A Regional Assessment of Changes in Plant–Plant Interactions Along Topography Gradients in Tunisian Sebkhas

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ABSTRACT

Facilitation among plants in dry ecosystems is crucial for diversity and ecosystem functioning and stability. However, the importance of facilitation in extremely stressful conditions is highly debated. We attempt to separate the effects of direct non-resource and resource stress factors on plant–plant interactions by assessing changes in facilitation and competition along salinity gradients at two contrasting levels of aridity. We selected eight saline depressions (hereafter sebkhas) in the wet and dry Mediterranean arid climate of North Africa, from central Tunisia to the Libyan border 500 km south-eastward. In each sebkha, we transplanted at four positions along the salinity gradient induced by topography, both in open areas and below dominant shrubs, three target species with contrasting tolerances to salinity stress. Target plant survival, soil electrical conductivity and moisture were recorded before and after the dry summer season in all treatments. Shrubs decreased salinity and drought stresses in all treatments, and facilitation was the dominant interaction. However, we found a strong collapse of facilitation along the salinity gradient, due to a dramatic mortality of the three target species both with and without neighbours above their threshold of salinity tolerance. Increasing aridity induced an earlier collapse of facilitation along the gradient. The three target species had contrasting responses to neighbours, with the least stress-tolerant species being facilitated and the two most stress-tolerant ones negatively affected by neighbours. Our study shows that disentangling resource and non-resource stresses along gradients and controlling for target species effects help understanding variation in plant–plant interactions under highly stressful conditions.

Key words: Collapse of facilitation; competition; drought; Mediterranean climate; non-resource factors; resource factors; salinity.

HIGHLIGHTS

- Regional experiment manipulating neighbours along salinity gradients in arid climate.
- Facilitation collapses in the most saline condi-
• Increasing aridity induces a stronger collapse of facilitation.

INTRODUCTION

Facilitation, the positive effect of an organism on another one (Callaway 2007), is widely recognized as an important biotic interaction occurring frequently in stressful environmental conditions from a variety of terrestrial and coastal marine ecosystems (Bruno and others 2003; He and others 2013; Soliveres and Maestre 2014; Michalet and Pugnaire 2016; Bulleri and others 2018). Facilitation might be due to the direct amelioration of abiotic conditions (salinity, cold, drought or oligotrophy) or to indirect positive effects, through reduced herbivory or competition (Callaway 2007). Facilitation is crucial in severe environments since it enhances local diversity by allowing stress-intolerant species to live in extreme habitats (Bruno and others 2003; Liancourt and others 2005a; Michalet and others 2006). Facilitation also affects the diversity of other organisms (Lortie and others 2016), enhances ecosystem functioning (Soliveres and others 2015; Wright and others 2017) and ecosystem stability (Kéfi and others 2007). Additionally, facilitation can be used as an engineering tool for restoring degraded habitats (Gómez-Aparicio and others 2004; Padilla and Pugnaire 2006).

However, changes in the intensity and importance of competition and facilitation along environmental gradients in water-stressed conditions are a highly debated issue (Maestre and others 2005, 2009; Michalet 2007; He and Bertness 2014; Michalet and others 2014a, b; Soliveres and others 2015). The pioneer stress-gradient hypothesis (SGH) facilitation model, considering that competition should switch to facilitation with increasing environmental severity (Bertness and Callaway 1994), was first supported by a number of experiments in water-stressed systems (Gómez-Aparicio and others 2004; Stultz and others 2007; Mohamed and others 2013). However, other studies showed that competition may conversely increase with increasing water stress in dry systems (Tielborger and Kadmon 2000; Maestre and Cortina 2004; Armas and Pugnaire 2005, a process now called the switchback to competition, Michalet and others 2014a, b). Additionally, facilitation may collapse (that is, decrease until neutral interactions) at the extreme of environmental gradients (Kitzberger and others, 2000). Thus, several authors have argued that the relationship between plant–plant interactions is not linear, as proposed by the pioneer SGH model, but unimodal, with a maximum of facilitation at intermediate position along environmental gradients (Michalet and others 2006; Holmgren and Scheffer 2010; Verwijmeren and others 2013). Additionally, Michalet and others (2014a) stressed the importance to separate two alternatives to the SGH, namely the collapse of facilitation and the switchback to competition, because they are likely to be due to different processes, a decrease in facilitative effects for the former and a change in the response of the target species in particular environmental conditions for the latter.

Recent research conducted since the beginning of that debate has provided conflicting results, with either support of the SGH, in particular along cold stress gradients (Cavieres and others 2014; Pugnaire and others 2015, and see the meta-analysis of He and others 2013) or of one of the other two models proposing an unimodal facilitation curve (Forey and others 2010; Le Bagousse-Pinguet and others 2014; Michalet and others 2013; Butterfield and others 2016; O’Brien and others 2017; Zhang and others 2018; Berdugo and others 2019). Thus, as suggested by Michalet and Pugnaire (2016), it is time considering that there is no unique model of facilitation–environment relationship and concentrating our research efforts towards assessing under which environmental and biotic conditions any of the three proposed models is expected to occur. This is crucial for understanding the role of facilitation for ecosystem functioning and stability in dry environments.

Michalet (2007) and Maestre and others (2009) proposed in particular, that non-resource direct gradients (that is, factors that are directly affecting the physiology of a plant species but that are not consumable, sensu Austin and Smith 1989) that should support the SGH and collapse of facilitation models (that is, only facilitation and neutral interactions in stressful and extremely stressful conditions, respectively), whereas resource gradients (for example, water availability gradients) were more likely to show a switchback to competition at the extreme of the stress gradient, consistent with the McArthur and Wilson (1967) theory. Several authors also highlighted the importance of the functional strategy of the species involved in the interactions (Liancourt and others 2005a; Michalet and others 2006; Maestre and others 2009). Indeed, stress-intolerant species are more likely to be facilitated and stress-tolerant ones more sensitive to competition (Forey and others 2010; Liancourt and others 2017; Qi and others 2018).
Another crucial point is the necessity, when possible, to assess variation in biotic interactions along direct environmental gradients and to avoid complex gradients involving several direct factors interacting with each other (He and Bertness 2014). For example, Leong and others (2019) have shown that isolating resource gradients provides SGH support in pitcher plants. Several studies have indeed shown that the interaction between drought stress and disturbance induces a collapse of facilitation (Forey and others 2010; Maalouf and others 2012; Le Bagousse-Pinguet and others 2014; Verwijmeren and others 2019). In arid ecosystems subjected to high evaporation rates, salinity stress also often interacts with water stress along complex topography or elevation gradients, since salt accumulates in soils with increasing climatic drought (Fariña and others 2017; Chaieb and others 2019). We suggest that discrepancies among studies on the shape of the facilitation–environment curve in water-stressed systems may in part be due to the occurrence of an interaction between these two direct stresses. In other words, studies conducted along salinity gradients are more likely to support the SGH or eventually the collapse of facilitation model since salinity is a non-resource direct factor, whereas studies conducted along water gradients are more likely to support the switchback to competition model because water availability is a direct resource factor.

The overall goal of our study was to disentangle the role of salinity and drought stresses in the variation in plant–plant interactions along complex environmental stress gradients in arid ecosystems. Since salinity and drought stresses interact across complex topographic and climatic gradients (Fariña and others 2017; Chaieb and others 2019), we set up an experimental design at the regional scale where we assessed the effect of salinity on plant–plant interactions along topographic gradients in different climatic conditions. We selected eight continental saline depressions (locally called sebkhas) from both the upper and lower arid climates (sensu Emberger 1958) of North Africa, from central Tunisia to the Libyan border, 500 km south-eastward. Within each sebkha, we transplanted along the salinity gradient induced by topography three dominant species having contrasting tolerances to salinity stress, both below the dominant shrubs and in adjacent open areas. Electrical conductivity and soil moisture were also measured in all treatments in order to identify the effects of the dominant shrubs on these two direct stress factors. We aimed to answer the following questions: (1) what is the relationship between facilitation and salinity? (2) Are changes in facilitation with increasing salinity due to changes in the effects of dominant shrubs on salinity? (3) Does increasing water stress with increasing aridity alter the relationship between facilitation and salinity? (4) Does the tolerance to salinity of the target species alter the relationship between facilitation and salinity?

**Materials and Methods**

**Study Sites and Target Species**

The study was conducted in eight sebkhas from central Tunisia in the Mediterranean arid climate (Figure 1 and Appendix 1 of ESM). The Mediterranean arid and Saharan climates occupy about 75% of the total surface of Tunisia, in the central and southern parts of the country, at the northern edge of the Sahara Desert (Figure 1). In the arid climate of Tunisia, mean annual precipitation is between 100 and 300 mm and mean annual temperature is 19.0°C with 4.6°C in winter (December to February) and 37.0°C in summer (June to August). Following the bioclimatic classification of Emberger (1958), the Mediterranean arid climate has been divided in two sub-climates, upper and lower arid (hereafter wet and dry arid, respectively), with mean annual precipitation between 200 and 300 mm and 100 and 200 mm, respectively. The year of our experiment, climate was wetter in the winter, and drier in the spring and summer than average years, but these anomalies were approximately the same in the wet and dry arid climates (winter: +92% and +78%, spring –31% and –41% and summer –53% and –100%, for the wet and dry arid, respectively, see Appendix 2 of ESM). Thus, the climate treatment applied during our experiment was representative of average differences in aridity occurring in Tunisia between these two sub-climates. In contrast, differences in humidity between the wet and dry seasons during the course of our experiment were higher the year of our experiment than average in both the wet and dry arid.

All sebkhas, whose size ranges between 10 and 300 km², had similar lentil-like shapes and habitat organization. The most important central part, with no vegetation, can be inundated during the wetter winter season during very wet years. The central part is surrounded at the edge of the sebkhas by several circular belts of shrubby halophytic vegetation. Chaieb and others (2019) distinguished four habitats in relation to elevation and salinity stress, from the central part to the edge of the sebkhas at the ecotone with crop fields. Soil texture is highly
influenced by the presence of sand throughout the sebkhas, although there is a slight but significant increase in clay from the uppermost habitat (H4) to the lowest one (H1) (from 6.25 ± 0.8% of clay, 12.25 ± 0.7% of silt and 81.5 ± 1.2% of sand in H4, that is, loamy sand texture, to 18 ± 1.5, 14.5 ± 0.5 and 67.5 ± 1.2 in H1, respectively, that is, sandy loam texture, n = 8). Habitat 1, the most saline and lowest habitat, has very low vegetation cover (<20%) and is occupied by only one species, Halocnemum strobilaceum (Amaranthaceae), a C3 shrub present from the Mediterranean area to western Asia (Liu 1985) and known as a highly salinity-tolerant species (Redondo-Go´mez and others 2010). Habitat 2, also only occupied by H. strobilaceum, has higher cover (20–50%) and biomass. Habitat 3 is dominated by Arthrocnemum macrostachyum (Amaranthaceae), a C3 shrub, known as a salt-accumulator (Redondo-Gómez and others 2010). Other shrub species associated with A. macrostachyum are Suaeda mollis, Limoniastrum monopetalum and Atriplex halimus. Habitat 4 is co-dominated by several shrub species (Salsola tetran-dra, Zygophyllum album, Reaumuria vermiculata and Nitraria retusa) and Lygeum spartum (Poaceae), a widespread rhizomatous perennial grass native of the Mediterranean region and known to be tolerant to salinity. Human disturbance due to agriculture is very low in sebkhas because of the very low soil fertility and palatability of halophytic shrubs, which make them an excellent natural system for studying the effects of stress in arid climates, in particular in developing countries like Tunisia where disturbance is overall very high. Only low grazing by sheep can be observed at the upper edge of habitat 4 (Chaieb and others 2019), but we disregarded those grazed areas.

Experimental Design

Late August 2016, we selected for the climate treatment four sebkhas (blocks) in the wet arid climate and four sebkhas in the dry arid climate (Figure 1). Each block was separated from each other by at least 50 km, as shown in Figure 1. Each block was split into four main plots for the habitat treatment, with four levels of salinity along an increasing gradient of salinity stress from H4 to H1.
Each plot had a size of approximately 500 m², and plots were separated from each other by 10–20 metres in average, since the whole topographic gradient was in general not longer than 200 metres (Chaieb and others 2019). Thus, there was a total of 32 main plots (2 climates × 4 blocks × 4 habitats). We applied a target and a neighbour treatment within each main plot, with three individuals of each target species planted with and without neighbours. For the target treatment, we selected three dominant species of the four habitats for transplantation in the whole experimental design. The three target species have increasing tolerances to salinity stress from L. spartum to A. macrostachyum and H. strobilaceum (Chaieb and others 2019). In each of the 32 main plots, we randomly selected nine subplots in open areas without vegetation (with at least 1 m distance from the centre of the open plot to the nearest shrub) and nine subplots covered by a dominant shrub individual. Subplots were separated from each other by a minimum distance of two metres. Thus, in each main plot, there were three replicates of each target species, transplanted with and without neighbours in 18 subplots. The selected neighbour species were H. strobilaceum in H1 and H2, A. macrostachyum in H3 and S. tetrandra in H4. Transplanted individuals were grown from rooted cuttings of the three species, collected in early September 2016 from their own habitats in at least two sebkhas of each climate condition. Collected individuals had an average above-ground height of 15 cm and were harvested in mesh bags 20 cm deep and 10 cm diameter filled with the soil of each corresponding habitat. Individuals in mesh bags were first transplanted in the common garden of the Tunisian Ministry of Environment at Sfax, the main town at the vicinity of our sites. Transplants were grown there until mid-November 2016 with irrigation every 15 days with 20 mm of tap water per transplant. Mid-November 2016, all species had formed roots in the nursery. Thus, they were transplanted at this date in all treatments, after removing the mesh bags but not the soil surrounding roots, and the experiment lasted until late September 2017. Transplants with neighbours were transplanted beneath northern edge of the shrub canopy, and each transplant was watered after transplantation with 1 l of tap water. The results of Appendix 3 of ESM show that salinity level around the roots of the transplants of the three target species was similar at time of transplantation in the plots, since salinity strongly decreased in the common garden due to watering of the transplants with tap water during 2.5 months.

Data Collection and Interaction Index Calculation

We recorded survival of all target individuals early April 2017, at the end of the wet season and late September 2017, after the dry season (Appendix 2 of ESM). Although transplants were measured at the beginning of the experiment, we did not record biomass nor measured them at the end of the experiment, since survival was too low, in particular in the most saline habitats of the dry arid climate. Survival rates were calculated in each subplot, in terms of per cent of the three individuals transplanted per treatment combination and per block.

Soil water content and soil salinity were measured at 5 cm distance from each transplant individual, early April and late September 2017, using a Wet Sensor HH2 moisture meter (Delta-T Devices, Cambridge, England). Only soil salinity data in the spring and soil water content in the summer are shown here since soil salinity was too high in the most saline plots in the summer for our probe and soil water content similarly high in all plots in the spring.

We used the relative interaction index (RII) (Armas and others 2004) to assess the effect of the dominant shrubs on the target species in all treatments. This index is based on the comparison of target performance with and without neighbours: $RII = (A - B)/(A + B)$.

A is the per cent survival with neighbours in a main plot and B the per cent survival without neighbours in the same plot. Values of RII vary between −1 and 1, with negative values indicating competition and positive ones facilitation.

Statistical Analyses

We used a generalized linear mixed-effects model (GLMM) to test the effects of the climate, salinity, target, neighbour treatments and their interactions on spring survival. Salinity, the continuous variable, was log-transformed before analyses to meet parametric model assumptions and to homogenize the gradient to avoiding aggregated point patterns. We used two forms of salinity in this analysis (log (salinity) and (log salinity)^2) to eventually detect nonlinear relationships (Maalouf and others 2012). Since only linear effects were significant in the GLMM, nonlinear ones are not shown in Table 1.

To test the effects of the climate, habitat, target treatments and their interactions on RII survival calculated at the two seasons, we used a split–split-plot mixed ANOVA model with blocks as a random
effect, climate as main treatment, habitat as sub-treatment and target as sub-sub-treatment. We also used a split–split-plot mixed analysis of variance (ANOVA) to test the effect of the climate, habitat, neighbour treatments and their interactions on salinity in spring season and soil moisture in summer season, with blocks as a random effect, climate as main treatment, habitat as sub-treatment and neighbour as sub-sub-treatment. Soil moisture and salinity variables were log-transformed to meet normality before ANOVAs.

Data analysis was done in R version 3.3.3 (R Core Team 2017). The GLMM model used for survival was performed with the function glmer and the package lme4. The split–split-plot mixed ANOVAs used for RII, salinity and soil moisture variables were performed with the function AOV and the package agricolae (De Mendiburu 2017) for linear mixed effect models. We used Tukey HSD tests after ANOVAs when necessary. In addition, sample t-tests were used to test if RII values were different from zero.

**RESULTS**

Climate, habitat and neighbour treatments all had highly significant effects on spring salinity, with higher values in dry arid climate, towards the centre of the sebkhas and in the open subplots than in wet arid climate, at the edge of the sebkhas and in the shrub subplots, respectively (Figure 2 and Table 1). However, there was a highly significant habitat by neighbour interaction, because in both climates the neighbour effect was not significant in H4 only (see Tukey tests, Figure 2). Additionally, the neighbour effect was stronger towards the

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**Table 1. Results of the Split–Split-Plot ANOVAs on the Effects of the Climate, Habitat, Neighbour Treatments and Their Interactions on Soil Salinity in Spring and Soil Moisture in Summer.**

| Effects                      | df | Salinity in spring | Soil moisture in summer |
|------------------------------|----|--------------------|------------------------|
|                              |    | F      | p        | F      | p        |
| Climate                      | 1  | 17.3   | < 0.001  | 80.4   | < 0.001  |
| Error (block)                | 7  |         |          |        |          |
| Habitat                      | 3  | 268.1  | < 0.001  | 458.7  | < 0.001  |
| Climate × habitat            | 3  | 4.8    | 0.006    | 0.3    | 0.020    |
| Error (block/subplot)        | 21 |         |          | 105.6  | < 0.001  |
| Target                       | 2  | 747    | < 0.001  |         |          |
| Climate × target             | 2  | 9.5    | 0.003    | 0.3    |          |
| Habitat × target             | 6  | 54.9   | < 0.001  | 5.5    | < 0.001  |
| Climate × habitat × target   | 6  | 10.2   | < 0.001  | 1.1    | 0.003    |
| Residual error               | 448|        |          |        |          |

Significant ($p < 0.05$) effects are indicated in bold.

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Figure 2. Means ($n = 4, \pm SE$) of spring soil salinity (electrical conductivity) with and without neighbour in the four habitats of the two climate conditions. Letters show the results of the HSD Tukey test for the climate × habitat × neighbour interaction ($p < 0.001$, Table 3) in the split–split-plot ANOVA.

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centre of the sebkhas in the dry arid climate (highly significant climate by habitat by neighbour interaction, Figure 2 and Table 1). Overall, these results show that effects on salinity did not collapse in the most severe environmental conditions (centre of the sebkhas in dry arid climate).

Climate, habitat and neighbour treatments had all highly significant effects on summer soil mois-
ture, with higher values in wet arid climate, towards the centre of the sebkhas and in the shrub subplots, than in dry arid climate, at the edge of the sebkhas and in open subplots, respectively (Figure 3 and Table 1). However, there was a weakly significant climate by habitat interaction, because the climate effect was not significant in H3 (see Tukey test). There was also a significant habitat by neighbour interaction, because differences in soil moisture between shrub and open subplots increased from the edge towards the centre of the sebkhas (results of Tukey test not shown).

In spring, salinity strongly decreased survival in all treatments (highly significant salinity effect in Table 2 and Figure 4 and see Appendix 4 of ESM for results for each species). There was a highly significant climate effect due to overall higher survival in wet arid than dry arid climate. There was a highly significant climate by neighbour interaction, with overall targets, lower survival with neighbours than in the open in wet arid climate, but higher survival with neighbours than in the open in dry arid climate (Figure 4). However, there was a weakly significant climate by neighbour by salinity interaction, because the negative effect of neighbours on survival in wet arid climate occurred only in low saline conditions and switched to positive in high-saline conditions, while the positive effect of neighbours in dry conditions tends to vanish with increasing salinity (Figure 4). There was also a highly significant salinity by target interaction because the decrease in survival with increasing salinity was stronger for L. spartum than for the two shrubs, with almost no survivors for the grass at the highest salinity level (Appendix 4 of ESM). Finally, there was a significant climate by salinity by target interaction because the positive effect of neighbours on survival in dry arid climate was only observed for the two shrub species, while there was no effect of neighbours in dry arid climate for the grass species whose survival was only affected by salinity (Appendix 4 of ESM).

In spring, habitat had a significant effect on RII survival, for the three targets and in both climates, with higher RII values at mid positions along the topography gradient than in the two extreme habitats (Table 3, Figure 5 and results of the Tukey test for the habitat effect). However, there was a highly significant climate by habitat interaction because the highest RII value was observed in H2 in wet arid climate but in H3 in dry arid climate (see the results of the Tukey test in Figure 5). In addition, the results of sample t-tests show that facilitation was only significant in H2 in the wet arid climate and in H3 in the dry arid one. Overall, these results show evidence that facilitation peaked at a

Figure 3. Means ($n = 4$, ± SE) of summer soil moisture with and without neighbours in the four habitats of the two climate conditions. Letters show the results of the HSD Tukey test for the climate × habitat interaction ($p = 0.012$, Table 3) in the split-split-plot ANOVA.
medium position along the topography gradient and collapsed towards the centre of the sebkhas, and in particular in the dry arid climate, consistent with results of Figure 5. The absence of a significant three-way interaction suggests that this interaction between the climate and habitat treatments was valid over all species at that date.

In late summer, only the target treatment had a (weakly) significant single effect on RII survival, with higher RII values for _L. spartum_ than for the two shrub species (Table 3, Figure 6 and result of the Tukey test not shown). There was a highly significant habitat by target interaction because over all climate conditions, RII decreased from the edge to the centre of the sebkhas for _L. spartum_, but rather peaked at intermediate position for the two shrub species. However, this effect was particularly strong only in H4 of the wet arid climate, with significant facilitation for the grass and significant competition for the two shrub species (significant climate by habitat by target interaction, Table 3 and results of Tukey and t tests in Figure 6). Overall, these results highlight that the collapse of facilitation increased and became more species-specific from the spring to the summer seasons.

**DISCUSSION**

In response to our first question, we found that the relationship between plant–plant interactions and salinity was unimodal with maximum facilitation at intermediate salinity levels and a collapse of facilitation at extreme salinity levels. In response to our second question, this collapse was not due to a decrease in the facilitative effect of the shrubs, but rather occurs because salinity stress exceeded the threshold tolerance of the target species even below shrubs. Increasing aridity did not change the shape of the curve of plant–plant interaction along the

| Effects                        | Survival                        | df | Chisq | P     |
|-------------------------------|---------------------------------|----|-------|-------|
| Climate                       |                                 | 1  | 34    | <0.001|
| Salinity                      |                                 | 1  | 174.8 | <0.001|
| Neighbour                     |                                 | 1  | 0.3   | 0.551 |
| Target                        |                                 | 2  | 0.4   | 0.807 |
| Climate × salinity            |                                 | 1  | 1.4   | 0.422 |
| Climate × neighbour           |                                 | 1  | 26.5  | <0.001|
| Salinity × neighbour          |                                 | 1  | 9.0   | 0.678 |
| Climate × target              |                                 | 2  | 13.2  | 0.967 |
| Salinity × target             |                                 | 2  | 5.2   | <0.001|
| Neighbour × target            |                                 | 2  | 9.8   | 0.007 |
| Climate × salinity × neighbour|                                 | 1  | 5.5   | 0.021 |
| Climate × salinity × target   |                                 | 2  | 4.1   | 0.289 |
| Climate × neighbour × target  |                                 | 2  | 1.8   | 0.472 |
| Salinity × neighbour × target |                                 | 2  | 3.23  | 0.235 |
| Climate × salinity × neighbour × target |                 | 2  | 8.1   | 0.008 |

Significant (p < 0.05) effects are indicated in bold.
salinity gradient, but it does induce an earlier collapse of facilitation along the gradient. Finally, the three target species had contrasting responses to neighbours, in particular after the summer drought and in the least saline habitat. Separating direct non-resource and resource stresses on plant–plant interactions, by assessing changes in similar climatic conditions. Separating drought from salinity stress for plants is not easy, since it is known that a major consequence of NaCl stress is the loss of intra-cellular water and both stresses have in common to induce an osmotic stress (Mahajan and Tuteja 2005). Thus, when salinity stress is increasing in constant climatic conditions, drought stress may increase as well for plants. Additionally, we are aware that drought stress also varied within each climate condition since the salinity gradient was set up along a complex gradient of topography. Thus, an experiment conducted in controlled conditions of salinity and moisture availability might certainly be useful to really separating both stresses. However, increasing drought stress along the topography gradient within each climate cannot explain the negative effect of increasing salinity on plant survival since

Figure 5. Means ± SE (n = 4) of relative interaction index (RII survival) calculated in spring for the three pooled target species in the four habitats of the two climate conditions. Letters show results of the HSD Tukey test for the climate × habitat interaction (p < 0.001, Table 2) in the split–split-plot ANOVA. Results of one-sample t tests are shown below bars: *, p < 0.05.

The Collapse of Facilitation Along the Salinity Gradient

In our study, we attempt separating the effects of direct non-resource from direct resource stresses on plant–plant interactions along a salinity gradient in similar climatic conditions. Separating drought from salinity stress for plants is not easy, since it is known that a major consequence of NaCl stress is the loss of intra-cellular water and both stresses have in common to induce an osmotic stress (Mahajan and Tuteja 2005). Thus, when salinity stress is increasing in constant climatic conditions, drought stress may increase as well for plants. Additionally, we are aware that drought stress also varied within each climate condition since the salinity gradient was set up along a complex gradient of topography. Thus, an experiment conducted in controlled conditions of salinity and moisture availability might certainly be useful to really separating both stresses. However, increasing drought stress along the topography gradient within each climate cannot explain the negative effect of increasing salinity on plant survival since

Table 3. Results of the Split–Split-Plot ANOVAs on the Effects of the Climate, Habitat, Target Treatments and Their Interactions on RII Survival Calculated in Spring and Summer.

| Effects                           | df | RII in spring |                      | RII in summer |                      |
|-----------------------------------|----|---------------|----------------------|---------------|----------------------|
|                                   |    | F             | p                    | F             | p                    |
| Climate                           | 1  | 1.3           | 0.2                  | 0.3           | 0.5                  |
| Error (block)                     | 7  | 7             |                      |               |                      |
| Habitat                           | 3  | 4.5           | **0.005**            | 1.9           | 0.1                  |
| Climate × habitat                 | 3  | 6.8           | < **0.001**          | 4.9           | **0.031**            |
| Error (block/subplot)             | 21 |               |                      |               |                      |
| Target                            | 2  | 1.3           | 0.2                  | 3.6           | **0.030**            |
| Climate × target                  | 2  | 0.4           | 0.6                  | 3.9           | **0.020**            |
| Habitat × target                  | 6  | 0.7           | 0.6                  | 5.4           | < **0.001**          |
| Climate × habitat × target        | 6  | 0.2           | 0.9                  | 3.5           | **0.003**            |
| Residual error                    | 448|               |                      |               |                      |

Significant (p < 0.05) effects are indicated in bold.

Figure 5. Means ± SE (n = 4) of relative interaction index (RII survival) calculated in spring for the three pooled target species in the four habitats of the two climate conditions. Letters show results of the HSD Tukey test for the climate × habitat interaction (p < 0.001, Table 2) in the split–split-plot ANOVA. Results of one-sample t tests are shown below bars: *, p < 0.05.
the most saline habitat where survival was the lowest was also the wettest.

We did not find a switchback to competition in the most saline habitats likely because salinity is not a direct resource gradient, consistent with refined facilitation theory (Michalet 2007; Maestre and others 2009; Michalet and others 2014a). Facilitation vanished in the most saline habitat, at both seasons, in both climatic conditions and for the three target species, consistent with the collapse of facilitation theory (Michalet and others 2006). However, facilitation did not collapse because of a decrease in the facilitative effects of shrubs on abiotic conditions, as predicted by Michalet and others (2006, 2014a). Our measurements of soil electrical conductivity showed that shrubs still strongly decreased salinity stress in the most saline habitats. Additionally, results on survival rates showed that all target plants (even below shrubs) died in the spring above a threshold of salinity that was observed in the most saline habitat of both climatic conditions. Thus, our study highlights that the collapse of facilitation along a direct non-resource gradient can occur even when nurses still ameliorate the environment, as shown by Qi and others (2018) for two target species along an intertidal salinity gradient.

He and Bertness (2014) argued that SGH exceptions occur when weak stress gradients or stresses outside of species’ niches are examined, multiple stresses co-occur cancelling out their effects, temporally dependent effects are involved, or results are improperly analysed. Indeed, Qi and others (2018) showed with modelling and field results that weak gradients are unlikely to support the SGH and Leong and others (2019) that isolating resource gradients provide SGH support in pitcher plants. As argued above, our gradient was not complex since we could separate salinity from drought stresses. Additionally, our salinity gradient was not weak, with electrical conductivity varying from less than 5 ms cm\(^{-1}\), where target spring survival was close to 100%, to close to 80 ms cm\(^{-1}\), where most plants died. Third, we did not examine the effect of salinity outside species’ niches, since our most saline habitat was naturally colonized by the most stress-tolerant target species, \textit{H. strobilaceum}, whose facilitative response also vanished at this stressful end of its niche. Fourth, life-history stage of our target species did not shift across our salinity gradient since we used transplants of sim-

Figure 6. Means ± SE (\(n = 4\)) of relative interaction index (RII survival) calculated in summer for the three target species in the four habitats of the two climate conditions. Letters show results of the HSD Tukey test for the climate × habitat × target interaction (\(p = 0.003\), Table 2) in the split-split-plot ANOVA. Results of one-sample \(t\) tests are shown above or below bars: *, \(p < 0.05\).
ilar stages in all habitats. Thus, the collapse of facilitation found in our study could not be explained by any of these four reasonable exceptions. It is likely that alternatively supports of the SGH might result from assessing gradients that exclude the most extreme portions of the stress gradient or complex gradients involving stress factors that may cancel each other (Pugnaire and others 2015). Finally, studies assessing intraspecific interactions along gradients with abrupt end, as often occurring in intertidal systems (for example, mussel beds or chord grass communities, Figure 1B, C in He and Bertness 2014) and some terrestrial ones (for example, tree lines, Fajardo and McIntire 2011), cannot detect a collapse of facilitation since the nurse and target die at the stressful end of their niche in close association.

One can also argue that the important mortality that occurred early in the experiment might have impeded to straightforwardly test the collapse of facilitation. It is true that a higher number of replicates of transplants or an early replacement of dead individuals would have strengthened our conclusions. However, it should be noted that mortality was only high for *Lygeum spartum*, the species least tolerant to salinity, in particular in the most saline habitats. In contrast, the two halophytic shrubs, and in particular *Halocnemum strobilaceum*, survived well in the early spring in the wet arid climates even in the most saline habitats, due to the facilitation of the shrub (see Appendix 4 of ESM). This was likely due the high winter rainfall that occurred right after transplantation (see Appendix 2 of ESM). In addition, most facilitative experiments have shown that increasing facilitation in stressful environments is due to the high mortality of target plants in no-neighbours plots, a process called environmental severity effect (Michalet and others 2014b). Thus, a high mortality in open plots is not a condition impeding the test of facilitation models. Finally, an increasing mortality occurring with neighbours at extreme of environmental gradients is specifically the evidence of a collapse of facilitation, as early found in Argentina by Kitzberger and others (2000) for natural seedlings of an austral conifer.

Other soil properties than salinity might have influenced the survival of transplants in interaction with the effect of neighbours. There were in particular significant differences in soil texture across the topography gradient due to erosion, with slight increase in clay content from the upper most habitat (H4) to the lowest one (H1, see methods). However, soil texture is overall sandy in Tunisian sebkhas (from loamy sand in H4 to sandy loam in H1, Chaieb and others 2019). In addition, *L. spartum*, the species which survived the least in the lowest habitats, could not have been negatively influenced by an excess of clay since it is known to occur on soils of contrasting soil textures (including clay soils) throughout the Mediterranean Basin (Spampinato and others 2018).

**The Effect of Increasing Aridity**

Our design was not set up in order to straightforwardly assess the effect of increasing water stress since we had only two points along the drought gradient in similar conditions of low salinity, the H4 position from the two climate conditions. Additionally, the increase in aridity from wet to dry arid climate was likely too low to test facilitation models as argued above. However, it is interesting to notice that the increase in water stress from wet to dry arid climate at the H4 position did not induce an increase in competition, but a decrease in competition or an increase in facilitation, depending on the season and the target species. Since the dry arid climate in central Tunisia is very dry (less than 200 mm per year) as compared to other facilitation studies, we can consider that this habitat is close to the stressful end of the water stress gradient. Thus, this increase in RH from wet to dry arid climate at the H4 position does not support the switchback to competition model (Maestre 2007; Maestre and others 2009; Malkinson and Tielborger 2010) and the results of many experiments conducted in dry environments (Tielborger and Kadmon 2000; Maestre and Cortina 2004; Armas and Pugnaire 2005; Noumi and others 2016). We argue that this might be explained by two main reasons. First, we did not measure growth due to low survival in most treatments and several studies have shown that competition for water is more likely to be observed for growth than for survival (Liancourt and others 2005b; Forey and others 2010). Second, we assessed plant–plant interactions only with the observational method, comparing the performance of a target with neighbours to that of a target transplanted in naturally open areas. Several authors have shown that using the observational method rather than the removal method in the same system exacerbates facilitative interactions, due to including long-term positive effects of neighbours in the former only (Maestre and others 2003, 2005; Michalet 2006; Michalet and others 2015; Noumi and others 2016). Indeed, our soil water measurements showed that in all treatments there was more available water below shrubs than in open areas. This might also suggest that shrubs...
from arid saline depressions are not good competitors for water, in contrast to grass neighbours (Maestre and others 2003; Maestre and Cortina 2004).

However, our system allows us to assess the interactive effects of increasing aridity and salinity, comparing the curve of interactions along the salinity gradient, either spatially in the two climates or temporally before and after the summer drought. Both of these comparisons showed that increasing aridity induced an earlier collapse of facilitation along the salinity gradient, with no more facilitation in H1 and H2 for all target species after the summer drought in the dry arid climate, whereas in the wet arid climate, facilitation was still present in H1 before the summer drought and in H2 after the summer drought. Several experiments have shown that the interaction between drought stress and disturbance induced collapses of plant–plant interactions (Forey and others 2010; Soliveres and others 2011; Maalouf and others 2012; Le Bagousse-Pinguet and others 2014; Verwijmeren and others 2019), consistent with models (Forey and others 2010; Qi and others 2018). Thus, this effect has been taken into account in most refined facilitation theories (Michalet and others 2006; Maestre and others 2009). However, this species effect did not change the fundamental shape of collapse of facilitation, but just induced an earlier collapse along the salinity gradient for L. spartum, the grass target least tolerant to salinity stress than for the two most tolerant shrubs.

**CONCLUSIONS**

Assessing the impact on plant–plant interactions of salinity along topographic gradients in two conditions of aridity allowed us to disentangle effects of direct non-resource and resource stresses on variation in facilitation along complex environmental gradients, a highly debated issue in ecology. Consistent with refined facilitation theories, we found a strong collapse of facilitation along the salinity gradient. However, this decrease in facilitation was not due to a collapse of the mitigating effects of shrub nurses on salinity stress, but to exceeding the threshold of salinity tolerance of target species even in the presence of neighbours. Increasing aridity through space or time induced an earlier collapse of facilitation, as rather observed when disturbance and stress interact along complex environmental gradients. Although we could assess with our design the direct effect of water stress in absence of salinity stress only at two positions along the drought gradient, we did not observe a switchback to competition at the extreme end of this resource stress gradient as predicted by refined facilitation theories. This is likely due to the observational method exacerbating long-term facilitation and/or the low negative effect of halophytic shrubs on soil water availability. Finally, we found contrasting target species responses to neighbours, mostly after the summer drought in the wet arid climate, consistent with refined facilitation theories. Our results are consistent with studies showing that the collapse of facilitation at extremes of drought and...
disturbance gradients threatens diversity and ecosystem functioning and stability (Kefi and others 2007; Verwijmeren and others 2013, 2019; Berdugo and others 2019, 2020).

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