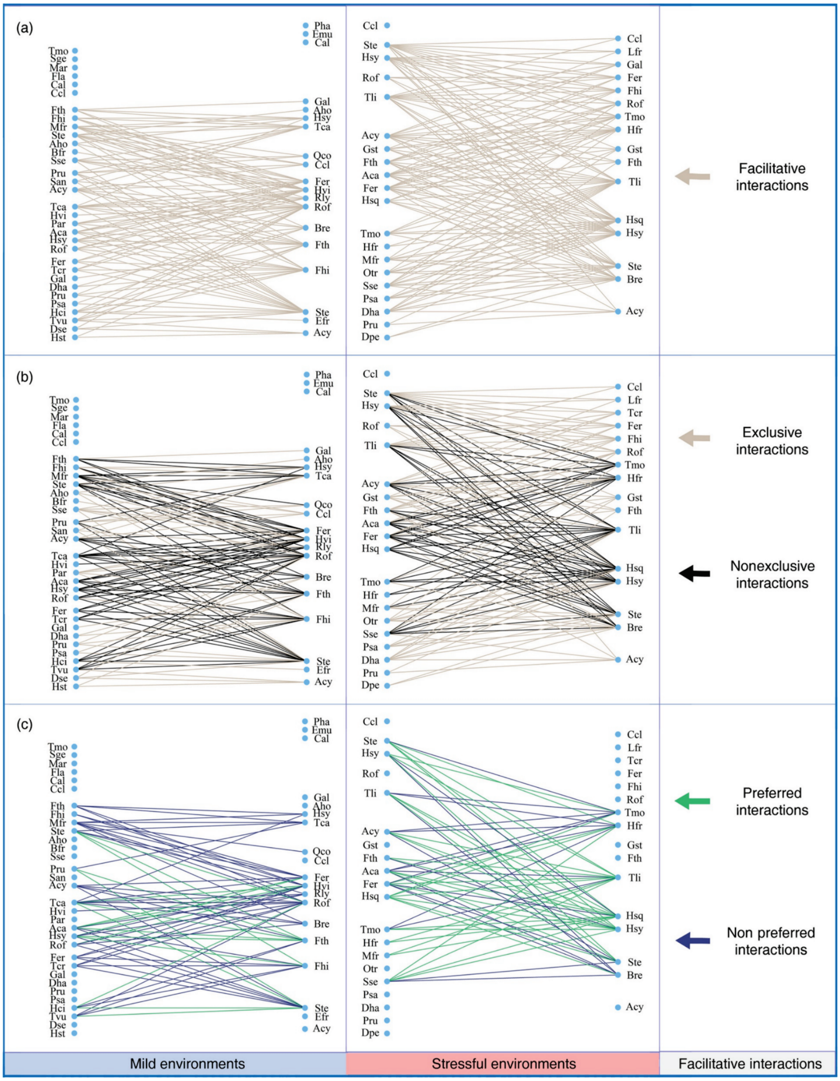
(a)
Pha Emu Cal Gal Aho Hsy Tca Qco Ccl Fer Hyi Rly Rof Bre Fth Fhi Ste Efr Acy
Tmo Sge Mar Fla Cal Ccl Fth Fhi Mfr Ste Aho Bfr Sse Pru San Acy Tca Hvi Par Aca Hsy Rof Fer Tcr Gal Dha Pru Psa Hci Tvu Dse Hst
Ccl Ste Hsy Rof Tli Acy Gst Fth Aca Fer Hsq Tmo Hfr Mfr Otr Sse Psa Dha Pru Dpe
Ccl Lfr Gal Fer Fhi Rof Tmo Hfr Gst Fth Tli Hsq Hsy Ste Bre Acy
Facilitative interactions
(b)
Pha Emu Cal Gal Aho Hsy Tca Qco Ccl Fer Hyi Rly Rof Bre Fth Fhi Ste Efr Acy
Tmo Sge Mar Fla Cal Ccl Fth Fhi Mfr Ste Aho Bfr Sse Pru San Acy Tca Hvi Par Aca Hsy Rof Fer Tcr Gal Dha Pru Psa Hci Tvu Dse Hst
Ccl Ste Hsy Rof Tli Acy Gst Fth Aca Fer Hsq Tmo Hfr Mfr Otr Sse Psa Dha Pru Dpe
Ccl Lfr Tcr Fer Fhi Rof Tmo Hfr Gst Fth Tli Hsq Hsy Ste Bre Acy
Exclusive interactions
Nonexclusive interactions
(c)
Pha Emu Cal Gal Aho Hsy Tca Qco Ccl Fer Hyi Rly Rof Bre Fth Fhi Ste Efr Acy
Tmo Sge Mar Fla Cal Ccl Fth Fhi Mfr Ste Aho Bfr Sse Pru San Acy Tca Hvi Par Aca Hsy Rof Fer Tcr Gal Dha Pru Psa Hci Tvu Dse Hst
Ccl Ste Hsy Rof Tli Acy Gst Fth Aca Fer Hsq Tmo Hfr Mfr Otr Sse Psa Dha Pru Dpe
Ccl Lfr Tcr Fer Fhi Rof Tmo Hfr Gst Fth Tli Hsq Hsy Ste Bre Acy
Preferred interactions
Non preferred interactions
Mild environments
Stressful environments
Facilitative interactions

Figure 2. **(a)** A meta web representation combining the facilitation patterns detected in the four studied sites in mild (left) and stressful (right) environments. Nodes in the left column represent recruit species, whereas nodes in the right column are the nurse species. Lines connecting nurse and recruit species represent significant facilitative interactions **(b)** Interactions dissimilarity patterns in the meta web can be due to interactions between unique species of local networks, so all the changes in these interactions among networks are due to species turnover (grey arrows). Conversely, we also found interactions that can occur in more than one local network (black arrows). **(c)** Among the latter, we can identify interactions occurring in more (preferred ones; green arrows) or in less (non-preferred ones; fuchsia arrows) than half of its possibilities, being the latter those identified as rewiring cases. Species' full names related to codes are in Appendix 3, Table S2.

Rewiring and abiotic conditions

Network dissimilarity analyses suggested a differentiated pattern between networks in different edaphic environments. Networks from mild environments showed higher dissimilarity of interactions (mean β_{WN}±SE; 0.855±0.033) than networks in stressful environments (0.734±0.033) (F= 6.796, p=0.026; Figure 3 a, b). Interestingly, this greater dissimilarity of interactions was not equally distributed between rewiring (β_{OS}) and species turnover (β_{ST}). While rewiring (β_{OS}) was significantly lower in the stressful environments (0.270±0.068) than in the mild environments (0.623±0.068) (F=13.613; p=0.004; Figure 3 a, c), species turnover (β_{ST}) was higher in stressful (0.463±0.054) than in mild environments (0.232±0.054) (F=9.189; p=0.013; Figure 3 a, d). Remarkably, this occurred even though the dissimilarity in species composition (β_S) did not differ among environments (F=3.783; p=0.080), and in any case, showed a tendency to be greater in mild (0.441±0.023) than in stressful (0.378 ± 0.023) environments. Similar results were obtained with Fründ's alternative approach. See Appendix 3, Figure S5 for details.

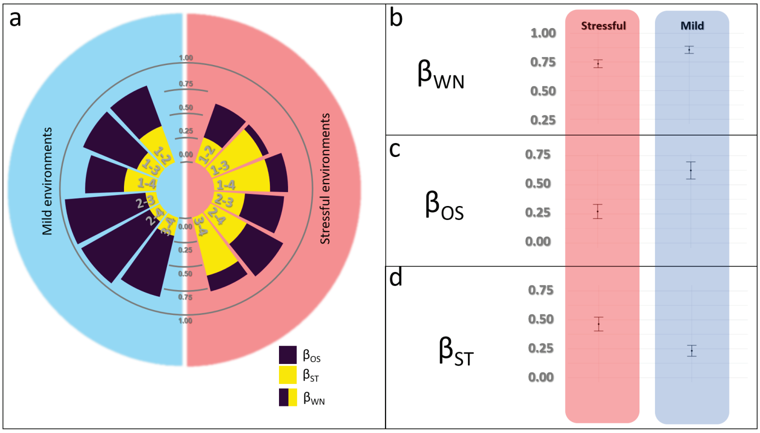

Figure 3. Results of networks comparison across sites in two contrasting edaphic environments. The circle bar plot **(a)** summarises the dissimilarity of interactions decoupled in rewiring and species turnover ($\beta_{WN} = \beta_{OS} + \beta_{ST}$), being the numbers within each bar the specific pair of sites being compared. **(b)** shows the overall dissimilarity of interactions (β_{WN}) between mild and stressful environments **(c)** shows differences between environments in the rewiring component (βOS), while **(d)** refers to species turnover (β_{ST}) comparison between environments.

Constraints to rewiring

Rewiring is phylogenetically constrained in mild communities, i.e., recruits rewire preferentially beneath non-preferred canopies that are phylogenetically closer to the preferred ones than expected by the null model (t = −3.635; df = 16; p= 0.002; CI 95% (-104.814,-27.588); Figure 4a left). On the contrary, this phylogenetic constraint does not occur in communities from stressful environments (t = -1.188; df = 19; p= 0.249, CI 95% (-55.891,15.411), Figure 4a right). On the other hand, the rewiring constraints in stressful environments are governed by the gypsum affinity of the nurses, i.e., facilitated plants rewire with nurse species with higher levels of gypsum affinity than expected by chance (t = 2.608; df = 19, p= 0.017; CI 95% (0.038,0.344); Figure 4b right). In contrast, this functional limitation does not seem to apply in communities from mild environments (t = -0.718; df = 13; p= 0.486; CI 95% (-0.102,0.051); Figure 4b left). These results show consistency when checked with other preference thresholds. See Appendix 3, Table S1 for the results of a sensitivity test checking multiple thresholds.

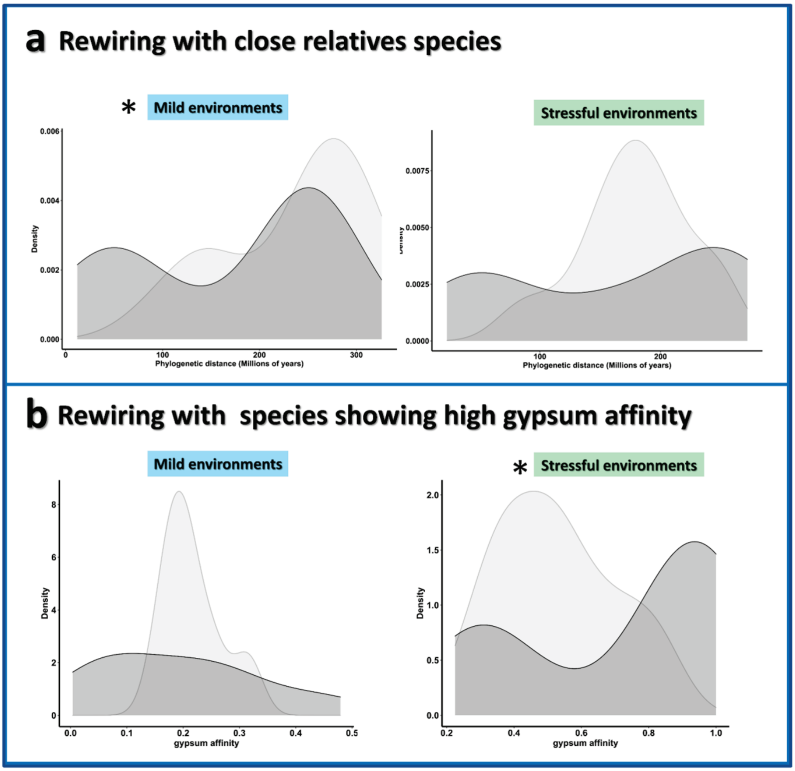

Figure 4. (a) Density plots comparing the observed (dark grey) and expected (light grey) distribution of the minimum phylogenetic distances between non-preferred and their closest preferred nurse species. **(b)** Density plots comparing the observed (dark grey) and expected (light grey) distribution of the average gypsum affinity of non-preferred nurses. The observed distribution is significantly different from the expected by the null model.

Discussion

The observed dissimilarity pattern of interactions sheds light on how these communities subjected to different stress levels may respond to changes in community composition. In both environments, interaction dissimilarity was high, suggesting that the facilitative interactions can shift with high freedom across sites. Our results showed that interaction dissimilarity was slightly but significantly lower in stressful than in mild environments. However, much of this dissimilarity is explained by species turnover across communities, causing the appearance and disappearance of multiple interactions, a common pattern in our networks of stressful systems. Conversely, the dynamics reversed when we focused on the pool of shared species across sites, showing that rewiring was of less magnitude in stressful environments than in mild ones. These spatial variations have implications for understanding how the structure of these communities will vary under a species loss scenario.

On the one hand, we found a higher turnover in stressful environments even though species composition dissimilarities between mild and stressful environments did not differ. The higher reliance on facilitation of the recruiting species in stressful environments results in concomitant changes in interactions with the gain or loss of nurse species. On the other hand, rewiring was low in stressful environments. Below we discuss that in addition to stressful conditions, other phylogenetic and functional specific patterns may explain the limitations to rewiring patterns.

Limitations on rewiring patterns

Despite the high rewiring capacity found in mild environments, we still identified constraints to the establishment of interactions with non-preferred nurse species. Specifically, we observed a pattern where the recruiting species interacted with the non-preferred nurse species that were close relatives of their preferred nurse species. That seems logical under the assumption that closely related species tend to share similar traits, so the traits that make a nurse suitable for certain species could also apply to similar species (Gómez et al., 2010). Indeed, this phylogenetic restriction to rewiring seems to apply to other mutualistic networks, such as those established between plants and fungi (Montesinos-Navarro et al., 2019), suggesting that it could be a pattern widely distributed in nature. Conversely, we found no functional limitations related to gypsum affinity for rewiring in mild environments, which is logical since the specific traits that sustain facilitative interactions in stressful environmental contexts may be meaningless in these milder environments (Butterfield & Callaway 2013).

In contrast, we did not find any phylogenetic constraint to rewiring in stressful environments. In these systems, the harshness of the abiotic conditions has shaped a plant community harboring species with specific adaptations (Palacio et al., 2007).

These specialized traits have emerged at different points in evolutionary history (Moore et al., 2014; Escudero et al., 2015), which prevents finding phylogenetic constraints to rewiring because species with varying gypsum affinity levels can be found within the same clades of the community (Appendix 3, Figure S6). Indeed, in these systems, rewiring occurred with non-preferred species showing higher gypsum affinity levels than expected independently of the phylogenetic relatedness. Among the potential mechanisms underlying this pattern might be the fact that some gypsum-specialized species seem to be better nurses than stress-sensitive species (Saiz et al., 2014; Foronda et al., 2019), which may promote that many species depend on the presence of these nurses to survive (Verdú & Valiente-Banuet, 2008).

Ecological implications

This paper emphasizes the critical role that well-adapted species play in the stability of plant communities. When communities are subjected to high-intensity stressors and recruitment on bare soil is highly undermined, facilitative interactions are established with those species that have mechanisms to deal adequately with this stressful condition (Saiz et al., 2014; Foronda et al., 2019). This fact fosters the appearance of benefactor species facilitating multiple species (Verdú & Valiente-Banuet, 2008). A role that the gypsum specialists assume as they are the only ones that can guarantee the survival of facilitated species. However, that limits rewiring with other less-specialized species since they may not provide the needed benefits. For that reason, retaining these specialist nurse species is vital in preserving the system's stability since their disappearance could be catastrophic for the entire community (Valiente-Banuet & Verdú, 2013). This is especially relevant in fragmented stressful edaphic environments where many specialized species are endemisms whose isolation makes them more vulnerable to extinction (Corlett & Tomlinson 2020).

In contrast, in mild environments where the reliance on facilitation is less prominent, the rewiring capacity seems limited by nurse traits that must be phylogenetically conserved. It is worth noticing that the phylogenetically constrained rewiring found in these environments does not respond to recruits' necessity for particular traits as those found in the stressful environments, but to other general traits evolutionary conserved that are not homogeneous for all recruit species necessarily. These less stringent facilitation requirements could open a gap for a more prominent contribution of rewiring in structuring these communities, since facilitative effects are not so shaped by the traits of specific taxa.

Nevertheless, these results should be interpreted with caution as our results are based on a space-by-time substitution approach using multiple sites' snapshots. Ideally, it would be interesting to monitor this process over time within each subsite. However, assessing biological interactions in the field requires intense samplings, so conducting these studies in the long term under field conditions might become unfeasible.

Conclusions

We provide evidence of a distinct pattern in the rewiring of facilitation-driven communities at different stress levels. On the one hand, in highly stressful environments where plants strongly depend on facilitation, rewiring is limited to growing beneath habitat-specialized species. However, when environments are milder, rewiring, although of less magnitude, is still limited to occur with nurse species relatively close to their preferred ones.

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RESULTS SYNTHESIS

This Ph.D. project unveils how the interplay between abiotic conditions, plant traits, and biotic interactions shapes the assembly of natural plant communities. Our results show that species inhabiting stressful soils exhibit contrasting strategies to cope with such conditions. While some species have traits to overcome such constraints, others, rarer and stress-sensitive species, depend on facilitation for their maintenance, giving rise to multispecific patches where species balance their performance. These facilitative interactions appear to rely on the presence of nurses which differ in their specific traits, limiting the rewiring capacity of beneficiary species to occur with particular nurses. These rewiring constraints varied depending on the environment, resulting in a complex set of assembly rules finally shaping plant communities. The following paragraphs highlight the key results found in each chapter:

Chapter 1

Firstly, chapter 1 demonstrated niche segregation between well-adapted and stress-sensitive species co-occurring in a community. Secondly, we identified that this segregation is related to specific responses to overcome severe gypsum constraints regarding particular chemical and physical stresses derived from gypsum presence. Thirdly, the presence of these particular traits was related to the gypsum affinity of the species, so species displaying a higher gypsum affinity (i.e., larger cover in gypsum than in the surrounding environments) tended to have these traits while stress-sensitive species did not. Finally, we did not identify trade-offs between having these traits and the ability of these species to respond to other general constraints of a semi-arid environment.

Chapter 2

Chapter 2 shows that plant communities growing on gypsum relied strongly on facilitation. We evidenced facilitation occurring at the juvenile stage, showing that facilitation enhanced the establishment of rare species, which also tend to be those with low affinity to the stressful environment. Then, when plants become adults, plant-plant interactions may also enhance species coexistence, as those plants that grow within vegetation patches show smaller size differences among species than the same conspecific plants growing isolated. This pattern suggests that plant cohabiting vegetation patches could be presenting a more equitable use of resources favoring their persistence.

Chapter 3

In this last chapter, we provide empirical evidence showing that facilitated species tend to rewire with nurse species that show traits similar to their preferred nurses. Additionally, we demonstrated that in gypsum sites, beneficiary plants tend to rewire with nurse species with a high gypsum affinity, while in non-gypsum sites, they tend to rewire to nurses that are closely related to their preferred nurses. Elucidating general patterns in the rewiring of facilitative processes in contrasted environments can be critical to predict how natural communities may react to future perturbation.

GENERAL DISCUSSION AND CONCLUSIONS

Plant diversity within a community is maintained by a delicate equilibrium based on the compatibility of each species with the abiotic environment and with other species (Kraft et al., 2015; Figure 1). These two elements constantly change in sign and relative intensity, hindering our understanding of how

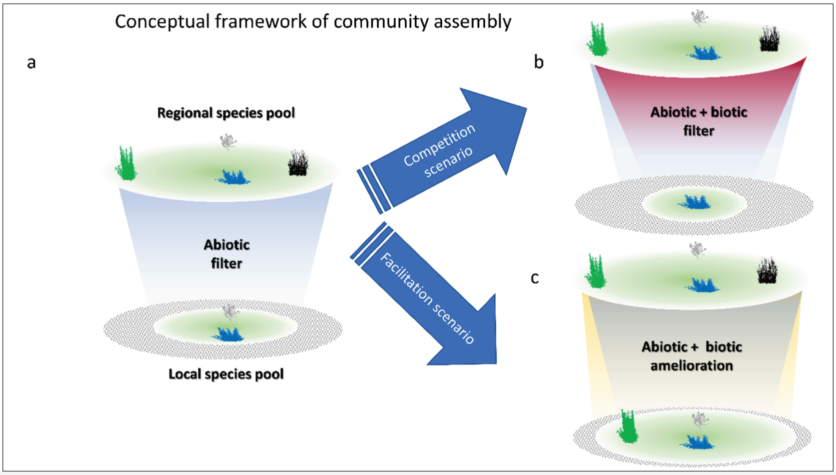

Figure 1. Simplified framework of community assembly rules. Wild natural communities result from a combination of processes that sample from the regional pool of species, a subset of co-occurring species (Holyoak, Leibold, & Holt, 2005). **(a)** Species from the local pool are a subset of the regional pool species capable of overcoming the imposed abiotic filter of the environment. However, biotic interactions can still modulate the final community. On the one hand, **(b)** biotic interactions can aggravate the effect of the abiotic filter, thus giving place to poorer communities. On the other hand, **(c)** biotic interactions can alleviate the harmful abiotic condition allowing the presence of species that could otherwise be lost.

communities maintain diversity and will respond to future changes (Medeiros, Boege, Del-Val, Zaldívar-Riverón, & Saavedra, 2020).

The three chapters of the thesis contribute to understanding how species are assembled and survive in natural communities driven by facilitation. Our studied plant communities harbor a diversity of species differing in their response to stressful conditions. These functional differences determine each species' survival strategy. While some species show specific traits to overcome the limitations imposed by gypsum (species with high gypsum affinity), others that do not have those traits, rely more on establishing facilitative interactions with the neighboring species (i.e., stress-sensitive species). Moreover, within plant patches, species show a more similar performance than their conspecifics growing isolated, suggesting a more equitative use of resources driven by plant-plant interactions, with a potential impact on species coexistence. Finally, we found that the rewiring of facilitative interactions is not random, but it tends to occur with nurse species that possess a high affinity for the gypsum substrate in gypsiferous habitats. That suggests that the presence of species adapted to a given environment may be critical to maintaining stress-sensitive species diversity. However, in surrounding less stressful limestone lithology, the reconnection tends to occur with nurse species related to preferred nurses, suggesting that functional traits may differ among nurses and that facilitative interactions have some specificity for several phylogenetically conserved traits.

The species and the abiotic filter

Species do not perceive their environment in the same way but rather depend on each species' traits and how these traits interact with the environment. Therefore, species are subjected to different strain levels for the same given stress, resulting in different deviations from their ecological optimum (Liancourt, Le Bagousse-Pinguet, Rixen, & Dolezal, 2017). In this regard, our studied communities harbor a pool of species with different responses to the gypsum's abiotic filter on plant life.

Specifically, we found that species with a high gypsum affinity, i.e., those that have more cover in gypsum than in less stressful surrounding environments, follow a strategy involving major water acquisition from deeper soil layers, suggesting an ability to penetrate hard soil layers, while also accumulate Ca, Mg, and S, which may prevent chemical toxicity. Within the group of species with these traits, we found the gypsophytes species, species exclusively inhabiting gypsum outcrops, but also certain gypsovags species (i.e., those inhabiting both gypsum and other habitats) that showed better performance in the gypsum outcrops than in the surrounding milder environments. In contrast, the species without those traits showed a substantial decrease in relative cover in gypsum compared to less stressful surrounding limestone habitats, suggesting a solid abiotic filter for these gypsovags (low gypsum affinity, stress-sensitive species).

Contrary to our expectation, our results did not identify any clear cost of harboring gypsum-tolerance traits concerning macronutrient acquisition or water use efficiency that could explain the absence of these edaphic-specialist species beyond the limit of gypsum soils. However, the lack of evidence is not necessarily evidence of absence. Indeed, it has been demonstrated that gypsophytes species can grow out of gypsum, even displaying a better performance under controlled circumstances (Cera, Montserrat-Martí, Ferrio, Drenovsky, & Palacio, 2020), which could suggest a relevant role of the biotic neighborhood in explaining the natural filter. However, a complete understanding of the principles that avoid the expansion of gypsophytes species beyond the limit of gypsum remains obscure and deserves further research.

Facilitative effects on plant communities

The functional segregation between gypsum-adapted and gypsum-sensitive species may promote plants' spatial associations, as interspecific competence is undermined due to niche segregation among functionally different species (Bulleri, Bruno, Silliman, & Stachowicz, 2016). In that circumstance, it is to be expected that edaphic-specialist (i.e., gypsum-adapted species) act as nurses for species without specific traits to deal with gypsum, explaining the wide richness of stress-sensitive species that coexist with edaphic-specialist species in these habitats (Escudero, Palacio, Maestre, & Luzuriaga, 2015; Moore, Mota, Douglas, Olvera, & Ochoterena, 2014; Mota et al., 2008; Mota, Sánchez-Gómez, & Guirado, 2011; Murdy, 1968; Sianta & Kay, 2019).

Indeed, our results indicate that species with low gypsum affinity, which tend to be less abundant, rely more on facilitation than those with high gypsum affinity, which tend to be more abundant. In literature, some studies have shown that facilitation favors rare species (Calatayud et al., 2020; Soliveres, Maestre, Berdugo, & Allan, 2015), although the mechanisms by which this pattern emerges were not identified, while others have evidenced that facilitation is more important for species living out of their ecological optimum (stress-sensitive; Gross et al., 2009; Le Bagousse-Pinguet et al., 2014; Liancourt et al., 2005; Qi et al., 2018). Our findings contribute to integrating these two perspectives, demonstrating that species with low edaphic affinity may be scarce in a specific environment because they live outside their ecological optimum, and these, in turn, tend to benefit from living in association with neighboring species.

The above-mentioned facilitative effects were measured at an early ontogenetic stage. However, biotic interactions can change in sign and intensity in unpredictable ways throughout ontogeny due to changes in the interacting plants, the abiotic environment, or indirect effects of the neighborhood (Castillo, Verdú, & Valiente-Banuet, 2010; Hirn et al., 2022; Schöb, Armas, & Pugnaire, 2013), blurring facilitation's long-lasting effects. Indeed, facilitation can only ensure interacting species coexistence when the benefits of the association compensate for the prejudices along the live-span of the interacting plants (Valiente-Banuet & Verdú, 2013).

In this regard, we showed that adult species growing associated within a vegetation patch displayed smaller size asymmetries among them than their conspecifics growing isolated. Although we cannot identify the specific mechanisms behind this effect, it should be mediated by direct or indirect interactions with the neighboring plants.

The size asymmetry reduction could have profound implications for species assembly since a similar size could potentially result in a more equalized fitness (Younginger, Sirová, Cruzan, & Ballhorn, 2017), one of the major forces favoring species coexistence (Chesson, 2000). Multiple mechanisms could be implied in the reduction of these asymmetries. For instance, neighboring plants can exchange nitrogen and water or share beneficial mycorrhizae (Montesinos-Navarro, Verdú, Querejeta, Sortibrán, & Valiente-Banuet, 2016; Montesinos-Navarro, Verdú, Querejeta, & Valiente-Banuet, 2017, 2019; Sortibrán, Verdú, & Valiente-Banuet, 2019). All these potential benefits must be added to the observed trait differentiation between species that could minimize niche resource overlapping, maximizing potential positive effects since interspecific competition would be minimized (Bulleri et al., 2016; Sánchez-Martín et al., 2021).

Limitations on facilitation's rewiring patterns

Although many species rely on facilitation to survive, these species are in a fragile position due to the changing nature of biotic interactions. Facilitation depends on a delicate balance between interacting species traits and the abiotic conditions (Poisot, Stouffer, & Gravel, 2015), a balance altered by the global change which is pushing natural systems into uncharted territory (Komatsu et al., 2019). Amongst the harmful effects inflicting natural systems, the loss of diversity is the most striking and is often preceded by the loss of biological interactions (Janzen, 1974). However, species that rely on facilitation can at least partially compensate for these changes by their rewiring capacity, i.e., the ability to switch nurses that might provide robustness to facilitation networks.

In this regard, we show that rewiring is not random, meaning that not all species can act as nurses. We show that rewiring tendencies differ depending on the environment. In this regard, we demonstrated that rewiring in stressful gypsum environments tends to occur with nurse species displaying a high gypsum affinity. In contrast, gypsum affinity traits do not play a significant role in less stressful limestone systems, where rewiring tends to occur with phylogenetically close relatives to the preferred nurses.

Our results suggest that habitat-specialized species play a fundamental role in facilitative interactions, so the maintenance of these species should be prioritized in conservation policies. Furthermore, we find that functional or phylogenetic redundancy could be a good asset for community stability, as the absence of a good nurse could be palliated by the presence of a similar species.

Future avenues

Although our approach has shed some light on the mechanisms shaping natural communities, we are barely scratching the surface of a complex interplay of processes. We have demonstrated that facilitation plays a fundamental role in the assembly of natural communities. However, to fully understand how communities are assembled, we need to delve deeper into the following points we consider critical to understand the static and dynamic picture of natural communities.

Firstly, we need to understand why certain species cannot survive in nature outside their local optimum (i.e., specialists), although they thrive well under controlled circumstances (Cera et al., 2020). Specifically, we need to address the role played by biotic interactions in this pattern. It could be hypothesized that in the absence of the abiotic filter gypsum inflicts on communities, other species with a broader niche would outperform specialists. A deeper understanding of these unexplored processes may improve our current predictions of how species may be affected by changes in community composition.

Secondly, we must explore the neglected realm of indirect interactions, which is critical to understanding the biotic neighborhood (Schöb et al., 2013; Simmons et al., 2019). These interactions could shed light on the subtle mechanisms that enable the survival of multispecific patches and how species modify their role depending on their neighbors constituting the patches. To that effect, the new artificial intelligence tools provide an extraordinary chance to understand how these higher-order interactions shape community diversity by boosting, attenuating, or reversing the effect of direct interactions (Hirn et al., 2022). In this regard, observational studies will prevail over unfeasible experimental designs as they more efficiently capture the complex reality of natural systems.

Finally, a deeper understanding of potential general patterns in the rewiring of interactions will contribute to designing conservation plans and restoring projects of plant communities, allowing the applicability of this knowledge to other managed or natural ecosystems. Understanding which factors limit plant development and which traits provide the best nursing effects in each system is also critical to designing special and targeted efforts to increase systems' resilience, thus diminishing the harmful impacts of the current crisis of biodiversity loss, fueled by global change drivers (Ceballos et al., 2015; Cowie, Bouchet, & Fontaine, 2022; Kolbert, 2014).

Conclusions

This Ph.D. project identifies essential elements to understand how species are assembled in natural communities. We demonstrated a diversity of strategies that arise among co-occurring species to survive under the same environmental context. While some species have specific traits to deal with the edaphic limitations of the system, others do not, resulting in functional differences among species in the communities. That gives place to systems strongly shaped by facilitation that result in spatial associations that endure throughout the ontogeny of plants. The realization of facilitative interactions depends on both the local environment stress and the presence of species with traits that can mitigate these stresses. Finally, functional or phylogenetic redundancy among species is key to maintaining the stability of facilitation networks sustaining the diversity of these communities.

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APPENDIX 1

Table S1

Trait	model	coefficient	T-value	P-value
d-excess	gls(d-excess~g+heigh,correlation=corPagel(1,tree))	26.66±4.82	5.52	0.0004***
$\delta^{18}O$	gls(d18O~g+heigh,correlation=corPagel(1,tree))	-4.025±2.07	-1.94	0.08º
$\delta^{13}C$	gls(d13C~g+heigh,correlation=corPagel(1,tree))	-1.58±0.60	-2.65	0.03*
C	gls(C~g,correlation=corPagel(1,tree))	-0.001±0.0006	-2.22	0.05º
N	gls(N~g,correlation=corPagel(1,tree))	-8.88E-6±1.82E-5	-0.49	0.63
P	gls(P~g,correlation=corPagel(1,tree))	-0.03±0.02	-1.54	0.15
K	gls(K~g,correlation=corPagel(1,tree))	-1.80±0.97	-1.86	0.09
Ca	gls(Ca~g,correlation=corPagel(1,tree))	1.52±0.57	2.66	0.02*
S	gls(S~g,correlation=corPagel(1,tree))	0.50±<0.01	97082.62	<0.001**
Mg	gls(Mg~g,correlation=corPagel(1,tree))	0.02±<0.01	1044.7	<0.001**

Table S1. This table shows the results obtained for each variable with univariate phylogenetic models (pgls) and its correlation with g (gypsum affinity). All the isotopic variables include height as a co-factor since it is a significant factor for the model. For the rest of the variables height is not significant, and it has been removed for simplicity. Univariate results supported the prediction of the PCA where the significant factors are those with higher loading in the PCA (ºmarginally significant at p 0.1,*Significant at p 0.05, ** Significant at p 0.01,*** significant at p 0.001)

APPENDIX 2

Figure S1

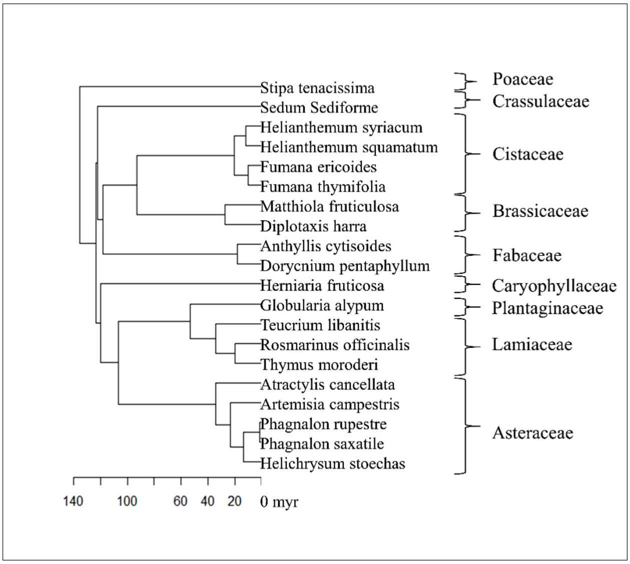

Figure S1. Phylogenetic relationships among the studied species. Plant families appear in the figure. The units of the axis scale are millions of years (myr).

Figure S2

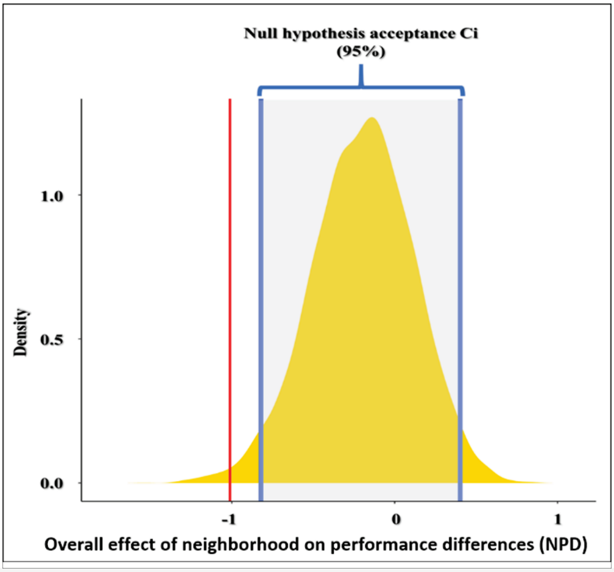

Figure S2. The observed NPD (red line) is significantly lower than expected by the null model. The two blue lines represent the 95% credible interval of the null model. The null model reshuffled 10,000 times the plant's position (non-associated/associated), keeping the data structure unchanged regarding the species identity and locality where it was found.

APPENDIX 2

Table S1

Stressful sub-locality-species	C max (cm^2)	C median (cm^2)	C min (cm^2)	Ø max (cm)	Ø median (cm)	Ø min (cm)
Crevillente-*Artemisia campestris*	471.24	139.02	37.70	24.49	13.30	6.93
Crevillente-*Atractylis cancellata*	219.91	32.99	12.57	16.73	6.48	4.00
Crevillente-*Fumana ericoides*	801.11	212.06	56.55	31.94	16.43	8.49
Crevillente-*Fumana thymifolia*	777.54	93.46	1.57	31.46	10.91	1.41
Crevillente-*Globularia alypum*	1801.70	333.79	19.63	47.90	20.62	5.00
Crevillente-*Helianthemum squamatum*	551.35	56.55	6.28	26.50	8.49	2.83
Crevillente-*Helianthemum syriacum*	549.78	43.20	7.07	26.46	7.42	3.00
Crevillente-*Helichrysum stoechas*	282.74	47.12	4.71	18.97	7.75	2.45
Crevillente-*Herniaria fruticosa*	267.04	60.87	7.85	18.44	8.80	3.16
Crevillente-*Stipa tenacissima*	35.34	17.28	7.07	6.71	4.69	3.00
Crevillente-*Teucrium libanitis*	4618.14	176.71	11.00	76.68	15.00	3.74
Crevillente-*Thymus moroderi*	1060.29	71.47	3.93	36.74	9.54	2.24
Monóvar-*Anthyllis cytisoides*	650.31	169.65	49.48	28.77	14.70	7.94
Monóvar-*Atractylis cancellata*	141.37	49.48	19.63	13.42	7.94	5.00
Monóvar-*Diplotaxis harra*	91.89	9.42	0.79	10.82	3.46	1.00
Monóvar-*Fumana ericoides*	69.12	25.13	9.42	9.38	5.66	3.46
Monóvar-*Fumana thymifolia*	392.70	38.88	3.93	22.36	7.04	2.24
Monóvar-*Helianthemum squamatum*	415.48	81.68	12.57	23.00	10.20	4.00
Monóvar-*Helianthemum syriacum*	490.87	54.98	3.14	25.00	8.37	2.00
Monóvar-*Helichrysum stoechas*	282.74	38.48	7.85	18.97	7.00	3.16
Monóvar-*Herniaria fruticosa*	84.82	49.48	15.71	10.39	7.94	4.47
Monóvar-*Matthiola fruticulosa*	12.57	5.11	1.57	4.00	2.55	1.41
Monóvar-*Phagnalon saxatile*	376.99	64.40	9.42	21.91	9.06	3.46
Monóvar-*Salsola genistoides*	157.08	86.39	28.27	14.14	10.49	6.00
Monóvar-*Stipa tenacissima*	117.81	23.56	3.14	12.25	5.48	2.00
Monóvar-*Teucrium libanitis*	534.07	141.37	9.42	26.08	13.42	3.46
Petrer-*Anthyllis cytisoides*	361.28	51.05	4.71	21.45	8.06	2.45
Petrer-*Atractylis cancellata*	40.84	28.27	12.57	7.21	6.00	4.00
Petrer-*Cistus clusii*	1114.48	95.43	4.71	37.67	11.02	2.45
Petrer-*Coronilla minima*	109.96	22.38	7.07	11.83	5.34	3.00
Petrer-*Dorycnium pentaphyllum*	314.16	75.40	3.93	20.00	9.80	2.24
Petrer-*Fumana ericoides*	157.08	21.21	1.57	14.14	5.20	1.41
Petrer-*Fumana thymifolia*	395.84	35.34	6.28	22.45	6.71	2.83

Stressful sub-locality-species	C max (cm^2)	C median (cm^2)	C min (cm^2)	Ø max (cm)	Ø median (cm)	Ø min (cm)
Petrer-*Helianthemum squamatum*	530.93	31.42	3.14	26.00	6.32	2.00
Petrer-*Helianthemum syriacum*	678.58	19.63	1.57	29.39	5.00	1.41
Petrer-*Helichrysum stoechas*	204.20	56.55	5.89	16.12	8.49	2.74
Petrer-*Herniaria fruticosa*	439.82	51.05	12.57	23.66	8.06	4.00
Petrer-*Lithodora fruticosa*	1225.22	112.70	9.42	39.50	11.98	3.46
Petrer-*Matthiola fruticulosa*	43.20	4.71	0.79	7.42	2.45	1.00
Petrer-*Stipa tenacissima*	94.25	37.70	6.28	10.95	6.93	2.83
Petrer-*Teucrium capitatum*	449.25	31.42	7.85	23.92	6.32	3.16
Petrer-*Teucrium libanitis*	2073.45	193.21	15.71	51.38	15.68	4.47
Petrer-*Thymus moroderi*	478.31	42.41	4.71	24.68	7.35	2.45
Salinas-*Atractylis cancellata*	31.42	14.14	4.71	6.32	4.24	2.45
Salinas-*Cistus clusii*	307.09	184.57	70.69	19.77	15.33	9.49
Salinas-*Fumana ericoides*	172.79	28.27	3.93	14.83	6.00	2.24
Salinas-*Fumana hispidula*	615.75	235.62	40.84	28.00	17.32	7.21
Salinas-*Fumana thymifolia*	117.81	9.42	0.39	12.25	3.46	0.71
Salinas-*Helianthemum squamatum*	251.33	27.49	3.14	17.89	5.92	2.00
Salinas-*Helianthemum syriacum*	142.94	23.56	0.79	13.49	5.48	1.00
Salinas-*Herniaria fruticosa*	157.08	42.41	3.14	14.14	7.35	2.00
Salinas-*Matthiola fruticulosa*	32.99	9.42	3.14	6.48	3.46	2.00
Salinas-*Stipa tenacissima*	192.42	38.48	3.93	15.65	7.00	2.24
Salinas-*Teucrium libanitis*	376.99	54.98	1.57	21.91	8.37	1.41

Table S1. Maximum, median, and minimum specific plant coverage values (Cmax, Cmedian, and Cmin) and diameters (Ømax, Ømedian, and Ømin) across stressful sub-localities. The 1% of the littlest plant for each species in a sub-locality was excluded to ensure that all plant were adults. Diameters were calculated considering coverage as the area of a plant with a perfect circular form.

APPENDIX 3

Figure S1

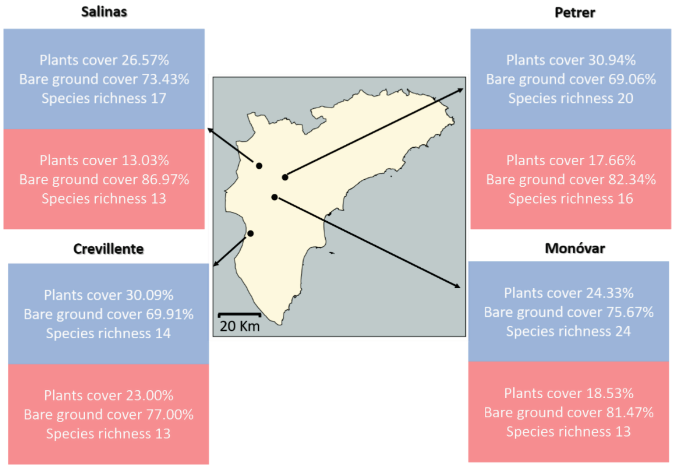

Figure S1. Site plant community basic information. Each textbox refers to a study site. Within each study site, boxes provide the information referring to the mild (blue boxes) and the stressful (red boxes) subsite. For each community, it is provided the % of plant cover, % of bare ground cover, and species richness, but only those species with 20 or more recruits in the subsite or adults with a relative cover greater than 0.5% are considered.

Figura S2

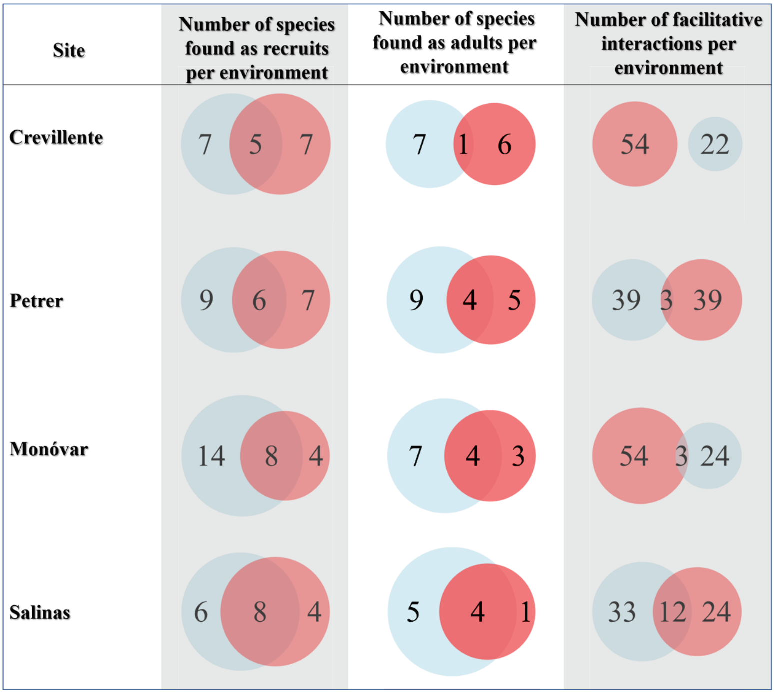

Figure S2. Variation in species richness at the level of recruit species, adult species, and the number of facilitative interactions per environment. Every combination represents a Venn diagram showing the number of species or interactions occurring in the mild (blue circles) or in the stressful (red circles) environment within each site. The crossing areas between the two circles quantify the number of species or interactions co-occurring in both subsites.

Figure S3

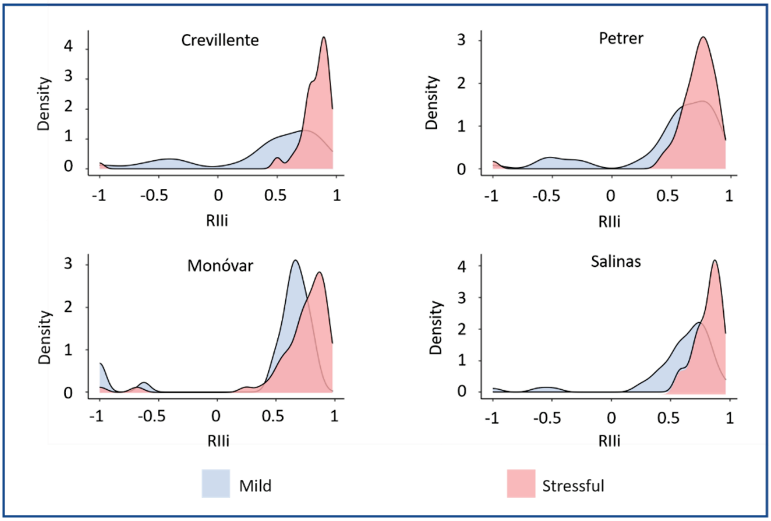

Figure S3. Density plots of the significant RIIi detected in each of the four sites from the stressful (red) and the mild (blue) environments. RIIi is an interaction intensity index that compares the recruiting intensity of one species (j) growing beneath a specific nurse (i) compared with the same recruit species growing on the bare ground. RIIi negative values suggest competition (i.e., recruits grow preferentially in the bare ground), whereas positive values suggest facilitation (i.e., the recruit species grow preferentially beneath specific nurse i).

Figure S4

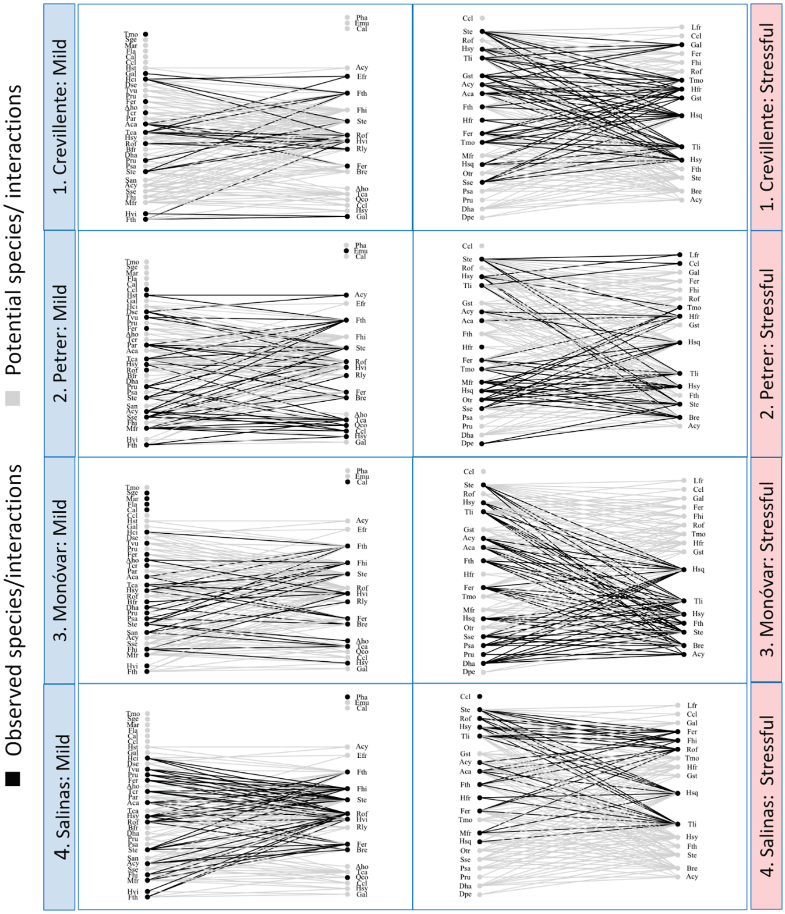

Figure S4. The type of environment separates facilitation networks for each site: in blue (mild environments; communities growing in limestone) and in red (stressful environments; communities growing in gypsum). Nodes in the left column represent recruit species, whereas nodes in the right column are the nurse species. The facilitation network of each site (black) is embedded in the meta web, containing all the potential species (both recruit and nurse) and interactions from each site, separating mild and stressful environments. Species' full names related to codes are in Appendix 3, Table S2.

Figure S5

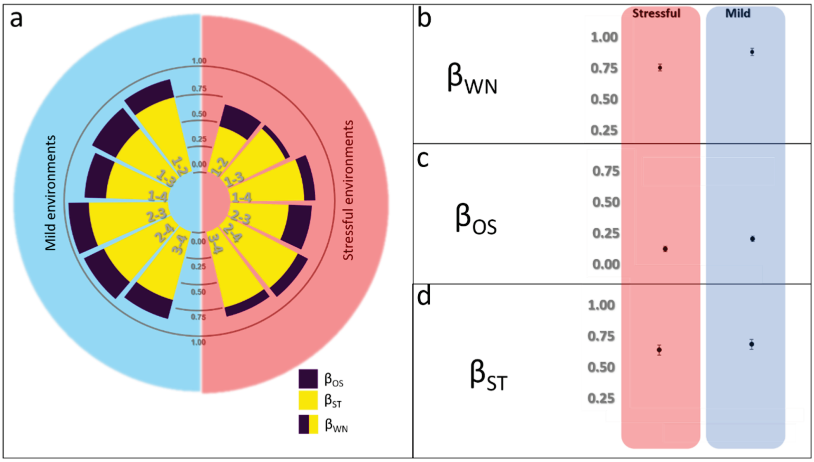

Figure S5. Results of networks comparison across sites in two contrasting edaphic environments following Fründ approach. The circle bar plot **(a)** summarizes the dissimilarity of interactions decoupled in rewiring and species turnover (βWN= βOS+ βST), being the numbers within each bar the specific pair of sites being compared, **(b)** shows the overall dissimilarity of interactions (βWN) between mild and stressful environments, **(c)** shows differences between environments in the rewiring component (βOS), while **(d)** refers to species turnover (βST) comparison between environments. Compared to figure 3 of chapter 3, the species turnover component βST has gained much importance and shows no differences between environments (stressful 0.635±0.039, mild 0.680±0.039, F= 0.673, P= 0.430), while rewiring has reduced it. However, the results remain qualitatively the same regarding rewiring (βOS), which is more constrained in stressful sites compared to mild sites (stressful 0.123±0.022, mild 0.205±0.022, F= 6.355, P= 0.030). The same occurs with overall dissimilarity (βWN; stressful 0.758±0.029, mild 0.884±0.029, F= 9.010, P= 0.013).

Figure S6

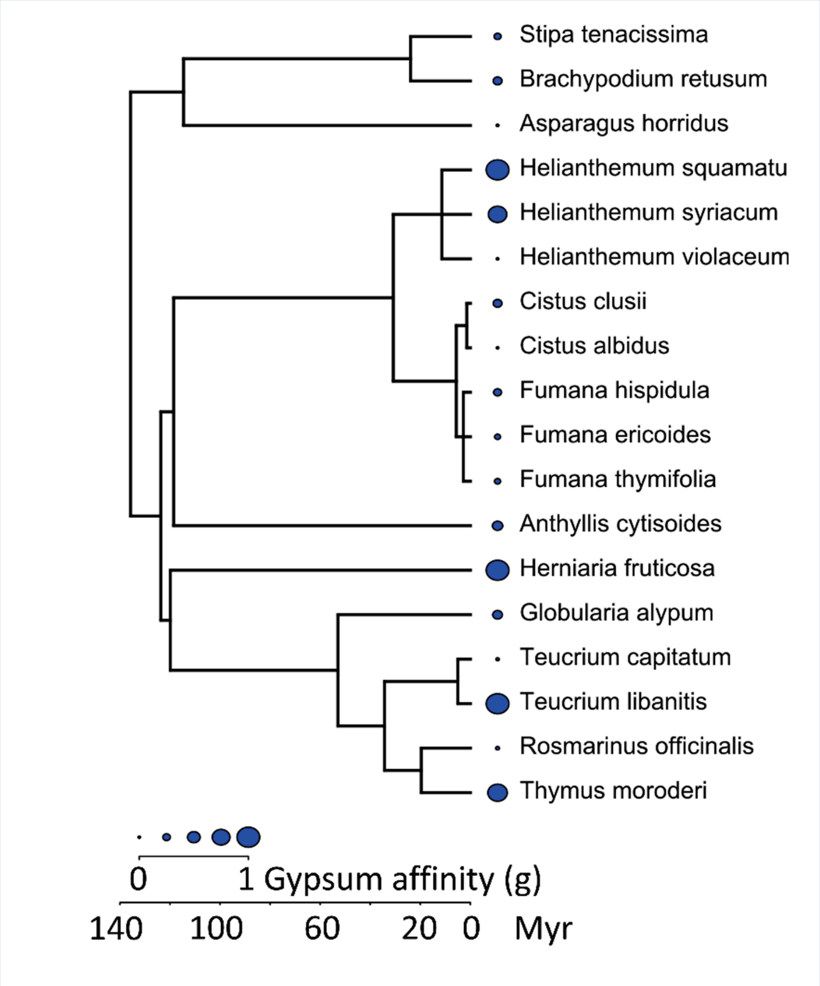

Figure S6. Phylogenetic relationship of the studied nurse species along with their gypsum affinity values. The size of the circle is proportional to the degree of gypsum affinity. Tree branches' length represent the chronological time in Millions of years (Myr).

Table S1

Environment	Level of preference	Type of constriction	CI 95%
Mild	>0.5	phylogenetic	(-104, -27)
Mild	>=0.66	phylogenetic	(-97, -25)
Mild	>=0.75	phylogenetic	(-129, -67)
Mild	>0.5	Functional	(-0.10, 0.05)
Mild	>=0.66	Functional	(-0.11, 0.00)
Mild	>=0.75	Functional	(-0.13, -0.02)
Stressful	>0.5	phylogenetic	(-55, 15)
Stressful	>=0.66	phylogenetic	(-31, 44)
Stressful	>=0.75	phylogenetic	(-46, 27)
Stressful	>0.5	Functional	(0.05, 0.34)
Stressful	>=0.66	Functional	(0.08,0.36)
Stressful	>=0.75	Functional	(0.09,0.32)

Table S1. The sensitivity test shows that the results of the t-tests assessing functional and phylogenetic constriction in the rewiring of interactions are consistent when we alter the proportion of times that a specific facilitated-nurse facilitative interaction must be observed to define a specific nurse as preferred (Level of preference). CI refers to the 95% confidence intervals of our estimated difference between the observed and expected values, using the null model described in methods. When CI includes 0, the observed value is similar to that expected by chance, and when the lower CI>0 or the higher CI<0 , that the observed is higher or lower, respectively, than expected by chance. The thresholds used in the main text are highlighted in gray.

Table S2

Species	Code
Anthyllis cytisoides	Acy
Asparagus horridus	Aho
Atractylis cancellata	Aca
Brachypodium retusum	Bre
Bupleurum fruticescens	Bfr
Cistus clusii	Ccl
Dipcadi serotinum	Dse
Diplotaxis harra	Dha
Dorycnium pentaphyllum	Dpe
Ephedra fragilis	Efr
Fumana ericoides	Fer
Fumana hispidula	Fhi
Fumana thymifolia	Fth
Globularia alypum	Gal
Gypsophila struthium	Gst
Helianthemum cinereum	Hci
Helianthemum squamatum	Hsq
Helianthemum syriacum	Hsy
Helianthemum violaceum	Hvi
Helichrysum stoechas	Hst
Herniaria fruticosa	Hfr
Lithodora fruticosa	Lfr
Matthiola fruticulosa	Mfr
Ononis tridentata	Otr
Paronychia argentea	Par
Phagnalon rupestre	Pru
Phagnalon saxatile	Psa
Pinus halepensis	Pha
Polygala rupestris	Pru
Quercus coccifera	Qco
Rhamnus lycioides	Rly
Rosmarinus officinalis	Rof
Sedum sediforme	Sse
Sideritis angustifolia	San
Stipa tenacissima	Ste
Teucrium capitatum	Tca
Teucrium carolipaui	Tcr
Teucrium libanitis	Tli
Thymus moroderi	Tmo
Thymus vulgaris	Tvu

Table S2. Studied species included in the facilitation networks

RESUMEN EXPANDIDO EN CASTELLANO

Introducción

En la naturaleza, la capacidad de supervivencia de las especies está fuertemente determinada por cómo perciben su entorno abiótico y por cómo responden al mismo (Lewis et al., 2017). Además, las especies no viven aisladas, sino ligadas en una red de interacciones con otras especies de las que pueden depender en menor o mayor grado (Vizentin-Bugoni, Debastiani, Bastazini, Maruyama, & Sperry, 2020). Estas interacciones bióticas, actuando en sinergia con los factores abióticos (Lewis et al., 2017), condicionan la diversidad de las comunidades vegetales (Bairey, Kelsic, & Kishony, 2016; Bruno, Stachowicz, & Bertness, 2003; Bulleri, Bruno, Silliman, & Stachowicz, 2016; Chesson, 2000; Hirn et al., 2022). Comprender los procesos que subyacen al ensamblaje de las interacciones bióticas puede resultar fundamental para comprender cómo estas contribuyen al mantenimiento de la diversidad y a incrementar su resiliencia frente a los cambios ambientales (Valladares, Bastias, Godoy, Granda, & Escudero, 2015).

Las comunidades vegetales albergan especies que se enfrentan a las presiones ambientales de manera diferente (Corlett &

Tomlinson, 2020; Valiente-Banuet, Rumebe, Verdu, & Callaway, 2006). Mientras que algunas especies poseen adaptaciones específicas para hacer frente a los factores de estrés ambiental (Corlett & Tomlinson, 2020), otras carecen de las mismas y, sin embargo, pueden sobrevivir en la comunidad beneficiándose de las interacciones bióticas con especies vecinas (Baraza et al., 2006; Bertness & Callaway, 1994; Callaway, 2007; Schlau, Huxman, Mooney, & Pratt, 2021). Por lo tanto, el éxito de cada especie dependerá de sus rasgos y cómo éstos le permiten sobrevivir en cada ambiente. La coexistencia de especies con estrategias diferentes puede ser resultado de su segregación funcional, que puede reducir la competencia entre ellas, favoreciendo así su persistencia en la comunidad (Navarro-Cano, Goberna, Valiente-Banuet, & Verdú, 2021; Schöb, Macek, Pistón, Kikvidze, & Pugnaire, 2017; Verdú, Gómez, Valiente-Banuet, & Schöb, 2021).

Sin embargo, tanto las especies adaptadas a un determinado ambiente, como las que dependen de otras especies para sobrevivir, pueden encontrar dificultades para establecerse y prosperar. Por un lado, las adaptaciones que permiten a ciertas especies prosperar en ciertas circunstancias estresantes pueden reducir su supervivencia en otros hábitats (Corlett & Tomlinson, 2020; Futuyma & Moreno, 1988; Palacio et al., 2007; Rajakaruna, 2018). Por ejemplo, muchas especies adaptadas a suelos con condiciones estresantes quedan restringidas a estos ambientes (Escudero, Palacio, Maestre, & Luzuriaga, 2015; Sianta & Kay, 2019). Esto puede resultar crítico si algunas poblaciones de estas especies desaparecen, ya que su capacidad de recolonización desde otros hábitats adyacentes está muy limitada (Corlett, 2020; Corlett & Tomlinson, 2020). Por su parte, las especies sin estas adaptaciones pueden seguir una estrategia más oportunista, ya que pueden recolonizar rápidamente desde fuentes adyacentes (Büchi & Vuilleumier, 2014). Sin embargo, para prosperar en un contexto ecológico estresante, es probable que necesiten a otras especies para subsistir (Graff & Aguiar, 2017; Liancourt, Le Bagousse-Pinguet, Rixen, & Dolezal, 2017). Esta dependencia se puede materializar mediante interacciones de facilitación fundadas sobre una intrincada, y a menudo impredecible, relación de factores abióticos y bióticos (Poisot, Stouffer, & Gravel, 2015).

Las interacciones de facilitación se dan cuando unas especies, denominadas nodrizas, alteran las condiciones de su entorno circundante, atenuando los filtros abióticos para otras especies beneficiarias que encuentran una ventana de oportunidad para sobrevivir en entornos inadecuados (Brooker et al., 2008; Bruno et al., 2003; Soliveres, Maestre, Berdugo, & Allan, 2015). Las interacciones de facilitación dependen de un equilibrio entre los rasgos de las especies interactuantes y el entorno (Navarro-Cano et al., 2021; Poisot et al., 2015). En consecuencia, la facilitación se ve favorecida cuando las especies que interactúan segregan sus rasgos funcionales para adquirir recursos, lo que reduce la competencia interespecífica (Navarro-Cano, Goberna, & Verdú, 2019; Valiente-Banuet & Verdú, 2013).

Tradicionalmente, el papel de la facilitación en la vertebración de la diversidad se ha contemplado desde la perspectiva de las mejoras ambientales que favorecen el establecimiento de especies sensibles al estrés (Liancourt, Callaway, & Michalet, 2005) y, posteriormente, la concurrencia de plantas adultas de especies funcionalmente diferentes debido a la baja competencia entre ellas (Butterfield & Briggs, 2011; Cavieres & Badano, 2009). Más recientemente, también se han aportado evidencias empíricas de intercambio de recursos entre plantas adultas, aportando otro mecanismo menos conocido de facilitación entre plantas (Montesinos-Navarro, Verdú, Querejeta, Sortibrán, & Valiente-Banuet, 2016; Montesinos-Navarro, Verdú, Querejeta, & Valiente-Banuet, 2019). Esto podría resultar esencial para comprender el rol de la facilitación en el ensamblaje de comunidades naturales, ya que puede resultar en mecanismos que promuevan un uso más equitativo de los recursos entre las especies de la comunidad favoreciendo su coexistencia en estadios adultos, donde los efectos de mejora abiótica pierden intensidad.

Las interacciones interespecíficas de diferentes signos e intensidades interconectan a las especies dentro de las comunidades, dando lugar a estructuras con implicaciones ecológicas, es decir, redes (Bascompte, 2007, 2010; CaraDonna et al., 2017). Las interacciones interespecíficas dentro de las redes ecológicas no son componentes estáticos, sino dinámicos que varían en el tiempo y el espacio (CaraDonna et al., 2021; Poisot et al., 2015; Trøjelsgaard & Olesen, 2016; Tylianakis & Morris, 2017). Esta flexibilidad a la hora de materializar interacciones (reconexión), proporciona a las especies beneficiarias la ventaja de reconectarse con diferentes especies nodrizas, reduciendo posibles cascadas de coextinción ante eventos de desaparición de estas últimas.

La reconexión de las interacciones ecológicas puede estar limitada, ya que ciertas interacciones entre especies son altamente improbables, y existen algunas evidencias de patrones generales en esta reconexión, como por ejemplo, una tendencia a reconectarse con especies emparentadas con los interactuantes preferidos (Montesinos-Navarro, Díaz, Torres, Caravaca, & Roldán, 2019; Raimundo, Guimarães, & Evans, 2018). La magnitud de la reconexión en las interacciones de facilitación puede ser crítica para el mantenimiento de las especies facilitadas, pues puede proporcionar un conjunto de nodrizas alternativas donde las especies beneficiarias pueden prosperar. A pesar de su importancia a la hora de evaluar cómo las comunidades vegetales pueden responder a cambios ambientales, no se conocen completamente los procesos que limitan la reconexión de las interacciones de facilitación y cómo estos patrones pueden cambiar en distintos ambientes y en comunidades con especies con rasgos distintos.

Justificación de la tesis y objetivos generales

Comprender los fundamentos que rigen el ensamblaje de las comunidades vegetales es esencial para anticipar cómo las especies podrían verse afectadas por los cambios ambientales. La diversidad de especies dentro de una comunidad depende de las variables abióticas locales, de cómo las especies responden a ellas y de las interacciones bióticas que se producen dentro de la comunidad (Lewis et al., 2017). Las interacciones biológicas son cruciales para mantener la diversidad, ya que su pérdida puede dar lugar al desencadenamiento de cascadas de coextinción, con los consiguientes efectos sobre la diversidad de toda la comunidad (Janzen, 1974; Valiente-Banuet et al., 2015). A pesar de los avances en este área, el rompecabezas sigue incompleto, lo que limita nuestra capacidad para evaluar cómo sobreviven múltiples especies que conviven en comunidades naturales, particularmente en ambientes estresantes, donde muchas especies sensibles al estrés están al borde de la extinción local (Rajakaruna, 2018).

Esta tesis explora el ensamblaje de especies de plantas en dos ambientes con niveles de estrés contrastados con el fin de desvelar cómo la interacción entre las condiciones abióticas, los rasgos de las plantas y las interacciones bióticas moldean las reglas de ensamblaje de la comunidad de estos sistemas. En concreto, nuestros objetivos son (a) explorar distintas estrategias para prosperar en estos entornos, y posibles contrapartidas de invertir en cada una de ellas, (b) mecanismos potenciales de concurrencia a largo plazo entre especies de plantas adultas en estas comunidades, y (c) las limitaciones para reconectarse a distintas especies de nodrizas en estos dos ambientes.

Específicamente, proponemos que las especies con rasgos específicos para superar factores abióticos adversos podrán prosperar en esos entornos estresantes, mientras que las especies sensibles al estrés, dependerán de otras especies para establecerse. Además, exploramos mecanismos por los que estas interacciones de facilitación pueden persistir a largo plazo (es decir, hasta la edad adulta de ambas especies). En concreto, proponemos que uno de ellos es que especies interactuantes tengan desempeños más similares si crecen juntas que si crecen aisladamente. Finalmente, buscamos patrones generales en la reconexión de las redes de facilitación, detectando limitaciones para reconectarse a distintas especies de nodrizas en distintos ambientes. Proponemos que, en ambientes estresantes, las reconexiones serán más frecuentes entre nodrizas con adaptaciones específicas a ese estrés, mientras que en ambientes menos estresantes la reconexión ocurrirá entre especies emparentadas con las nodrizas preferidas y, por tanto, similares funcionalmente a éstas en múltiples rasgos.

Métodos

Sitios de Estudio

Desarrollamos los estudios relacionados con los tres capítulos de esta tesis en comunidades vegetales establecidas sobre cuatro afloramientos yesíferos (entorno estresante) y sus entornos calizos circundantes (entorno benigno). Este sistema constituye un mosaico ambiental con presencia de contrastes de diversidad y niveles de estrés. Se muestrearon cuatro localidades con dos subsitios en cada una (hábitat yesífero y no-yesífero), separados por unas pocas decenas de metros, lo que minimiza los cambios en las condiciones climáticas entre los pares dentro de cada localidad. En concreto, las localidades se sitúan a lo largo del valle del Vinalopó, en el sureste semiárido de la Península Ibérica. Aquí, las comunidades vegetales se caracterizan por una vegetación dispersa, generalmente agrupada en parches multiespecíficos, compuestos principalmente por caméfitos y arbustos pequeños donde dominan las interacciones de facilitación (Delalandre & Montesinos-Navarro, 2018; Montesinos-Navarro, Storer, & Perez-Barrales, 2019).

Los ambientes seleccionados resultan idóneos para evaluar nuestros objetivos desde una triple perspectiva. En primer lugar, estudiamos comunidades vegetales que prosperan en dos niveles de estrés contrastados (entornos benignos y estresantes). Ambos entornos se ven sometidos a un estrés climático similar como consecuencia de un clima semiárido. A esto, se suma un estrés litológico, principalmente en los afloramientos de yeso, donde la estructura y composición química del yeso puede limitar el desarrollo de las plantas ($CaSO_4 \cdot 2H_2O$; Escudero et al., 2015).

En segundo lugar, en los afloramientos de yesos coexisten dos tipos de especies. Especies adaptadas a esta litología, con rasgos específicos que les permiten sobreponerse a los estreses particulares del yeso, y otras especies que no tienen rasgos específicos para hacer frente al estrés del yeso (es decir, especies sensibles al estrés).

En tercer lugar, los hábitats yesíferos se presentan como afloramientos aislados envueltos por una matriz de otras litologías, principalmente calizas. Esta estructura geográfica, en forma de archipiélagos de hábitats yesíferos, podría haber favorecido, entre las especies adaptadas, a aquellos linajes con una baja capacidad de dispersión (Escudero et al., 2015; Isnard, L'huillier, Rigault, & Jaffré, 2016; Silveira et al., 2019; Spasojevic, Damschen, & Harrison, 2014), ya que las especies que invierten en dispersión a larga distancia podrían ver peligrar su éxito si las semillas salen de los límites de su hábitat óptimo (Schenk, 2013). Debido a la dificultad de dispersión a través de los entornos circundantes no-yesíferos, la recolonización de una población localmente extinguida se vuelve más improbable, lo que podría resultar en una extinción a largo plazo (Corlett & Tomlinson, 2020). Este escenario sería crítico para aquellas especies que dependen de nodrizas extintas, a menos que puedan reconectarse con otras especies de la comunidad, lo que mitigaría el efecto de extinción en cascada causado por la extinción de especies clave.

Muestreo

Nuestro diseño de muestreo comprendía 80 parcelas (150x150 cm) en cada uno de los 8 subsitios, excepto en un subsitio en el que se muestrearon 79 parcelas. En cada subsitio, las 80 parcelas se distribuyeron aleatoriamente hasta ocupar 1 ha, evitando los lugares de difícil accesibilidad. Muestreamos las cuatro localidades entre abril de 2019 y febrero de 2020.

En primer lugar, identificamos todas las plantas adultas dentro de cada parcela y medimos su cobertura. También estimamos la cobertura de suelo desnudo. Además, identificamos si cada planta crecía sola (es decir, no asociada) o en un parche con otros individuos (es decir, asociada). En segundo lugar, identificamos todos los juveniles dentro de las parcelas e identificamos si estaban reclutando en el suelo desnudo o debajo de una planta adulta, identificando la especie adulta. Se consideraron juveniles todas aquellas plantas comprendidas entre el estadio de plántula (con al menos las dos primeras hojas) hasta plantas pequeñas (<15% en altura en comparación con los adultos promedio) que no mostraban presencia de estructuras reproductivas ni lignificación en la base del tallo.

Definiendo las características de las especies de la comunidad

Para caracterizar las especies de nuestras comunidades y como estás interactúan calculamos una serie de índices y métricas. En primer lugar, calculamos el denominado índice de afinidad al yeso, que determina el grado de afinidad de cada especie por este sustrato. La afinidad al yeso (g) es una variable continua con un rango [0, 1] que mide el éxito de las especies en el suelo yesífero en comparación con los ambientes circundantes no yesíferos. Este tipo de métrica nos permite identificar el filtro abiótico que los afloramientos de yeso imponen a la vida vegetal.

En segundo lugar, evaluamos la intensidad de las interacciones entre especies en las comunidades estudiadas. Para ello, utilizamos el índice de intensidad relativa de interacción (RII en adelante) definido en Armas, Ordiales, & Pugnaire (2004). Los signos y la intensidad de la interacción entre dos plantas pueden deducirse comparando la supervivencia, el crecimiento o la reproducción de plantas, que crecen asociadas a otras frente a las que no lo están. El RII da valores simétricos alrededor de cero y con límites definidos [-1,1]; siendo los valores positivos un indicio de que estar asociado a otra planta aumenta la eficiencia biológica (es decir, facilitación) y los negativos, un indicio de que la reduce (es decir, competencia; Armas et al., 2004). En esta tesis doctoral se utilizó la densidad de reclutamiento (es decir, número de reclutas por cm^2 de especie nodriza o suelo desnudo) como proxi de eficiencia biológica.

En tercer lugar, Abordamos los factores que limitan la reconexión de las interacciones de facilitación, evaluando cómo las especies beneficiarias cambian de nodriza cuando sus preferidas no están presentes mediante un sistema de sustitución espacio-temporal. Para ello, identificamos aquellas interacciones de facilitación que tienden a ocurrir frecuentemente cuando los dos interactuantes están presentes (es decir, interacciones preferidas) y aquellas que no suelen realizarse, incluso cuando las dos especies están presentes (es decir, interacciones no preferidas). Así identificamos el conjunto de nodrizas preferidas y no preferidas para cada especie facilitada.

Por último, calculamos las distancias filogenéticas entre especies de nuestras comunidades para informar a los análisis efectuados a lo largo de esta tesis. Las especies comparten una historia evolutiva en mayor o menor grado (Revell, 2010), de forma que especies emparentadas tenderán a parecerse y cuanto más similares sean dos especies, mayor será la probabilidad de que compartan rasgos o respuestas similares para enfrentarse a su contexto ambiental (Pausas & Verdú, 2010). Para considerar las relaciones filogenéticas entre las especies encontradas, construimos el árbol filogenético podando el mega árbol "GBOTB.extended.tre" que incluye 74.533 especies, resultantes de la combinación de dos mega árboles previamente publicados (Smith & Brown, 2018; Zanne et al., 2014).

Análisis estadísticos

En el Capítulo 1, utilizamos un enfoque multivariante para identificar conjuntos de rasgos que conforman estrategias de respuesta de las especies vegetales a las limitaciones específicas impuestas por el yeso vs. estrategias de respuesta a otras limitaciones menos específicas (es decir, estreses más generales como la aridez). A continuación, utilizando modelos mixtos filogenéticamente informados, examinamos cómo las estrategias derivadas del análisis multivariado se relacionan con la afinidad al yeso de cada especie. En el Capítulo 2, utilizamos, en primer lugar, un modelo mixto bayesiano filogenéticamente informado para examinar la relación existente entre la afinidad de los reclutas por el yeso, su rareza local y su dependencia en la facilitación. En segundo lugar, comprobamos si las asimetrías de tamaño entre las especies de la comunidad se reducen cuando se asocian, y comparamos las diferencias de tamaño observadas con un modelo nulo basado en simulaciones teóricas. Por último, en el Capítulo 3, analizamos las redes de facilitación de las comunidades estudiadas y cuantificamos su disimilitud entre localidades. Además, investigamos los patrones que limitan las reconexiones en los procesos de facilitación utilizando modelos nulos basados en simulaciones para desvelar si las relaciones filogenéticas o la afinidad al yeso explican los patrones de reconexión con nodrizas no preferidas.

Resultados

Esta tesis doctoral revela cómo la interacción entre las condiciones abióticas, los rasgos de las plantas y las interacciones bióticas moldea el ensamblaje de las comunidades vegetales naturales. Nuestros resultados muestran que las especies que habitan en suelos estresantes exhiben estrategias contrastadas para hacer frente a tales condiciones. Mientras que algunas especies poseen rasgos para superar tales limitaciones (Capítulo 1), otras, más raras y sensibles al estrés, dependen de la facilitación para su mantenimiento, dando lugar a parches multiespecíficos en los que las especies equilibran su desempeño (Capítulo 2). Estas interacciones facilitadoras parecen depender de la presencia de nodrizas que difieren en sus rasgos, lo que limita la capacidad de reconexión de las especies beneficiarias a un conjunto limitado de nodrizas. Estas limitaciones de reconexión varían en función del ambiente, dando lugar a un complejo conjunto de reglas de ensamblaje que finalmente moldean las comunidades vegetales (Capítulo 3).

Discusión

Los tres capítulos de la tesis contribuyen a comprender cómo se ensamblan y subsisten las especies en comunidades naturales regidas por la facilitación. Las comunidades vegetales estudiadas albergan una diversidad de especies que difieren en su respuesta a condiciones estresantes. Las diferencias funcionales entre las especies que concurren determinan la estrategia de supervivencia de cada una de ellas. Mientras que algunas especies encuentran un hábitat adecuado en condiciones de estrés (es decir, especies con alta afinidad al yeso), otras, sensibles al estrés, dependen más del establecimiento de interacciones facilitadoras con las especies vecinas. Además, en los parches de vegetación donde múltiples especies vegetales interactúan, los individuos de diferentes especies muestran un desempeño más similar que sus congéneres creciendo aislados, sugiriendo un uso de los recursos más equitativo regulado por las interacciones entre plantas. Por último, observamos que la reconexión de las interacciones de facilitación no es al azar, sino que en hábitats yesíferos tiende a ocurrir con especies nodrizas que poseen una alta afinidad por el sustrato en yeso. Esto sugiere que la presencia de especies adaptadas a un determinado ambiente puede ser crítica para mantener la diversidad de especies sensibles al estrés. Sin embargo, en litologías calizas circundantes menos estresantes, la reconexión tiende a darse con especies de nodrizas emparentadas con las nodrizas preferidas, sugiriendo que los rasgos funcionales pueden diferir entre nodrizas y las interacciones de facilitación tienen cierta especificidad por varios rasgos conservados filogenéticamente.

La especie y el filtro abiótico

Las especies no perciben su entorno de la misma manera, sino que esta percepción depende de los rasgos de cada especie y de cómo estos rasgos interactúan con el entorno (Liancourt et al., 2017). En este sentido, las comunidades estudiadas albergan un conjunto de especies que presentan diferentes respuestas al filtro abiótico que el yeso impone a la vida vegetal. Esta diversidad de respuestas depende de la presencia de rasgos particulares para superar las severas condiciones ambientales del yeso, y la efectividad de estas estrategias condicionará el éxito de las especies en el ambiente estresante en comparación con ambientes más benignos (afinidad con el yeso).

En concreto, observamos que las especies con una alta afinidad al yeso, es decir, aquellas que tienen más cobertura en yeso que en entornos circundantes menos estresantes, muestran una mayor adquisición de agua de las capas más profundas del suelo, sugiriendo una mayor capacidad para penetrar en capas duras del mismo, al tiempo que acumulan Ca, Mg y S, lo que puede evitar la toxicidad química. Dentro del grupo de especies que poseen estos rasgos, encontramos las especies gipsófitas, especies que habitan exclusivamente en afloramientos de yeso, pero también ciertas especies gipsovagas (es decir, aquellas que habitan tanto en el yeso como en otros hábitats) que mostraron un mejor desempeño en los afloramientos de yeso que en los ambientes circundantes más benignos. Por el contrario, las especies que no poseían esos rasgos mostraron una disminución sustancial de la cobertura relativa en el yeso en comparación con los hábitats calizos circundantes menos estresantes, lo que sugiere un fuerte filtro abiótico para estos gipsovagos poco afines al yeso y sensibles al estrés.

En contra de lo que esperábamos, nuestros resultados no identificaron ningún coste asociado a la presencia de rasgos de tolerancia al yeso. Así, especies con alta afinidad al yeso, también muestran niveles altos de adquisición de macronutrientes y eficiencia en el uso del agua. De forma que la especialización a suelos yesíferos no explica la ausencia de estas especies de especialistas-edáficos más allá del límite del yeso.

Los efectos de la facilitación en las comunidades vegetales

La segregación funcional entre especies adaptadas al yeso y otras sensibles a este estrés, puede promover las interacciones de facilitación. Esto ocurre porque la competencia interespecífica se ve debilitada a causa de la segregación de nicho entre especies funcionalmente diferentes (Bulleri et al., 2016). En esas circunstancias, es esperable que las especies adaptadas al yeso actúen como nodrizas de las especies sin estas adaptaciones, explicando la amplia riqueza de especies sensibles al estrés que coexisten con especialistas edáficos en estos hábitats (Escudero et al., 2015; Moore, Mota, Douglas, Olvera, & Ochoterena, 2014; Mota et al., 2008; Mota, Sánchez-Gómez, & Guirado, 2011; Murdy, 1968; Sianta & Kay, 2019).

Nuestros resultados también indican que las especies localmente raras tienden a ser especies sensibles al estrés (es decir, especies con baja afinidad al yeso), y dependen más de la facilitación que las especies bien adaptadas y localmente abundantes (es decir, aquellas con alta afinidad por el yeso). Atendiendo a la bibliografía, ciertos estudios han demostrado que la facilitación favorece a las especies raras (Calatayud et al., 2020; Soliveres et al., 2015) aunque no se identifican los mecanismos por los que emerge este patrón, mientras que otros trabajos han evidenciado que la facilitación es más importante para las especies que viven fuera de su óptimo ecológico (Gross et al., 2009; Le Bagousse-Pinguet et al., 2014; Liancourt et al., 2005; Qi et al., 2018). Nuestros hallazgos contribuyen a integrar estas dos perspectivas, demostrando que la rareza local y el encaje ambiental pueden estar relacionados. Las especies con baja afinidad edáfica pueden ser escasas en un entorno específico porque viven fuera de su óptimo ecológico, y a su vez, éstas pueden beneficiarse de vivir asociadas a especies adaptadas a este ambiente.

Los efectos facilitadores mencionados se midieron en una fase ontogenética temprana. Sin embargo, las interacciones bióticas pueden cambiar de signo e intensidad de forma impredecible a lo largo de la ontogenia debido a cambios en las plantas que interactúan, el entorno abiótico o efectos indirectos de la vecindad (Castillo, Verdú, & Valiente-Banuet, 2010; Hirn et al., 2022; Schöb, Armas, & Pugnaire, 2013), lo que dificulta la identificación de efectos netos a largo plazo de las interacciones entre plantas. De hecho, la facilitación solo puede asegurar la coexistencia de las especies que interactúan cuando los beneficios de la asociación compensan sus perjuicios a lo largo de la vida de las plantas interactuantes (Valiente-Banuet & Verdú, 2013).

En este sentido, también demostramos que las plantas adultas que crecen asociadas dentro de un parche de vegetación muestran menos asimetrías de tamaño entre especies que sus congéneres creciendo aislados. La reducción de la asimetría de tamaño podría tener profundas implicaciones para el ensamblaje de especies, ya que un tamaño similar podría resultar potencialmente en un fitness más igualado (Younginger, Sirová, Cruzan, & Ballhorn, 2017), una de las principales fuerzas que favorecen la coexistencia de especies (Chesson, 2000). Múltiples mecanismos podrían estar implicados en la reducción de estas asimetrías. Por ejemplo, las plantas vecinas pueden intercambiar nitrógeno y agua o compartir micorrizas beneficiosas (Montesinos-Navarro et al., 2016; Montesinos-Navarro, Verdú, Querejeta, & Valiente-Banuet, 2017; Montesinos-Navarro, Valiente-Banuet, & Verdú, 2019; Sortibrán, Verdú, & Valiente-Banuet, 2019). Todos estos potenciales beneficios deben sumarse a la diferenciación de rasgos observada entre especies, que podría minimizar el solapamiento de recursos de nicho, maximizando los potenciales efectos positivos, ya que se minimizaría la competencia interespecífica (Bulleri et al., 2016; Sánchez-Martín et al., 2021).

Limitaciones en los patrones de reconexión de la facilitación

La pérdida de interacciones de facilitación puede tener consecuencias devastadoras sobre la diversidad de las comunidades vegetales (Janzen, 1974; Valiente-Banuet et al., 2015). Sin embargo, las especies que dependen de la facilitación pueden compensar, al menos parcialmente, estas pérdidas si existe reconexión en las interacciones de facilitación, es decir flexibilidad para reclutar debajo de otras especies nodrizas cuando las preferidas no están presentes, lo que podría dotar a las comunidades de una mayor robustez frente a la pérdida de especies.

A este respecto, hemos demostrado que la reconexión no es al azar, lo que significa que no todas las especies pueden actuar como nodrizas. La identidad de las especies nodrizas dependerá del ambiente y de los rasgos de las especies. El ambiente define las presiones abióticas del sistema y, en consecuencia, el conjunto de rasgos que podrían ser adaptativos en ese sistema. Esto a su vez puede condicionar que ciertas especies puedan o no actuar como nodrizas, lo que variará en función de las condiciones de estrés. En este sentido, comprobamos que la reconexión en ambientes estresantes de yeso tiende a ocurrir con especies nodrizas que muestran una alta afinidad por el yeso. Por el contrario, los rasgos de afinidad al yeso no desempeñan un papel significativo en sistemas calcáreos menos estresantes, en los que la reconexión tiende a ocurrir con parientes filogenéticamente cercanos a las nodrizas preferidas.

Nuestros resultados sugieren que las especies especialistas juegan un papel fundamental en las interacciones de facilitación, por lo que el mantenimiento de estas especies debería ser prioritario en las políticas de conservación. Además, encontramos que la redundancia funcional o filogenética podría ser relevante para la estabilidad de la comunidad, ya que la ausencia de nodrizas preferidas podría paliarse con la presencia de una especie similar.

Conclusiones

En esta tesis doctoral se han identificado elementos esenciales para comprender cómo se ensamblan las especies en comunidades naturales. Hemos puesto de manifiesto la diversidad de estrategias que surgen entre las especies que coexisten para subsistir en un mismo contexto ambiental. Mientras que algunas especies pueden estar adaptadas a estreses edáficos específicos, otras persiguen una estrategia más oportunista, siendo más dependientes de las interacciones de facilitación, sobre todo con especies de especialistas edáficos. Esto da lugar a sistemas fuertemente condicionados por la facilitación, cuyos efectos perduran a lo largo de la ontogenia de las plantas. En estos sistemas, la redundancia funcional o filogenética de especies es clave para evitar el colapso de las redes de facilitación que sostienen la diversidad de estas comunidades.

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