

Direct and indirect consequences of dominant plants in arid environments

Diego Alejandro Sotomayor Melo

A Dissertation submitted to the Faculty of Graduate Studies in Partial
Fulfillment of the Requirements for the Degree of Doctor of Philosophy

Graduate Program in Geography
York University
Toronto, Ontario

April 2016

© Diego Alejandro Sotomayor Melo, 2016

Abstract

In arid environments, dominant woody plants such as shrubs or trees, usually facilitate a high density of species in their understories. This phenomenon is composed by a series of direct and indirect effects from the dominant plant to the understory species, and among understory species. The aim of this project was to determine these direct and indirect consequences of dominant plant-plant facilitation in a collection of field sites along the coastal Atacama Desert. The following objectives and hypotheses were examined in this project: (1) to summarize and contextualize the breadth of research on indirect interactions in terrestrial plant communities; (2) that the positive effects of dominant plants on understory communities are spatiotemporally scale dependent, from micro- to broad-scale spatial effects, and from within-seasonal to among-year temporal effects; (3) that dominant plants via their different traits determine the outcome of plant-plant interactions; (4) that dominant plants determine the outcome of interactions amongst understory species and that their responses are species-specific; and (5) that facilitation by dominant plants generates sufficiently different micro-environmental conditions that lead to consistent differences in seeds traits of understory plants.

Overall, we found that multiple factors determine the outcome of plant-plant interactions along the field sites studied in this project. These factors impact both the direct and indirect effects of dominant woody plants on their understory communities and include species-specific traits of both the dominant and understory species, and the spatial and temporal environmental gradients that manifest their effects at different scales.

Dominant plants usually facilitate increased species richness and density of plants in

their understory, that in turn mediates effects amongst these species. However, these direct effects seem to have a limit given that at extremely stressful environmental conditions they tend to change to neutral and even competitive effects of canopies on their understories. This provides evidence that positive effects of dominant plants collapse under extreme spatiotemporal stress. Although we did not find evidence of evolutionary effects of top-down facilitation, the methodology proposed here represents a contribution to test the conditions under which these results hold. Overall, this project illustrates the importance of understanding the multiple drivers that determine the outcome of biotic interactions.

To my family : Cecilia, Alejandro,
Sergio and Dieguito

Acknowledgements

I would like to thank my supervisor Dr. Chris Lortie for the opportunity to come work with him in Canada. I am extremely grateful for your encouragement, guidance and patience over all of these years.

I would like to thank Dr. Taly Drezner for her valuable comments and suggestions as my co-supervisor throughout the completion of my degree. I thank Dr. Rick Bello for his valuable comments and for being part of my supervisory committee. I also would like to thank Drs. Suzanne MacDonald, Sapna Sharma and Lonnie Aarssen (Queen's U) for being part of my examining committee.

Many thanks to all the people who contributed to this project. In Peru: Pr. Francisco Villasante, Italo Revilla, Birggeth Flores, Jessica Turpo, Paola Medina, Anthony Pauca and Alejandro Sotomayor. In Chile: Dr. Julio Gutiérrez, Dr. Francisco Squeo, Pr. Gina Arancio, Danny Carvajal, Patricio García, Eric Anacona and Fernanda Delgado. In Canada, members of the Lortie Lab: Laurent Lamarque, Ryan Spafford and Alex Filazzola. I especially thank the Comunidad Campesina de Atiquipa for allowing me entrance to their private reserve and their support during fieldwork. I also thank the Chilean Government for allowing me entrance to Parque Nacional Fray Jorge.

I thank all the Lortie lab members who made my life as a graduate student a memorable experience: Laurent Lamarque, Ryan Spafford, Dan Masucci, Anya Reid,

Alex Filazzola, Amanda Liczner, Ally Ruttan, and Taylor Noble. I would also like to thank the Faculty and students of the Department of Geography that have welcomed me during my stay at York University. I thank Yvonne Yim for her disposition and help during all these years in the Program.

This project was possible due to several research and fieldwork grants from the Faculty of Graduate Studies at York University. I also thank Chris Lortie's NSERC Discovery Grant for travel funding support.

I thank my family: Cecilia, Alejandro, Sergio and Dieguito for their love, unconditional support and constant encouragement during my studies.

Table of Contents

Abstract	ii
Dedication	iv
Acknowledgements	v
Table of Contents	vii
List of Tables	xi
List of Figures	xvi
Chapter 1	1
Abstract	1
Introduction	2
Methods	8
Results	11
Discussion	15
Conclusions	23
Acknowledgements	24
References	24
Chapter 2.....	30
Abstract	31
Introduction	32
Methods	35
<i>Desert localities and study sites</i>	35
<i>Dominant plants and vegetation surveys</i>	36
<i>Statistical analyses</i>	39
Results	40

Discussion	44
Concluding remarks	49
Acknowledgements	50
References	50
Chapter 3.....	55
Abstract	56
Introduction	57
Methods	61
<i>Study site, plot selection, and dominant species</i>	61
<i>Vegetation surveys and microsite measurements</i>	63
<i>Subordinate plant-plant interaction outcomes by microsite</i>	64
<i>Statistical analyses</i>	67
Results	68
<i>Microhabitat differentiation</i>	68
<i>Species-specific effects of dominant plants on their understory</i>	71
<i>Subdominant plant-plant interactions as affected by dominant plants</i>	73
Discussion	79
Concluding remarks	82
Acknowledgements	83
References	83
Chapter 4	88
Abstract	89
Introduction	90
Methods	94
<i>Study site and species</i>	94
<i>Vegetation surveys and target understory species</i>	95
<i>Neighborhood removal experiment</i>	96
<i>Statistical analyses</i>	99

Results	101
<i>Surveys of plant density</i>	101
<i>Neighborhood removal experiment</i>	104
Discussion	110
Acknowledgements	114
References	115
Chapter 5	119
Abstract	120
Introduction	121
Methods	125
<i>Study site and species</i>	125
<i>Plant density</i>	126
<i>Seed mass and viability</i>	127
<i>Seed germination trials</i>	127
<i>Statistical analyses</i>	130
Results	131
<i>Plant density</i>	131
<i>Seed mass and viability</i>	131
<i>Seed germination</i>	135
Discussion	138
Acknowledgements	142
References	142
Synthesis	146
Aim and over-arching hypothesis	147
Summary of major findings	150
Implications for ecological and biogeographical theory	157
Implications for methodology	162
Implications for coexistence in stressful environments	163

Implications for applied ecology, global change and desertification	165
Conclusion	166
References	167
Appendices	171
Appendix 1	172
Appendix 2	173
Appendix 3	186
Appendix 4	187
Appendix 5	192
Appendix 6	193
Appendix 7	194
Appendix 8	195
Appendix 9	196
Appendix 10	197
Appendix 11	198
Appendix 12	199
Appendix 13	200
Appendix 14	201

List of Tables

Chapter 1

Table 1. Main hypotheses tested regarding indirect interactions in terrestrial plants along with a concise definition and examples of reference articles 6

Chapter 2

Table 1. Study sites within each desert region along with their climatic characteristics. De Martonne AI was calculated using mean annual temperature and annual precipitation extracted from WorldClim (Hijmans et al. 2005) for each site. Rainfall data are means and annual totals within brackets. * mean data from Sotomayor & Jimenez 2008 for Atiquipa, and yearly data from a local weather station; mean data for Romeral from Almeyda 1950, for Fray Jorge from Madrigal et al. 2011, and annual data from www.ceazamet.cl. 38

Table 2. Dominant plant species surveyed along the Atacama Desert in Southern Peru and North-Central Chile. Traits presented include plant height (m), life form, presence of thorns (thorniness), and the site where each species was sampled. * denotes endemic species (Marticorena et al. 2001). 39

Table 3. Summary of GLMMs contrasting RILs of species richness and plant density among desert localities, dominant plants and gradients in three different years along the Atacama Desert. P-values <0.05 are bolded and indicate significant differences. 41

Chapter 3

- Table 1.** Summary of statistical models contrasting species richness and plant density (plants.m⁻²) between open microsites and dominant plant microsites in Atiquipa, Southern Peru. P-values <0.05 indicate significant differences 69
- Table 2.** Summary of statistical models contrasting plot level species richness and standardized effects sizes (SES) of C-scores between open microsites and dominant plant microsites in Atiquipa, Southern Peru. P-values <0.05 indicate significant differences 70

Chapter 4

- Table 1.** Summary of the four possible indirect outcomes based on direct and indirect interaction indices. Greater-than or lower-than symbols indicate significant differences that are considered to determine the respective indirect outcome. Adapted from Michalet et al. (2015a, b). 99
- Table 2.** Summary of generalized linear mixed models (GLMMs) for the differences in plant density between microsites from surveys in Atiquipa, Southern Peru. Bolded values denote significance at $P < 0.05$ 102
- Table 3.** Summary of parameter estimates (slope) from GLMMs examining the effect of neighborhood density on the density of two target species under shrubs and in the open. Density is number of plants per m⁻². Observed probability functions were exponential. Bolded values denote significance at $P < 0.05$ 103
- Table 4.** Summary of GLMMs for plant height, fruit production and biomass following a neighborhood removal treatment in Atiquipa, Southern Peru. Entire

herbaceous neighborhoods were removed at two microsites: under *C. spinosa* and open adjacent microsites. Bolded values denote significance at $P < 0.05$ 106

Table 5. Summary of indirect interaction effects and outcomes in a desert tree-understorey assemblage tested using herbaceous neighborhood removals under the canopy and in open. The four possible indirect outcomes are (see Table 1 and Methods for details): indirect facilitation, additional facilitation, indirect competition, and additional competition 107

Chapter 5

Table 1. Micro-habitat data for open (O) and understorey (U) conditions obtained from field observations at Atiquipa, Southern Peru. Field values are presented $\pm 1SE$, and values utilized to program the growth chambers are between brackets 129

Table 2. Summary of GLMMs of plant density, seed mass and viability for five annual species found in both understorey and open micro-habitats (i.e. seed sources). Chi-square values are presented along with the corresponding p-values. Bolded values indicate statistically significant differences ($p < 0.05$) 132

Table 3. Summary of GLMMs of seed germination under controlled conditions (growth chambers). Micro-habitat refers to the chamber conditions, while source corresponds to the micro-habitat where seeds were collected. Chi-square values are presented along with corresponding p-values. Bolded values indicate statistically significant differences ($p < 0.05$) 133

Table 4. Summary of post hoc chi-square contrasts for seed germination of five annual species. False discovery rate corrected p-values are presented. Combinations of seed source (open (O) and understorey (U)) and simulated micro-habitat (open (O) and understorey (U)) were contrasted with their respective reciprocal treatment. Bolded values indicate statistically significant values ($p < 0.05$) along with the corresponding treatment with the higher final germination rate	134
---	-----

Synthesis

Table 1. Summary of findings from the study of direct and indirect consequences of plant-plant interactions	151
--	-----

Appendices

Appendix 3. Summary of findings for the indirect plant interactions literature, including hypotheses tested and information about experimental procedures, target species and field sites	186
Appendix 5. Summary of GLMMs contrasting species richness among microsites and gradients in three different years along the Atacama Desert. P-values < 0.05 are bolded and indicate significant differences	192
Appendix 6. Summary of GLMMs contrasting plant density among microsites and gradients in three different years along the Atacama Desert. P-values < 0.05 are bolded and indicate significant differences	193

Appendix 9. Study sites along with their climatic characteristics (Chapter 4). De Martonne AI was calculated using mean annual temperature and annual precipitation extracted from WorldClim (Hijmans et al. 2005) for each site 196

Appendix 10. Summary of Akaike Information Criterion (AIC) values for probability distribution functions fitted to the response variables analyzed in different models in Chapter 4. Lower AIC values indicate a better fit 197

List of Figures

Chapter 1

- Figure 1.** Synthetic framework for indirect interactions in plant communities showing the frequency of hypotheses tested to date. This framework nests hypotheses into a trophic chain while aggregating the models of Wootton (1994) and Callaway (2007). It also depicts a hypothetical relationship where higher complexity of interactions would be supported in more productive and benign environments. Dashed lines indicate indirect effects, solid lines represent direct interactions, and dotted lines indicate future directions for studies 10
- Figure 2.** Geographical distribution of studies on indirect interactions involving terrestrial plants 12
- Figure 3.** Distribution of studies according to ecosystem type: a) type of interaction tested (trophic chain (TC), shared defense (SD), associational resistance (AR), indirect facilitation (IF), exploitative competition of facilitation (ECF), and apparent competition (AC)); b) number of trophic levels studied; c) number of target species tested. Asterisks (*) denote significant differences between groups ($p < 0.05$) 14

Chapter 2

Figure 1. Relative interaction indices (RIIs) with ± 1 SE confidence intervals for species richness of two dominant plant microsites in relation to open microsites at the peak of the growing seasons of 2011, 2012 and 2013 (vertical panels) at three desert locations (horizontal panels) along the Atacama Desert. Stars indicate significantly different from zero RII values obtained from one sample t-tests (* $P < 0.05$, ** $P < 0.01$). 42

Figure 2. Relative interaction indices (RIIs) with ± 1 SE confidence intervals for plant density of two dominant plant microsites in relation to open microsites at the peak of the growing seasons of 2011, 2012 and 2013 (vertical panels) at three desert locations (horizontal panels) along the Atacama Desert. Stars indicate significantly different from zero RII values obtained from one sample t-tests (* $P < 0.05$, ** $P < 0.01$). 43

Chapter 3

Figure 1. Absolute differences along with standard errors between microhabitat conditions for two dominant plant microsites in relation to open microsites at noon (12 hrs.) during the growing season of 2012 (August-October) in Atiquipa, Southern Peru 72

Figure 2. Relative interaction indices (RII) along with ± 1 SE confidence intervals for species richness of two dominant plant microsites in relation to open microsites across the growing seasons of 2011 and 2012 in Atiquipa, Southern Peru. Left-hand panels correspond to 2011, and right-hand panels to 2012. Upper

panels correspond to the 1 m² microscale, mid panels to the 0.25 m² microscale, and lower panels to the 0.0625 m² microscale. Stars indicate significantly different from zero RII values obtained from one sample t-tests (* P < 0.05, ** P < 0.01)

..... 74

Figure 3. Relative interaction indices (RII) along with ± 1 SE confidence intervals for plant density of two dominant plant microsites in relation to open microsites across the growing seasons of 2011 and 2012 in Atiquipa, Southern Peru. Left-hand panels correspond to 2011, and right-hand panels to 2012. Upper panels correspond to the 1 m² microscale, mid panels to the 0.25 m² microscale, and lower panels to the 0.0625 m² microscale. Stars indicate significantly different from zero RII values obtained from one sample t-tests (* P < 0.05, ** P < 0.01)

..... 75

Figure 4. Principal Components Analysis (PCA) ordinations of the species composition of two dominant plant species and open microsites at different spatial micro-scales along the growing seasons of 2011 and 2012 in Atiquipa, Southern Peru. Points are centroids with bi-directional 95% confidence error bars. Results of two-way ANOVAs on the effects of microscale (S), microsite (M), and their interaction are shown in each panel: * P < 0.05, ** P < 0.01. Year: left-hand panels correspond to 2011, and right-hand panels to 2012. Month: upper panels correspond to September, mid-upper panels to October, mid-lower panels to November, and lower panels to December of their respective years

Figure 5. Plot level species richness (left hand y-axis, bars) and standardized effect size (SES) values of the C-score (right hand y-axis, dots) with ± 1 SE

confidence intervals for two dominant plant microsites and open microsites across the growing seasons of 2011 and 2012 in Atiquipa, Southern Peru. Left-hand panels correspond to 2011, and right-hand panels to 2012. Upper panels correspond to the 1 m² microscale, mid panels to the 0.25 m² microscale, and lower panels to the 0.0625 m² microscale 78

Chapter 4

Figure 1. Relative interaction indices (RII) \pm 1SE for plant density of the target annual species from censuses at the peak of the growing season in Atiquipa, southern Peru. Stars indicate significantly different from zero RII values obtained from one sample t-tests (* $P < 0.05$, ** $P < 0.01$) 104

Figure 2. Plant height (cm) + 1SE, fruit production (number of fruits. plant⁻¹) + 1SE, and final biomass (g) + 1SE of two target annual species (*Fuertesimalva peruviana* and *Plantago limensis*) after an herbaceous neighborhood removal experiment (N+ = neighborhood intact, N- = neighborhood removed) conducted during two years in Atiquipa, southern Peru. Stars (*) indicate significant differences between removal treatments in a microhabitat ($P < 0.05$) 108

Figure 3. Relative interaction indices (RII) \pm 1SE for plant height, number of fruits. plant⁻¹ (fruits), and final biomass of two target annual species (*Fuertesimalva peruviana* and *Plantago limensis*) after an herbaceous neighborhood removal experiment conducted during two years in Atiquipa, southern Peru. DN is the direct interaction of the neighbors, DC the direct interaction of the canopy, and IC is the indirect interaction of the canopy mediated by herbaceous neighbors. Stars

indicate significantly different from zero RII values obtained from one sample t-tests (* $P < 0.05$, ** $P < 0.01$). P -values on each graph indicate the result of one-way ANOVAs comparing RIIs for each species, with significantly different RIIs not sharing the same letter 109

Chapter 5

Figure 1. Relative interaction indices for plant density of five annual plant species growing in open and understorey micro-habitats in Atiquipa, Southern Peru. Means and standard errors are shown 135

Figure 2. Relative interaction indices ($RII \pm SE$) for seed germination of five annual plant species collected in open and understorey micro-habitats. Seeds from both sources were germinated under simulated open and understorey conditions in growth chambers. (a) Final germination rates. (b) Numbers of days required to 50% germination 137

Synthesis

Figure 1. Location of the study sites along the Atacama Desert 149

Figure 2. Synthetic framework of the plant-plant interactions concepts examined in this project. Solid lines denote direct effects, dashed lines indirect effects, and the numbers between brackets indicate the chapter that included such concepts 156

Appendices

Appendix 1. PRISMA Flow Diagram for the identification of studies included in the systematic review	172
Appendix 7. Absolute differences along with standard errors between microhabitat conditions for two dominant plant microsites in relation to open microsites at noon (12 hrs.) during the growing season of 2012 (August-October) in Atiquipa, Southern Peru. a. Differences in temperature (°C). b. Differences in relative humidity (%)	194
Appendix 8. Vapor pressure deficit (VPD) along with standard errors for three microsites associated with two dominant plants and nearby open microsites at noon (12 hrs.) during the growing season of 2012 (August-October) in Atiquipa, Southern Peru. The three microsites correspond to the understory of <i>C. spinosa</i> and <i>R. armata</i> , and open nearby microsites. a. Overall averages per microsite; b. Seasonal trend according to Julian Day. VPD was calculated using Hartman (1994)'s equation based on Temperature (°C), and Relative Humidity (%)	195
Appendix 11. Density (mean \pm SE) of five annual species in open and understorey micro-habitats at Atiquipa, southern Peru. Asterisks denote statistically significant differences	198
Appendix 12. Seed attributes of the five studied species. (a) Seed mass (\pm SE) in open and understorey micro-habitats. (b) Seed viability (\pm SE) using Tetrazolium tests for seeds collected in open and understorey micro-habitats. Asterisks denote statistically significant differences	199

Appendix 13. Final germination rates (\pm SE) for seeds of five annual species that were collected in two different micro-habitats (i.e. source: open and understorey, different line patterns) and germinated under two different simulated micro-habitat conditions (i.e. micro-habitat: open and understorey) 200

Appendix 14. Numbers of days required to 50% germination (\pm SE) for seeds of five annual species that were collected in two different micro-habitats (i.e. source: open and understorey, different line patterns) and germinated under two different simulated micro-habitat conditions (i.e. micro-habitat: open and understorey) 201

Chapter 1

Indirect interactions in terrestrial plant communities

Published as:

Sotomayor, D. A. & Lortie, C. J. 2015. Indirect interactions in terrestrial plant communities: emerging patterns and research gaps. *Ecosphere* 6: art103.

ABSTRACT

Indirect interactions occur when the effect of one species on another is mediated by a third species. These interactions occur in most multi-species assemblages and are diverse in their mechanistic pathways. The interest in indirect interactions has increased exponentially over the past three decades, in recognition of their importance in determining plant community dynamics and promoting species coexistence. Here, we review the literature on indirect interactions among plants published since 1990, using a novel synthetic framework that accounts for and classifies intervening species and mechanisms within trophic networks. The objectives of this review are: (1) to identify the geographical regions and ecosystem types where indirect interactions have been examined; (2) to summarize the information on the number of trophic levels examined in studies of indirect interactions; (3) to test whether the frequency of indirect interactions varies across environmental gradients; and (4) to identify the experimental approaches most commonly used in studies of indirect interactions. Studies examining indirect interactions in plants have been conducted primarily in the Northern Hemisphere, with a focus on grasslands and forests. The majority of studies (67%) examined 2 trophic levels. Indirect facilitation and apparent competition are the interactions that have been most frequently examined, with the latter being reported more frequently in relatively productive environments. Other indirect interactions reported include associational resistance, exploitative competition or facilitation, shared defenses, and trophic cascades. Generally, field experiments tested indirect interactions based on single target species. While the majority of studies on indirect interactions dealt with basic ecology issues, several studies have dealt with such interactions in the context of

biological invasions (18%) and rangeland management (12%). This review allowed us identifying a number of research needs, including the study of non-feeding interactions and that for more realistic complex designs, explicitly testing indirect interactions across different trophic levels, in geographical regions that have been under-examined to date, and in stressful ecosystems.

Keywords: Apparent competition; associational resistance; herbivory; indirect facilitation; multi-species interactions; systematic review; trait-mediated indirect effects.

INTRODUCTION

Net interactions between two species are the outcome of both direct and indirect effects of each species on the other (Bruno et al. 2003, Lortie et al. 2004, Callaway 2007). While direct positive and negative plant interactions have received considerable attention (Aarsen 1992, Silvertown 2004, Schenk 2006, Callaway 2007, Brooker et al. 2008), comparatively few studies have examined indirect interactions, possibly due to the challenges posed by the need to use sets of three or more species vs. those of testing pair-wise interactions (Strauss 1991, Wootton 1994, Callaway 2007).

Indirect interactions occur when the strength or direction of interactions between two species changes in the presence of a third species (Strauss 1991, Wootton 1994, Callaway and Pennings 2000, Callaway 2007). For instance, plant-plant interactions are mediated by herbivores (e.g., Beguin et al. 2011, Vesterlund et al. 2012), pollinators (e.g. Moeller 2004), mycorrhizal fungi (e.g. Facelli et al. 2010), soil microbes (e.g.

Johnson et al. 2003), or another plant species (e.g. Schöb et al. 2013). Trait-mediated indirect effects can also occur when interactions among plants change the traits of the interacting species thereby altering interactions with other species at different trophic levels (Abrams 1995, Werner and Peacor 2003, Ohgushi et al. 2013).

Indirect interactions occur virtually in all multi-species assemblages and can play an important role in the assembly and coexistence of species, and promote diversity in complex communities (Levine 1976, Miller 1994, Levine 1999) or in non-transitive interaction networks (Aarsen 1992, Brooker et al. 2008) by mitigating strong direct effects (Berlow 1999).

A number of important hypotheses are associated with indirect interactions. These include commonly studied feeding interactions, yet most indirect effects correspond to non-feeding interactions (Kéfi et al. 2012). Indirect interactions include apparent competition, indirect facilitation, exploitative competition and facilitation, associational resistance, trophic cascades and shared defenses (see Table 1 for definitions of common terms and references). Apparent competition is defined as an antagonistic interaction that occurs when the effects of one plant species on the other are manifested through a common consumer such as an herbivore (Chaneton et al. 2010, Recart et al. 2013). Indirect facilitation is defined as a positive interaction that occurs when the effects of one plant species on the other occur through a common competitor, as for example in networks of competing plants (Callaway and Pennings 2000, Schöb et al. 2013). Plants can also mediate effects between consumers resulting

in apparent competition when an herbivore negatively alters the resource offer to other herbivores via changes in the phenotype of the plant (Kaplan et al. 2011), or conversely in indirect facilitation when these changes result in positive effects on the other herbivores (Vesterlund et al. 2012). When plants represent resources (e.g. seeds) without changing their phenotype the interaction is termed exploitative competition or facilitation (*sensu* Wootton 1994) depending on its outcome for the consumer species (Beard et al. 2013). Hence, indirect feeding interactions have been documented through a number of different mechanisms.

Associational resistance is defined as a positive interaction in which the influence of one plant on the other decreases the likelihood of the beneficiary species being detected by a consumer (Barbosa et al. 2009). This occurs when palatable beneficiaries are associated closely with unpalatable species (Callaway et al. 2005, Graff et al. 2013). Shared defense occurs in a similar interaction context, but the nearby unpalatable species presents adaptations to repel herbivores such as spines (Vanderberghe et al. 2009). Trophic cascades are strong interactions within food webs (Polis et al. 2000) and involve more than two trophic levels such as predators, herbivores and plants (Polis et al. 2000, Schmitz et al. 2000). These indirect interactions occur when plants change their resource offer (e.g. chemical composition) to herbivores that in turn affect their predators (Laws and Joern 2013). They can take place at the species or population level when a subset of the community is involved in the interaction, but also at the community level when they alter substantially the distribution of organisms or biomass of the entire system (Polis et al. 2000). Trophic cascades can also be conceptualized as

top-down or bottom-up, when regulation within the interaction is exerted by an upper-level predator or the primary producers, respectively (Pace et al. 1999).

Table 1. Main hypotheses tested regarding indirect interactions in terrestrial plants along with a concise definition and examples of reference articles.

Hypotheses tested	N (%)	Definition	Reference article(s)
Apparent competition	89 (41.6)	Antagonistic interactions occurring when the negative effects of one plant species on the other occur through a common consumer, such as an herbivore. Plants can also mediate these interactions through changes in their resource offer.	Burger and Louda (1994); Recart et al. (2013)
Indirect facilitation	76 (35.5)	Positive interactions occurring when the positive effects of one plant species on the other occur through a common competitor. Plants can also mediate these interactions through changes in their resource offer.	Callaway and Pennings (2000); Schöb et al. (2013)
Exploitative competition and facilitation	20 (9.3)	Negative or positive interactions occurring when two species interact through resource consumption where the resource is a plant species.	Samson et al. (1992), Vesterlund et al. (2012)
Associational resistance	19 (8.9)	Positive interactions occurring when a palatable beneficiary is spatially clustered with nearby unpalatable species making it undetectable for herbivores.	Mulder and Ruess (1998); Graff et al. (2013)
Trophic cascades	9 (4.2)	Positive or negative interactions spanning more than two trophic levels. Plants mediate these interactions through changes in their resource offer (e.g. chemical composition) to herbivores that in turn affect their predators.	Harri et al. (2008); Laws and Joern (2013)
Shared defense	1 (0.5)	Positive interactions occurring when a palatable beneficiary is protected by a nearby unpalatable species with adaptations to repel herbivores.	Vanderberghe et al. (2009)

Two general frameworks have been proposed to conceptualize indirect interactions in ecology. Wootton (1994) proposed a framework to categorize these interactions using five hypotheses or mechanistic pathways: (1) interspecific competition, (2) trophic cascades, (3) apparent competition, (4) indirect mutualism via interference, and (5) indirect mutualism via exploitation. This framework characterizes indirect effects in terms of the mechanisms involved in the interaction, but fails to describe the function played by the intervening species. Alternatively, Callaway (2007) proposed six forms of positive indirect interactions focusing on plants and on the intervening organisms: (1) herbivore mediated interactions, (2) reproductive feedback and pollinator interactions, (3) disperser mediated interactions, (4) mycorrhizae interactions, (5) microbe interactions and (6) interactions involving competing terrestrial plants. While useful, these classification systems do not allow to distinguish between multiple mechanisms that can operate simultaneously, particularly for non-trophic interactions such as plant competition or facilitation (Kéfi et al. 2012), and provide only partial depictions of the networks of conceptual effects within a community because mechanistic pathways or intervening organisms are considered, but not an integration of both sets of elements.

The study of indirect interactions may provide important information on ecological and evolutionary processes, yet, appreciation of the full scope of their impacts is limited (Wootton 2002, Brooker et al. 2008, Allesina & Levine 2011, McIntire and Fajardo 2014). The primary purpose of this study is to summarize and contextualize the research on indirect interactions within the proposed framework as a mechanism that

may contribute to the development of ecological theory. The following specific objectives were addressed using a systematic review: (1) to identify the geographic and ecosystem extent of indirect interactions in terrestrial ecological communities; (2) to summarize the information on the number of trophic levels studied when examining indirect interactions in different ecosystems; (3) to determine whether the frequency of indirect interactions varies across large environmental gradients, and (4) to describe and compare the most common experimental designs and statistical techniques used to examine indirect interactions in plant communities.

To review the recent literature (i.e. within the last 25 years) on indirect interactions, we classified indirect interactions based on a novel conceptual framework that synthesizes previous research efforts. Our framework explicitly incorporates interacting species and their hypothesized interactions both within and across trophic levels (Fig. 1) and provides a more comprehensive view of indirect interactions nested within trophic relationships. This framework includes non-feeding interactions as proposed by Kéfi et al. (2012) such as indirect facilitation or trait-mediated indirect interactions.

METHODS

To review the field of indirect interactions in terrestrial plants we conducted a systematic review of the literature published between 1990 and July 2014 using the ISI Web of Science (WoS), Scopus, and Google Scholar. We used a combination the following keywords: “indirect”, “plant”, “interaction”, “competition”, “facilitation”. The first three

words were used together in combination with the last two words in separate queries (i.e. indirect* plant* interaction* competition OR indirect* plant* interaction* facilitation). We included literature published over the past 25 years as the study of indirect interactions is relatively young and indirect effects are clearly defined (Wootton 2002). However, we recognize the existence of previous articles examining indirect interactions even with different terminology predating this window of publications but focused on papers that clearly describe the same set of processes.

We identified 490 research articles obtained from the WoS, which were screened in order to assess their relevance. Searches in both Scopus and Google Scholar were conducted to complement the WoS search (Appendix 1). The following inclusion criteria were used: (1) studies explicitly dealing with indirect interactions in terrestrial ecosystems (i.e. 3 or more species reported in the interaction); (2) studies describing the results of experiments specifically designed to test effects of indirect interactions versus proposals of indirect interactions in discussion; and (3) primary empirical research reported (i.e., not reviews). Papers complying with these criteria were processed to extract data on (1) type of interaction tested; (2) number of species tested as targets, where target is defined as the species on which measurements of performance were taken; (3) role of the species involved in the interaction considering not only target species, but also species that were removed or mentioned by the authors as members of the interaction; (4) type of experiment and number of field sites; (5) type of ecosystem and geographical location of the study; and (6) type of

measurements and statistical analysis performed. These characteristics provide a thorough assessment of the scope of the literature to date.

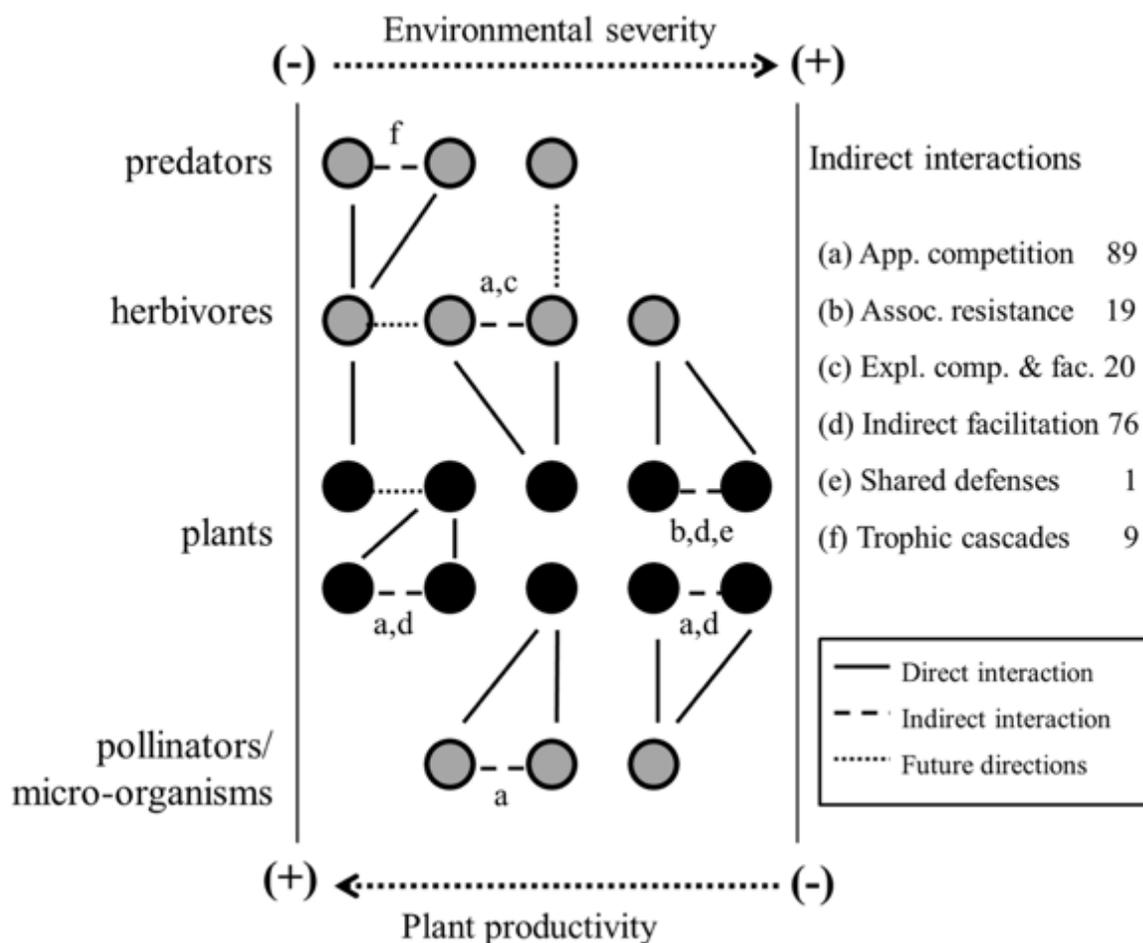


Figure 1. Synthetic framework for indirect interactions in plant communities showing the frequency of hypotheses tested to date. This framework nests hypotheses into a trophic chain while aggregating the models of Wootton (1994) and Callaway (2007). It also depicts a hypothetical relationship where higher complexity of interactions would be supported in more productive and benign environments. Dashed lines indicate indirect effects, solid lines represent direct interactions, and dotted lines indicate future directions for studies.

A total of 214 articles published in 53 different journals were included in this review (Appendix 2). The majority of studies (49%) were published in the last 5 years (2009-2014). Two journals published several studies on indirect interactions (*Ecology*: 19%; *Oecologia*: 13%), while 28 journals published only a single article on indirect interactions for terrestrial plants.

A regression model was fit to the number of publications per year, and contingency table and chi-square analyses were used to test for biases in the distribution of the number of studies associated with particular hypotheses, geographic regions, ecosystem types, trophic structures and number of target species tested. Using the proposed framework, studies were categorized following three general categories: plant-plant, plant-animal, and plant-pollinator interactions. We included plant-pollinator interactions in a different category from plant-animal interactions because the former represents non-feeding interactions (Kéfi et al. 2012).

RESULTS

The number of publications on this topic has increased exponentially within the last 25 years ($r^2 = 0.78$, $p < 0.01$). The majority of studies were conducted in the Northern Hemisphere (85%, $\chi^2 = 105.1$, $p < 0.01$) with a high number of publications originating from North America and Europe (Fig. 2), while indirect interactions in South America, Africa, Asia, and the tropical regions have been understudied (or at least under-reported in the peer-reviewed literature) (Fig. 2, Appendix 3). Indirect interactions have been

most frequently examined in forests and grasslands (45.3% of studies), while comparatively few studies have been conducted in stressful ecosystems such as deserts, alpine ecosystems, and salt marshes (Fig. 3, $\chi^2 = 195.1$, $p < 0.01$).

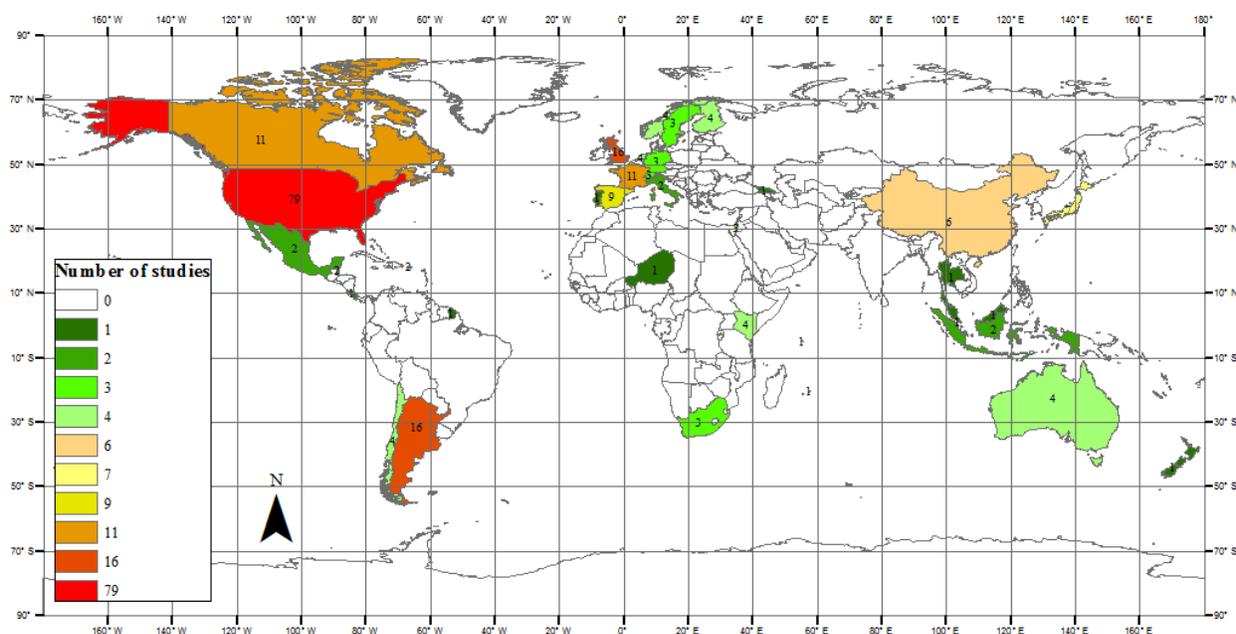


Figure 2. Geographical distribution of studies on indirect interactions involving terrestrial plants.

The majority of studies on indirect interactions have focused on plant-animal interactions (70%), followed by studies dealing with plant-plant (20%) and plant-pollinator (10%) interactions ($\chi^2 = 130.6$, $p < 0.01$). Six main hypotheses on indirect interactions have been tested (Table 1). Apparent competition and indirect facilitation have been most frequently tested to date ($\chi^2 = 195.1$, $p < 0.01$). Apparent competition has been more frequently tested in relatively productive environments such as forests ($\chi^2 = 46.6$, $p < 0.01$) and grasslands ($\chi^2 = 20.7$, $p < 0.01$), or under high resource levels

in controlled experiments ($\chi^2 = 29.7$, $p < 0.01$). On the other hand, positive effects such as indirect facilitation and associational resistance were not more frequently reported in less productive environments such as alpine ecosystems ($\chi^2 = 2$, $p = 0.57$), deserts ($\chi^2 = 1.7$, $p = 0.43$) and salt marshes ($\chi^2 = 5.7$, $p = 0.06$) (Fig. 3a).

The majority of studies dealt with two trophic levels across all ecosystem types (Fig. 3b, $\chi^2 = 114.8$, $p < 0.01$), but more complex studies included three levels in more productive environments such as agricultural ecosystems, forests, and grasslands (Fig. 3b). The method most frequently used was the single-target approach (37.7%, $\chi^2 = 100.6$, $p < 0.01$), and 75% of studies examining indirect interactions used less than five target species (Fig. 3c). There is no clear trend between ecosystem productivity and the number of target species utilized (Fig. 3c), although significantly more single-target studies were reported from agricultural systems ($\chi^2 = 10.1$, $p = 0.02$), grasslands ($\chi^2 = 51.9$, $p < 0.01$), and under greenhouse/laboratory conditions ($\chi^2 = 22.9$, $p < 0.01$).

Most studies were conducted in the field (75%, $\chi^2=166.3$, $p < 0.01$) and were also manipulative (73%, $\chi^2 = 154.1$, $p < 0.01$) (Appendix C). Single-site approaches were used in 74% of field conducted studies ($\chi^2 = 731.8$, $p < 0.01$), while studies reporting research from more than five field sites were particularly rare (11% of field studies). Experimental studies conducted exclusively in laboratories and/or greenhouses represented 15% of the total articles analysed in this review.

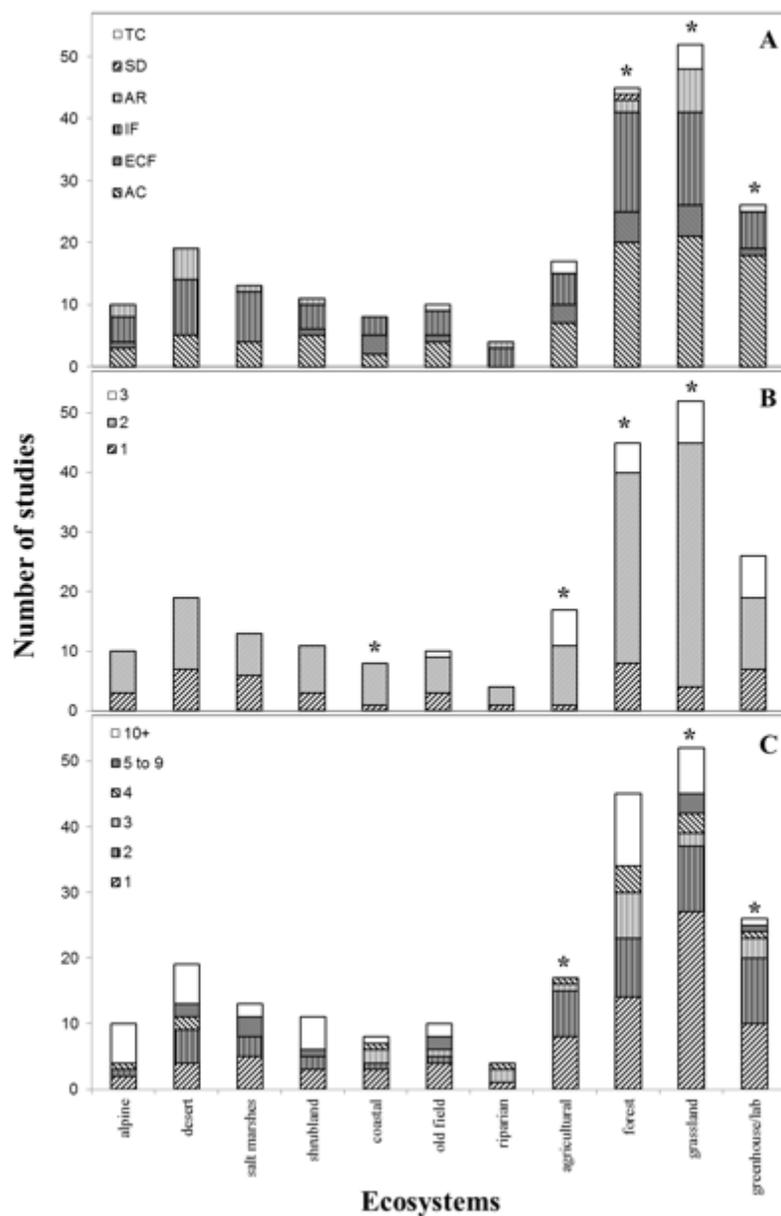


Figure 3. Distribution of studies according to ecosystem type: a) type of interaction tested (trophic chain (TC), shared defense (SD), associational resistance (AR), indirect facilitation (IF), exploitative competition of facilitation (ECF), and apparent competition (AC)); b) number of trophic levels studied; c) number of target species tested. Asterisks (*) denote significant differences between groups ($p < 0.05$).

DISCUSSION

Indirect interactions are very frequent mechanisms and can play an important role in the coexistence of species and in promoting species diversity (Brooker et al. 2008, McIntire and Fajardo 2014). Indirect interactions provide stabilizing effects within communities when they co-occur with and influence direct interactions with opposing effects (Berlow 1999), or within intransitive interacting species networks (Miller 1994, Levine 1999, Allesina and Levine 2011). Indirect interactions occur at multiple trophic levels producing higher complexity than in single trophic levels thus also affecting ecosystem functioning (Duffy et al. 2007). The influence of indirect interactions can thus scale from population to ecosystem-level impacts.

This systematic review is the first to formally synthesize the literature on indirect interactions in terrestrial plant communities, providing a quantitative summary of the scope of published research on the topic to date. Despite the potential limitations of knowledge synthesis tools such as publication bias or the “file drawer problem” (Fanelli 2012), this review has allowed us identifying the major research gaps in this field and provides directions for future research.

First, we showed a clear geographic and ecosystem bias, with the majority of studies being conducted in North America and Europe, and in mesic ecosystems, consistent with trends found for ecological research in general (Martin et al. 2012). Indirect interactions have, however, been reported in most geographic regions and ecosystems in the world, and new studies from regions and ecosystem types that have been under-examined can provide important insights into the mechanisms and

processes underlying indirect interactions. In particular, tropical and arid environments provide excellent opportunities for research on indirect interactions, as they maintain high biodiversity, their evolutionary speed is high compared to temperate regions (see Hillebrand 2004, Ward 2009), and they are important determinants of global biogeochemical processes (Millennium Ecosystem Assessment 2005).

The organizational framework proposed in this study (Fig. 1) effectively contextualized the current state of research through an explicit visualization of all logical pathways of indirect interactions occurring in plant communities. This allowed us to pinpoint specific pathways that have received considerable attention within the literature to date. The majority of studies have examined plant-animal feeding interactions to test hypotheses including apparent competition between plants mediated by a common consumer (Burger and Louda 1994, Recart et al. 2013) and positive effects that result from herbivore protection, such as associational resistance or shared defenses (Vandenberghe et al. 2009, Graff et al. 2013). Studies dealing with interactions exclusively among at least 3 plant species are relatively scarce (21% of the studies analyzed). These interactions at the base of the trophic structure have the capacity to influence overall plant diversity (Tielbörger and Kadmon 2000, Cuesta et al. 2010) and community composition by mitigating the effects of strong competitors and facilitating coexistence in networks of competing plants (Callaway and Pennings 2000, Schöb et al. 2013). Plant-pollinator interactions have received the least attention (ca. 10% of studies analyzed) and have mainly tested hypotheses of plant-plant facilitation through shared pollinators (Johnson et al. 2003, Moeller 2004), although negative effects between

invasive plant species and native species have also been tested (Morales and Traveset 2009, Gibson et al. 2012). New research efforts should address the reported gaps and focus on indirect effects exclusively among plants and on non-feeding interactions such as plant-pollinator effects taking into consideration the main pathways identified within the organizational framework in order to design more comprehensive studies.

Less studied interactions include indirect effects mediated through plant phenotypic plasticity (i.e. trait-mediated indirect effects) in response to two or more herbivores (Kaplan et al. 2011), or even more complex effects scaling-up in the trophic chain to predators (Harri et al. 2008, Laws and Joern 2013). Plastic responses of plant species to multiple environmental factors or other species have been demonstrated to be prevalent on natural communities (Werner and Peacor 2003, Miner et al. 2005), hence their incorporation on studies of indirect interactions is critical. Plasticity adds a new layer of complexity to the study of indirect interactions as different phenotypes may interact in a different way with other species (Abrams 1995, Utsumi et al. 2010). Tracking the effects of plasticity scaling-up in the trophic chain to herbivores and predators (Fig. 1) can be accomplished by studying different populations across the range of the target species or by manipulating environmental conditions in order to extend the limits of plastic responses. Plastic responses can be either beneficial or costly to the target plant, given that one herbivore or other plant may increase or decrease the interaction with a subsequent herbivore or plant (Valladares et al. 2007), and this should be accounted for in further studies.

Plant local adaptation can also influence indirect effects given that different genotypes may interact differently with other plants or herbivores, which also have important evolutionary consequences by altering the pattern of fitness interactions between genotypes (Biere and Tack 2013). The incorporation of local adaptation to indirect interactions studies is crucial for the development of evolutionary theory as it might be responsible of co-evolutionary processes, additional spatial and temporal variation and ultimately affect the strength and direction of natural selection (Fordyce 2006). Moreover, intra-specific variability, either as a result of plasticity or different genotypes derived from local adaptation, should be incorporated into indirect interactions studies because of its ecological consequences (see Aschehoug and Callaway 2014), and also because of its evolutionary consequences. The latter are built upon the amount of genetic variability and how this is transferred vegetatively to other individuals, or sexually to the next generations.

Studies reporting negative indirect effects were more frequent in mesic environments, while studies reporting positive indirect effects in extreme ecosystems too limited in empirical scope to explore the opposite trend. This nonetheless provides partial support for the stress gradient hypothesis (Bertness and Callaway 1994), which postulates that positive effects should be more common under highly stressful biotic or abiotic conditions, while competitive interactions should be more common in relatively more benign environments. A viable set of hypotheses is that more complex chains of interactions should be supported in more benign and productive environments given that more resources are available to spread through the trophic chain, or that indirect

effects resulting from non-feeding interactions with a lower energetic cost (e.g. herbivore protection, pollination) should be more frequent in less productive or more stressful ecosystems (Fig. 1). Testing these hypotheses requires more information on indirect interactions in regions and ecosystems that have been under-examined to date. The assessment of the conditionality and context-dependence of indirect interactions will require testing indirect interactions along regional and environmental gradients, and thus developing studies to be undertaken at multiple sites.

Research on indirect effects may have been limited by difficulties in testing the mechanisms that may be involved in these interactions (Callaway 2007) and that specific literature on the design of experiments aimed at examining indirect interactions is relatively scarce. Strauss (1991) proposed the following two basic designs to assess indirect interactions: (1) removal or exclusion experiments that manipulate species with supposed strong effects in the community, and (2) construction of artificial communities using density as a variable to determine non-linear responses. The first design, the most commonly used to date (e.g. Callaway and Pennings 2000, Tielbörger and Kadmon 2000, Cuesta et al. 2010), has the limitation of assessing only the effects of the removed or excluded species at their naturally occurring density that can be solved by manipulating that factor (second design) or by replicating the experiment in different years. Importantly, the majority of these approaches used are to date based on a relatively restricted number of target species - usually one. This highlights the need for multiple target-species designs to better examine networks of interactions common in all communities, as even weak effects of species interactions might be important for the

structure of naturally occurring assemblages (Berlow 1999). New protocols that include removals or exclusions embedded in the manipulation of other factors should be designed. Ecologists should move beyond the single-target approach and consider the proposed framework as a model for structuring future experiments.

Because of the importance of indirect interactions as a mechanism of species coexistence and in the assembly of plant communities, the study of indirect interactions can have important implications in the control of invasive species, both in natural and agricultural ecosystems. Studies dealing with invasive species represented 18% of the publications analyzed in this review. In general, exotic species introduced outside their native range mostly experience direct interactions, but also become members of large networks of resident species interacting through indirect pathways at different trophic levels (Mitchell et al. 2006). Indirect effects may play an important role in determining the successful establishment and spread of invasive plants or the resistance of native plant communities to plant invasions (White et al. 2006). The release from natural enemies has long been considered as a mechanism promoting the successful establishment of invasive species and explaining their superior performance in their non-native range (Keane and Crawley 2002; Enemy Release Hypothesis). The release from specialized herbivores could also result in the selection for an increased competitive ability in alien plants (Evolution of Increased Competitive Ability hypothesis; Blossey and Nötzold 1995, Callaway and Ridenour 2004) with positive effects on seed production. However, exotic species can also acquire new enemies that negatively impact seed production and/or seed mortality (Vanhellemont et al. 2014). Indirect

interactions, such as apparent competition, may provide invasive plant species with a competitive advantage over native species (Marler et al. 1999, White et al. 2006, Orrock et al. 2008). For instance, invasive plants can indirectly outcompete natives by increasing the pressure of shared consumers on native plants (Dangremond et al. 2010, Recart et al. 2013). However, they can also contribute to the maintenance of native diversity through the reduction of consumer pressure when unpalatable invasive plants provide refuges from herbivory to native plants (Atwater et al. 2011). Competition for shared pollinators may also affect the outcomes of the introduction of exotic plants. Exotic species have been reported to reduce pollination of native plants by attracting more pollinators (Morales and Traveset 2009, Gibson et al. 2012), however at early stages of invasion, native plant communities are able to tolerate these competitive effects via changes in the plant-pollinator network (Kaiser-Bunbury et al. 2011).

The effects of invasions on higher trophic levels via increases in herbivore populations may also be important, but to date have been rarely studied (but see Lau 2013). Indirect effects have also been examined to improve our understanding of the efficacy of the use of biocontrol agents to control invasive populations, with studies showing that the presence of alternative hosts decreased the effectiveness of biological control, while increasing the richness of a particular guild of natural enemies can reduce the density of a widespread group of herbivorous pests and increase crop yields (Cardinale et al. 2003). Overall, the importance of indirect interactions relative to direct interactions, such as resource competition, in promoting successful invasions is largely unknown (Gioria and Osborne 2014, but see Palladini and Maron 2013). Additional

studies are required to examine the role of indirect interactions in promoting plant invasions and how indirect effects may be manipulated to control plant invasions.

The effects of indirect interactions may also have important implications in rangeland management. Studies dealing with this topic represented 12% of the literature included in this review, and show that the incorporation of indirect effects into management is important to develop best practices. Grazers in general, and particularly livestock, can alter plant community composition through indirect effects when palatable plants associate with unpalatable plants (Callaway et al. 2005, Graff et al. 2013), or can exert strong control on plant communities through direct and indirect effects (Beguin et al. 2011, Versterlund et al. 2012). Future studies should address the mechanistic pathways of herbivore effects on plant communities in order to better inform management practices.

Importantly, the effects of climate change on the net outcome of indirect interactions are still largely under-explored (Brooker 2005, McIntire and Fajardo 2014) and have only been studied once in the literature included in this review (see Auer and Martin 2013). This represents a critical gap as new climate regimes will change the physiology and fitness of plants (Kirschbaum 2004, Brooker 2005), which in turn will change the intensity and importance of indirect effects as they propagate through trophic structures (Woodward et al. 2010). Moreover, the potential effects of other global changes, such as changes in nutrient cycling and fragmentation, on indirect interactions

should be examined to develop better models projecting future community composition and ecosystem functioning.

CONCLUSIONS

Here, we proposed a synthetic framework that allows for a more readily characterization of direct and indirect effects within networks and encourages more effective examinations of causality. This synthetic framework can then be used for the interpretation of the available literature, the design of new studies and the development of ecological theory improving our ability to understand species interactions by better addressing all the players within an ecosystem together rather than in isolation. Overall, this framework is an important contribution to the literature as it identifies dominant pathways of indirect effects, assists in the determination of relevant players and casual relationships in a network of interactions, and highlights the importance of interactions at the plants trophic level as they drive the dynamics of plant communities and ecosystems.

The most frequently studied indirect interactions to date were consumer-mediated indicating that non-feeding interactions such as plant trait-mediated interactions, interactions within networks of competing plants, and trophic cascades need to be incorporated into research in this field. Experimental approaches were also relatively limited because studies commonly used single target species and single study sites. Incorporating multiple study sites along regional and environmental gradients will allow for a better understanding of the context-dependency of indirect interactions, as

well as the potential effects of plasticity and adaptation of plant species on indirect interactions. By complementing this systematic review with a conceptual framework illustrating all possible interaction pathways, a number of gaps were identified and recommendations for future studies on indirect effects were made. Even for the most studied consumer-mediated interactions, additional information on their relationship with environmental gradients is required and can be important to predict the effects of global changes on the direction and intensity of indirect interactions. Future studies should also assess the relative importance of indirect interactions in comparison to that of direct interactions. If environmental conditions have the potential to alter competitive hierarchies or physiological/phenotypic responses between interacting plants (Brooker 2005) or even at higher trophic levels, an improved understanding of their effects and scope in a wide range of biomes represents a critical step forward to predict community responses to global change drivers and develop appropriate strategies to maintain ecosystem services perhaps capitalizing on networks of interacting species.

ACKNOWLEDGEMENTS

This research was funded by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada to CJL and York University Faculty of Graduate Studies salary support to DAS. The comments of two anonymous reviewers and the subject Editor greatly improved the quality of this manuscript.

REFERENCES

Aarssen, L. W. 1992. Causes and consequences of variation in competitive ability in plant communities. *Journal of Vegetation Science* 3:165-174.

- Abrams, P. A. 1995. Implications of dynamically variable traits for identifying, classifying and measuring direct and indirect effects in ecological communities. *American Naturalist* 146:112-134.
- Allesina, S., and J. M. Levine. 2011. A competitive network theory of species diversity. *Proceedings of the National Academy of Sciences USA* 108:5638-5642.
- Aschehoug, E. T., and R. M. Callaway. 2014. Morphological variability in tree root architecture indirectly affects coexistence among competitors in the understory. *Ecology* 95:1731-1736.
- Atwater, D. Z., C. M. Bauer, and R. M. Callaway. 2011. Indirect positive effects ameliorate strong negative effects of *Euphorbia esula* on a native plant. *Plant Ecology* 212:1655-1662.
- Auer, S. A. K., and T. E. Martin. 2013. Climate change has indirect effects on resource use and overlap among coexisting bird species with negative consequences for their reproductive success. *Global Change Biology* 19:411-419.
- Barbosa, P., J. Hines, I. Kaplan, H. Martinson, A. Szczepaniec, and Z. Szendrei. 2009. Associational resistance and associational susceptibility: having right or wrong neighbors. *Annual Review in Ecology, Evolution and Systematics* 40:1-20.
- Beard, K. H., C. A. Faulhaber, F. P. Howe, and T. C. Edwards Jr. 2013. Rodent-mediated interactions among seed species of differing quality in a shrub-steppe ecosystem. *Western North American Naturalist* 73:426-441.
- Beguín, J., D. Pothier, and S. D. Cote. 2011. Deer browsing and soil disturbance induce cascading effects on plant communities: a multilevel path analysis. *Ecological Applications* 21:439-451.
- Berlow, E. L. 1999. Strong effects of weak interactions in ecological communities. *Nature* 398:330-334.
- Bertness, M. D., and R. M. Callaway. 1994. Positive interactions in communities. *Trends in Ecology and Evolution* 9:191-193.
- Biere, A., and A. J. M. Tack. 2013. Evolutionary adaptation in three-way interactions between plant, microbes and arthropods. *Functional Ecology* 27:646-660.
- Blossey, B., and R. Nötzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *Journal of Ecology* 83:887-889.
- Brooker, R. W. 2005. Plant-plant interactions and environmental change. *New Phytologist* 171:271-284.
- Brooker, R. W. et al. 2008. Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology* 96:18-34.
- Burger, J. C., and S. M. Louda. 1994. Indirect versus direct effects of grasses on growth of a cactus (*Opuntia fragilis*) - insect herbivory versus competition. *Oecologia* 99:79-87.
- Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* 18:119-125.
- Callaway, R. M. 2007. *Positive interactions and interdependence in plant communities*. Springer, Dordrecht, The Netherlands.
- Callaway, R. M., D. Kikodze, M. Chiboshvili, and L. Khetsuriani. 2005. Unpalatable plants protect neighbors from grazing and increase plant community diversity. *Ecology* 86:1856-1862.

- Callaway, R. M., and S. C. Pennings. 2000. Facilitation may buffer competitive effects: indirect and diffuse interactions among salt marsh plants. *American Naturalist* 156:416-424.
- Callaway, R. M., and W. M. Ridenour. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* 2:436-443.
- Cardinale, B. J., C. T. Harvey, K. Gross, and A. R. Ives. 2003. Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. *Ecology Letters* 6:857-865.
- Chaneton, E. J., C. N. Mazía, and T. Kitzberger. 2010. Facilitation vs. apparent competition: insect herbivory alters tree seedling recruitment under nurse shrubs in a steppe-woodland ecotone. *Journal of Ecology* 98:488-497.
- Cuesta B., P. Villar-Salvador, J. Puertolas, J. M. R. Benayas, and R. Michalet. 2010. Facilitation of *Quercus ilex* in Mediterranean shrubland is explained by both direct and indirect interactions mediated by herbs. *Journal of Ecology* 98:687-696.
- Dangremond, E. M., E. A. Pardini, and T. M. Knight. 2010. Apparent competition with an invasive plant hastens the extinction of an endangered lupine. *Ecology* 91:2261-2271.
- Duffy, J. E., B. J. Cardinale, K. E. France, P. B. McIntyre, E. Thébault, and M. Loreau. 2007. The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecology Letters* 10:522-538.
- Facelli E., S. E. Smith, J. M. Facelli, H. M. Christophersen, and F. A. Smith. 2010. Underground friends or enemies: model plants help to unravel direct and indirect effects of arbuscular mycorrhizal fungi on plant competition. *New Phytologist* 185:1050-1061.
- Fanelli, D. 2012. Negative results are disappearing from most disciplines and countries. *Scientometrics* 90:891-904.
- Fordyce, J. A. 2006. The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *Journal of Experimental Biology* 209:2377-2383.
- Gibson, M. R., D. M. Richardson, and A. Pauw. 2012. Can floral traits predict an invasive plant's impact on native plant-pollinator communities? *Journal of Ecology* 100:1216-1223.
- Gioria, M., and B. A. Osborne. 2014. Resource competition in plant invasions: emerging patterns and research needs. *Frontiers in Plant Science* 5:501. doi: 10.3389/fpls.2014.00501.
- Graff, P., F. Rositano, and M. R. Aguiar. 2013. Changes in sex ratios of a dioecious grass with grazing intensity: the interplay between gender traits, neighbour interactions and spatial patterns. *Journal of Ecology* 101:1146-1157.
- Harri, S. A., J. Krauss, and C. B. Muller. 2008. Trophic cascades initiated by fungal plant endosymbionts impair reproductive performance of parasitoids in the second generation. *Oecologia* 157:399-407.
- Hillebrand, H. 2004. On the generality of the latitudinal diversity gradient. *American Naturalist* 163:192-211.
- Johnson, S. D., C. I. Peter, L. A. Nilsson, and J. Ågren. 2003. Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. *Ecology* 84:2919-2927.

- Johnson, S. N., A. E. Douglas, S. Woodward, and S. E. Hartley. 2003. Microbial impacts on plant-herbivore interactions: the indirect effects of a birch pathogen on a birch aphid. *Oecologia* 134:388-396.
- Kaiser-Bunbury, C. N., T. Valentin, J. Mougai, D. Matatiken, and J. Ghazoul. 2011. The tolerance of island plant-pollinator networks to alien plants. *Journal of Ecology* 99: 202-213.
- Kaplan, I., S. Sardanelli, B. J. Rehill, and R. F. Denno. 2011. Toward a mechanistic understanding of competition in vascular-feeding herbivores: an empirical test of the sink competition hypothesis. *Oecologia* 166:627-636.
- Keane, R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution* 17:164-170.
- Kéfi, S., et al. 2012. More than a meal... integrating non-feeding interactions into food webs. *Ecology Letters* 15:291-300.
- Kirschbaum, M. U. F. 2004. Direct and indirect climate change effects on photosynthesis and transpiration. *Plant Biology* 6:242-253.
- Lau, J. A. 2013. Trophic consequences of a biological invasion: do plant invasions increase predator abundance? *Oikos* 122:474-480.
- Laws, A. N., and A. Joern. 2013. Predator-prey interactions in a grassland food chain vary with temperature and food quality. *Oikos* 122:977-986.
- Levine, J. M. 1999. Indirect facilitation: evidence and predictions from a riparian community. *Ecology* 80:1762-1769.
- Levine, S. H. 1976. Competitive interactions in ecosystems. *American Naturalist* 110:903-910.
- Lortie, C. J., R. W. Brooker, P. Choler, Z. Kikvidze, R. Michalet, F. I. Pugnaire, and R. M. Callaway. 2004. Rethinking plant community theory. *Oikos* 107: 63-70.
- Marler, M. J., C. A. Zabinski, and R. M. Callaway. 1999. Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. *Ecology* 80:1180-1186.
- Martin, L. J., B. Blossey, and E. Ellis. 2012. Mapping where ecologists work: biases in the global distribution of terrestrial ecological observations. *Frontiers in Ecology and the Environment* 10:195-201.
- McIntire, E. J. B., and A. Fajardo. 2014. Facilitation as a ubiquitous driver of biodiversity. *New Phytologist* 201:403-416.
- Millennium Ecosystem Assessment. 2005. *Ecosystems and well-being: synthesis*. Island Press, Washington DC, USA.
- Miller, T. E. 1994. Direct and indirect species interactions in an early old-field plant community. *American Naturalist* 143:1007-1025.
- Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Parrilla, and R. A. Relyea. 2005. Ecological consequences of phenotypic plasticity. *Trends in Ecology and Evolution* 20:685-692.
- Mitchell, C. E. et al. 2006. Biotic interactions and plant invasions. *Ecology Letters* 9:726-740.
- Moeller, D. A. 2004. Facilitative interactions among plants via shared pollinators. *Ecology* 85:3289-3301.

- Morales, C. L., and A. Traveset. 2009. A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. *Ecology Letters* 12:716-728.
- Mulder, C. P. H., and R. W. Ruess. 1998. Effects of herbivory on arrowgrass: Interactions between geese, neighboring plants, and abiotic factors. *Ecological Monographs* 68:275-293.
- Ohgushi, T., O. Schmitz, and R. D. Holt. 2013. Trait-mediated indirect interactions: ecological and evolutionary perspectives. Cambridge University Press, New York.
- Orrock, J. L., M. S. Witter, and O. J. Reichman. 2008. Apparent competition with an exotic plant reduces native plant establishment. *Ecology* 89:1168-1174.
- Pace, M. L., J. J. Cole, S. R. Carpenter, and J. F. Kitchell. 1999. Trophic cascades revealed in diverse ecosystems. *Trends in Ecology and Evolution* 14:483-488.
- Palladini, J. D., and J. L. Maron. 2013. Indirect competition for pollinators is weak compared to direct resource competition: pollination and performance in the face of an invader. *Oecologia* 172:1061-1069.
- Polis, G. A., A. L. W. Sears, G. R. Huxel, D. R. Strong, and J. Maron. 2000. When is a trophic cascade a trophic cascade? *Trends in Ecology and Evolution* 15:473-475.
- Recart, W., J. D. Ackerman, and A. A. Cuevas. 2013. There goes the neighborhood: apparent competition between invasive and native orchids mediated by a specialist florivorous weevil. *Biological Invasions* 15:283-293.
- Samson, D. A., T. E. Philippi, and D. W. Davidson. 1992. Granivory and competition as determinants of annual plant diversity in the Chihuahuan Desert. *Oikos* 65:61-80.
- Schenk, H. J. 2006. Root competition: beyond resource depletion. *Journal of Ecology* 94:725-739.
- Schmitz, O. J., P. A. Hambäck, and A. P. Beckerman. 2000. Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. *American Naturalist*: 155:141-153.
- Schöb, C., C. Armas, and F. I. Pugnaire. 2013. Direct and indirect interactions co-determine species composition in nurse plant systems. *Oikos* 112:1371-1379.
- Strauss, S. Y. 1991. Indirect effects in community ecology: their definition, study and importance. *Trends in Ecology and Evolution* 6:206-210.
- Silvertown, J. 2004. Plant coexistence and the niche. *Trends in Ecology and Evolution* 19:605-611.
- Tielbörger, K., and R. Kadmon. 2000. Indirect effects in a desert plant community: is competition among annuals more intense under shrub canopies? *Plant Ecology* 150:53-63.
- Utsumi, S., Y. Ando, and T. Miki. 2010. Linkages among trait-mediated indirect effects: a new framework for the indirect interaction web. *Population Ecology* 52:485-497.
- Vanhellemont, M., L. Baeten, A. Smets, J. Mertens, and K. Verheyen. 2014. Spatio-temporal variation in seed predation by a native weevil in the invasive *Prunus serotina*. *Flora* 209: 541-546.
- Valladares, F., E. Gianoli, and J. M. Gómez. 2007. Ecological limits to plant phenotypic plasticity. *New Phytologist* 176:749-763.
- Vandenberghe, C., C. Smit, M. Pohl, M. Buttler, and F. Frelechoux. 2009. Does the strength of facilitation by nurse shrubs depend on grazing resistance of tree saplings? *Basic and Applied Ecology* 10:427-436.

- Vesterlund, S-R., O. Suominen, R. Bergström, K. Danell, and I-L. Persson. 2012. The impact of simulated moose densities on conifer aphids along a productivity gradient. *Ecography* 35:105-112.
- Ward, D. 2009. *The biology of deserts*. Oxford University Press, New York, USA.
- Werner, E. E., and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84:1083-1100.
- White, E. M., J. C. Wilson, and A. R. Clarke. 2006. Biotic indirect effects: a neglected concept in invasion biology. *Diversity and Distributions* 12:443-455.
- Woodward, G. et al. 2010. Ecological networks in a changing climate. *Advances in Ecological Research: Ecological Networks* 42:71-138.
- Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. *Annual Review in Ecology and Systematics* 25:443-466.
- Wootton, J. T. 2002. Indirect effects in complex ecosystems: recent progress and future challenges. *Journal of Sea Research* 48:157-172.

Chapter 2

Dominant plant effects across large spatial and temporal gradients

Manuscript entitled:

The collapse of the stress-gradient hypothesis under increasing spatiotemporal stress in the Atacama Desert

Sotomayor, D. A. & Lortie C. J.

ABSTRACT

Positive plant interactions are most frequent under stressful environmental conditions. However, it has been recently proposed that positive interactions can collapse at extremely high levels of stress within these environments. This hypothesis is controversial. We examined the geographical and temporal drivers of positive plant interactions as environmental stress increased spatiotemporally within the Atacama Desert. Three independent study regions were surveyed for three years to test the following specific predictions: 1) the frequency of facilitation will increase with environmental severity (spatial) but collapses at the most stressful conditions, and 2) the frequency of facilitation will increase in more severe growing seasons (temporal) within a region but will also collapse in relatively extreme years. The intensity of positive interactions was also examined in each instance. Within each desert region, we surveyed a total of five sites along regional elevation gradients during three consecutive years of increased drought. Plant density and species richness were surveyed under dominant plants and adjacent, open microsites. There were no significant differences in interaction intensity or frequency within the elevational gradients sampled in each region. Positive interactions however decreased in intensity and frequency between regions at the most arid regions suggesting spatial collapse of plant facilitation at larger scales within the Atacama Desert. Increased temporal stress through drought increased the intensity and frequency of facilitation only in the 'least' environmentally stressed region sampled. Increased temporal stress did lead to collapse in the two more extreme regions supporting the prediction that combined spatiotemporal stressors are important in desert plant communities. These findings show that spatiotemporal variation in stress

is a component of positive plant interactions and thus likely fundamental to community assembly and resilience.

Keywords: coexistence, collapse, facilitation, functional group, net interactions, North-Central Chile, nurse plant, positive interactions, species specificity, stress gradient hypothesis, Southern Peru, temporal scale, competition.

INTRODUCTION

Plant communities are shaped by a variety of drivers. Biotic interactions can act as filters that determine whether species persist within a given environment in concert with abiotic limitations (Brooker et al. 2008, Lortie et al. 2004, McIntire & Fajardo 2014). The effects of positive interactions (facilitation) have been well documented and include recruitment, growth, and spatial associations of beneficiary species (for comprehensive reviews see: Bruno et al. 2003, Flores & Jurado 2003, Callaway 2007, Brooker et al. 2008, Filazzola & Lortie 2014). Moreover, positive interactions have the potential to drastically change ecosystems (Xu et al. 2015) or promote divergent evolutionary dynamics (Kefi et al. 2008). Nonetheless, the intensity if not importance of facilitation can in turn be expressed through traits, distributions, and population dynamics of the intervening species (Maestre et al. 2003, Soliveres et al. 2010, Tielbörger & Kadmon 2000, He et al. 2013). The Stress Gradient Hypothesis (SGH) proposes a monotonic increase of the frequency of positive interactions with increasing stress (Bertness & Callaway 1994). Several syntheses have examined the spatial component of this hypothesis on gradients from regional to global scales (Maestre et al. 2005, Lortie &

Callaway 2005, He et al. 2013, Soliveres & Maestre 2014). Temporal variation in environmental stress is less studied (Tielborger & Kadmon 2000, Miriti 2007, Soliveres et al. 2010, Biswas & Wagner 2014). Even fewer instances concurrently examined the spatial and temporal dynamics of facilitation (Soliveres & Maestre 2015). The complexity of positive interactions does not end there. The traits of benefactor species can also mediate the outcome via indirect effects such as herbivore protection or interactions with pollinators (Barbosa et al. 2009, Sotomayor & Lortie 2015). Examining each component separately is a valid building block for the concept of facilitation in plant communities, but an integrated experiment, even mensurative, on stress gradients through time and space is an important empirical alternative to regional synthesis tests of this hypothesis.

Plant facilitation has been frequently studied within stressful ecosystems. In arid environments, dominant plants such as shrubs or trees frequently nurse a high density of understory plant species (Franco & Nobel 1989, Flores & Jurado 2003, Filazzola & Lortie 2014). The more benign conditions within the canopy of these plants (Franco & Nobel 1989, Callaway 2007, Pugnaire et al. 2011) increases the local species pool due to microclimatic amelioration in key environmental factors (Pugnaire et al. 1996, Tielbörger & Kadmon 2000b, Flores & Jurado 2003, Filazzola & Lortie 2014). However, it has been recently proposed that there is a limit to the capacity of dominant plants to facilitate within extremely harsh environments. The facilitation collapse hypothesis as it has been termed has been debated (Maestre et al. 2005, Lortie & Callaway 2005, Michalet et al. 2013, Lopez et al. 2013, Pugnaire et al. 2015). The first formulation of the

SGH predicted a monotonic increase in the intensity of positive interactions with increased environmental stress, but Michalet et al. (2006) detected that positive effects decreased towards the most stressful end of an environmental gradient. Resource limitation at extremes are proposed to become more important than positive interactions and leads to collapse (Michalet et al. 2014a). Collapse has been supported empirically for arid environments (Maestre et al. 2005, 2006, Miriti 2006). However, several syntheses of the SGH did not detect a collapse on very large-scale gradients (Lortie & Callaway 2006, Lopez et al. 2013, He et al. 2013). There are numerous explanations, and the purpose of this study is not to engage in this debate but to use these ideas to examine this new, extended hypothesis of the SGH for a set of desert environments and address some of the broad research gaps in plant facilitation research. Importantly, most tests of both hypotheses have been conducted with spatial stress gradients (but see Miriti 2007, Biswas & Wagner 2014) and often with intensity and not frequency as proposed by the hypotheses. Hence, shifts in the intensity and frequency of positive interactions through time and on more extreme gradients within ecosystems are novel and critical advancements for theory and potential community resilience.

Extreme stress can lead to dominant plants not being able to provide positive effects for understory communities. A decrease in the intensity of positive effects provided by dominant plants can, in turn, reduce the frequency of positive effects and impact overall species richness and community structure. We used a regionally replicated, multi-year observational study within the Atacama Desert to test for a collapse of plant facilitation at extremes with relatively extreme levels of stress in both

space and time. We examined the following predictions: 1) the frequency and intensity of facilitation will increase as environmental severity increases at both the regional and whole desert level (spatial prediction), but it will collapse at extremely stressful conditions; and (2) the frequency and intensity of facilitation will increase when year-to-year environmental conditions increase environmental severity (temporal prediction), but extreme temporal stress (drought) will lead to a collapse of positive interactions. This ecosystem is one of the most arid deserts in the world (Ward 2009) and thus a perfect candidate to explore the context dependency of facilitation and possible collapse.

METHODS

Desert localities and study sites

The Atacama Desert includes the tropics and subtropics along the Pacific coast of South America. Within this desert, we studied 3 independent regions - Atiquipa in Southern Peru and Romeral and Fray Jorge in North-Central Chile (Table 1). All three regions are coastal deserts with a wet winter season between July and November typically characterized by high moisture due to fog (about 90% on average). Approximately 70% of the mean annual rainfall occurs between these winter months (Novoa & Lopez 2001, Sotomayor & Jimenez 2008). The long-term annual rainfall mean is 185 mm at Atiquipa (Sotomayor & Jimenez 2008), 124 mm/year at Romeral (Almeyda 1950), and 130 mm at Fray Jorge (Madrigal et al. 2011) (Table 1). All years sampled had annual rainfall below the mean with drought increasing by year sampled (Table 1). Aridity conditions are lower at Atiquipa due to the amelioration provided by the high

humidity present (fog) especially during the growing season with more than 40 l/m²/day of fog captured in a fog-meter in that time period (Sotomayor & Jimenez 2008). Fog is a major determinant of plant community structure in similar environments (Stanton et al. 2014). Both Romeral and Fray Jorge occur at lower elevations where fog is less important (Rundel et al. 1991). The plant community at each desert locality includes a few large woody species (i.e. mainly shrubs and trees) and a diversity of herbaceous plants wherein annuals are the most common life form (Marticorena et al. 2001, Sotomayor & Jimenez 2008).

Within each desert region, we selected five sites (100 x 100 m) separated by at least 2 km but no more than 5 km. These sites corresponded to an elevation gradient within each region (Table 1). This selection was supported with a previous field survey along the extension of each region (Appendix 4). That survey systematically sampled 1-km grids using one 20 x 20 m quadrat per grid to determine plant cover and species composition within each of these grids (Sotomayor & Lortie 2016). These data were then combined with climate data from WorldClim (Hijmans et al. 2005) and used to quantitatively determine the study plots. By combining ground-truthed data with climate parameters (e.g. temperature, precipitation, and seasonality) via multivariate statistics (i.e., direct ordination analyses), we were able to estimate environmental gradients within our field site (Lortie 2010).

Dominant plants and vegetation surveys

We chose two dominant perennial woody species at each site (Table 2). At each site, we surveyed one dominant plant with spines and one without. In Atiquipa, we sampled the understories of *Caesalpinia spinosa* Molina (Kuntze) and *Randia armata* (Sw.) DC.; at Romeral, we surveyed *Haplopappus parvifolius* (DC.) Gay, *Flourensia thurifera* (Molina) DC., and *Senna cumingii* (Hook. Et Arn.) H. S. Irwin & Barneby (Fabaceae); and at Fray Jorge, we surveyed *Porlieria chilensis* I. M. Johnst., *Gutierrezia resinosa* (Hook. Et Arn.) S. F. Blake, *Pleocarphus revolutus* D. Don, *F. thurifera* and *S. cumingii*.

During three growing seasons (2011, 2012 and 2013), we surveyed triplets comprising of one plot located in the understory of each of two dominant species and a plot in open nearby spaces. Plots were 0.25 m² in size. At each site in Atiquipa, we surveyed 10 triplets, and in the Chilean sites, we surveyed 8 triplets per site. Microsites within each triplet were separated from each other by ca. 2m, and each set of replicate samples was separated from each other by at least 5m. The abundance of each of the species within the plots was recorded and used to calculate total plant species abundance and richness. The strength of the effect of dominant plants on species richness and plant density was estimated using the Relative Interaction Index (RII) calculated as follows (Armas et al. 2004):

$$\text{RII} = \frac{D_u - D_o}{D_u + D_o} \quad (1)$$

The terms D_u and D_o corresponded to the density of plants in understory and open microsites, respectively. This index varies from -1 to +1 with positive effects being > 0 and negative effects < 0 on the density of these species.

Table 1. Study sites within each desert region along with their climatic characteristics.

De Martonne AI was calculated using mean annual temperature and annual precipitation extracted from WorldClim (Hijmans et al. 2005) for each site. Rainfall data are means and annual totals within brackets. * mean data from Sotomayor & Jimenez 2008 for Atiquipa, and yearly data from a local weather station; mean data for Romeral from Almeyda 1950, for Fray Jorge from Madrigal et al. 2011, and annual data from www.ceazamet.cl.

Region	Rainfall (mm/year)*	Site	Geographical location (LS, LW)		Elevation (m)	DeMartonne AI
Atiquipa	185 (167.3, 137.4)	1	15.763	74.369	741	0.177
		2	15.785	74.392	921	0.141
		3	15.774	74.385	1042	0.179
		4	15.732	74.372	1092	0.403
		5	15.748	74.388	1124	0.288
Romeral	124 (114.9, 70.7, 39.8)	1	29.808	71.274	32	3.108
		2	29.783	71.262	109	3.173
		3	29.763	71.254	170	3.185
		4	29.781	71.245	150	3.145
		5	29.769	71.216	335	3.319
Fray Jorge	130 (160, 62.1, 75.6)	1	30.705	71.636	61	4.808
		2	30.694	71.633	127	5.000
		3	30.678	71.646	145	4.903
		4	30.658	71.665	229	5.118
		5	30.647	71.661	258	5.238

Table 2. Dominant plant species surveyed along the Atacama Desert in Southern Peru and North-Central Chile. Traits presented include plant height (m), life form, presence of thorns (thorniness), and the site where each species was sampled. * denotes endemic species (Marticorena et al. 2001).

Species	Family	Height (m)	Life form	Thorniness	Site
<i>Caesalpinia spinosa</i>	Fabaceae	4-5	tree	No	Atiquipa 1-5
<i>Randia armata</i>	Rubiaceae	2-3	shrub	Yes	Atiquipa 1-5
<i>Happlopappus parvifolius</i> *	Asteraceae	3-4	shrub	Yes	Romeral 1-5
<i>Flourensia thurifera</i> *	Asteraceae	2-3	shrub	No	Romeral 2,4; Fray Jorge 4
<i>Senna cumingii</i> *	Fabaceae	3-4	shrub	No	Romeral 1,3,5;;Fray Jorge 5
<i>Porlieria chilensis</i> *	Zygophyllaceae	4-5	shrub	Yes	Fray Jorge 1-4
<i>Gutierrezia resinosa</i> *	Asteraceae	1-2	shrub	No	Fray Jorge 2,3,5
<i>Pleocarphus revolutus</i> *	Asteraceae	1-2	shrub	No	Fray Jorge 1

Statistical analyses

Plant density and species richness estimates were analyzed separately per desert locality using Generalized Linear Mixed Models (GLMMs) with microsite (3 levels, dominant plant species collapsed by their presence or absence of thorns, and the open microsite) and gradient (5 levels) as fixed factors in a fully factorial design, and year as a repeated measures variable (random factor, 3 years) (Bates et al. 2015) (Appendix 5 and 6). As RII_{species} represent effect size measurements, a single GLMM was used per response variable (i.e. RII_{species} richness and RII_{plant density}) that included desert locality (3 levels), dominant plant (2 levels) and gradient (5 levels) as fixed factors in a fully factorial design. In these models, year was treated as a repeated-measures effect (random variable). Pair-wise post hoc comparisons using Chi-

square tests were done when model effects were $p < 0.05$. All RIIs ($RII_{\text{species richness}}$, $RII_{\text{plant density}}$) were analyzed using one sample t-tests to test that there were significantly different from zero and ascertain frequencies of positive, negative or neutral interactions (Michalet et al. 2014b). As a control, we also analyzed the effects of canopy size on their respective interaction intensity (RII) using regression models for each response variable. Canopy size was calculated using the formula of the volume of a semi-sphere, $1/3\pi r^3$, where r was the largest radius of the dominant plant. Contingency tables and Chi-square tests by regional gradients, between regions, and by year within region were used to test differences in the frequency of canopy effects (Zar 1999). All statistics were done in R v3.2.3 (R Core Team 2015), using the package lme4 (Bates et al. 2015).

RESULTS

The intensity of the effects measured with the Relative Interaction Indices (RIIs) of the dominant plants surveyed was significantly different amongst desert regions but not within each regional gradient for either community structure measure (Table 3). Between regions, the positive effects on richness were greatest at Atiquipa (Atiquipa-Romeral contrast, Chi-square = 30.69, $p < 0.001$; Atiquipa-Fray Jorge contrast, Chi-square = 27.27, $p < 0.001$), whilst Fray Jorge and Romeral did not differ from one another (Chi-square = 0.62, $p = 0.43$) (Figure 1). The positive effects on density were also greatest at Atiquipa (Atiquipa-Romeral contrast Chi-square = 4.49, $p = 0.03$; Atiquipa-Fray Jorge contrast Chi-square = 8.57, $p = 0.003$). In this instance, the positive effects on density by dominant plants at Romeral were also higher than those in Fray Jorge (Chi-square = 7.04, $p = 0.008$) (Figure 2). The frequency of positive effects did

not significantly differ on gradients within regions for either community structure measure (species richness Atiquipa Chi-square=12.86, $p=0.12$, Romeral Chi-square=12.59, $p=0.13$, Fray Jorge Chi-square=7.08, $p=0.53$, Figure 1; plant density, Atiquipa Chi-square=4.55, $p=0.80$, Romeral Chi-square=14.93, $p=0.06$, Fray Jorge Chi-square=6.24, $p=0.62$, Figure 2). Between regions, the frequency of positive effects was greatest at Atiquipa, predominantly neutral at Romeral, and negative at Fray Jorge for both measures (species richness Chi-square=34.67, $p<0.0001$, Figure 1; plant density Chi-square=58.98, $p<0.0001$, Figure 2).

Table 3. Summary of GLMMs contrasting RII of species richness and plant density among desert localities, dominant plants and gradients in three different years along the Atacama Desert. *P*-values <0.05 are bolded and indicate significant differences.

Source	DF	RII species richness		RII plant density	
		Chi-Square	P-value	Chi-Square	P-value
Dominant plant	1	2.57	0.1088	0.46	0.4966
Desert	2	67.26	<.0001	17.92	0.0001
Gradient	4	8.24	0.0832	1.51	0.8249
Dominant*Desert	2	1.75	0.4163	0.82	0.6635
Desert*Gradient	8	16.13	0.0405	4.07	0.3971
Dominant*Desert*Gradient	8	4.89	0.7691	4.52	0.8072
Year	2	0.28	0.8686	4.17	0.1243
Desert*Year	4	14.32	0.0063	24.96	<.0001
Dominant*Year	2	1.79	0.4075	0.05	0.9738

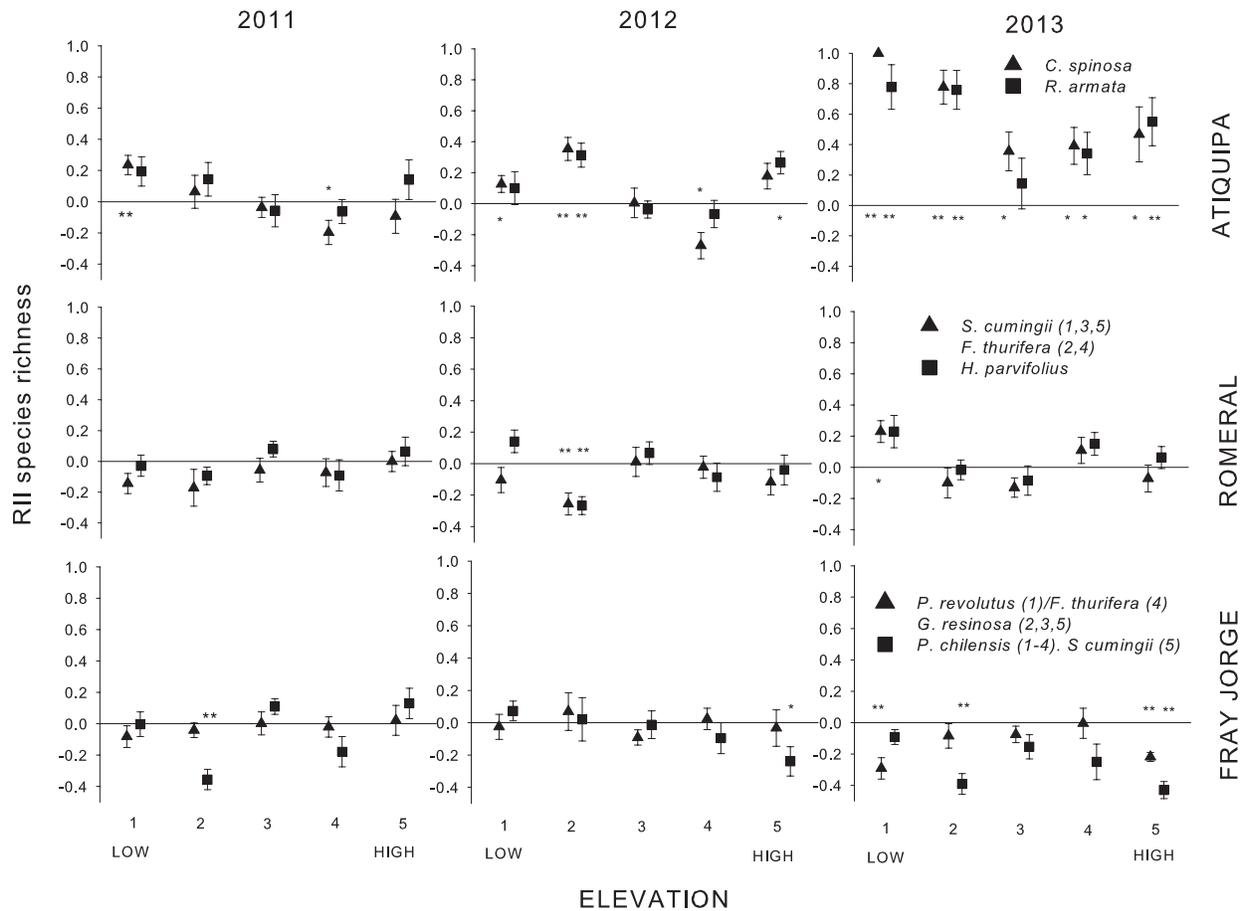


Fig. 1 Relative interaction indices (RIIs) with ± 1 SE confidence intervals for species richness of two dominant plant microsities in relation to open microsities at the peak of the growing seasons of 2011, 2012 and 2013 (vertical panels) at three desert locations (horizontal panels) along the Atacama Desert. Stars indicate significantly different from zero RII values obtained from one sample t-tests (* $P < 0.05$, ** $P < 0.01$).

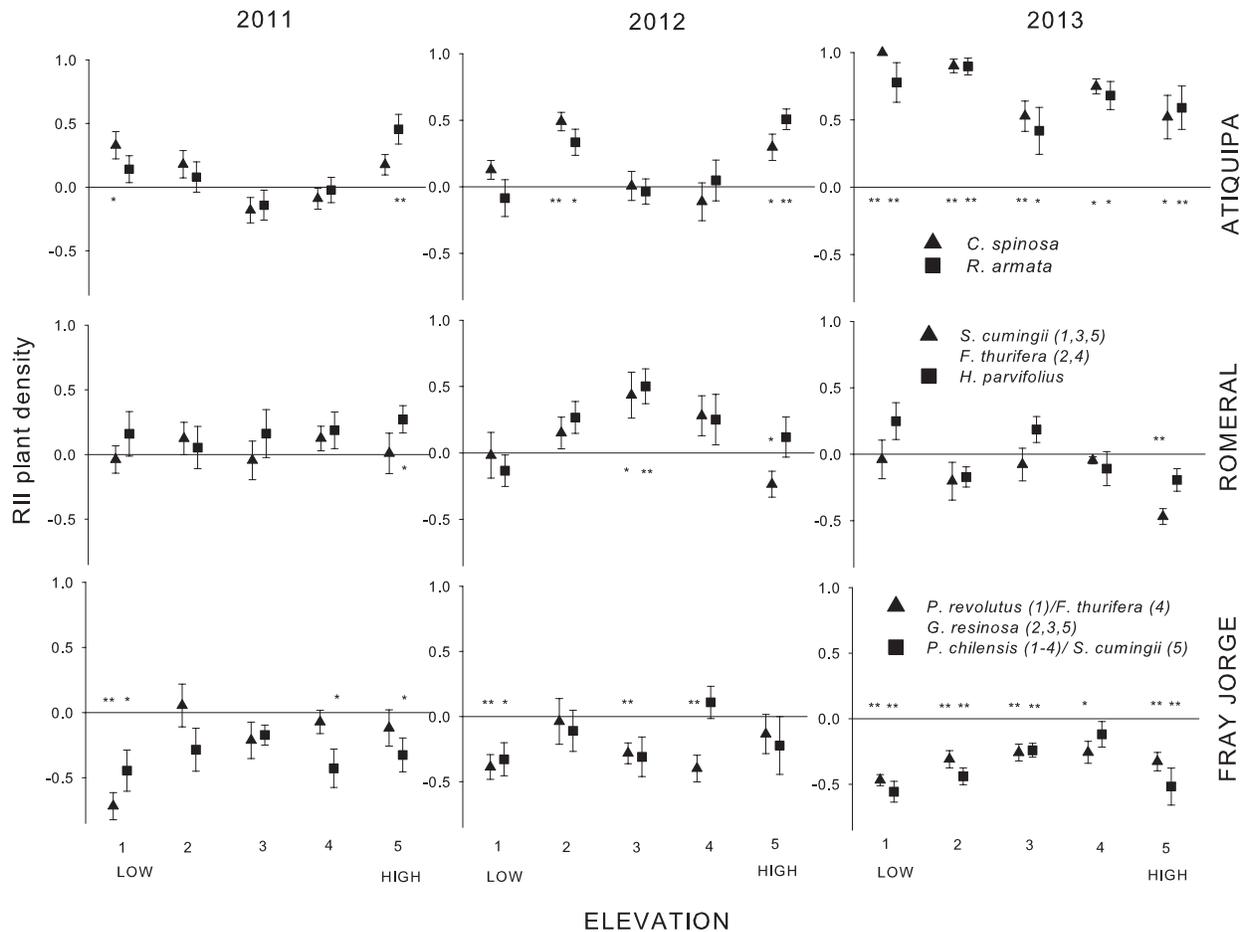


Fig. 2 Relative interaction indices (RIIs) with ± 1 SE confidence intervals for plant density of two dominant plant microsites in relation to open microsites at the peak of the growing seasons of 2011, 2012 and 2013 (vertical panels) at three desert locations (horizontal panels) along the Atacama Desert. Stars indicate significantly different from zero RII values obtained from one sample t-tests (* $P < 0.05$, ** $P < 0.01$).

The intensity of positive effects both increased then collapsed on each of the three regional stress gradients as drought levels increased with time (Table 3). At Atiquipa, the intensity of facilitation increased with temporal increased stress with RIIs of 2013 significantly higher than those on the previous two years for both community

structure measures (species richness 2013-2011 contrast Chi-square=39.08, $p < 0.001$, 2012-2011 contrast Chi-square = 24.81, $p < 0.001$, Figure 1; plant density 2013-2011 contrast Chi-square=19.17, $p < 0.001$, 2012-2011 contrast Chi-square = 16.64, $p < 0.001$, Figure 2). At Fray Jorge, the intensity of effect size measures significantly decreased from 2011 to 2013 only for species richness RIs (2013-2011 contrast Chi-square = 4.19, $p = 0.04$; 2013-2012 Chi-square = 4.42, $p = 0.03$; Figure 1). At Romeral, the intensity of positive effects for plant density collapsed with increasing temporal stress from 2012 to 2013 (Chi-square = 4.07, $p = 0.04$). The frequency of positive effects increased only at Atiquipa for both measures (species richness Chi-square=13.29, $p < 0.01$, Figure 1; plant density Chi-square=13.93, $p < 0.01$, Figure 2). The frequency of canopy effects did not change between years remaining neutral at Romeral (species richness Chi-square=6.22, $p = 0.18$, Figure 1; plant density Chi-square=3.32, $p < 0.51$, Figure 2). At Fray Jorge, the frequency of canopy effects for species richness remained neutral across years (Chi-square=3.75, $p = 0.44$; Figure 1), and remained frequently negative for plant density across years (Chi-square=6.79, $p = 0.14$; Figure 2). Finally, there were no differences in facilitation intensity between dominant plant species (Table 3), nor their size (as volume) was significant for the intensity of canopy effects for either community structure measure (species richness $t = -1.52$, $p = 0.12$; plant density $t = -0.99$, $p = 0.32$).

DISCUSSION

The conditionality of positive (and negative) effects at the extremes of environmental gradients is an important topic for community ecology because it

challenges our precision in modeling and testing for net interactions. This is a novel development to the SGH, and the concept of collapse is fundamental for a broader understanding of global change scenarios that include changing biotic interactions. In the Atacama Desert, we found that under the most extreme stress in space and time the positive effects on species richness and plant density provided by woody dominant plants varied from increasingly positive to negative depending on the specific region. Within each region on elevational gradients, we did not find evidence of spatial stress differences mediating interactions, but at the whole desert level, we found that contrasts from the least to most arid regions varied from positive to a collapse in facilitation. The effects of temporal stress also depended on the region but supported the SGH and collapses hypotheses. The collapse of plant facilitation with increasing stress along large-scale regional spatial gradients and temporally through drought is thus a very reasonable hypothesis for plant community ecological theory. These findings suggest that both mechanisms associated with collapse of positive interactions and the implications for community resilience should be resolved to better direct future change scenario predictions.

Spatial and temporal dynamics drive of the outcome of biotic interactions. Spatial stress gradients can determine interactions at multiple scales (Lopez et al. 2009, Tewksbury & Lloyd 2001, Pescador et al. 2014). Similarly, temporal stress gradients impact the outcome of interactions (Biswas & Wagner 2014, Sthultz et al. 2007, Schiffers & Tielbörger 2006). However, these drivers are typically studied separately. Here, we concurrently studied those gradients and found that the frequency and

intensity of positive effects provided by dominant plants collapsed along large-scale spatial stress gradients, showed no response within regional spatial gradients, and temporal responses to stress depended on the region existing environmental stress level. Hence, temporal community dynamics is anchored on spatial environmental variation, as has been shown by Sthultz et al. 2007 and leRoux et al. 2013. This, however, requires further examination using manipulative experiments because the latter have been shown to provide contrasting outcomes on the importance of spatiotemporal dynamics for community structure (Metz & Tielbörger 2016). The storage-effect hypothesis for species coexistence that proposes coexistence in dynamic environments through temporal differences in competitive abilities and through persistence in unfavorable periods is an important connection to the collapse of facilitation hypothesis (Chesson 2000). Here, we found such temporal differences in the frequency and intensity of canopy effects, which would be in turn mediated by the seed banks of the annuals studied. Overall, these findings underscore the importance of understanding the spatial and temporal dynamics of interactions as these allow for coexistence and can be used to develop better predictive models of community dynamics.

The outcome of biotic interactions by context is a foundational topic in ecology. In particular within population and community ecology, a change in the outcome of first competition and now facilitation on gradients is critical theory development. The divergent outcomes by region detected in this arid ecosystem supports two opposing views of the effects of extreme stress levels: (1) a collapse or change to competitive

interactions as supported by Maestre et al. (2005, 2006) and Miriti (2006); or (2) a monotonic increase in the frequency and intensity of positive effects with increasing stress (aridity) (Lortie & Callaway 2006, Lopez et al. 2013, He et al. 2013). Although these outcomes have been interpreted as divergent, it is also likely that due to limitation as a consequence of stress (White 2001), the collapse of facilitative interactions could be a more widespread outcome under extremely stressful environmental conditions. Temporal changes in the intensity and sign of interactions have also been reported showing that there were competitive/neutral interactions during favorable conditions and positive interactions during stressful conditions (Biswas & Wagner 2014, Sthultz et al. 2007, Tielborger & Kadmon 2000). Both the primary SGH as proposed by Bertness and Callaway (1994) and the collapse of positive hypothesis (Michalet et al. 2006) were supported in this study. The mechanisms that we did not study herein such as water limitation are likely candidates for support of both hypotheses. The form of facilitation and primary limitation within a region can most certainly shift the frequency and intensity of facilitation from monotonic increase to collapse. Facilitation from the benefit of growing close to another species can emerge when competition for water is relatively low (Holmgren et al. 1997; Michalet et al. 2014a, Pugnaire et al. 2015) or if the benefactor species directly increases water availability to the facilitated species (Zou et al. 2005; Maestre et al. 2009; Prieto et al. 2010). At Atiquipa, the fog capture provided by dominant plants likely alleviated extreme stresses and changed reduced critical water limitations; whilst at Romeral and Fray Jorge, the absence of this water income results in intense competition for the only source of water, i.e. rainfall. Consequently, the form and magnitude of limitations can potentially mediate how plant facilitation changes

on gradients. Eventual collapse of positive interactions under extreme stresses likely depends on the main stressor of the ecosystem and it is reasonable to suggest that there are different 'collapse thresholds' for different resources within and between high-stress ecosystems.

The outcome of biotic interactions is not only shaped by direct environmental variation. The functional traits associated with benefactor species are often important in determining the net facilitative effect (Aschehoug & Callaway 2014, Callaway 2007, Lortie et al. 2016) and form of facilitation (Filazzola & Lortie 2014) primarily due to their capacity to mediate the distribution of resources. Positive plant interactions have also been proposed to be species specific (Callaway 2007), and contrasting trait sets between species further these concepts. Dominant plant size within species in this study was not however related to respective biotic effects, which does not support previous research showing this simple trait as a proxy for facilitation (Pugnaire et al. 1996, Miriti 2006, leRoux et al. 2013). Usually, size of dominant plants is tested in terms of ontogenic categories, which was not done in our study. In arid environments, herbivory also has an important role for plant communities (Graff et al. 2013, Karban 2007, Madrigal et al. 2013). Hence dominant plants with herbivore deterrent structures, such as spines, can provide protected habitats for understory plants (Vandenberghe et al. 2009, Atwater et al. 2011, Sotomayor & Lortie 2015). Here, there was no evidence of thorns determining the outcome of the effects provided by the dominant plants studied. Arguably, the extreme stress conditions (increasing drought for three years) for the duration of this study reduced the likelihood of detecting consumer-pressure effects.

This suggests that herbivore protection at very high stress conditions is not an active mechanism of positive plant-plant interactions in deserts. These different dominant plants represent biologically similar microsites for understory species to survive within this arid ecosystem (i.e. microsites with ameliorated environmental stress) when resources (e.g. water) are the most limiting driver of survival. Limitation trumps regulation when resources are extremely scarce (White 2001). Species-specific positive effects (Aschehoug & Callaway 2014, Callaway 2007) can thus manifest via different mechanistic pathways (Filazzola & Lortie 2014) and consequently structure plant communities differently. The trait sets associated with increasing environmental stress is likely one of the most important topics we can further examine in foundation species in any contemporary ecosystem, not just in deserts.

CONCLUDING REMARKS

The SGH and collapse of interactions hypothesis under extreme stress were both supported in this study. Nonetheless, it is possible that stress on some of the gradients studied was not extreme enough, and that collapse of interactions is more widespread at extreme environmental conditions. The most arid regions included in this study showed a collapse in positive interactions, and there was also a collapse of positive effects when conditions became drier and more stressful with time. Our findings show that extreme stress induced by spatial or temporal environmental variation can collapse the positive effects of dominant plants as a result of a decrease in the intensity of positive effects and ultimately a decrease in the frequency of such interactions. The effects of the two dominant species with different traits did not differ from one another

suggesting that species-specific positive effects are likely dependent on the specific facilitation mechanism. This study extends ecological theory by including evidence from one of the most arid places reported to date, and explicitly testing both frequency and intensity of interactions as they are modified by environmental stress.

ACKNOWLEDGEMENTS

This research was funded by an NSERC DG to CJL. York University FGS salary supported DAS. We also want to thank Danny Carvajal, Julio Gutiérrez, Patricio García, and Italo Revilla for their help during fieldwork. We also want to recognize the community of Atiquipa for allowing us the entrance to their private reserve.

REFERENCES

- Almeyda, E. 1950. Pluviometría de las zonas del desierto y las estepas cálidas de Chile. Editorial Universitaria S. A, Santiago, CL.
- Armas, C., Ordiales R. & Pugnaire, F. I. 2004. Measuring plant interactions: a new comparative index. *Ecology* 85: 2682-2686.
- Armas, C., Rodríguez-Echeverría, S. & Pugnaire, F.I. 2011. A field test of the stress-gradient hypothesis along an aridity gradient. *Journal of Vegetation Science* 22: 818-827.
- Aschehoug, E. T. & Callaway R. M. 2014. Morphological variability in tree root architecture indirectly affects coexistence among competitors in the understory. *Ecology* 95: 1731-1736.
- Atwater, D. Z., Bauer, C. & Callaway, R. M. 2011. Indirect positive effects ameliorate strong negative effects of *Euphorbia esula* on a native plant. *Plant Ecology* 212: 1655-1662.
- Barbosa, P., Hines, J., Kaplan, I., Martinson, H., Szczepaniec, A. & Szendrei, Z. 2009. Associational resistance and associational susceptibility: having right or wrong neighbors. *Annual Review in Ecology, Evolution and Systematics* 40: 1-20.
- Bates, D., Maechler, M., Bolker, B. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1-48.
- Bertness, M. D. & Callaway, R. M. 1994. Positive interactions in communities. – *Trends in Ecology and Evolution* 9: 187-191.
- Biswas, S. R. & Wagner, H. H. 2014. A temporal dimension to the stress gradient hypothesis for intraspecific interactions. *Oikos* 123: 1323-1330.

- Brako, L. & Zarucchi, J. L. 1993. *Catalogue of the flowering plants and gymnosperms of Peru*. Monographs in Systematic Botany Vol. 45. Missouri Botanical Garden, St. Louis
- Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. J., Cavieres, L. A., Kunstler, G., Liancourt, P., Tielborger, K., (...) & Michalet, R. 2008. Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology* 96: 18-34.
- Bruno, J. F., Stachowicz, J. J., & Bertness, M. D. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* 18: 119-125.
- Callaway, R. M. 2007. *Positive Interactions and Interdependence in Plant Communities*. Springer, New York.
- Cavieres, L. A. & Badano, E. I. 2009. Do facilitative interactions increase species richness at the entire community level? *Journal of Ecology* 97: 1181-1191.
- Chamberlain, S. A., Bronstein, J. L. & Rudgers J. A. 2014. How context dependent are species interactions? *Ecology Letters* 17: 881-890.
- Chesson, P. 2000. Mechanisms of maintenance of species diversity. *Annual Review in Ecology and Systematics* 31:343-366.
- Cipriotti, P. A. & Aguiar, M. A. 2015. Is the balance between competition and facilitation a driver of the patch dynamics in arid vegetation mosaics? *Oikos* 124: 139-149.
- Flores, J. & Jurado, E. 2003. Are nurse-protégé interactions more common among plants from arid environments? *Journal of Vegetation Science* 14: 911-916.
- Franco, A. C. & Nobel, P. S. 1989. Effect of nurse plants on the microhabitat and growth of cacti. *Journal of Ecology* 77: 870-886.
- Graff, P., F. Rositano, and M. R. Aguiar. 2013. Changes in sex ratios of a dioecious grass with grazing intensity: the interplay between gender traits, neighbour interactions and spatial patterns. *Journal of Ecology* 101:1146–1157.
- He, Q. & Bertness, M. D. 2014. Extreme stresses, niches and positive species interactions along stress gradients. *Ecology* 95: 1437-1443.
- He, Q. Bertness, M. D. & Altieri, A. H. 2013. Global shifts towards positive species interactions with increasing environmental stress. *Ecology Letters* 16: 695-706.
- Hijmans, R. J., Cameron, S. E. Parra, J. L., Jones, P. G. & Jarvis, A. 2005. Very High Resolution Interpolated Climate Surfaces for Global Land Areas. *International Journal of Climatology* 25: 1965-1978.
- Holmgren, M., Stapp, P., Dickman, C., Gracia, C., Graham, S., Gutiérrez, J. et al. 2006. Extreme climatic events shape arid and semiarid ecosystems. *Frontiers in Ecology and the Environment* 4: 87-95.
- Karban, R. 2007. Experimental clipping of sagebrush inhibits seed germination of neighbours. *Ecology Letters* 10: 791-797.
- Kéfi S., van Baalen M., Rietkerk M. et al. 2008. Evolution of local facilitation in arid ecosystems. *American Naturalist* 172, E1-E17.
- Kikvidze, Z. & Armas, C. 2010. Plant interaction indices based on experimental plant performance data. In Pugnaire, F. I. (ed.) *Positive plant interactions and community dynamics*. Pp 17-38. CRC Press, Boca Raton, USA.
- le Roux, P. C., Shaw, J. D. & Chown, S. L. 2013. Ontogenetic shifts in plant interactions vary with environmental severity and affect population structure. *New Phytologist* 200: 241-250.

- Lopez, R. P., Larrea-Alcazar, D. M. & Ortuno, T. 2009. Positive effects of shrubs on herbaceous species richness across several spatial scales: evidence from the semiarid Andean subtropics. *Journal of Vegetation Science* 20: 728-734.
- Lopez, R. P., Valdivia, S., Rivera, M. L. & Rios, R. S. 2013. Co-occurrence patterns along a regional aridity gradient of the subtropical Andes do not support stress gradient hypothesis. *PLoS ONE* 8: e58518.
- Lortie, C. J. 2010. Synthetic analysis of the stress-gradient hypothesis. In Pugnaire, F. I. (ed.) *Positive Plant Interactions and Community Dynamics*. Pp 125-148. CRC Press, Boca Ratón, USA.
- Lortie, C. J., Brooker, R. W., Choler, P., Kikvidze, Z., Michalet, R., Pugnaire, F. I. & Callaway, R. M. 2004. Rethinking plant community theory. *Oikos* 107: 433-438.
- Lortie, C.J. & Callaway, R.M. 2006. Re-analysis of meta-analysis: support for the stress-gradient hypothesis. *Journal of Ecology* 94: 7-16.
- Lortie, C. J., Filazzola, A. & Sotomayor, D. A. 2015. Functional assessment of animal interactions with shrub-facilitation complexes: a formal synthesis and conceptual framework. *Functional Ecology* 30: 41-51.
- Madrigal, J., Kelt, D. A., Meserve, P. L., Gutierrez, J. R. & Squeo, F. A. 2011. Bottom-up control of consumers leads to top-down indirect facilitation of invasive annual herbs in semiarid Chile. *Ecology* 92: 282-288.
- Maestre, F.T., Valladares, F. & Reynolds, J.F. 2005. Is the change of plant-plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. *Journal of Ecology* 93: 748-757.
- Maestre, F.T., Valladares, F. & Reynolds, J.F. 2006. The stress-gradient hypothesis does not fit all relationships between plant-plant interactions and abiotic stress: further insights from arid environments. *Journal of Ecology* 94: 17-22.
- Maestre, F.T., Callaway, R.M., Valladares, F. & Lortie, C.J. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology* 97: 199-205.
- Marticorena, C., Squeo, F. A., Arancio, G. & Muñoz M. 2001. Catálogo de la Flora Vasculare de la IV Región de Coquimbo. In: Squeo, F. A. et al. (eds.) *Libro Rojo de la Flora Nativa y de los Sitios Prioritarios para su Conservación: Región de Coquimbo*. Pp 105-142. Ed. Universidad de La Serena.
- McIntire, E. J. B. & Fajardo, A. 2014. Facilitation as a ubiquitous driver of biodiversity. *New Phytologist* 201: 403-416.
- Metz, J. & Tielbörger, K. 2016. Spatial and temporal aridity gradients provide poor proxies for plant-plant interactions under climate change: a large-scale experiment. *Functional Ecology* 30: 20-29
- Michalet, R., Brooker, R. W., Cavieres, L. A., Kikvidze, Z., Lortie, C. J., Pugnaire, F. I., Valiente-Banuet, A. & Callaway, R. M. 2006. Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? *Ecology Letters* 9: 767-773.
- Michalet, R., Le Bagousse-Pinguet, Y., Maalouf, J.P. & Lortie, C.J. 2014a. Two alternatives to the stress-gradient hypothesis at the edge of life: the collapse of facilitation and the switch from facilitation to competition. *Journal of Vegetation Science* 25: 609-613.

- Michalet, R., Schöb, C., Lortie, C. J., Brooker, R. W. & Callaway, R. M. 2014b. Partitioning net interactions among plants along altitudinal gradients to study community responses to climate change. *Functional Ecology* 28: 75-86
- Miriti, M. N. 2006. Ontogenetic shift from facilitation to competition in a desert shrub. *Journal of Ecology* 94: 973-979.
- Miriti, M. N. 2007. Twenty years of changes in spatial association and community structure among desert perennials. *Ecology* 88: 1177-1190.
- Novoa, J. E. & Lopez, D. 2001. IV Región: El Escenario Geográfico Físico. In: Squeo, F. A. et al. (eds.) Libro Rojo de la Flora Nativa y de los Sitios Prioritarios para su Conservación: Región de Coquimbo. Pp 13-28. Ed. Universidad de La Serena.
- Padilla, F.M. & Pugnaire, F. I. 2006. The role of nurse plants in the restoration of degraded environments. *Frontiers in Ecology and the Environment* 4:196-202
- Pescador, D. S., Chacón-Labela, J., de la Cruz, M. & Escudero, A. 2014. Maintaining distances with the engineer: patterns of coexistence in plant communities beyond the patch-bare dichotomy. *New Phytologist* 204: 140-148.
- Pugnaire, F. I., Hasse, P., Puigdefábregas J., Cueto, M., Clark, S. C. & Incoll, L. D. 1996. Facilitation and succession under the canopy of the leguminous shrub, *Retama sphaerocarpa*, in a semi-arid environment in south-east Spain. *Oikos* 76: 455-464.
- Pugnaire, F. I., Armas, C., & Maestre, F. T. 2011. Positive plant interactions in the Iberian Southeast: mechanisms, environmental gradients, and ecosystem function. *Journal of Arid Environments* 75: 1310-1320.
- Pugnaire, F. I., Zhang, L., Li R. & Luo, T. 2015. No evidence of facilitation collapse in the Tibetan plateau. *Journal of Vegetation Science* 26: 233-242.
- R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rundel P., Dillon M., Palma B., Money H., Gulmon L. & Ehleringer J. 1991. The phytogeography and ecology of the coastal Atacama and Peruvian deserts. *Aliso*. 13: 1-49.
- Schiffers, K. & Tielbörger, K. 2006. Ontogenetic shifts in interactions among annual plants. *Journal of Ecology* 94: 336-341.
- Schöb, C., Armas, C. & Pugnaire, F. I. 2013. Direct and indirect interactions co-determine species composition in nurse plant systems. *Oikos* 122: 1371-1379.
- Soliveres, S., DeSoto, L., Maestre, F. T. & Olano, J. M. 2010. Spatio-temporal heterogeneity in abiotic factors modulate multiple ontogenetic shifts between competition and facilitation. *Perspectives in Plant Ecology, Evolution and Systematics* 12: 227-234.
- Soliveres, S. & Maestre, F. T. 2014. Plant-plant interactions, environmental gradients and plant diversity: a global synthesis of community level studies. *Perspectives in Plant Ecology, Evolution and Systematics* 16: 154-163.
- Sotomayor D. A. & Lortie, C. J. 2015. Indirect interactions in terrestrial plant communities: emerging patterns and research gaps. *Ecosphere* 6: art 103
- Sotomayor, D. A. & Jimenez, P. 2008. Condiciones meteorológicas y dinámica vegetal del ecosistema costero Lomas de Atiquipa (Caravelí-Arequipa) en el sur del Perú. *Ecología Aplicada* 7: 1-8.
- Stanton, D. E., Armesto, J. A. & Hedin, L. O. 2014. Ecosystem properties self-organize in response to a directional fog-vegetation interaction. *Ecology* 95: 1203-1212.

- Sthultz, C. M., Gehring, C. A. & Whitham, T. G. 2007. Shifts from competition to facilitation between a foundation tree and a pioneer shrub across spatial and temporal scales in a semiarid woodland. *New Phytologist* 173: 135-145.
- Taylor, C. M. and Lorence, D. H. 1993. On the status of *Randia armata* (Sw.) DC. (Rubiaceae: Gardenieae). *Taxon* 42: 865-867.
- Tewksbury, J. J. & Lloyd, J. D. 2001. Positive interactions under nurse-plants: spatial scale, stress gradients and benefactor size. *Oecologia* 127: 425-434.
- Tielbörger, K. & Kadmon, R. 2000. Temporal environmental variation tips the balance between facilitation and interference in desert plants. *Ecology* 81:1544-1553.
- Vandenberghe, C., Smit, C., Pohl, M., Buttler, M. & Frelechoux, F. 2009. Does the strength of facilitation by nurse shrubs depend on grazing resistance of tree saplings? *Basic and Applied Ecology* 10: 427-436.
- Ward D. 2009. *The Biology of Deserts*. Oxford University Press, New York.
- White, T. C. R. 2001. Opposing paradigms: regulation or limitation of populations? *Oikos* 93: 148-152.
- Xu, C., Van Nes, E. H., Holmgren, M., Kefi, S. & Scheffer, M. Local facilitation may cause tipping points on a landscape level preceded by early-warning indicators. *American Naturalist* 186: E81-E90.
- Zar, J. H. 1999. *Biostatistical analysis*. Fourth Edition. Prentice Hall. USA. 663 p.

Chapter 3

Dominant plant effects at micro-scales and within seasons

Submitted to *Journal of Vegetation Science* as:

A spatiotemporal analysis of plant facilitation on arid plant community dynamics

Sotomayor, D. A., Lortie, C. J. & Revilla I.

ABSTRACT

Questions: The outcome of biotic interactions can frequently be determined by environmental severity, and variance in severity is scale and time dependent. The following questions on this topic are examined: does the spatial scale within the canopy of dominant plants change the sign of interactions? Is there temporal variation in positive effects similar to the stress gradient hypothesis? Do dominant plants mediate indirect effects within understory communities in comparison to non-canopied microsites?

Location: Coastal desert of Atiquipa, Southern Peru

Methods: We examined the spatiotemporal variation in positive interactions by studying the temporal and spatial effects of two dominant woody species, the spiny shrub *Randia armata* and the tree *Caesalpinia spinosa*, at three micro-scales over two growing seasons on subordinate plant communities. In each census, plant density and species richness with microsite temperature and relative humidity were measured.

Results: There were consistent differences in temperature and relative humidity under the canopies of dominant plants relative to open microsites. Both spatial and temporal scales tested significantly influenced the outcome of interactions between dominants and associated understory plants. Dominant plants had species-specific positive effects by increasing species richness and plant density. However, the strength of their positive effects was not different between dominants. The composition of subordinate plant communities was also dependent on both spatial and temporal scales. The relative strength of positive effects increased at the end of the growing season, but it was not influenced by spatial scale. Indirect effects within understory communities were not

mediated by the micro-scales tested within the canopy or by time suggesting weak indirect effects within understory communities that contribute to the annual-plant dynamics.

Conclusions: Collectively these findings illustrate the importance of exploring spatial and temporal effects when examining plant facilitation and support recent developments moving beyond simple, two-phase contrasts in deserts.

Keywords: coexistence, facilitation, indirect effects, net interactions, nurse plant, positive interactions, spatial scale, species specificity, stress gradient hypothesis, Southern Peru, temporal scale.

Nomenclature: Brako & Zarucchi (1993)

Running head: Spatiotemporal analysis of facilitation

INTRODUCTION

The relative importance of processes that structure plant communities and maintain biological diversity has been a primary focus in community ecology. Ecological dynamics has been characterized via spatial and temporal patterns at different scales (Levin 1992, Sthultz et al. 2007, Hastings 2010, Biswas & Wagner 2014). The relative severity of environmental gradients depends on seasonality and on the life-stage of the plant communities within a season (Tielbörger & Kadmon 2000a, Miriti 2006, Schiffers & Tielbörger 2006, le Roux et al. 2013). Biotic interactions can act as filters determining

whether species persist within a given environment (Brooker et al. 2008, Lortie et al. 2004, McIntire & Fajardo 2014). However, the spatial effects associated with these interactions are either lost at increasing scales (Araujo & Rozenfeld 2014) or yield opposing outcomes over extended sampling time periods (Tielbörger & Kadmon 2000a, Sthultz et al. 2007). The spatial scale of biotic interactions has been explored to some extent (Tewksbury & Lloyd 2001, Lopez et al. 2009, Araujo & Rozenfeld 2014), and their temporal scale as well using multi-year studies in relation to ontogeny of perennial plants (Tielborger & Kadmon 2000a, Miriti 2006, 2007, Soliveres et al. 2010). However, less is known about within season changes in interactions (but see Sthultz et al. 2007, Biswas & Wagner 2014), especially for non-perennial life forms such as annual plants. Responses to these drivers can be manifested on single measures such as species richness but can also be analyzed through community structure and population dynamics (Gómez-Aparicio et al. 2004, Cavieres & Badano 2009). Rarely, these outcomes are tested with both sets of measures (but see Soliveres & Maestre 2014). A more accurate estimate with the use of multiple response variables of spatiotemporal changes in species interactions will better inform our understanding of the structure and dynamics of plant communities and allow for more realistic prediction models (Lortie & Svenning 2015).

Mutualism represents the most variable species interaction (Chamberlain et al. 2014) likely due to resource exchanges that can carry both costs and benefits to the partners in the interaction (Jones et al. 2012). This variability can be manifested through the interacting species, their geographical distribution, and their temporal dynamics

(Maestre et al. 2003, Sandel 2015, Soliveres et al. 2010, Tielbörger & Kadmon 2000a). The local effects of positive interactions (facilitation) have been well documented and include recruitment, growth, and spatial associations of beneficiary species (for comprehensive reviews see: Bruno et al. 2003, Flores & Jurado 2003, Callaway 2007, Brooker et al. 2008, Filazzola & Lortie 2014). Despite substantial evidence that the magnitude and sign of species interactions vary along environmental gradients (Bertness & Callaway 1994, Michalet et al. 2006, He et al. 2013), the dynamics of this variation requires further characterization (Chamberlain et al. 2014, Soliveres et al. 2015). Most importantly, the scale (both spatial and temporal) used to test for plant-plant interactions can determine the patterns and processes that are subsequently reported (Leibold et al. 2004, Soliveres et al. 2010, Sthultz et al. 2007). Large-scale spatial effects related to stress gradients have been shown to influence the outcome of interactions (Bertness & Callaway 1994, He et al. 2013). However, benefactor species can also generate micro-scale stress-related dynamics even within their canopies (Koyama et al. 2015, Pescador et al. 2014), but these dynamics have remained unexplored although they have the potential of drastically changing arid systems structure (Xu et al. 2015) and evolutionary dynamics (Kefi et al. 2008). Stress manifested through time can also affect interaction outcomes (Biswas & Wagner 2014, le Roux, et al. 2013, Tielbörger & Kadmon 2000a), but a more comprehensive test needs to test both spatial scale and time such as inter-annual variation concurrently.

Dominant plants in arid environments frequently facilitate a high density of understory plant species (Franco & Nobel 1989, Tielbörger & Kadmon 2000b Flores &

Jurado 2003) that collectively display an increase in competitive interactions within the canopy (Pages & Michalet 2003, Schöb et al. 2013 - but see Tielbörger & Kadmon 2000b). Understory plant interaction networks also vary from clearly demarcated competitive to intransitive hierarchies (Soliveres et al. 2011). Increased niche segregation and competition intransitivity within the canopy therefore reduces competitive exclusion (Laird & Schamp 2006), and enhances overall local species richness. The more benign conditions often found under dominant plants (Franco & Nobel 1989, Pescador et al. 2014) increases the local species pool due to microclimate amelioration that expands the niches of stress intolerant species within the system by providing a higher degree of moisture, nutrients or mycorrhizae in comparison to open areas (Pugnaire et al. 1996, Drezner 2007, Tielbörger & Kadmon 2000b, Flores & Jurado 2003, Filazzola & Lortie 2014). However, these effects within arid understory communities have remained less understood (but see Schob et al. 2013, Soliveres et al. 2011). Disentangling these relatively less studied and localized effects of dominant plants on understory communities and among understory species informs species coexistence (Brooker et al. 2008, Callaway 2007, Cipriotti & Aguiar 2015, McIntire & Fajardo 2014).

This study examines the spatiotemporal dynamics of positive interactions focusing on the within and between seasonal effects and concurrently on the micro-scale effects of two different dominant woody species on their subordinate plant communities. We hypothesized that the positive effects of dominant desert plants on understory communities are spatiotemporally scale dependent. The effects examined

include both direct consequences of the dominant plant on understory species (e.g. increased understory species richness, increased abundance, and increased diversity) and indirect effects via changing competitive hierarchies amongst understory species (niche segregation and/or competition intransitivity). We examined the following questions: 1) how do spatial scale (micro-scale by varying distance from the dominant plant) within understory microsites changes the sign and strength of interactions with understory plants as well as their structure?; 2) what is the temporal effect of dominant plants and its interaction with micro-scale on understory plant communities?; and 3) how do dominant plants mediate indirect effects between understory species? We predicted that micro-scale even within dominant plants determines the outcome of species interactions, that interactions through time will follow stress-gradient dynamics (Bertness & Callaway 1994) in that less environmentally limiting seasons have reduced frequencies of facilitation, and, finally that dominant plants will mediate indirect effects via an increase in competitive interactions when environmental conditions are less stressful as a product of micro-habitat amelioration. The answers to these questions advance positive interactions theory and improve our understanding on the effects of dominant plants in arid environments for understory communities.

METHODS

Study site, plot selection, and dominant species

Atiquipa, Southern Peru (15°S, 74°W) is a coastal desert with a wet winter season between July and November typically characterized by high moisture due to fog (about 90% on average). Approximately 70% of the annual rainfall mean (i.e. 200 mm)

occurs between these months (Sotomayor & Jimenez 2008). Annual rainfall average for the period 2009-2012 was 185 mm, with 2011 receiving 167.3 mm, while 2012 received 137.4 mm. The plant community includes a few large woody species and a diversity of herbaceous plants wherein annuals are the most common life form. In order to select five sites within the desert that represented an environmental gradient, we conducted a survey along its range. This survey systematically sampled 1-km grids using one 20 x 20 m quadrat per grid to determine plant cover and species composition within each of these grids. These data were then combined with climate information from the database WorldClim (Hijmans et al. 2005) and used to quantitatively determine the plots to be used for this study. By combining ground-truthed data with climate parameters (e.g. temperature, precipitation, and seasonality) via multivariate statistics (i.e., direct ordination analyses), we were able to properly determine the complex environmental gradients within our field site (Lortie 2010) and sampled along these. Five sites (100 x 100 m) were selected using this procedure and were separated by at least 2 km from one another, but no more than 5 km. These sites also corresponded to a natural elevation gradient regionally ranging from 741 m asl to 1124 m asl, with each site approximately every 100 m in elevation.

Based on previous surveys (Sotomayor, per. obs.), we chose two dominant perennial woody species for this study that co-occurred at all 5 sites. One of those dominant plant species was the locally abundant 4-5 m tall tree *Caesalpinia spinosa* Molina (Kuntze) (Fabaceae). This tree has a high understory of about 2 m, and it is native to Peru. *Caesalpinia* can also be found in various places of South America such

as Bolivia, Colombia, Ecuador and Venezuela, tolerating dry climates and poor soils (Brako & Zarucchi 1993). The other dominant plant we selected was *Randia armata* (Sw.) DC. (Rubiaceae), a spiny 2-3 m tall shrub with low understory of about 0.5 m, also native to Peru, but with a wide distribution in America ranging from Mexico to Argentina and with presence in moist and dry forests (Taylor & Lorence 1993).

Vegetation surveys and microsite measurements

During two growing seasons (2011 and 2012), 100 plots located in the understory of the 2 dominant plant species (50 under *C. spinosa*, and 50 under *R. armata*) and 50 plots in open nearby spaces were surveyed in triplets at Atiquipa, Southern Peru. Each triplet contained one of each microsite, with microsites separated from each other ca. 2m, and triplets separated from each other at least 5m. These 150 plots were distributed along the 5 sites, with 10 of each species per microsite per site. The abundance of each of the species within the plots was recorded and used to calculate total plant species abundance and richness. These data were collected monthly during each growing season from September-December in 2011 and again in 2012 at three spatial scales within each plot both under canopies and in open microsites: 0.0625 m², 0.25 m² and 1 m². These three quadrats at different spatial scales were nested within each other using the axis of the dominant plant or one of the corners of the plot for open microsites. Temperature and relative humidity were collected during the 2012 season using HOBO U-23 Pro-V2 loggers located at the soil level within each of the three microsites under study and inside the finest-scale quadrat within the shrub or open microsite plot. A total of 9 data loggers were utilized with 3 replicates per microsite

placed randomly. Loggers were placed in the sites with the highest and lowest elevation as well as in the site at mid elevation. Data were recorded every 15 minutes between the beginning of August and the end of October (growing season). Vapour pressure deficit was also calculated based on both temperature and relative humidity (Hartman 1994).

The strength of the effect of dominant plants on species richness and plant density was estimated using the Relative Interaction Index (RII) calculated as follows (Armas et al. 2004):

$$\text{RII} = \frac{D_u - D_o}{D_u + D_o} \quad (1)$$

The terms D_u and D_o corresponded to the density of plants in understory and open microsites, respectively. This index varies from -1 to +1 with positive effects being > 0 and negative effects < 0 on the density of these species.

Subordinate plant-plant interaction outcomes by microsite

In order to assess the effect of dominant plants on the competitive outcomes of their understory species, we measured changes in competition intransitivity and the degree of niche segregation within the community by using guild structure null models of species co-occurrences (Gotelli & Graves 1996, Gotelli et al. 2010) and their relationship to the site-level richness found within canopies and open plots at each site (Soliveres et al. 2011). These null models are organized *a priori* by groups of ecological significance (plots per microsite in this case), and test the role of competition in

structuring the community within each of these groups independently (Gotelli & Graves 1996). Competitive intransitivity and transitivity produce less co-occurrence due to small-scale competitive exclusion, however networks with a marked hierarchy in competition have a few dominant species and, therefore, a reduced local richness, while intransitive networks generate high species turnover and therefore, a high local richness (Laird & Schamp 2006). We estimated species co-occurrence using the C-score index (Gotelli & Graves 1996). This metric has been used to estimate transitivity and community structure (e.g. Dullinger et al. 2007, Soliveres et al. 2011) and was calculated for each pair of species as:

$$\text{C-score} = \frac{(R_i - S)}{(R_j - S)} \quad (2)$$

The terms R_i and R_j are the number of total occurrences for species i and j , and S is the number of quadrats in which both species occur (Gotelli & Graves 1996). This score is then averaged over all possible pairs of species in the matrix (Gotelli 2000). The C-score is related to the competitive exclusion concept of “checkerboardness”, i.e., how many of the possible species pairs in a given community never appear in the same quadrat together. Thus, positive and large values of this index indicate that competition may be the prevalent mechanism determining the co-occurrence patterns observed (Gotelli 2000). To determine the strength of co-occurrence in a sample, the observed C-score value is compared against a set of null models that serve as a baseline for what a community unstructured by species interactions would look like, i.e. a null model (Connor & Simberloff 1979). The C-score is calculated utilizing presence/absence

matrices, hence it is sensitive to changes in interspecific co-occurrence patterns, and is independent of intraspecific interactions. As the values of the C-score are dependent on the number of species and co-occurrences observed within each plot, we calculated a standardized effect size (SES) as $(I_{\text{obs}} - I_{\text{sim}})/S_{\text{sim}}$, where I_{obs} is the observed value of the C-score, and I_{sim} and S_{sim} are the mean and standard deviation, respectively, of this index obtained from simulations (Gotelli & Entsminger 2006). We used the software EcoSim version 7.72 with 'fixed rows-equiprobable columns' null models and ran 5000 simulations (Gotelli & Entsminger 2006). Standardized effect size (SES) values of the C-score less than zero suggest prevailing higher co-occurrence (spatial aggregation) whereas SES values greater than zero indicate lower co-occurrence (spatial segregation) amongst the species within a community.

To assess the relationship between co-occurrence and local diversity, we compared SES values with the site-level species richness found in each microsite. This comparison has the following four possible outcomes (Soliveres et al. 2011): (1) dominant plants concurrently reduce SES and increase site-level species richness compared to open microsities: dominant plants would be promoting the occurrence of understory species via niche segregation (reduced SES indicates reduced competition), with positive effects on the overall site-level species richness; (2) dominant plants increase both SES and site species richness: dominant plants would be increasing microsite-scale competition, but species with competitive advantages vary among plots within sites, generating high species turnover, and therefore increasing site-level species richness (intransitivity); (3) dominant plants increase SES, but reduce site-level

species richness: competitive exclusion would be the dominant interaction amongst understory species, and a smaller set of competitive winners dominate all plots, resulting in reduced site-level species richness; and (4) dominant plants do not affect SES, regardless of their effects on site-level species richness: changes in the competitive outcomes are not an important factor modulating the effect of dominant plants on site-level richness. We calculated SES and site-level species richness for the 3 micro-scales surveyed using the 10 plots per microsite available in each of the 5 sites. All 5 plots were treated as replicates within the desert and for each of the evaluations conducted in this study resulting in 360 experimental units (3 microsities x 3 micro-scales x 5 sites x 8 evaluations across 2 years).

Statistical analyses

Microclimatic data were analyzed with general linear mixed models (GLMMs) using time (Julian day of the measurement) as a repeated measures factor (random variable) and microsite (2 dominant plant microsities and open microsities) as a fixed factor (Littell et al. 2006). Species richness and plant density estimates were also analyzed using GLMMs with time as a repeated measures variable (nested structure with 4 monthly evaluations in each of 2 years) and the following fully crossed fixed factors: microsite as described above (3 levels), micro-scale with 3 levels, and site with 5 levels. Principal Components Analysis (PCA) ordinations along with significance tests (999 permutations) were used to examine the differences among plant communities associated with the 3 microsities (2 dominant plants and open plots) under study and the 3 micro-scales evaluated independently per months and years evaluated using each

species abundance. Site-level species richness and C-scores SES were also analyzed using GLMMs with time as a repeated measures variable (random factor). In all GLMMs, Tukey post-hoc tests were conducted for pair-wise comparisons (Zar 1999). Ordinations were conducted with the software R, package *vegan* (R Core Team 2014), and GLMMs were done with the software JMP version 12 (SAS Institute Inc. 2015). All RIIs ($RII_{\text{species richness}}$, $RII_{\text{plant density}}$) were analyzed using one sample t-tests to test that there were significantly different from zero (Michalet et al. 2014). RIIs were also analyzed with GLMMs with micro-scale and microsite as fixed factors and time, with its nested structure, as a repeated measures effect (random variable).

RESULTS

Microhabitat differentiation

The temperatures under both dominant woody species were significantly cooler than the open microsites throughout the growing season of 2012 (Figure 1, Appendix 7, $F_{(2,182)} = 22.29$, $p < 0.01$). The dominant canopy cooling effect was greatest at midday throughout the season (*C. spinosa*: $14.86 \pm 1.35^\circ\text{C}$, *R. armata*: $14.79 \pm 1.35^\circ\text{C}$, and open: $25.89 \pm 1.35^\circ\text{C}$), but there were no differences in temperature between the two shrub microsites (Tukey HSD $p < 0.05$). There were no differences in relative humidity between the two dominant species or with the open microsites (Figure 1, Appendix 7, $F_{(2,182)} = 3.12$, $p = 0.11$). Vapour pressure deficit was the highest in open microsites (Appendix 8).

Table 1. Summary of statistical models contrasting species richness and plant density (plants.m⁻²) between open microsites and dominant plant microsites in Atiquipa, Southern Peru. *P*-values <0.05 indicate significant differences.

Source	DF	Species richness			Plant density		
		DF _{Den}	F Ratio	<i>P</i> -value	DF _{Den}	F Ratio	<i>P</i> -value
Microscale	2	2714	1130.36	<0.0001	2967	295.39	<0.0001
Site	4	2658	68.68	<0.0001	2477	41.71	<0.0001
Microsite	2	2805	240.83	<0.0001	2841	121.22	<0.0001
Microscale*Site	8	2772	5.75	<0.0001	2316	5.58	<0.0001
Microscale*Microsite	4	2992	21.31	<0.0001	2764	1.01	0.4024
Site*Microsite	8	2799	64.49	<0.0001	2292	39.66	<0.0001
Year	1	2741	5.25	0.0220	2819	500.66	<0.0001
Month	3	2402	218.10	<0.0001	2756	328.93	<0.0001
Year*Month	3	2295	22.02	<0.0001	2679	110.48	<0.0001
Year*Microsite	2	2761	16.23	<0.0001	2957	0.98	0.3768
Month*Microsite	6	2654	7.94	<0.0001	2534	3.29	0.0032
Microscale*Month	6	2459	10.44	<0.0001	2648	25.05	<0.0001

Table 2. Summary of statistical models contrasting plot level species richness and standardized effects sizes (SES) of C-scores between open microsites and dominant plant microsites in Atiquipa, Southern Peru. *P*-values <0.05 indicate significant differences.

Source	Species richness				SES		
	DF	DF _{Den}	F Ratio	<i>P</i> -value	DF _{Den}	F Ratio	<i>P</i> -value
Microsite	2	160	24.75	<0.0001	162.1	1.36	0.2592
Microscale	2	176.2	327.85	<0.0001	166.3	3.22	0.0423
Microsite*Microscale	4	153.2	6.05	0.0002	153.2	3.21	0.0144
Year	1	248	37.16	<0.0001	256.3	0.27	0.6064
Month	3	171	7.39	0.0001	165.2	1.22	0.3035
Year*Month	3	165.9	10.86	<0.0001	176.9	0.66	0.5791
Year*Microsite	2	144.2	15.44	<0.0001	157.2	4.98	0.0080
Year*Microscale	2	159.3	2.28	0.1055	169.9	0.52	0.5940
Microsite*Month	6	156.6	1.02	0.4143	153.7	1.70	0.1241
Month*Microscale	6	149.3	5.99	<0.0001	154.8	0.33	0.9209
Year*Microsite*Month	6	144.4	2.20	0.0462	157.3	2.57	0.0211
Year*Month*Microscale	6	149.5	3.46	0.0031	161.2	0.62	0.7102
Plant density	1	302.2	104.93	<0.0001	238.9	0.73	0.3948

Species-specific effects of dominant plants on their understory

Both dominant species had significantly more species and higher plant densities in their understory in comparison to open microsites (Table 1, Tukey HSD $p < 0.05$), but *R. armata* had the highest species richness and plant densities. Species richness per unit area was higher at the 1 m² scale with 8.08 ± 0.07 species/quadrat while plant density was the lowest with 129.35 ± 3.64 plants/m². Differences between dominant plants at the studied microscales were significant for species richness but not for plant density (Tukey HSD $p < 0.05$). There were significant differences between sites in both species richness and plant density, but these did not coincide with the elevation gradient identified. At the end of the growing season, both dominant species microsites had significantly higher species richness and plant density than open microsites. This pattern was consistent amongst years, but 2011 had overall higher species richness and plant density (Table 1).

The strength of positive effects for species richness (Figure 2) and plant density (Figure 3) was not significantly different between dominant plants ($F_{(1,992)} = 0.83$, $p = 0.36$; $F_{(1,1377)} = 2.81$, $p = 0.09$; respectively), but changed from neutral effects at the beginning of the growing season to strong positive effects at the end of the growing season in both years ($F_{(3,630)} = 64.88$, $p < 0.01$; $F_{(3,1059)} = 103.59$, $p < 0.01$; respectively). However, facilitative effects occurred earlier in the season during 2012 and were on average stronger that year for both species richness (Figure 2) and plant density (Figure 3). These patterns were consistent for all micro-scales and for both dominant species

communities ($F_{(2,920)} = 0.25$, $p = 0.78$ for species richness; $F_{(2,1579)} = 2.96$, $p = 0.05$ for plant density). Communities from the understory of dominant plants were more similar to each other than to open microsite communities (Figure 4). The communities differed by spatial scale (Figure 4), although these differences diminished by the end of the growing season of both years, mainly for the smallest micro-scales (Figure 4, lower panels).

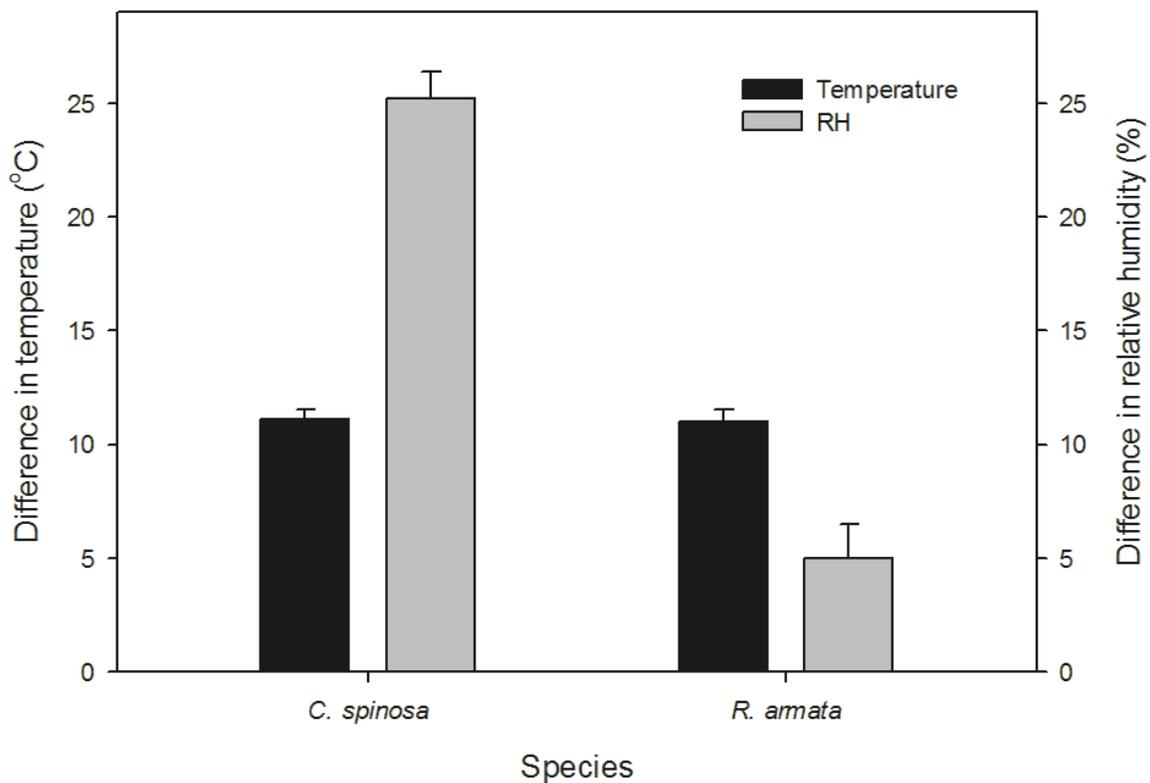


Fig. 1 Absolute differences along with standard errors between microhabitat conditions for two dominant plant microsites in relation to open microsites at noon (12 hrs.) during the growing season of 2012 (August-October) in Atiquipa, Southern Peru.

Subdominant plant-plant interactions as affected by dominant plants

Dominant plants did not affect SES, neither did time, but micro-scale did significantly influence co-occurrence patterns (Table 2) with higher SES values at the largest micro-scale in understory microsites, and the lowest at the smallest scale in open microsites (Tukey HSD $p < 0.05$). All SES values indicate lower co-occurrence and means that there was spatial segregation amongst subdominant species within all communities (Figure 5). Site-level species richness was different among microsites, micro-scales, and time (Table 2). Both *R. armata* and *C. spinosa* had significantly higher species richness than open microsites (Figure 5).

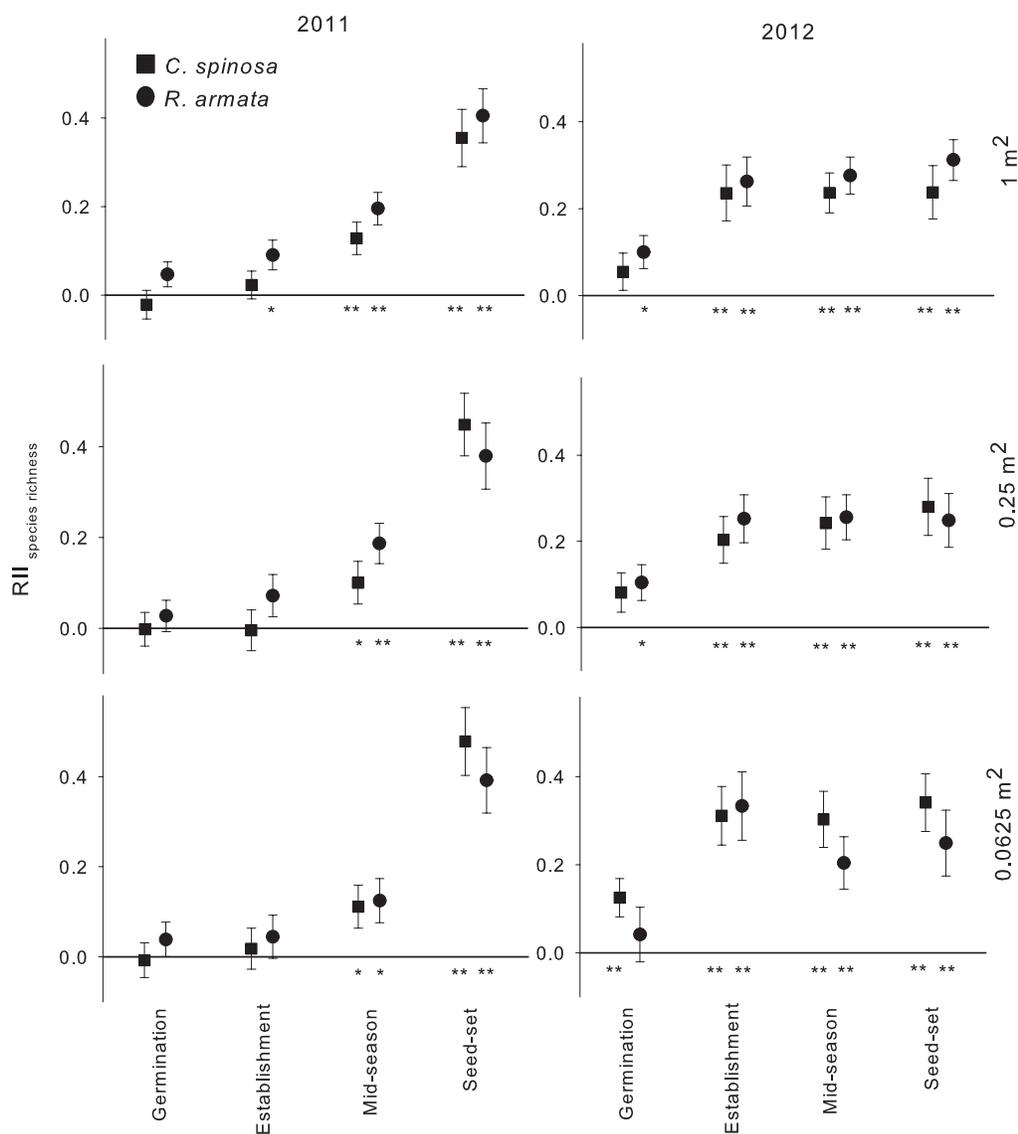


Fig. 2 Relative interaction indices (RII) along with ± 1 SE confidence intervals for species richness of two dominant plant microsites in relation to open microsites across the growing seasons of 2011 and 2012 in Atiquipa, Southern Peru. Left-hand panels correspond to 2011, and right-hand panels to 2012. Upper panels correspond to the 1 m² microscale, mid panels to the 0.25 m² microscale, and lower panels to the 0.0625 m² microscale. Stars indicate significantly different from zero RII values obtained from one sample t-tests (* $P < 0.05$, ** $P < 0.01$).

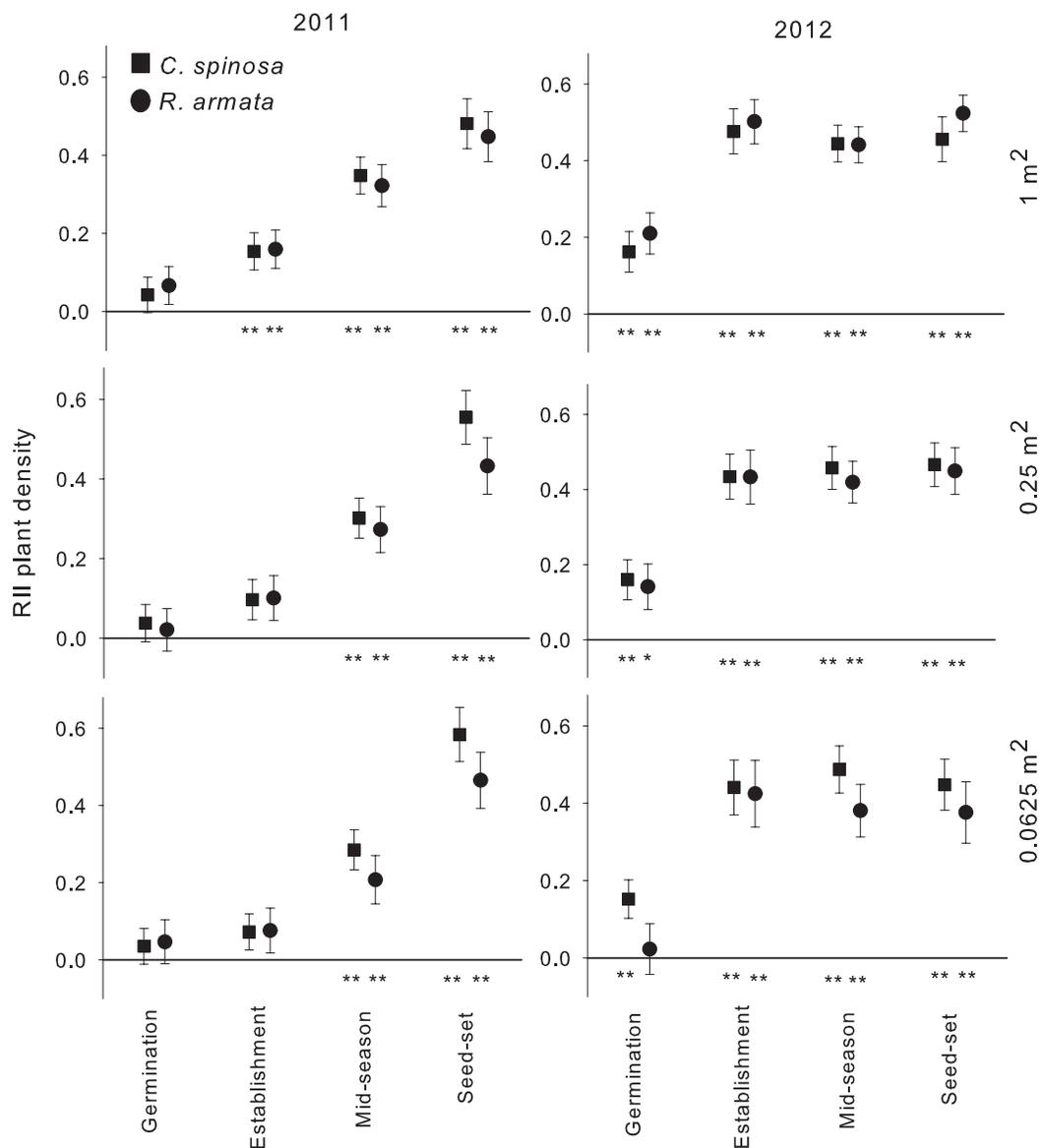
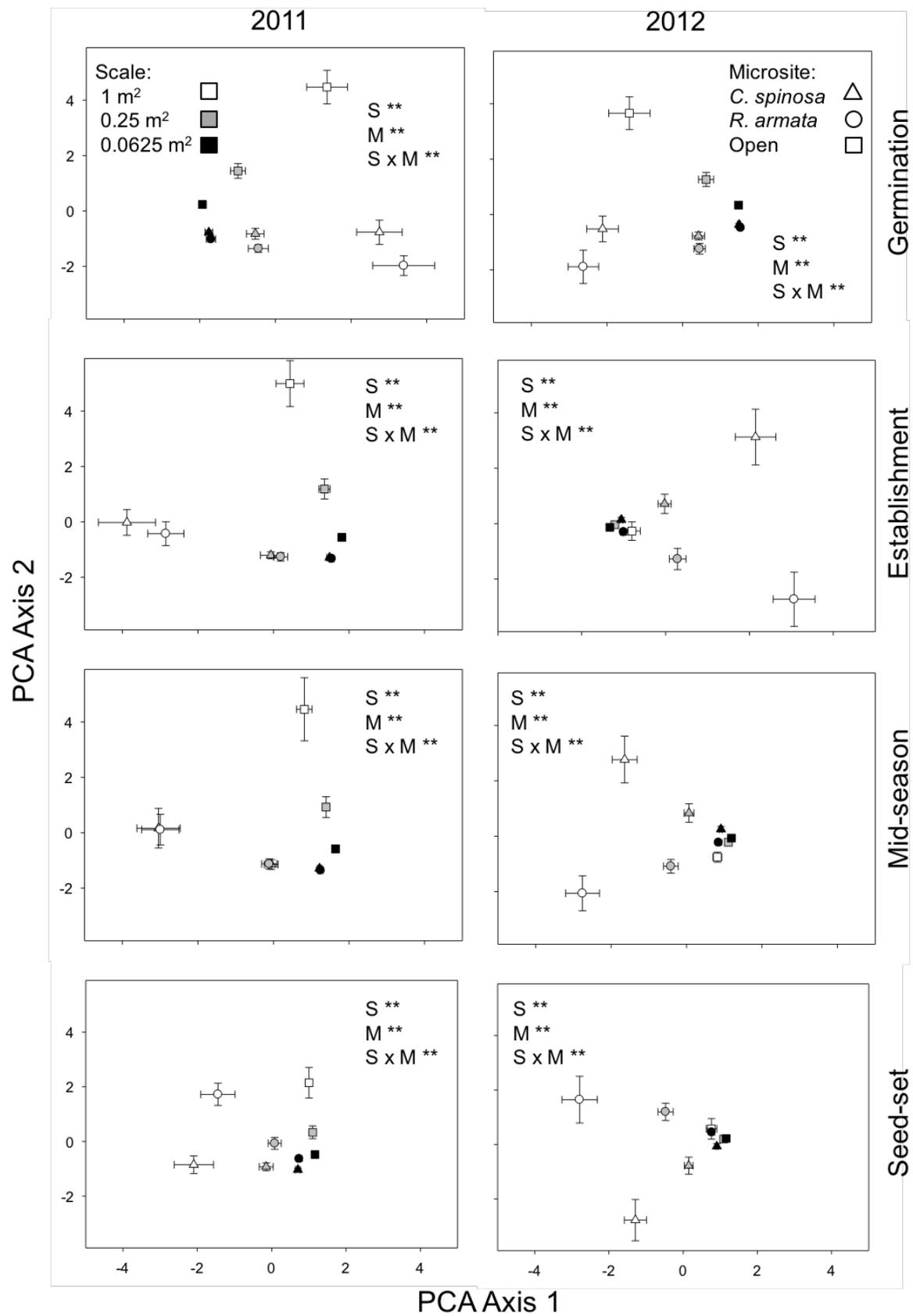


Fig. 3 Relative interaction indices (RII) along with ± 1 SE confidence intervals for plant density of two dominant plant microsites in relation to open microsites across the growing seasons of 2011 and 2012 in Atiquipa, Southern Peru. Left-hand panels correspond to 2011, and right-hand panels to 2012. Upper panels correspond to the 1 m² microscale, mid panels to the 0.25 m² microscale, and lower panels to the 0.0625 m² microscale. Stars indicate significantly different from zero RII values obtained from one sample t-tests (* $P < 0.05$, ** $P < 0.01$).

Fig. 4 Principal components analysis (PCA) ordinations of the species composition of two dominant plant species and open microsites at different spatial micro-scales along the growing seasons of 2011 and 2012 in Atiquipa, Southern Peru. Points are centroids with bi-directional 95% confidence error bars. Results of two-way ANOVAs on the effects of microscale (S), microsite (M), and their interaction are shown in each panel: * $P < 0.05$, ** $P < 0.01$. Year: left-hand panels correspond to 2011, and right-hand panels to 2012. Month: upper panels correspond to September, mid-upper panels to October, mid-lower panels to November, and lower panels to December of their respective years.



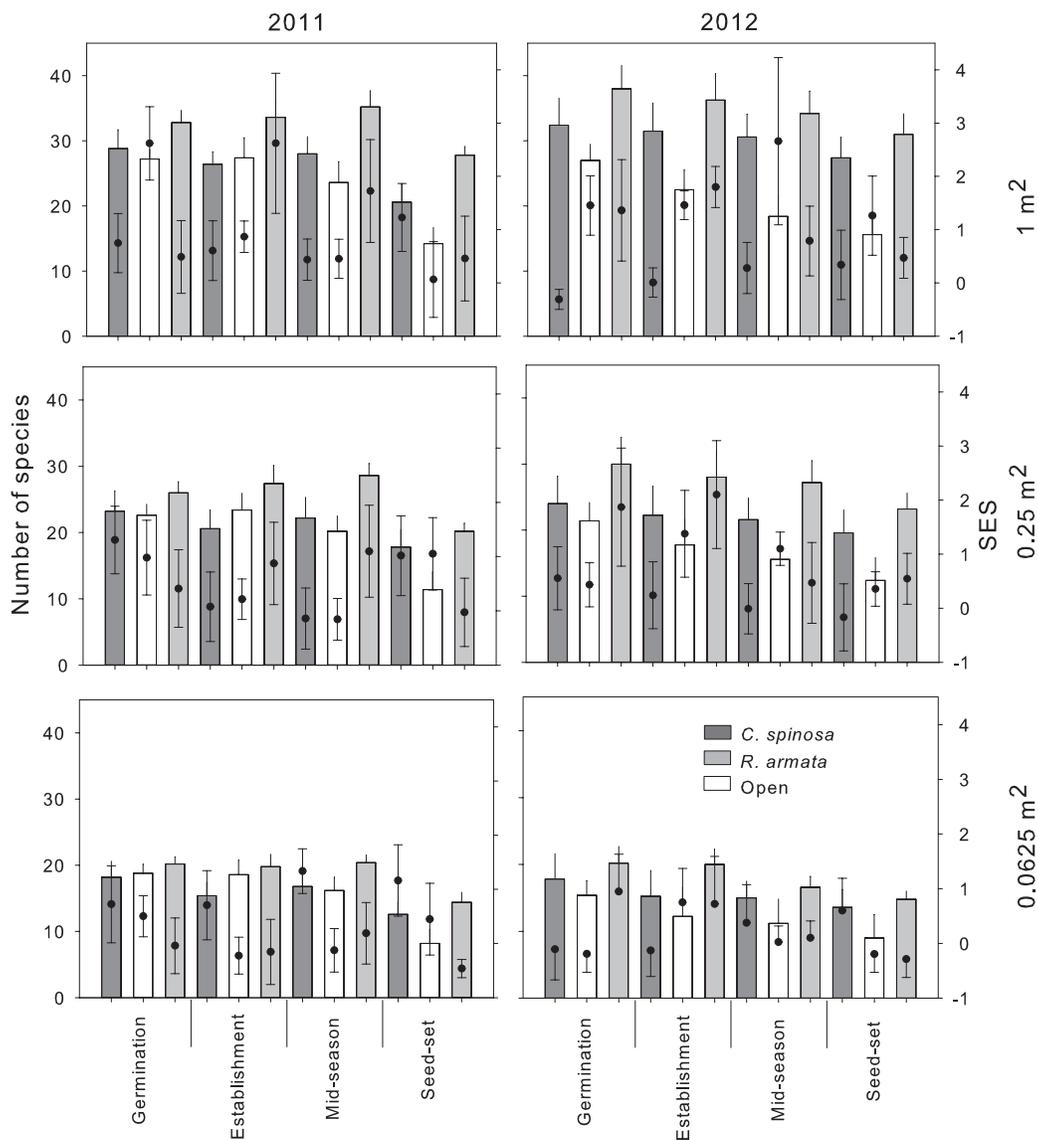


Fig. 5 Plot level species richness (left hand y-axis, bars) and standardized effect size (SES) values of the C-score (right hand y-axis, dots) with ± 1 SE confidence intervals for two dominant plant microsites and open microsites across the growing seasons of 2011 and 2012 in Atiquipa, Southern Peru. Left-hand panels correspond to 2011, and right-hand panels to 2012. Upper panels correspond to the 1 m² microscale, mid panels to the 0.25 m² microscale, and lower panels to the 0.0625 m² microscale.

DISCUSSION

The hypothesis that the positive effects of dominant desert plants on understory communities are spatiotemporally scale-dependent was clearly supported in this study in time but not at the relatively fine spatial scales examined. Interactions between dominant plants and their understory communities changed from neutral to positive as the growing season progressed, but the strength of positive effects at different micro-scales within the canopy did not vary. We also found that the intensity of interactions amongst understory plants is not strong and that spatial segregation within understory communities is not affected by the canopies of dominant plants. These results were consistent for both dominant plants examined in this study. Taken together, these findings illustrate that at micro-scales the strength of positive effects is governed by the environmental conditions as they change through time and that coexistence in the understory is the result of weak interactions between the different members of the plant community that do not have a micro-scale or temporal signature. This evidence underscores the importance of exploring the impact of time and spatial scales on the net outcome of biotic interactions.

We found differences in both species richness and plant density in relation to micro-scale, however, in both response variables the strength of the effects did not change with increasing scale or spatial grain (*sensu* Sandel 2015). Overall positive effects (Lopez et al. 2009), changes in the strength and sign of interactions with increasing scale from strong positive effects at the site level to neutral effects at the

landscape level (Tewksbury & Lloyd 2001), or complex effects at micro-scales according to the functional type of the benefactor (Pescador et al. 2014, Koyama et al. 2015) have been reported previously. Our study adds to this evidence by reporting that the strength of positive effects at micro-scales within shrubs does not change, but disagrees with Pescador et al. (2014)'s "halo" around dominant plants in which facilitation changes to competition. Admittedly, our study reported positive interactions within a 1m radius of the dominant plant, and further distances might yield similar results to Pescador et al. (2014) results or beneficiary species-specific patterns (Koyama et al. 2015). Testing larger spatial scales around dominant plants can help define the importance of ameliorated microhabitats in high stress ecosystems.

The strength of positive effects was not significantly different when comparing dominant plants either for species richness or plant density, however, dominant plants facilitated different plant communities within their understory. It is surprising that the strength of positive effects provided by the two dominant plants is similar even though they provide significantly different microhabitats in terms of temperature and have different life histories. This suggests that these dominant plants represent biologically similar "refuges" for understory species to survive within this arid ecosystem. However, these "refuges" facilitate different plant communities and suggests that the different approaches can determine distinct aspects of the outcomes of biotic interactions (Soliveres & Maestre 2014, Tewksbury & Lloyd 2001). Species-specific positive effects (Aschehoug & Callaway 2014, Callaway 2007) can thus manifest via different mechanistic pathways (Filazzola & Lortie 2014) and consequently structure plant

communities differently. Further studies should examine the extent to which certain traits of dominant plants would be associated with stronger positive effects.

The strength of the positive effects provided by the dominant plants in this study increased at the end of the growing season in both years, although it was stronger in the second year that had lower rainfall. This change in the sign of net interactions is most likely due to an increase in the stressful conditions as the growing season progresses exacerbating the difference between the canopies of dominant plants relative to open sites. This pattern coincides with previous reports (Biswas & Wagner 2014, Sthultz et al. 2007, Tielborger & Kadmon 2000a, Schiffers & Tielborger 2006) in that competitive to neutral interactions predominated during favourable conditions and positive interactions during stressful conditions (SGH, Callaway & Bertness 1994). However, our study expands this research to annual plant communities increasing the applicability of the stress gradient hypothesis and incorporating short-term population dynamics along the full ontogeny of desert annuals (le Roux et al. 2013, Schiffers & Tielborger 2006). Beyond these refinements, these findings also support the storage-effect hypothesis for species coexistence that proposes coexistence in dynamic environments through temporal differences in competitive abilities (as demonstrated here via changes in the strength of interactions) and through persistence in unfavorable periods (Chesson 2000, Chesson et al. 2004). Understanding the temporal effects of dominant plants on the dynamics of annual species is important as it allows for a better description of coexistence on ecosystems of fluctuating stress such as deserts.

We predicted that dominant plants mediate indirect effects between understory species via an increase in competitive interactions but did not find substantial evidence to support this. This suggests that the changes in the competitive outcomes within understory plant communities are not an important factor mediating the overarching effect of dominants on site-scale species richness. This finding support the results reported by Tielbörger & Kadmon (2000b) and Soliveres et al. (2011), but also disagrees with the findings reported by Schöb et al. (2013), Soliveres et al. (2011) and Michalet et al. (2015). Our findings suggest that spatial segregation is common for herbaceous plants in this desert and that this mechanism for species coexistence is similar among the microsites (i.e. dominant plant vs. open microsites) and the micro-scales (i.e. spatial grain) studied. Mensurative experiments exploring interactions often generate lower estimates of interaction strengths (Kikvidze & Armas 2010) and removals of relatively high abundance subordinate species would be an excellent complement to this study. Nonetheless, facilitation of herbaceous plants by dominant woody species does not necessarily increase inter-specific competition within an ameliorated microhabitat in deserts.

CONCLUDING REMARKS

Our study highlights the importance of concurrent examination of spatial and temporal scales in studying positive biotic interactions to establish context dependency. Ecological theory, design, and analyses have sufficiently evolved to encompass complex effects that influence the outcome of species interactions. Micro-scales (i.e. fine spatial grain) did not have an effect on the outcome of interactions, but the strength

of positive effects changed consistently over the two years studied from neutral to positive as the growing season progressed. This expands the SGH to relatively short time spans as annuals go through ontogenic stages very rapidly and the seed banks also interact with stress through inter-annual storage processes. The effects of the two dominant species tested did not differ from one another suggesting that species-specific positive effects are likely dependent on the specific facilitation mechanism. Larger grains of spatial scale in combination with detailed seasonal surveys within the canopy communities could further refine our understanding of the scope of positive plant interactions in desert ecosystems.

ACKNOWLEDGEMENTS

This research was funded by an NSERC DG to CJL and York University FGS salary support to DAS. We also want to thank Briggeth Flores, Paola Medina, and Jessica Turpo for their help during field work; and the community of Atiquipa for allowing us the entrance to their private reserve.

REFERENCES

- Aschehoug, E. T. & Callaway R. M. 2014. Morphological variability in tree root architecture indirectly affects coexistence among competitors in the understory. *Ecology* 95: 1731-1736.
- Araújo, M. B. & Rozenfeld, A. 2014. The geographic scaling of biotic interactions. *Ecography* 37:1-10.
- Armas, C., Ordiales R. & Pugnaire, F. I. 2004. Measuring plant interactions: a new comparative index. *Ecology* 85: 2682-2686.
- Bertness, M. D. & Callaway, R. M. 1994. Positive interactions in communities. – *Trends in Ecology and Evolution* 9: 187-191.
- Biswas, S. R. & Wagner, H. H. 2014. A temporal dimension to the stress gradient hypothesis for intraspecific interactions. *Oikos* 123: 1323-1330.

- Brako, L. & Zarucchi, J. L. 1993. *Catalogue of the flowering plants and gymnosperms of Peru*. Monographs in Systematic Botany Vol. 45. Missouri Botanical Garden, St. Louis
- Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. J., Cavieres, L. A., Kunstler, G., Liancourt, P., Tielborger, K., (...) & Michalet, R. 2008. Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology* 96: 18-34.
- Bruno, J. F., Stachowicz, J. J., & Bertness, M. D. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* 18: 119-125.
- Callaway, R. M. 2007. *Positive Interactions and Interdependence in Plant Communities*. Springer, New York.
- Cavieres, L. A. & Badano, E. I. 2009. Do facilitative interactions increase species richness at the entire community level? *Journal of Ecology* 97: 1181-1191.
- Chamberlain, S. A., Bronstein, J. L. & Rudgers J. A. 2014. How context dependent are species interactions? *Ecology Letters* 17: 881-890.
- Chesson, P. 2000. Mechanisms of maintenance of species diversity. *Annual Review in Ecology and Systematics* 31:343-366.
- Chesson, P., Gebauer, R. L. E., Schwinning, S., Huntly, N., Wiegand, K., Ernest, M. S. K., Sher, A., Novoplansky, A., & Weltzin, J. F. 2004. Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. *Oecologia* 141: 236-53.
- Cipriotti, P. A. & Aguiar, M. A. 2015. Is the balance between competition and facilitation a driver of the patch dynamics in arid vegetation mosaics? *Oikos* 124: 139-149.
- Connor, E. F. & Simberloff, D. 1979. The assembly of species communities: chance or competition? *Ecology* 60: 1132-1140.
- Drezner, T.D. 2007. An analysis of winter temperature and dew point under the canopy of a common Sonoran Desert nurse and the implications for positive plant interactions. *Journal of Arid Environments*. 69: 554-568.
- Dullinger, S., Kleinbauer, I., Pauli, H., Gottfried, M., Brooker, R., Nagy, L., Theurillat, J-P., Holten, J. I, Abdaladze, O., (...) & Grabherr, G. 2007. Weak and variable relationships between environmental severity and small-scale co-occurrence in alpine plant communities. *Journal of Ecology* 95: 1284-1295.
- Flores, J. & Jurado, E. 2003. Are nurse-protégé interactions more common among plants from arid environments? *Journal of Vegetation Science* 14: 911-916.
- Franco, A. C. & Nobel, P. S. 1989. Effect of nurse plants on the microhabitat and growth of cacti. *Journal of Ecology* 77: 870-886.
- Gotelli, N. J. 2000. Null model analysis of species co-occurrence patterns. *Ecology* 81: 2606-2621.
- Gotelli, N. J. & Entsminger, G. L. 2006. EcoSim: Null Models Software for Ecology, Version 7. Acquired Intelligence Inc. and Kesity-Bear. Available at: <http://garyentsminger.com/ecosim.htm>.
- Gotelli, N. J. & Graves, G. R. 1996. *Null Models in Ecology*. Smithsonian Institution Press, Washington, DC.
- Gotelli, N. J., Graves, G. R. & Rahbek, C. 2010. Macroecological signals of species interactions in the Danish avifauna. *Proceedings of the National Academy of Sciences USA* 107: 5030-5035.
- Hartman, D. L. 1994. *Global Physical Climatology*. P 350. Academic Press, UK.

- Hastings, A. 2010. Time scales, dynamics, and ecological understanding. *Ecology* 91: 3471-3480.
- He, Q., Bertness, M. D. & Altieri, A. H. 2013. Global shifts towards positive species interactions with increasing environmental stress. *Ecology Letters* 16: 695-706.
- Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. & Jarvis, A. 2005. Very High Resolution Interpolated Climate Surfaces for Global Land Areas. *International Journal of Climatology* 25: 1965-1978.
- Jones, E. I., Bronstein, J. L. & Ferriere, R. 2012. The fundamental role of competition in the ecology and evolution of mutualisms. *Annals of the New York Academy of Science* 1256: 66-88.
- Kikvidze, Z. & Armas, C. 2010. Plant interaction indices based on experimental plant performance data. In Pugnaire, F. I. (ed.) *Positive plant interactions and community dynamics*. Pp 17-38. CRC Press, Boca Raton, USA.
- Koyama, A., Sasaki, T., Jamsran, U. & Okuro, T. 2015. Shrub cover regulates population dynamics of herbaceous plants at individual shrub scale on the Mongolian steppe. *Journal of Vegetation Science* 26: 441-451.
- Laird, R. A. & Schamp, B. S. 2006. Competitive intransitivity promotes species coexistence. *American Naturalist* 168: 182-193.
- le Roux, P. C., Shaw, J. D. & Chown, S. L. 2013. Ontogenetic shifts in plant interactions vary with environmental severity and affect population structure. *New Phytologist* 200: 241-250.
- Levin, S. A. 1992. The problem of pattern and scale in ecology: the Robert H. MacArthur Award Lecture. *Ecology* 73: 1943-1967.
- Leibold, M. A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J. M., Hoopes, M. F., Holt, R. D., Shurin, J. B., Law, R., Tilman, D., Loreau, M. & A. González, A. 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* 7: 601-613.
- Littell, R. C., Milliken, G. A., Stroup, W.W., Wolfinger, R. D. & Schabenberger, O. 2006. *SAS for Mixed Models*. SAS Institute Inc., Cary.
- Lopez, R. P., Larrea-Alcazar, D. M. & Ortuno, T. 2009. Positive effects of shrubs on herbaceous species richness across several spatial scales: evidence from the semiarid Andean subtropics. *Journal of Vegetation Science* 20: 728-734.
- Lortie, C. J. 2010. Synthetic analysis of the stress-gradient hypothesis. In Pugnaire, F. I. (ed.) *Positive Plant Interactions and Community Dynamics*. Pp 125-148. CRC Press, Boca Raton, USA.
- Lortie, C. J., Brooker, R. W., Choler, P., Kikvidze, Z., Michalet, R., Pugnaire, F. I. & Callaway, R. M. 2004. Rethinking plant community theory. *Oikos* 107: 433-438.
- Lortie, C. J. & Svenning, J-C. 2015. The diversity of diversity studies: retrospectives and future directions. *Ecography* 38: 330-334.
- Maestre, F. M., Bautista, S. & Cortina, J. 2003. Positive, negative, and net effects in grass-shrub interactions in Mediterranean semi-arid grasslands. *Ecology* 84: 3186-3197.
- McIntire, E. J. B. & Fajardo, A. 2014. Facilitation as a ubiquitous driver of biodiversity. *New Phytologist* 201: 403-416.
- Michalet, R., Brooker, R. W., Cavieres, L. A., Kikvidze, Z., Lortie, C. J., Pugnaire, F. I., Valiente-Banuet, A. & Callaway, R. M. 2006. Do biotic interactions shape both sides

- of the humped-back model of species richness in plant communities? *Ecology Letters* 9: 767-773.
- Michalet, R., Schöb, C., Lortie, C. J., Brooker, R. W. & Callaway, R. M. 2014. Partitioning net interactions among plants along altitudinal gradients to study community responses to climate change. *Functional Ecology* 28: 75-86
- Michalet, R., Brooker, R. W., Lortie, C. J., Maalouf, J-P. & Pugnaire F. I. 2015. Disentangling direct and indirect effects of a legume shrub on its understorey community. *Oikos* 124: 1251-1262.
- Miriti, M. N. 2006. Ontogenetic shift from facilitation to competition in a desert shrub. *Journal of Ecology* 94: 973-979.
- Miriti, M. N. 2007. Twenty years of changes in spatial association and community structure among desert perennials. *Ecology* 88: 1177-1190.
- Pages, J-P. & Michalet, R. 2003. A test of the indirect facilitation model in a temperate hardwood forest of the northern French Alps. *Journal of Ecology* 91: 932-940.
- Pescador, D. S., Chacón-Labela, J., de la Cruz, M. & Escudero, A. 2014. Maintaining distances with the engineer: patterns of coexistence in plant communities beyond the patch-bare dichotomy. *New Phytologist* 204: 140-148.
- Pugnaire, F. I., Hasse, P., Puigdefábregas J., Cueto, M., Clark, S. C. & Incoll, L. D. 1996. Facilitation and succession under the canopy of the leguminous shrub, *Retama sphaerocarpa*, in a semi-arid environment in south-east Spain. *Oikos* 76: 455-464.
- R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Sandel, B. 2015. Towards a taxonomy of spatial scale-dependence. *Ecography* 38: 358-369.
- SAS Institute Inc. 2015. JMP 12 Modeling and Multivariate Methods. SAS Institute Inc., Cary.
- Schiffers, K. & Tielbörger, K. 2006. Ontogenetic shifts in interactions among annual plants. *Journal of Ecology* 94: 336-341.
- Schöb, C., Armas, C. & Pugnaire, F. I. 2013. Direct and indirect interactions co-determine species composition in nurse plant systems. *Oikos* 122: 1371-1379.
- Soliveres, S., DeSoto, L., Maestre, F. T. & Olano, J. M. 2010. Spatio-temporal heterogeneity in abiotic factors modulate multiple ontogenetic shifts between competition and facilitation. *Perspectives in Plant Ecology, Evolution and Systematics* 12: 227-234.
- Soliveres, S., Eldridge, D. J., Maestre, F. T., Bowker, M. A., Tighe, M. & Escudero, A. 2011. Microhabitat amelioration and reduced competition among understorey plants as drivers of facilitation across environmental gradients: towards a unifying framework. *Perspectives in Plant Ecology, Evolution and Systematics* 13: 247-258.
- Soliveres, S. & Maestre, F. T. 2014. Plant-plant interactions, environmental gradients and plant diversity: a global synthesis of community level studies. *Perspectives in Plant Ecology, Evolution and Systematics* 16: 154-163.
- Soliveres, S., Smit, C. & Maestre F. T. 2015. Moving forward on facilitation research: response to changing environments and effects on the diversity, functioning and evolution of plant communities. *Biological Reviews* 90: 297-313.

- Sotomayor, D. A. and Jimenez, P. 2008. Condiciones meteorológicas y dinámica vegetal del ecosistema costero Lomas de Atiquipa (Caravelí-Arequipa) en el sur del Perú. *Ecología Aplicada* 7: 1-8.
- Sthultz, C. M., Gehring, C. A. & Whitham, T. G. 2007. Shifts from competition to facilitation between a foundation tree and a pioneer shrub across spatial and temporal scales in a semiarid woodland. *New Phytologist* 173: 135-145.
- Taylor, C. M. and Lorence, D. H. 1993. On the status of *Randia armata* (Sw.) DC. (Rubiaceae: Gardenieae). *Taxon* 42: 865-867.
- Tewksbury, J. J. & Lloyd, J. D. 2001. Positive interactions under nurse-plants: spatial scale, stress gradients and benefactor size. *Oecologia* 127: 425-434.
- Tielbörger, K. & Kadmon, R. 2000a. Temporal environmental variation tips the balance between facilitation and interference in desert plants. *Ecology* 81:1544-1553.
- Tielbörger, K. & Kadmon, R. 2000b. Indirect effects in a desert plant community: is competition among annuals more intense under shrub canopies? *Plant Ecology* 150: 53-63.
- Zar, J. H. 1999. *Biostatistical analysis*. Fourth Edition. Prentice Hall. USA. 663 p.

Chapter 4

Plant-plant direct and indirect interactions by dominant plant canopies

Submitted to *Oecologia* as:

Facilitation networks generate apparent competition in a South-American desert plant
community

Sotomayor, D. A. & Lortie, C. J.

ABSTRACT

Desert dominant plants commonly facilitate plant communities within their canopies. Although substantial research has examined the direct consequences of this effect, a mechanistic understanding of indirect effects mediated via beneficiary plants is still relatively limited. We tested the hypothesis that the net positive outcome of dominant plants on beneficiaries extends to a network of interactions including indirect competition or facilitation. To test this hypothesis, we aggregated two years of field surveys with a manipulative experiment in Atiquipa, Southern Peru. We surveyed the understory plant community of the dominant tree *Caesalpinia spinosa* and compared to open microsites. Field manipulations included removal of plant neighborhoods of two target annual species in the understory of *C. spinosa* and adjacent open microsites. We measured plant density in the surveys and in the removal experiment, plant height, fruit production, and biomass of the targets. In the surveys, target species density was dependent on understory neighbors' density. Neighborhood removal did not affect fruit production or biomass of *F. peruviana* but decreased its plant height in open microsites. Neighborhood removal around *P. limensis* increased its fruit production in understory microsites suggesting reduced apparent competition. Canopies had indirect negative effects on fruit production of *F. peruviana*, and an indirect competitive outcome on fruit production of *P. limensis*. Hence, understory plant neighbors can mediate the direct effects of dominant plants in deserts. The facilitative effects of dominant plants represent key coexistence mechanisms because they reduce stress and generate extended networks of interactions not necessarily present in the open.

Keywords: coexistence, indirect effects, nurse plant, positive interactions, species specificity, southern Peru.

INTRODUCTION

Interactions amongst plants are important drivers of community composition, productivity, and function (Lortie et al. 2004, McIntire and Fajardo 2014), but most research to date has explored direct interactions both negative (i.e. competition) and positive (i.e. facilitation) (Grime 1973, Michalet et al. 2006, Brooker et al. 2008, He et al. 2013, Sotomayor and Lortie 2015). However, indirect interactions have the potential to similarly influence key ecosystem properties (Callaway 2007, Brooker et al. 2008, Sotomayor and Lortie 2015). Indirect interactions occur when the effects of one species on another are mediated through a third species (Strauss 1991, Wootton 1994, Callaway 2007). These interactions occur in most multi-species assemblages and have been proposed as mechanisms that maintain species diversity by enabling coexistence (Wootton 1994, Callaway 2007, Sotomayor and Lortie 2015). The outcome of interactions is determined by the net sum of direct and indirect effects, and even weak interactions have been shown to magnify spatiotemporal change (Berlow 1999, Schöb et al. 2013). Interaction effects correspond to quantifiable vectors whereas outcomes represent the product of such vectors (Michalet et al. 2015a, b). Indirect negative effects can sometimes cancel out direct positive effects. This is likely important when species are aggregated by overarching positive effects such as under shrubs or trees in deserts (Flores and Jurado 2003, Filazzola and Lortie 2014). Indirect effects thus comprise a key component of interaction outcomes within communities and networks. This is

particularly important given the influence of ongoing global change on stressors within ecosystems in addition to species loss (Valiente-Banuet et al. 2015).

In arid environments, dominant plants commonly facilitate the presence of communities of understory plant species (i.e. the nurse plant effect) (Franco and Nobel 1989, Flores and Jurado 2003, Filazzola and Lortie 2014). Although substantial attention has been given to the direct consequences of these interactions in terms of species richness and abundance/biomass, the potential impact of facilitation on the outcome amongst understory species (i.e. indirect effects) have remained relatively under-explored (Tielbörger and Kadmon 2000, Pages and Michalet 2003, Brooker et al. 2008, Schöb et al. 2013). Net community facilitation has been documented but interactions within these protégé species also likely change and should be examined. Facilitation generates a clumped spatial pattern of understory species under dominant plants. This can lead to increases in the intensity of competition or apparent competition due to competition for resources in microsites within this limited area (Tielbörger and Kadmon 2000, Soliveres et al. 2011). Hence, in a system with several understory species, the net outcome of the dominant plant on the understory species can also be mediated to some extent by the understory itself (Golberg and Landa 1991, Levine 1999, Callaway and Pennings 2000). The increase in the intensity of competition amongst understory species can also promote indirect facilitation because less competitive species would be released from competition and able to persist in these complexes (Tielbörger and Kadmon 2000, Schöb et al. 2013). However, indirect facilitation is not only the outcome of interactions amongst a network of competitors as

negative indirect effects such as competition enhancement due to concurrent negative effects of competitors on a target species can also occur (Xiao and Michalet 2013). Hence, the outcome of the net interaction between direct and indirect effects strongly determines local plant species richness within deserts in addition to the direct effects of abiotic limitation and stressors.

Evidence to date suggests that the role of herbaceous neighbors in mediating indirect effects in dominant plant-understory species is equivocal. Manipulative and mensurative studies have sometimes found that shrubs do facilitate other plant species directly but that competition amongst the facilitated species does not increase in comparison to non-facilitated microsites (Tielbörger and Kadmon 2000, Soliveres et al. 2011). Shrubs also have been reported to increase competition amongst understory species and, consequently, promote indirect facilitation (Pugnaire et al. 1996, Pages and Michalet 2003, Schöb et al. 2013, Poulos et al. 2014) however it is also likely that these interactions have not been examined in sufficient detail. It has been suggested that context-dependency could account for these seemingly opposing roles of dominant plants (Soliveres et al. 2011, Michalet et al. 2015a), however a mechanistic understanding of this process is still in its development and requires further investigation (Callaway 2007, Michalet et al. 2015a). Moreover, the relative importance of a shifting balance between direct and indirect interactions within the understory/facilitated species has only recently become a topic of study (Schöb et al. 2013, Michalet et al. 2015a, b). Another key component in determining outcomes can be the life-history strategy of the subordinate plant species because fast growing annuals can respond strongly to

competition within canopies but more conservative stress-tolerant species may not (Rolhauser and Pucheta 2015). Overall, the effect of dominant plants on understory communities is likely context-dependent and effects are specific to the associated species. Measuring context and tracking species-specific responses to indirect interactions will begin to provide more robust estimates of the relative importance of indirect effects in stressful environments.

The purpose of this study was to experimentally examine the direct and indirect effects of dominant woody species on abundant annual plant species that occur in both open and canopied microsites using a combination of surveys and full neighborhood removal experiments. We propose that the facilitative outcome by dominant woody plants can be further decomposed into apparent facilitation and competition between the beneficiary species. The following predictions were tested to examine the role of direct facilitation and its extended effects: (1) the density of target species is concurrently influenced by herbaceous neighbors density effects and the tree canopy within understory microsites; (2) the intensity of negative interactions amongst non-manipulated annual plants is higher under dominant species because plant growth is favored by the relatively benign conditions leading to increased inter-specific annual interference (apparent competition); and that (3) the removal of herbaceous neighbors will increase the estimated relative growth and reproductive output of target annual species due to reduced apparent competition under canopies. Understanding the importance of dominant plants in mediating interactions in their understory will inform

estimates of coexistence mechanisms in ecosystems characterized by high stress conditions.

METHODS

Study site and species

Atiquipa in Southern Peru (15°S , 74°W) is a coastal desert with a wet winter season between July and November typically characterized by high moisture due to fog (about 90% on average). Approximately 70% of the annual rainfall mean (i.e. 200 mm) occurs between these months (Sotomayor and Jimenez 2008). Mean annual rainfall in 2011 was 167.3 mm and in 2012 was 137.4 mm. This was recorded with a Vantage Pro2 weather station installed locally ($15^{\circ}46'$, $74^{\circ}23'$). The plant community includes a few dominant woody species and a diversity of herbaceous plants wherein annuals are the most frequent life form. We conducted a survey along this desert in order to select five sites for experiments. This survey systematically sampled 1-km grids using 20 x 20 m quadrats to determine plant cover and species composition within each of these grids. These data were then combined with climate information from the database WorldClim (Hijmans et al. 2005) and used to quantitatively determine the sites to be used for this study. By combining ground-truthed data with climate parameters (e.g. temperature, precipitation, and seasonality) and with multivariate statistics, we were able to appropriately determine the complex environmental gradients within our field site (Lortie 2010) and sampled along these. Five sites (100 x 100 m each) were selected using this procedure and were separated by at least 2 km from one another (Appendix 9). These five sites belong to a private reserve that limits access by cattle and other

large herbivores. These sites also corresponded to a natural elevation gradient regionally and were chosen to represent the largest amount of environmental variability within this desert.

We chose one dominant perennial woody species for this study that was present in all five sites. This dominant plant species was the locally abundant 4-5 m tall tree *Caesalpinia spinosa* Molina (Kuntze) (Fabaceae). This tree has a relatively high understory of about 2 m, 4-5 m canopy diameter, and it is native to Peru, but can also be found in various countries of South America such as Bolivia, Colombia, Ecuador and Venezuela tolerating dry climates and poor soils (Brako and Zarucchi 1993).

Vegetation surveys and target understory species

During the two growing seasons of 2011 and 2012, plant density was surveyed at the following two paired microsites: 50 1x1 m plots located in the understory of *C. spinosa* and 50 1x1 m plots in open nearby spaces. Surveys were conducted at the peak of the growing season of each year at Atiquipa, Southern Peru. These plots were distributed along the 5 sites with 10 replicates in each site per microsite (i.e. understory and open). The abundance for each species within the plots (microsite) was recorded and was used to calculate plant species density. Two locally abundant understory species that occurred in both microsites were selected for further experimentation using these surveys. These understory species were the annuals *Fuertesimalva peruviana* (L.) Fryxell (Malvaceae) and *Plantago limensis* Pers. (Plantaginaceae). *F. peruviana* is a fast-growing erect herb with palmate leaves, that produces 100-200 flowers and is

distributed in Peru, Chile, and Bolivia; while *P. limensis* is a rosette-like herb with linear leaves, that produces 50-150 flowers and is endemic to Peru (Brako and Zarucchi 1993).

The strength of the *C. spinosa* effect on target species plant density was estimated using the Relative Interaction Index (RII) calculated as follows (Armas et al. 2004):

$$\text{RII} = \frac{D_u - D_o}{D_u + D_o} \quad (1)$$

The terms D_u and D_o corresponded to the density of plants in understory and open microsites, respectively. This index varies from -1 to +1 with positive effects being > 0 and negative effects < 0 on the density of these species.

Neighborhood removal experiment

A neighborhood removal experiment was conducted adapting the methodology from Tielbörger and Kadmon (2000) and Callaway and Pennings (2000). To examine the intensity of direct and indirect effects on the net outcome for understory plant communities, we utilized a fully-crossed factorial design, with microsite (i.e. under the canopy of *C. spinosa* and open), and removal treatment (i.e. neighborhood intact and neighborhood removed) as factors. Herbaceous plant neighbors within a radius of 30 cm of 2-3 individuals of each target species were removed in the “neighborhood removed” treatment plots. Each pair of treatments per microsite (i.e. neighborhood intact, neighborhood removed) was located in nearby sites, with the canopy treatments

located in different individual trees of *C. spinosa*. Treatment location was randomly selected, with 10 replicates per treatment in each of the 5 sites selected, totaling 40 experimental units per species in each site. At the peak of the growing season of both years (2011 and 2012) we measured the height and fruit production of each plant in this experimental system. At the end of the growing season of 2012 those entire plants were harvested, dried in an oven, and weighed using a Metler Toledo MX5 scale (0.1 μ g precision).

To estimate the strength of direct and indirect effects on the target species, we calculated the Relative Interaction Index (RII) as follows (Armas et al. 2004):

$$\text{RII} = \frac{R_{N+} - R_{N-}}{R_{N+} + R_{N-}} \quad (2)$$

The terms R_{N+} and R_{N-} corresponded to the response variable (i.e. plant height, fruit production and biomass) of the target species with the presence of a canopy neighbor only, presence of understory neighbors only, or both (N+) and with this neighbor absent or removed (N-). This index varies from -1 to +1 with positive effects being > 0 and negative effects < 0 on the response variable of the target species. This allowed us to calculate the following three RIIs: (1) RII_{DN} that is the direct interaction of herbaceous neighbors with the targets in the absence of the dominant plant; (2) RII_{DC} that is the direct interaction of dominant plants with the targets in the absence of herbaceous neighbors; and (3) RII_{IC} that is the indirect interaction of canopies with the target species mediated by herbaceous understory neighbors respectively (*sensu* Michalet et al. 2015b).

Michalet et al. (2015b) differentiated between indirect effects and indirect outcomes. Indirect effects occur when the interaction between dominant plants and target species is significantly altered by the presence of herbaceous neighbors (*sensu* Wootton 1994); this occurs when the indirect interaction index ($R_{II_{IC}}$) is significantly different from the direct interaction index ($R_{II_{DC}}$). When $R_{II_{IC}}$ is significantly higher than $R_{II_{DC}}$ the indirect effect is positive, and when $R_{II_{IC}}$ is significantly lower than $R_{II_{DC}}$ the indirect effect is negative. Indirect outcomes can be conceptualized in four cases (Table 1). Indirect facilitation (1) and additional additional facilitation (2) occur when both the indirect effect and the $R_{II_{IC}}$ are positive. In the former instance, the $R_{II_{DC}}$ is negative or neutral, whereas in the latter, the $R_{II_{DC}}$ is positive. Indirect competition (3) and additional competition (4) occur when both the indirect effect and the $R_{II_{IC}}$ are significantly negative. Indirect competition occurs when the $R_{II_{DC}}$ is positive or neutral, and additional competition occurs when the $R_{II_{DC}}$ is already significantly negative.

Table 1. Summary of the four possible indirect outcomes based on direct and indirect interaction indices. Greater-than or lower-than symbols indicate significant differences that are considered to determine the respective indirect outcome. Adapted from Michalet et al. (2015a, b).

Indirect effect	Direct RII (RII_{DC})		Indirect RII (RII_{IC})	Indirect outcome
Positive	Negative/Neutral	<	Positive	Indirect facilitation
Positive	Positive	<	Positive	Additional facilitation
Negative	Positive/Neutral	>	Negative	Indirect competition
Negative	Negative	>	Negative	Additional competition

Statistical analyses

All variables were fitted to probability distributions and tested for homogeneity of variances (Zar 1999, Zuur et al. 2010). The best probability distribution fit for all variables was exponential as estimated by Akaike Information Criterion (Appendix 10). Generalized linear mixed models (GLMMs) with a reciprocal link function were subsequently used to examine effects (McCulloch et al. 2008, SAS Institute Inc. 2015). Plant density estimates for the target species, other non-target species, and total species present in the quadrats across both years were analyzed separately using GLMMs to compare differences between microsites (under *C. spinosa* and open spaces) with site as a random effect. An additional variable was used to denote the temporal structure nested within microsites as a random effect (repeated measures effect), and year was treated as a fixed factor. To estimate if there was an effect of other

non-target species on the target species density from surveys, we used GLMMs with site as random effect and compared the parameter estimates between microsites of non-target species density for the significant relationships. The removal experiment was analyzed with fully orthogonal GLMMs using target species (*F. peruviana* and *P. limensis*), microsite (under *C. spinosa* and open spaces), and treatment (neighborhood intact and neighborhood removed) as main effects for all variables separately: fruit production, plant height and biomass. Site was included in the models as a random effect. For fruit production and plant height, an additional variable denoting the temporal structure associated with different years was nested within treatments as a random effect (repeated measures effect) and year was included as a fixed effect (McCulloch et al. 2008, SAS Institute Inc. 2015). Three-way interaction terms in these models were not included because the sample sizes needed to examine multilevel models were not sufficient (Maas and Hox 2006). Post-hoc comparisons were conducted using Chi-squared tests (Littell et al. 2006, SAS Institute Inc. 2015). All RIIs ($RII_{\text{plant density}}$, RII_{DN} , RII_{DC} , and RII_{IC} of plant height, number of fruits and biomass) were analyzed using one sample t-tests to test that there were significantly different from zero. All RIIs from the removal experiment (i.e. RII_{DN} , RII_{DC} , RII_{IC}) were compared per target species and response variable using one-way ANOVAs (Michalet et al. 2015b). Indirect outcomes were assigned using the conceptual framework developed herein (Table 1).

RESULTS

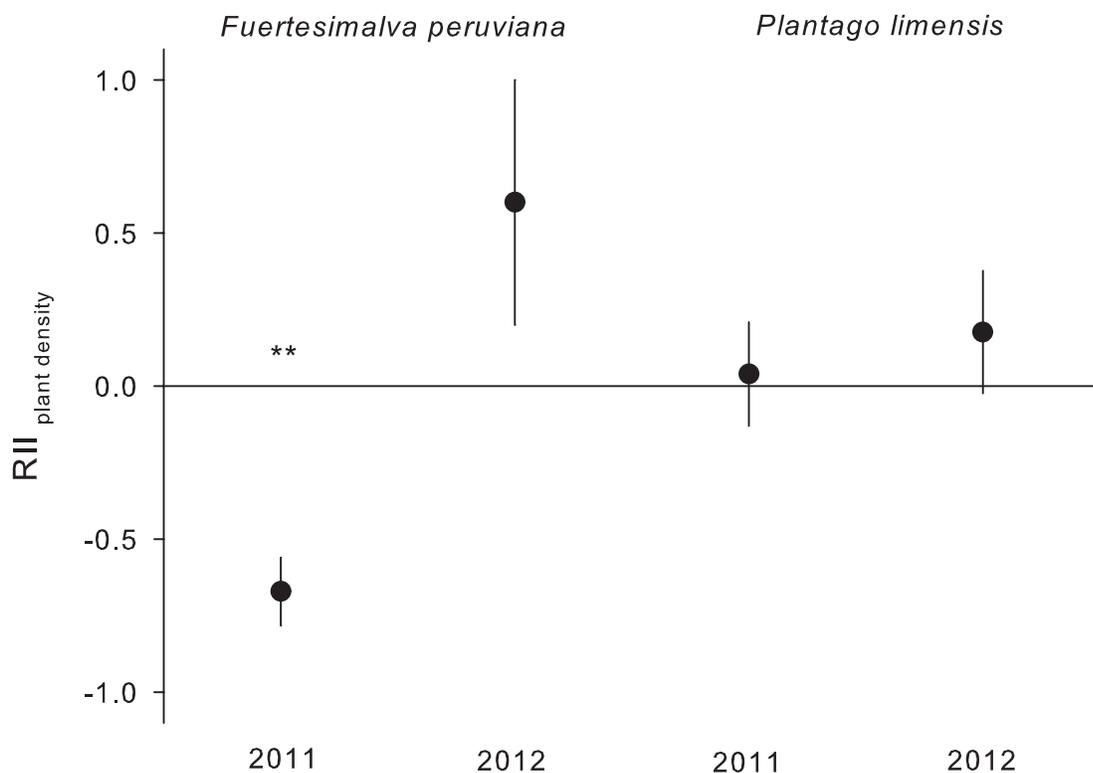
Surveys of plant density

We found significant differences in plant density of *F. peruviana*, *P. limensis*, other non-target species, and total plant density between microsites and between years (Table 2). During 2011, *F. peruviana* was negatively associated with *C. spinosa* whilst in 2012 this species was neutrally associated with that dominant plant (Figure 1). In both years, *P. limensis* was neutrally associated with the dominant plant (Figure 1). In both years, the density of *F. peruviana* was positively related with the density of its neighbors in understory microsites. Only in 2012 this relationship contrasted with that of open microsites (Table 3). The density of *P. limensis* was negatively related with the density of its neighbors in understory microsites in both years. This density correlation was positive in the open microsites only in 2012 (Table 3).

Table 3. Summary of parameter estimates (slope) from GLMMs examining the effect of neighborhood density on the density of two target species under shrubs and in the open. Density is number of plants per m⁻². Observed probability functions were exponential. Bolded values denote significance at $P < 0.05$.

Target species	Canopy			Open		
	Parameter			Parameter		
	estimate	Chi-square	<i>P-value</i>	estimate	Chi-square	<i>P-value</i>
2011						
<i>P. limensis</i>	-0.000079	7.153	0.0075	-0.000039	0.089	0.7653
<i>F. peruviana</i>	0.003883	15.809	<0.0001	0.000178	5.598	0.0180
2012						
<i>P. limensis</i>	-0.000403	4.688	0.0304	-0.001611	11.712	0.0006
<i>F. peruviana</i>	0.117373	46.376	<0.0001	-0.075261	3.354	0.0671

Fig. 1 Relative interaction indices (RII) \pm 1SE for plant density of the target annual species from censuses at the peak of the growing season in Atiquipa, southern Peru. Stars indicate significantly different from zero RII values obtained from one sample t-tests (* $P < 0.05$, ** $P < 0.01$)



Neighborhood removal experiment

Plant height, fruit production, and biomass were significantly influenced by the removal treatment (Table 4). Target plant height was significantly reduced due to neighborhood removal for *F. peruviana* in both years for open microsites (2011: Chi-squared = 7.09, $p < 0.05$; 2012: Chi-squared = 8.45, $p < 0.05$; Figure 2). *P. limensis* displayed the greatest effects following the removal treatments (Figure 2): (1) plant height was significantly different in both years and in open microhabitats after

neighborhood removal, shorter plants in 2011 (Chi-squared = 8.09, $p < 0.05$), and taller plants in 2012 (Chi-squared = 6.17, $p < 0.05$ for 2012); (2) increased fruit production per plant after neighborhood removal in understory microsites in both years (Chi-squared = 4.43, $p < 0.05$ for 2011, Chi-squared = 6.33, $p < 0.05$ for 2012; Figure 2); and finally (3) significantly greater biomass after neighborhood removal in open microhabitats in 2012 (Chi-squared = 10.05, $p < 0.05$).

In 2012, *F. peruviana* plant height was positively affected by direct interactions with canopies ($R_{II_{DC}}$) (Figure 3). Fruit production of *F. peruviana* was also favored by positive direct canopy interactions ($R_{II_{DC}}$), and there was an indirect negative effect because $R_{II_{IC}}$ was significantly lower than $R_{II_{DC}}$ (Figure 3, Table 5). In both years, the plant height of *P. limensis* was favored by positive indirect canopy interactions ($R_{II_{IC}}$). The fruit production of *P. limensis* in 2011 was not affected by direct canopy interactions ($R_{II_{DC}}$), but negatively affected by indirect canopy interactions ($R_{II_{IC}}$). This resulted in indirect competition mediated via plant neighbors (Table 5). Finally, the biomass of *P. limensis* was not impacted by direct canopy interactions ($R_{II_{DC}}$), although indirect canopy interactions were negative ($R_{II_{IC}}$, Figure 3).

Table 4. Summary of GLMMs for plant height, fruit production and biomass following a neighborhood removal treatment in Atiquipa, Southern Peru. Entire herbaceous neighborhoods were removed at two microsites: under *C. spinosa* and open adjacent microsites. Bolded values denote significance at $P < 0.05$.

Effect	Number of fruits/plant (d. f. = 379)		Plant height (d. f. = 390)		Biomass (d. f. = 257)	
	Chi-square	<i>P</i> -value	Chi-square	<i>P</i> -value	Chi-square	<i>P</i> -value
Year	4.03	0.0447	17.11	<0.0001	--	--
Species	1.9	0.1678	1.45	0.2282	37.25	<0.0001
Microsite	3.99	0.0455	0.02	0.8762	8.63	0.0033
Removal	5.77	0.0163	14.62	0.0001	6.71	0.0096
Microsite*Removal	0.89	0.3455	2.59	0.1072	2.89	0.0892
Species*Microsite	2.63	0.1047	0.01	0.901	0.07	0.7931
Species*Removal	1.06	0.3041	0.00	0.9707	6.13	0.0133
Year*Species*Microsite* Removal	0.03	0.8614	0.16	0.6869	--	--

Table 5. Summary of indirect interaction effects and outcomes in a desert tree-understory assemblage tested using herbaceous neighborhood removals under the canopy and in open. The four possible indirect outcomes are (see Table 1 and Methods for details): indirect facilitation, additional facilitation, indirect competition, and additional competition.

Target species	Measure	2011		2012	
		Indirect effect	Indirect outcome	Indirect effect	Indirect Outcome
<i>F. peruviana</i>	Plant height	--	--	--	--
	Fruit production	Negative	--	--	--
	Biomass			--	--
<i>P. limensis</i>	Plant height	--	--	--	--
	Fruit production	Negative	Indirect competition	--	--
	Biomass			--	--

Fig. 2 Plant height (cm) + 1SE, fruit production (number of fruits. plant⁻¹) + 1SE, and final biomass (g) + 1SE of two target annual species (*Fuertesimalva peruviana* and *Plantago limensis*) after an herbaceous neighborhood removal experiment (N+ = neighborhood intact, N- = neighborhood removed) conducted during two years in Atiquipa, southern Peru. Stars (*) indicate significant differences between removal treatments in a microhabitat ($P < 0.05$)

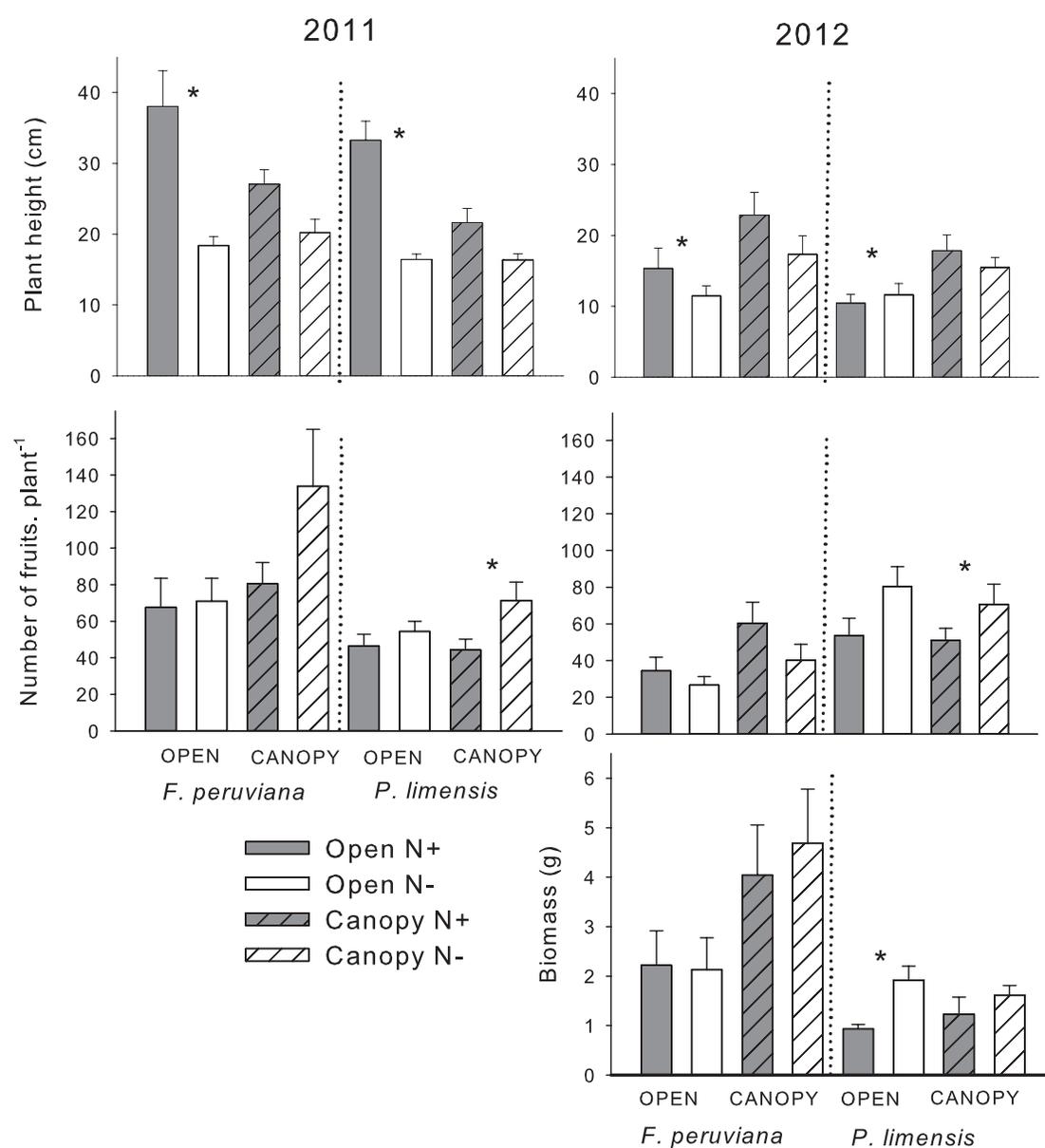
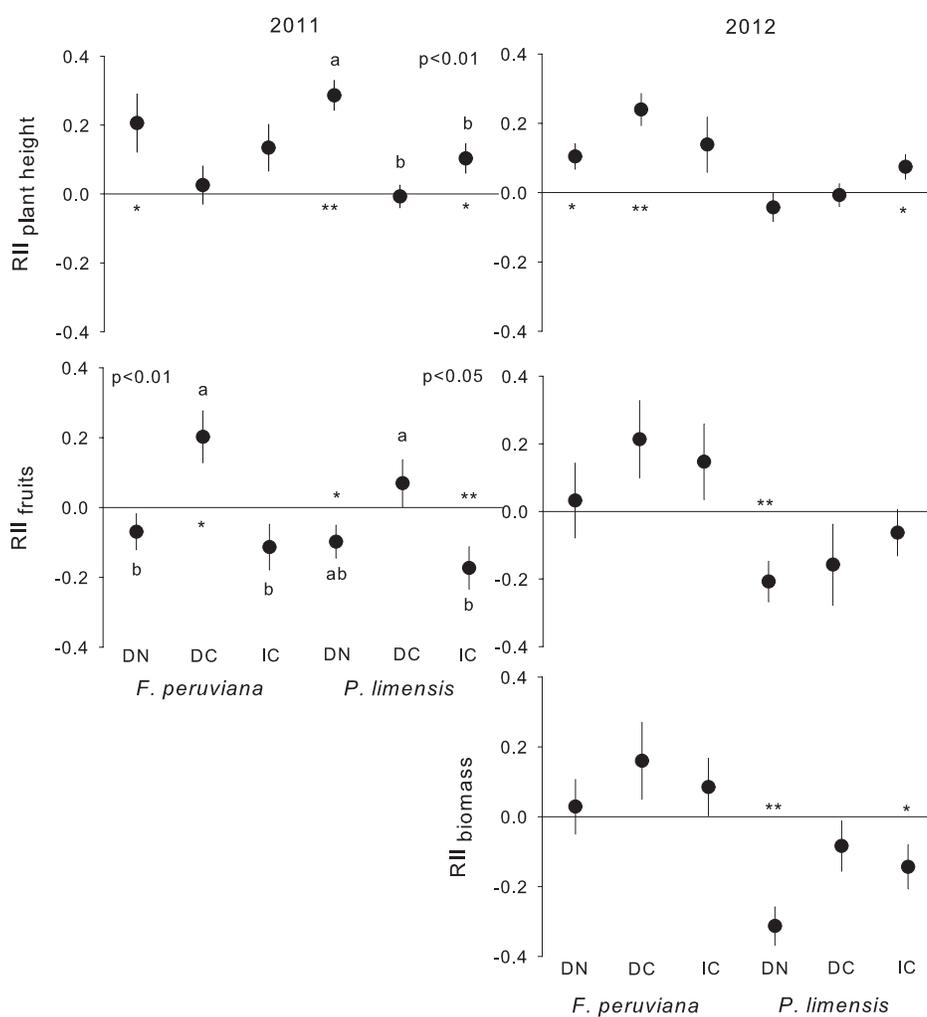


Fig. 3 Relative interaction indices (RII) \pm 1SE for plant height, number of fruits. plant⁻¹ (fruits), and final biomass of two target annual species (*Fuertesimalva peruviana* and *Plantago limensis*) after an herbaceous neighborhood removal experiment conducted during two years in Atiquipa, southern Peru. DN is the direct interaction of the neighbors, DC the direct interaction of the canopy, and IC is the indirect interaction of the canopy mediated by herbaceous neighbors. Stars indicate significantly different from zero RII values obtained from one sample t-tests (* $P < 0.05$, ** $P < 0.01$). P -values on each graph indicate the result of one-way ANOVAs comparing RIIs for each species, with significantly different RIIs not sharing the same letter.



DISCUSSION

Understanding the outcome of the effects of dominant plants on understory communities remains a challenge in community ecology because of the capacity for extended and unique effects within dominant-herbaceous assemblages. Apparent or indirect competition amongst understory species can be just as important as direct facilitation in determining their performance which has important theoretical and applied consequences for coexistence in stressful ecosystems (Chesson et al. 2004, Cuesta et al. 2010, Schöb et al. 2013) as well as their management (Gomez-Aparicio 2009, Caldeira et al. 2014). In surveys, both target species were generally detected evenly in the open and under woody dominants but were influenced by the abundance of other beneficiary plant species. This suggests that apparent interactions within understories mediate and change the sign of net outcomes for at least these two target species tested. Neighborhood removal and microsite had separate effects on plant height, fruit production, and biomass for both target species showing that plant neighborhoods had both direct effects on the targets and also mediated canopy effects on the targets. The removal of plant neighbors of *P. limensis* resulted in increased fruit production in understory microsites which suggests that removal reduced apparent competition in understory microsites for this particular species. Conversely, the removal of plants neighbors of *F. peruviana* did not have any effect on fruit production or final biomass, but decreased plant height in open microhabitats indicating no significant competition for this species in canopied microsites. Relative interaction indices (RIIs) suggest direct and indirect interactions were most frequent for the target species *P. limensis*. Integration of interaction indices and effects detected indirect competition only for the

target *P. limensis* suggesting that this species is more sensitive to plant-plant interactions relative to the other target tested. Hence, apparent competition can occur under the canopy of dominant benefactor species but is likely species specific. Importantly, these results provide evidence that plant neighbors in dominant plant-understory systems can mediate indirect effects for target species but are dependent on the specific species and traits examined.

Our main hypothesis in this study was that competition in the understory of dominant woody plants would be increased due to the positive effects brought by dominant plants in arid environments. We detected indirect competition mediated by understory neighbors for the target species *P. limensis*. An increase in competitive interactions in understory plant communities of arid environments has been reported previously (Pugnaire et al. 1996, Schöb et al. 2013, Poulos et al. 2014), and it is likely due to the increased density of plants within these microsites. Conversely, neighborhood-mediated effects of dominant plants did not influence the target species *F. peruviana*. A similar pattern has also been reported previously for this trend too (Tielbörger and Kadmon 2000, Soliveres et al. 2011). Stronger positive direct effects of plant canopies or increased niche segregation can weaken possible indirect effects and likely best explain this trend (Soliveres et al. 2011, Michalet et al. 2015). Our findings illustrate that indirect effects of dominant plants on understory species are thus species-specific. The different life-history strategies of the target species can be an explanation or possible tool to examine these differences (Pages and Michalet 2006, Rolhauser and Pucheta 2015). *F. peruviana*, the least affected species, is a fast-growing annual plant

thereby acting as a ruderal species (*sensu* Grime 1979) whereas *P. limensis* is as a more competitive annual species with a slower growth rate and a rosette-like architecture. Theory predicts that competitive species would be more affected by biotic interactions (Rolhauser and Pucheta 2015). These life-history classifications in addition to plant function group classifications (Pages and Michalet 2006, Michalet et al. 2015a) can serve as predictive tools for further studies of indirect effects within understory communities. Overall, these findings add to a growing body of research demonstrating that direct positive interactions are composed by both direct and indirect effects on the facilitated species, but we have also added the novel and likely critical finding that these effects are species-specific.

In this study, we combined field surveys and manipulations under field conditions and detected different outcomes associated with the performance response and species measured. These approaches tested different levels of organization. Field density surveys examine both community and population-level processes, and field removals test species-specific responses to the community. This is also a methodological distinction as the reported outcomes from different methods can vary significantly (Kikvidze and Armas 2010, Gomez-Aparicio 2012, Schöb et al. 2012). In our study, *P. limensis* was evenly associated with the dominant plant and open microsites. It showed consistent results between the two methodologies with negative neighborhood effects in both experiments. The second target species, *F. peruviana*, was also evenly associated with the dominant plant and open but showed positive associations in density with neighborhood density in the survey and generally no significant effects of neighborhood

removal. These differences between methodologies can be due to the fact that each approach would be measuring a different aspect of the interaction between plants and a different level of community organization (Dunne et al. 2004, Schöb et al. 2012). For instance, fruit production measurements after removal treatments would be integrating the final outcome of interactions of individual-based effects, or the net effect, whereas regression analyses on survey data at the peak of the growing season would provide a snapshot of the physiological status at a population level effect due to interactions. Moreover, measurements directly related to plant fitness, such as fruit production, would be yielding different results than those reflecting demographic processes, such as plant density (Tielbörger and Kadmon 2000, Lortie et al. 2016). Although the mensurative approach is more amenable for field studies, experimental manipulations, such as removals, provide a more accurate depiction of the effects among species (Díaz et al. 2003), and hence a combination of both should be used to better inform ecological theory and its applications. These two methodologies would explore different dynamics, with the mensurative approach capturing variation within the community more effectively and removal treatments capturing the effects of loss within the community.

Competition and facilitation are concurrently acting between neighboring plants in plant communities (Wright et al. 2014), and indirect effects within canopies can be as important as direct positive effects. The finding that dominant plants exert direct canopy effects and indirect neighborhood effects has important implications for coexistence under extreme conditions. As dominant plants can indirectly compete with some abundant understory species (i.e. apparent competition with *P. limensis*), this effect

could result in competitive release for less abundant species, which would promote resource partitioning as a mechanism of coexistence in this arid plant community (Tilman 1982, Chesson et al. 2004, Scheffer and van Nes 2006). Increased competition within understories likely acts as a mechanism promoting two-phase vegetation mosaics (i.e. understory and open microsites), because these microsites can sometimes regulate the emergence of dominant plants via direct and indirect effects in combination with other spatiotemporally variable mechanisms such as seed trapping or rainfall (Cipriotti et al. 2014, Cipriotti and Aguiar 2015). Importantly, understory microsites in stressful environments can open doors to invasions through facilitation and biotic acceptance (Flory and Bauer 2014, Badano et al. 2015), but indirect effects can filter and reduce invasions through biotic resistance associated with the subordinate community. Overall, this study underscores the importance of plant-plant indirect interactions for species coexistence in environments of stressful conditions, and that these interactions are composed by a series of direct and indirect effects. Global change will most certainly also influence indirect interactions by altering physiologies and competitive hierarchies (Brooker 2005, Valiente-Banuet et al. 2015).

ACKNOWLEDGEMENTS

This research was funded by salary and fieldwork financial support from the Faculty of Graduate Studies at York University to DAS and by a Discovery Grant of the National Sciences and Engineering Research Council of Canada to CJL. We also want to thank Italo Revilla, Briggeth Flores, Paola Medina, and Jessica Turpo for their help during fieldwork; and the community of Atiquipa for allowing us the entrance to their

private reserve. The suggestions of two anonymous reviewers and the subject Editor greatly improved the quality of our manuscript.

REFERENCES

- Armas C, Ordiales R, Pugnaire FI (2004) Measuring plant interactions: a new comparative index. *Ecology* 85:2682-2686
- Badano EI, Bustamante RO, Villarreal E, Marquet PA, Cavieres LA (2015) Facilitation by nurse plants regulates community invasibility in harsh environments. *J Veg Sci* 26:756-767
- Brako L, Zarucchi JL (1993) Catalogue of the flowering plants and gymnosperms of Peru. Monographs in Systematic Botany Vol. 45. Missouri Botanical Garden, St. Louis
- Brooker RW (2005) Plant-plant interactions and environmental change. *New Phytol* 171:271-284
- Brooker RW, Maestre FT, Callaway RM, Lortie CJ, Cavieres LA, Kunstler G, Liancourt P, Tielborger K, Travis MJM, Anthelme F, Armas C, Coll L, Corcket E, Delzon S, Forey E, Kikvidze Z, Olofsson J, Pugnaire FI, Quiroz CL, Saccone P, Schiffrers K, Seifan M, Touzard B, Michalet R (2008) Facilitation in plant communities: the past, the present, and the future. *J Ecol* 96:18-34
- Caldeira MC, Ibáñez I, Nogueira C, Bugalho MN, Lecomte X, Moreira A, Pereira JS (2014) Direct and indirect effects of tree canopy facilitation in the recruitment of Mediterranean oaks. *J Appl Ecol* 51:349-358
- Callaway RM (2007) Positive Interactions and Interdependence in Plant Communities. Springer, New York
- Callaway RM, Pennings SC (2000) Facilitation may buffer competitive effects: indirect and diffuse interactions among salt marsh plants. *Am Nat* 156:416-424
- Chesson P, Gebauer RLE, Schwinning S, Huntly N, Wiegand K, Ernest MSK, Sher A, Novoplansky A, Weltzin JF (2004) Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. *Oecologia* 141:236-253
- Cipriotti PA, Aguiar Ma, Wiegand T, Paruelo JM (2014) A complex network of interactions controls coexistence and relative abundances in Patagonian grass-shrub steppes. *J Ecol* 102:776-788
- Cipriotti PA, Aguiar MA (2015) Is the balance between competition and facilitation a driver of the patch dynamics in arid vegetation mosaics? *Oikos* 124:139-149
- Cuesta B, Villar-Salvador P, Puértolas J, Rey-Benayas JM, Michalet R (2010) Facilitation of *Quercus ilex* in Mediterranean shrubland is explained by both direct and indirect interactions mediated by herbs. *J Ecol* 98:687-696
- Díaz S, Symstad AJ, Chapin III FS, Wardle DA, Huenneke LF (2003) Functional diversity revealed by removal experiments. *Trends Ecol Evol* 18:140-146
- Dunne JA, Saleska SR, Fischer ML, Harte J (2004) Integrating experimental and gradient methods in ecological climate change research. *Ecology* 85:904-916

- Filazzola A, Lortie CJ (2014) A systematic review and conceptual framework for the mechanistic pathways of nurse plants. *Glob Ecol Biogeogr* 23:1335-1345
- Flores J, Jurado E (2003) Are nurse-protégé interactions more common among plants from arid environments? *J Veg Sci* 14:911-916
- Flory SL, Bauer JT (2014) Experimental evidence for indirect facilitation among invasive plants. *J Ecol* 102:12-18
- Franco AC, Nobel PS (1989) Effect of nurse plants on the microhabitat and growth of cacti. *J Ecol* 77:870-886
- Golberg DE, Landa K (1991) Competitive effect and response: hierarchies and correlated traits in the early stages of competition. *J Ecol* 79:1013-1030.
- Gomez-Aparicio L (2009) The role of plant interactions in the restoration of degraded systems: a meta-analysis across life-forms and ecosystems. *J Ecol* 97:1202-1214
- Grime JP (1973) Competitive exclusion in herbaceous vegetation. *Nature* 242:344-347
- Grime JP (1979) *Plant Strategies and Vegetation Processes*. John Wiley & Sons, Chichester
- He Q, Bertness MD, Altieri AH (2013) Global shifts towards positive species interactions with increasing environmental stress. *Ecol Lett* 16:695-706
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* 25:1965-1978
- Kikvidze Z, Armas C (2010) Plant interaction indices based on experimental plant performance data. In Pugnaire FI (ed.) *Positive plant interactions and community dynamics*. CRC Press, Boca Raton, pp 17-38
- Levine JM (1999) Indirect facilitation: evidence and predictions from a riparian community. *Ecology* 80:1762-1769
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O (2006) *SAS for mixed models*. SAS Institute Inc., Cary
- Lortie CJ, Brooker RW, Choler P, Kikvidze Z, Michalet R, Pugnaire FI, Callaway RM (2004) Rethinking plant community theory. *Oikos* 107:433-438
- Lortie CJ (2010) *Synthetic analysis of the stress-gradient hypothesis. Positive Plant Interactions and Community Dynamics*. C.R.C. Press, USA.
- Lortie CJ, Filazzola A, Welham C, Turkington R (2016) A cost benefit model for plant-plant interactions: a density series tool to detect facilitation. *Plant Ecol* doi: 10.1007/s11258-016-0604-y
- Maas CJM, Hox JJ (2005) Sufficient sample sizes for multilevel modeling. *Methodology* 1:86-92
- McCulloch CE, Searle SR, Neuhaus JM (2008) *Generalized, Linear, and Mixed Models*. Second Edition. Wiley. USA. 424 pp.
- McIntire EJB, Fajardo A (2014) Facilitation as a ubiquitous driver of diversity. *New Phytol* 201:403-416
- Michalet R, Brooker RW, Cavieres LA, Kikvidze Z, Lortie CJ, Pugnaire FI, Valiente-Banuet A, Callaway RM (2006) Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? *Ecol Lett* 9:767-773
- Michalet R, Brooker RW, Lortie CJ, Pugnaire FI (2015a) Disentangling direct and indirect canopy and soil effects of a legume shrub on its understory forb community. *Oikos* 124:1251-1262

- Michalet R, Chen S-Y, An L-Z, Wang X-T, Wang Y-X, Guo P, Ding C-C, Xiao S (2015b) Communities: are they groups of hidden interactions? *J Veg Sci* 26:207-218
- Pages J-P, Michalet R (2003) A test of the indirect facilitation model in a temperate hardwood forest of the northern French Alps. *J Ecol* 91:932-940
- Pages J-P, Michalet R (2006) Contrasted responses of two understorey species to direct and indirect effects of a canopy gap. *Plant Ecol* 187:179-187
- Poulos JM, Rayburn AP, Schupp EW (2014) Simultaneous, independent, and additive effects of shrub facilitation and understory competition on the survival of a native forb (*Penstemon palmeri*). *Plant Ecol* 215:417-426
- Pugnaire FI, Haase P, Puigdefábregas, Cueto M, Clark SC, Incoll LD (1996) Facilitation and succession under the canopy of the leguminous shrub, *Retama sphaerocarpa*, in a semi-arid environment in south-east Spain. *Oikos* 76:455-464
- Rolhauser AG, Pucheta E (2015) Annual plant functional traits explain shrub facilitation in a desert community. *J Veg Sci* in press doi:10.1111/jvs.12335
- SAS Institute Inc. (2015) JMP 12 Fitting Linear Models. SAS Institute Inc., Cary
- Scheffer M, van Nes EH (2006) Self-organized similarity, the evolutionary emergence of groups of similar species. *Proc Natl Acad Sci USA* 103:6230-6235
- Schöb C, Kammer PM, Kikvidze Z (2012) Combining observational and experimental methods in plant-plant interaction research. *Plant Ecol Divers* 5:27-36
- Schöb C, Armas C, Pugnaire FI (2013) Direct and indirect interactions co-determine species composition in nurse plant systems. *Oikos* 122:1371-1379
- Soliveres S, Eldridge DJ, Maestre FT, Bowker MA, Tighe M, Escudero A (2011) Microhabitat amelioration and reduced competition among understorey plants as drivers of facilitation across environmental gradients: towards a unifying framework. *Persp Plant Ecol Evol Syst* 13:247-258
- Sotomayor DA, Jimenez P (2008) Condiciones meteorológicas y dinámica vegetal del ecosistema costero Lomas de Atiquipa (Caravelí-Arequipa) en el sur del Perú. *Ecología Aplicada* 7:1-8
- Sotomayor DA, Lortie CJ (2015) Indirect interactions in terrestrial plant communities: emerging patterns and research gaps. *Ecosphere* 6: art 103
- Strauss SY (1991) Indirect effects in community ecology - their definition, study and importance. *Trends Ecol Evol* 6:206-210
- Tielbörger K, Kadmon R (2000) Indirect effects in a desert plant community: is competition among annuals more intense under shrub canopies? *Plant Ecol* 150:53-63
- Tilman D (1982) Resource competition and community structure. Princeton University Press, New Jersey
- Valiente-Banuet A, Aizen MA, Alcantara JM, Arroyo J, Cocucci A, Galetti M, Garcia MB, Garcia D, Gomez JM, Jordano P, Medel R, Navarro L, Obeso JR, Oviedo R, Ramirez N, Rey PJ, Traveset A, Verdú M, Zamora R (2015) Beyond species loss: the extinction of ecological interactions in a changing world. *Funct Ecol* 29:299-307
- Wootton JT (1994) The nature and consequences of indirect effects in ecological communities. *Annu Rev Ecol Syst* 25:443-466
- Wright A, Schnitzer SA, Reich PB (2014) Living close to your neighbors: the importance of both competition and facilitation in plant communities. *Ecology* 95:2213-2223

Xiao S, Michalet R (2013) Do indirect interactions always contribute to net indirect facilitation? *Ecol Model* 268:1-8

Zar JH (1999) *Biostatistical analysis*. Fourth Edition. Prentice Hall. USA. 663 p.

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. *Methods Ecol Evol* 1:3-14

Chapter 5

Dominant plant effects on ecotypic differentiation

Published as:

Sotomayor, D. A., Lortie, C. J. & Lamarque L. J. 2014. Nurse-plant effects on the seed biology and germination of desert annuals. *Austral Ecology* 39: 786-794.

ABSTRACT

Nurse-plants generally have positive effects on understorey species by creating more suitable conditions for stress intolerant plants relative to open micro-habitats. However, long-term effects of this plant-plant facilitation system have been rarely examined. Seeds of five desert annual species from Atiquipa coastal desert in Southern Peru were used to examine whether different microenvironmental conditions under the nurse-plants *Caesalpinia spinosa* Molina (Kuntze) lead to differences in seed biology and germinability of annual plants relative to open, canopy-free conditions. Seeds collected from plants associated with nurse-plants were predicted to be (i) larger due to more favourable growing conditions, (ii) more viable and with greater germination rates, (iii) less variable in size and viability due to reduced environmental heterogeneity, and (iv) to germinate faster to avoid apparent competition with other annuals. Seed attribute measurements and germination trials in growth chambers were used to test these predictions. Although the plant abundance of only 2 of 5 species was strongly facilitated by the nurse-plant, no significant differences were found in seed mass, viability, or relative variability between understorey and open micro-habitats for any of the species. Contrary to our predictions, final seed germination rates of seeds from open micro-habitats were higher, and the open micro-habitat treatment was more favourable for germination of seeds from both open and understorey environments. Taken together, these results suggest that plant-plant facilitation does not necessarily affect seed biology traits. Further studies addressing larger distribution ranges and/or density gradients of understorey species will illuminate the potential evolutionary effects of nurse-plants.

Keywords: differentiation, ecotypes, facilitation, germination, nurse-plant, positive effects, seeds, viability.

INTRODUCTION

Consequences of positive effects in plant communities (i.e. facilitation) have been widely demonstrated and incorporated into general ecological theory (Bruno *et al.* 2003; Callaway 2007; Brooker *et al.* 2008). Facilitation effects on the frequency of occurrence of species have been shown to occur in several ecosystems, although most frequently in stressful environments such as deserts (Bertness & Callaway 1994; Flores & Jurado 2003; Holzapfel *et al.* 2006). Nurse-plants have been championed by ecologists as clear examples of positive interactions in stressful environments (Maestre *et al.* 2003; Gomez-Aparicio *et al.* 2004). Effects of nurse-plants have usually been described in terms of an increase in abundance and/or diversity of understory plants compared to neighboring open environments, i.e. away from the nurses (Pugnaire *et al.* 1996; Holzapfel *et al.* 2006). Nurse-plants generate more benign habitats that allow stress intolerant species to persist under extreme environmental conditions (Liancourt *et al.* 2005; Maestre *et al.* 2009). Facilitation at local scales is likely more frequent in ecosystems subject to significant perturbation or abiotic limitation (Kéfi *et al.* 2008). Local facilitation depends on low dispersal ability of the target species, and this is a trait generally reported for desert annuals (Ellner & Shmida 1981; Venable *et al.* 2008; Ward 2009). Venable *et al.* (2008) demonstrated that the mean dispersal distances for these species are on average relatively small at less than one metre. Wilson (1993) showed

that the mean dispersal distance was 0.92 m for herbaceous species with morphological adaptations for wind dispersal, and 0.49 m for herbaceous species with no apparent dispersal mechanism. Moreover, nurse-plants usually act as seed traps and barriers for the dispersal of understorey species (Bullock & Moy 2004; Giladi *et al.* 2013). Low dispersal ability in stressful environments could potentially lead to ecotypic differentiation between plants from understorey and open micro-habitats. This hypothesis has however remained unexamined to date (but see Liancourt & Tielbörger 2011). Moreover, increased abundance of plants under nurse-plants can also lead to increased competition (or apparent competition), although evidence of this is equivocal to date (Tielbörger & Kadmon 2000; Seifan *et al.* 2010). Increased competition among understorey plant species associated with nurse-plants (i.e. apparent competition mediated by the nurse-plant by increasing negative effects among understorey plant species competing for abiotic resources) could in theory induce accelerated adaptive germination (Dyer *et al.* 2000; Goldberg *et al.* 2001) to avoid such competitive effects. Ecotypic differentiation and local adaptation following nurse-plant facilitation are therefore two important evolutionary processes that may occur in harsh environments. They nonetheless represent critical research gaps in plant interaction ecology (Callaway 2007; Brooker *et al.* 2008; Thorpe *et al.* 2011), and a close examination of changes in life-history traits of understorey annual plants is a pertinent starting point to address them.

Desert plant species cope with harsh conditions by avoiding them (i.e. annuals) or enduring them (i.e. shrubs) (Whitford 2002; Ward 2009). For annuals, seed

production represents their only link between one generation and the next, and seed germination is also critical (Pake & Venable 1996; Facelli *et al.* 2005). Germination of annuals has to be finely tuned with environmental cues to ensure that germinating plants will produce new seeds for the species to persist (Noy-Meir 1973; Venable 2007). To this end, many strategies have been described such as dormancy to remain latent until appropriate environmental conditions arise (Baskin & Baskin 2001), bet-hedging in which plants delay short-term germination in favour of long-term fitness increases (Venable & Lawlor 1980), or age structuring of seed banks (Chesson 2000; Adonkakis & Venable 2004). However, in the context of nurse-plants, little is known on whether the positive effects experienced by plants in the understory translate into increased seed germination capabilities due to improved micro-environmental conditions, and whether these traits would be evolutionary stable (Thorpe *et al.* 2011). Maternal effects expressed in seeds traits are commonly studied and well established (Roach & Wulff 1987; Galloway 2005; Donohue 2009). Increased size (Sultan 1996; Valencia-Díaz & Montaña 2005), germination fraction and viability (Baskin & Baskin 2001; Valencia-Díaz & Montaña 2005; Breen & Richards 2008) of seeds have been reported as consequences of the maternal plant experiences including environmental conditions. However, few studies track the effects of facilitation to the seeds (but see Liancourt & Tielbörger 2011), even for more evident facilitation consequences on seeds such as seed traps (Pugnaire & Lazaro 2000). Given that nurse-plants commonly facilitate annuals and that the seed bank is a critical tool for persistence, nurse-plant effects on seeds thus represent a critical question to explore longer-lasting consequences of plant facilitation such as ecotypic differentiation.

The purpose of this study was to determine the effects of nurse plants on seed biology and germination of understorey annual plant species relative to the same species growing in open micro-habitats. We hypothesized that facilitation by nurse-plants generates sufficiently different micro-environmental conditions that lead to consistent differences in seeds traits of understorey plants. We explored the following predictions to test this hypothesis: seeds collected from plants associated with the nurse-plant micro-habitat would (i) be larger due to more favourable growing conditions, (ii) have greater viability and germination rate (iii) have less variability in size and viability due to reduced environmental heterogeneity provided by nurses (buffering), and (iv) germinate faster due to potential apparent competition with other annuals. Whilst many studies have examined and documented the importance of nurse plant-plant interactions for understorey plant diversity and abundance (Callaway 2007; Brooker *et al.* 2008), few of them have explored the potential evolutionary implications for beneficiary species by examining other life-history stages such as seed viability and germination (but see Liancourt & Tielbörger 2011). Reciprocal common gardens are an important approach to study trait sets under sets of conditions that a species may be associated with, particularly when the habitats are very discrete (Hufford & Mazer 2003; Maron *et al.* 2004). A smaller-scale version of this approach is applied here using a reciprocal germination design in growth chambers programmed to emulate each set of conditions from field measurements (open versus understorey). This design identifies whether there is preliminary evidence for ecotypic differentiation in seed traits driven by nurse-plant facilitation.

METHODS

Study site and species

Seeds of 5 annual species were collected from Atiquipa, Southern Peru (15°S, 74°W). Atiquipa is a coastal desert with a wet winter season occurring between July and November and characterized by high moisture due to fog (about 90%) and with about 70% of the annual rainfall mean (e.g. 200 mm) falling between those months (Sotomayor & Jimenez 2008). One of the nurse-plant species of this location is the locally abundant 4-5m tall tree *Caesalpinia spinosa* Molina (Kuntze) (Fabaceae). This tree is native to Peru, where is abundant, but it can also be found in various places of South America such as Bolivia, Colombia, Ecuador and Venezuela tolerating dry climates and poor soils (Sprague 1931). The five understory annual species used in this study were selected because of their relatively high abundance and their contrasting relative patterns of abundance from high to low densities in the understory of nurse-plants. They were *Alonsoa meridionalis* (L. f.) Kuntze (Scrophulariaceae), *Cyperus hermaphroditus* (Jacq.) Standl. (Cyperaceae), *Fuertesimalva peruviana* (L.) Fryxell (Malvaceae), *Nassella mucronata* (Kunth) R.W. Pohl (Poaceae), and *Plantago limensis* Pers (Plantaginaceae). Both *Cyperus hermaphroditus* and *Nasella mucronata* correspond to grass-like species that grow up to 30-40cm tall and produce 80-150 seeds per plant. Meanwhile, *Alonsoa meridionalis* and *Fuertisimalva peruviana* can grow up to the size of little shrubs (about 80cm tall) and produce thousands of seeds from their numerous flowers (especially for *Alonsoa meridionalis*). *Plantago limensis* is a small annual rosette plant that grows up to 20-30 cm and produces 40-70 seeds per

individual. All 5 species are distributed along the Andes from 0 to about 4000 m asl in South America, although *Plantago limensis* is endemic to Peru (Brako & Zarucchi 1993).

Entire plants including their seeds were collected in December 2012 (end of spring/start of summer) across five sites separated by about 2 km within Atiquipa, right after seed set and plant senescence. Both understorey (i.e. under nurse-plant) and open micro-habitats were sampled in each site, where plants were harvested in pairs with one individual from understorey and one from open micro-habitat. Pairs of plants were carefully selected to span the whole species distribution range in each location, and were therefore at least 5 m apart to ensure the collection of different meta-populations considering average dispersal distances reported in the literature (see Wilson 1993; Venable *et al.* 2008). At least five pairs of plants (i.e. replicates) were collected in each site for each species, for a total of 125 pairs, i.e. 250 plants.

Plant density

Plant density of each species in each micro-habitat was recorded at the peak of the 2012 growing season (October) using 0.25 m² quadrats (n = 90): 9 quadrats per micro-habitat type paired across the five locations used for seed collection. The strength of nurse-plant effect on each understorey plant species density was reported via the Relative Interaction Index (RII), which was calculated as follows (Armas *et al.* 2004):

$$\text{RII} = \frac{D_u - D_o}{D_u + D_o} \quad (1)$$

The terms D_u and D_o corresponded to the density of plants in understorey and open micro-habitats, respectively. This index varies from -1 to +1 with positive effects being > 0 and negative effects < 0 on the density of these species.

Seed mass and viability

Because of their small size, seeds of all species were weighed in groups of 10 using a Metler Toledo MX5 scale (0.1 μg precision). A total of 200 seeds were weighed per micro-habitat per species. Seed viability was assessed via tetrazolium (Tz) tests, a valid method for both dormant and non-dormant embryos (Flemion & Poole 1948; Baskin & Baskin 2001). Embryos were dissected from 24-hour water-imbibed seeds, and then placed in a 1% solution of 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) for another 24 h before evaluation. They were considered viable when they had turned red or pink due to the reaction between TTC and hydrogen ions released by embryos during respiration (Baskin & Baskin 2001). This test was carried out on 25 randomly selected seeds per replicate, with 4 replicates per micro-habitat per species (200 seeds per species, 100 per micro-habitat).

Seed germination trials

Germination trials were conducted in growth chambers whose conditions simulated both open and understorey micro-habitats. Micro-habitat conditions were measured *in situ* using HOBO U-23 Pro-V2 loggers for temperature and humidity (two in each micro-habitat, understorey of *Caesalpinia spinosa*), and HOBO UA-002-64 loggers for light intensity and temperature (one in each micro-habitat). Loggers were installed in

the field site on August 2011, and recorded micro-habitat conditions during the whole growing season, i.e. until mid-October 2011. Data from the loggers were aggregated to obtain 2-hour means for temperature, relative humidity, and light intensity in order to program the growth chambers simulating our field site conditions (Table 1). Mean temperature and relative humidity were best simulated in the growth chambers, however light conditions, especially for the open maximum, did diverge from field site conditions (Table 1). Overall, the growth chambers were able to effectively simulate the general patterns of relative differences between open and understorey micro-habitats, i.e. warmer, less humid and with increased illuminance towards the middle of the day for open micro-habitats.

The growth chamber experiment was run in six growth chambers (Sanyo MLR-351H, Japan), with three of them simulating understorey micro-habitat conditions, and three simulating open space micro-habitat conditions. We utilized a full-factorial reciprocal design using 10 seeds per replicate and the following factors: 2 germination environments (open, understorey), 2 seed sources (open, understorey), and 5 species, with 10 replicates per treatment. This was a total of 200 experimental units tested. Seed germination was recorded every 2-3 days until no further changes were observed for at least one week (after 27 days in total). A seed was considered germinated when the radicle or coleoptile was visible by 1 to 2 mm.

Table 1. Micro-habitat data for open (O) and understory (U) conditions obtained from field observations at Atiquipa, Southern Peru. Field values are presented \pm 1SE, and values utilized to program the growth chambers are between brackets.

Hour of the day	Temperature ($^{\circ}$ C)		Relative humidity (%)		Illuminance (lx)*	
	O	U	O	U	O	U
0-4	12.5 \pm 0.1 (12.5)	12.2 \pm 0.1 (12.2)	84.5 \pm 0.6 (85)	86.1 \pm 0.4 (86)	0.0 \pm 0 (0)	0.0 \pm 0 (0)
4-8	11.8 \pm 0.1 (11.8)	11.7 \pm 0.1 (11.7)	85.1 \pm 0.6 (85)	86.6 \pm 0.4 (87)	93.9 \pm 10.5 (0)	68.1 \pm 20.8 (0)
8-10	12.5 \pm 0.1 (12.5)	12.6 \pm 0.2 (12.6)	86.0 \pm 0.9 (86)	87.1 \pm 0.7 (87)	2860.7 \pm 150.7 (2)	6402.3 \pm 1048.7 (3)
10-12	15.0 \pm 0.2 (15.0)	14.2 \pm 0.2 (14.2)	84.1 \pm 0.9 (84)	87.3 \pm 0.7 (87)	10862.0 \pm 594.9 (4)	4005.6 \pm 458.5 (2)
12-14	22.4 \pm 0.3 (22.4)	15.2 \pm 0.2 (15.2)	76.9 \pm 0.9 (77)	86.7 \pm 0.7 (87)	54855.2 \pm 2412.4 (5)	1247.3 \pm 30.6 (1)
14-16	24.9 \pm 0.4 (24.9)	15.1 \pm 0.2 (15.1)	72.2 \pm 1.0 (72)	86.2 \pm 0.7 (86)	59898.1 \pm 2629.5 (5)	866.5 \pm 19.2 (1)
16-18	20.8 \pm 0.3 (20.8)	14.5 \pm 0.2 (14.5)	75.4 \pm 1.0 (75)	85.7 \pm 0.7 (86)	19444.1 \pm 1120.2 (4)	373.5 \pm 17.0 (1)
18-20	15.3 \pm 0.2 (15.3)	13.3 \pm 0.1 (13.3)	82.7 \pm 0.9 (83)	86.0 \pm 0.6 (86)	993.9 \pm 128.3 (1)	27.3 \pm 2.7 (0)
20-24	13.4 \pm 0.1 (13.4)	12.6 \pm 0.1 (12.6)	85.1 \pm 0.6 (85)	86.5 \pm 0.4 (87)	0.0 \pm 0 (0)	0.0 \pm 0 (0)

* Illuminance in the growth chambers could only be programmed using "light steps"

(LS), corresponding to the number of light bulbs active at each step within the chamber.

At 0 LS no bulb is active (0 lx); 1 LS, 1 bulb on (~1800 lx); 2 LS, 2 bulbs on (~3400 lx); 3

LS, 3 bulbs on (~5000 lx); 4 LS, 9 bulbs on (~15000 lx); and 5 LS, 15 bulbs on or all

available (~22000 lx).

Statistical analyses

Generalized linear mixed models (GLMM) were used to test for differences in plant density, seed mass and seed viability among species and seed sources, including the species x source interaction. In order to test for differences in relative scaled variability between sources, we calculated coefficients of variation (CVs) for seed mass and seed viability estimates, and compared those using t-tests with species as replicates. Germination trial responses were condensed into final germination rate and number of days to 50% germination. These variables were also analyzed with GLMMs, using simulated micro-habitat, seed source, species and interaction terms as fixed factors. Seed mass was included as a covariate given that it can have an effect on germination (Maranon & Grubb 1993; Leishman & Westoby 1994). Pair-wise post-hoc comparisons were done using Chi-square tests (Littell *et al.* 2006; SAS Institute Inc. 2012). The post-hoc p-values were corrected for multiple comparisons using the false discovery rate procedure (Benjamini & Hochberg 1995). To document the strength of reciprocal effects of the simulated micro-habitat conditions we calculated RII using Equation 1 (Armas *et al.* 2004), wherein D_u corresponded to the condition when the simulated micro-habitat matched the seed source, and D_o corresponded to the condition when seed germination was assessed on the reciprocal simulated micro-habitat. The effect of the simulated micro-habitat on the germination of seeds collected from both micro-habitats was considered positive when $RII > 0$, and negative when $RII < 0$.

RESULTS

Plant density

Micro-habitats and species identity significantly influenced plant density estimates in the field (Fig. 1, Table 2, Appendix 11). *Cyperus hermaphroditus* and *Nassella mucronata* were more abundant in the understorey benefiting from strong facilitative effects by the nurse-plant *Caesalpinia spinosa*. However, *Alonsoa meridionalis* and *Fuertesilmalva peruviana* were more abundant in open micro-habitats whilst the abundance of *Plantago limensis* did not significantly differ between micro-habitats.

Seed mass and viability

There were no significant differences in seed mass between understorey and open micro-habitats, but there were significant differences among species (Table 2, Appendix 12). The percentage of viable seeds was not significantly different between micro-habitats or among species (Table 2, Appendix 12). There were no significant differences in the coefficients of variation for seed mass and seed viability between collection sources (seed mass: $t_8 = 0.7927$, $p = 0.4508$, seed viability: $t_8 = 0.1584$, $p = 0.8781$).

Table 2. Summary of GLMMs of plant density, seed mass and viability for five annual species found in both understorey and open micro-habitats (i.e. seed sources). Chi-square values are presented along with the corresponding p-values. Bolded values indicate statistically significant differences ($p < 0.05$).

Effect	Plant density (DF=440)		Seed mass (DF=190)		Seed viability (DF=30)	
	Chi-square	p-value	Chi-square	p-value	Chi-square	p-value
	Species	2223.62	<0.0001	97.25	<0.0001	4.78
Source	1514.66	<0.0001	0.05	0.8291	0.00	0.9756
Species*Source	2171.96	<0.0001	0.06	0.9996	0.05	0.9997

Table 3. Summary of GLMMs of seed germination under controlled conditions (growth chambers). Micro-habitat refers to the chamber conditions, while source corresponds to the micro-habitat where seeds were collected. Chi-square values are presented along with corresponding p-values. Bolded values indicate statistically significant differences ($p < 0.05$).

Effect	DF	Germination rate		Days to 50% germination	
		Chi-square	p-value	Chi-square	p-value
Seed mass	1	4.19	0.0405	0.39	0.5349
Micro-habitat	1	14.69	0.0001	1.01	0.3171
Source	1	12.63	0.0004	0.35	0.556
Species	4	116.74	<.0001	15.36	0.004
Micro-habitat*Source	1	6.28	0.0122	0.02	0.8885
Micro-habitat*Species	4	48.17	<.0001	2.88	0.5784
Source*Species	4	21.16	0.0003	0.29	0.9903
Micro-habitat*Source*Species	4	19.57	0.0006	0.31	0.9892

Table 4. Summary of post hoc chi-square contrasts for seed germination of five annual species. False discovery rate corrected p-values are presented. Combinations of seed source (open (O) and understorey (U)) and simulated micro-habitat (open (O) and understorey (U)) were contrasted with their respective reciprocal treatment. Bolded values indicate statistically significant values ($p < 0.05$) along with the corresponding treatment with the higher final germination rate.

Contrast	<i>Alonsoa meridionalis</i>	<i>Cyperus hermaphroditus</i>	<i>Fuertesimalva peruviana</i>	<i>Nassella mucronata</i>	<i>Plantago limensis</i>
By source					
O-O vs. O-U ¹	0.4592	0.0765	0.7850	0.7850	0.9731
U-U vs. U-O	0.2714	U-O 0.0420	0.2714	0.7850	0.4814
By simulated micro-habitat					
O-O vs. O-U ²	0.7850	0.2714	0.7850	0.2714	0.3963
U-U vs. U-O	0.3819	0.0600	0.2714	0.2714	0.7850
Home vs. away					
O-O vs. U-U	0.2714	O-O 0.0420	0.3819	0.8265	0.3819
O-U vs. U-O	0.7423	0.2714	0.9962	0.3819	0.2714

¹ By source, O-O vs O-U: seeds that germinated under open micro-habitat conditions and were collected in open micro-habitat vs. seeds that germinated understorey micro-habitat conditions and were collected in open micro-habitat.

² By micro-habitat, O-O vs O-U: seeds that germinated under open micro-habitat conditions and were collected in open micro-habitat vs. seeds that germinated under open micro-habitat conditions and were collected in understorey micro-habitat.

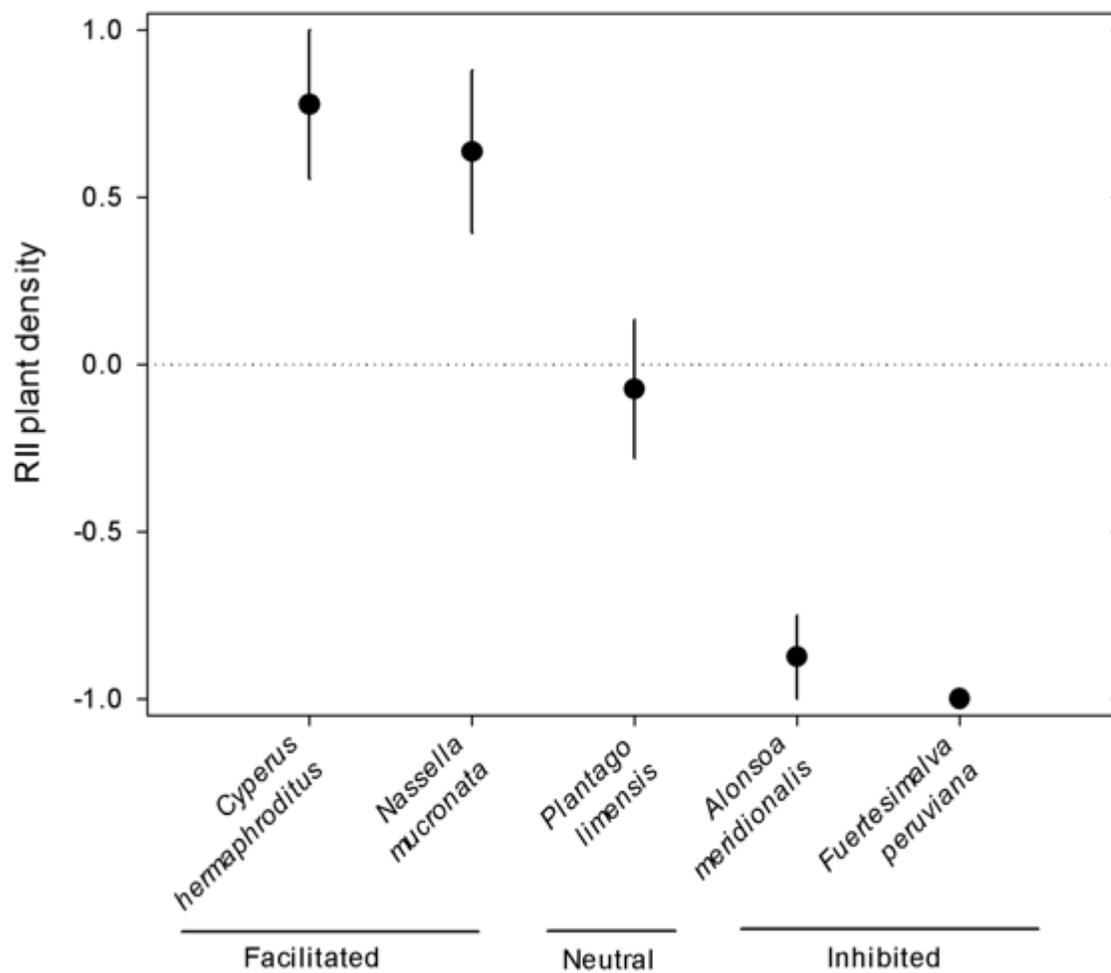


Fig 1. Relative interaction indices for plant density of five annual plant species growing in open and understorey micro-habitats in Atiquipa, Southern Peru. Means and standard errors are shown.

Seed germination

A total of 605 seeds germinated out of the 2000 seeds used in the experiment, i.e. 30.25% overall germination rate. However, germination rates significantly differed

among species (Table 3), although not between *Cyperus hermaphroditus* and *Fuertesimalva peruviana* (Chi-square = 2.4902, $p = 0.1146$). Total germination rates were 42.50% for *Plantago limensis*, 30.50% for *Nasella mucronata*, 18.25% for *Cyperus hermaphroditus* and 6.75% for *Fuertesimalva peruviana*. In addition, germination was significantly higher for seeds collected in open micro-habitats (i.e. significant source effect), and for all seeds regardless of source germinating in simulated open micro-habitat conditions (i.e. significant micro-habitat effect; Fig. 2a, Table 3). However, this result was mainly driven by *Cyperus hermaphroditus*, as effects of seed source and micro-habitat conditions did not differ for the other species (Table 4, Appendix 13). The number of days to 50% germination significantly differed among species (Fig. 2b, Table 3, Appendix 14), but there was no significant difference between sources or simulated micro-habitat conditions (Table 3).

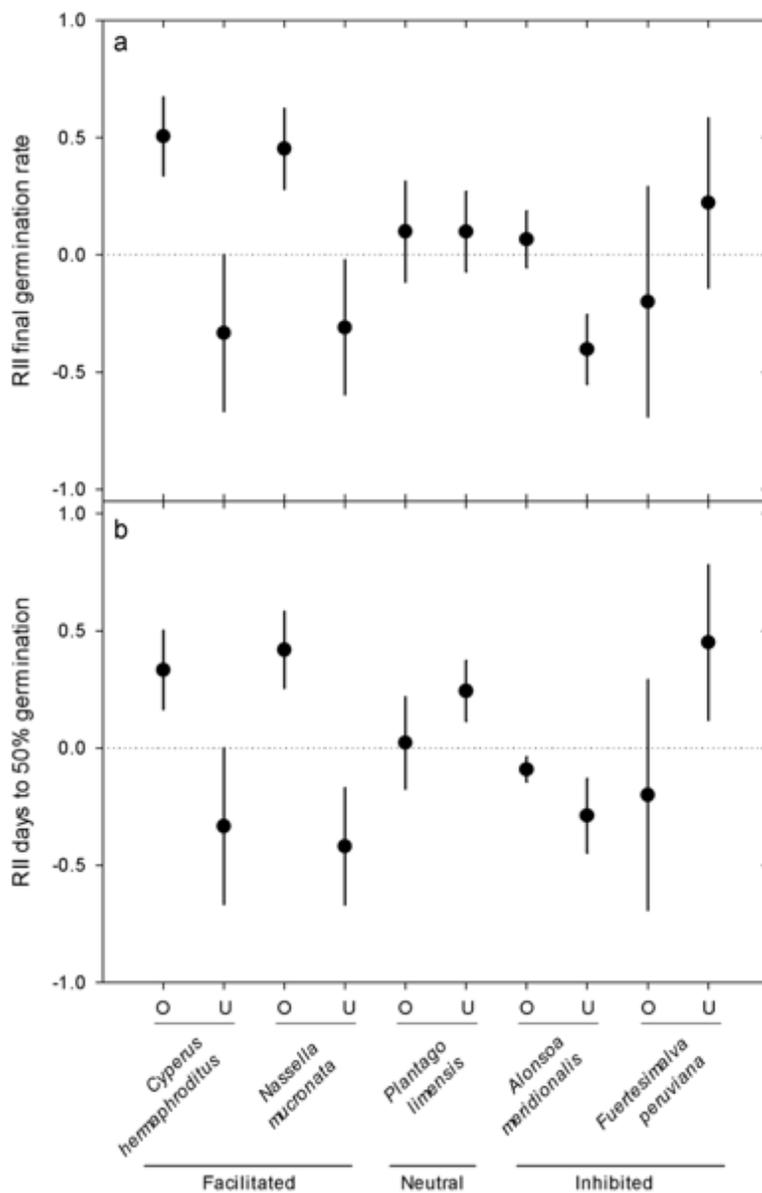


Fig 2. Relative interaction indices (RII \pm SE) for seed germination of five annual plant species collected in open and understorey micro-habitats. Seeds from both sources were germinated under simulated open and understorey conditions in growth chambers. (a) Final germination rates. (b) Numbers of days required to 50% germination.

DISCUSSION

We predicted that plant-plant facilitation would have positive effects on the seed biology and germination rates of understorey species. However, there was no evidence in these seed biology analyses or germination trials of plant facilitation effects on the seed life-stage trait set. Admittedly, only 2 of the 5 species experienced facilitation by nurse-plants at the plant life-stage and this necessarily limits the scope of our findings. Nonetheless, germination was significantly higher for seeds collected in open micro-habitats and most importantly for all species germinating in simulated open micro-habitats including the species that were facilitated by the nurse plant. Furthermore, these general differences were mainly driven by the annual species, *Cyperus hermaphroditus*, the most strongly facilitated species at the plant life-stage we studied, suggesting that plant facilitation does not shape seed biology. In addition, there was no evidence for reduced variability in seed size or viability, and germination was not accelerated for seeds from under nurse-plants. Even with potential maternal effects likely included in the seeds (Luzuriaga *et al.* 2005), no effects of nurse-plants were found on the net outcome of the measured traits. Overall, these results indicate that the effects of the improved micro-habitat conditions generated by nurse-plants had no consistent effects on the seed biology and germinability of understorey species, and do not thus support the hypothesis that relatively more fixed traits from an evolutionary perspective diverge between individuals of a population partitioned between understorey and open microsites. Selection processes associated with nurse-plant effects on annuals may be greater for vegetative traits.

Our study focused on the effects of nurse-plants, including both strongly facilitated and strongly inhibited plant species. The lack of differences in the traits measured suggests that seed biology traits are very conservative (Moles *et al.* 2005), or that they are controlled by a combination of complex genotype-environment relationships not entirely addressed in our experiment (Finch-Savage & Leubner-Metzger 2006). They also suggest that even while dispersal ability of understorey species is low (Venable *et al.* 2008; Giladi *et al.* 2013), ecotypic differentiation does not occur in this system. Local adaptation is not favoured in stressful conditions and plasticity is a more dominant strategy that allows desert plants to cope with highly unpredictable environmental conditions (Sultan & Spencer 2002, Chesson *et al.* 2004). Conversely, Liancourt & Tielbörger (2011) demonstrated local adaptation for plants from arid environments by subjecting individuals of the same species from Mediterranean conditions to arid field conditions. The contradiction in findings between studies may be related to the length of the gradients under study in that plants may adapt to a variety of conditions via plasticity of traits at local scales (this study), but they develop local adaptations when larger scales/gradients are considered (Liancourt & Tielbörger 2011). Although seed life-stages are critical for annuals, our study presents no evidence for consistent differences in performance between micro-habitats under controlled experimental conditions. Annual species that have more divergent population distributions with less gene flow may also be needed for nurse-plants to generate detectable selection processes, i.e. for species that are relatively rare in a system with a net balance toward nurse-plant associations.

We also predicted that the potential selective pressure of increased competition driven by higher plant densities under nurse-plants would lead to adaptive acceleration in germination (Dyer *et al.* 2000, Tielbörger & Kadmon 2000). We did not find evidence to this effect. One explanation is that the strength of apparent competition in the understory was insufficient to act as a selective process on seed biology characteristics. Generally, no effect of apparent competition on the demographic responses of understory plants has been demonstrated in other arid ecosystem studies (Tielbörger & Kadmon 2000, Soliveres *et al.* 2011) suggesting that either apparent competition is too infrequent or too weak in these contrasts. Alternatively, the net positive effect of shrubs could also neutralize changes in germination rates associated with avoiding annual plant-plant competition. In this sense, lack of response on seed biology traits could be related to their conservative nature (Moles *et al.* 2005), but could also be due to stabilizing selection generated by counter-directional interactions in a nurse-plant-annual system. A more powerful test of these predictions would be to either sample or generate annual plant density gradients to increase the likelihood that indirect effects are present/persistent enough to impact micro-evolutionary processes.

Germination was favoured for seeds collected in open micro-habitats, and most importantly, for all seeds regardless of species identity germinating under simulated open micro-habitats. This is a compelling finding and an opportunity to reconsider the context-dependency of nurse-plant effects generally assumed in the facilitation literature (Brooker *et al.* 2008; Le Bagousse-Pinguet *et al.* 2013; McIntire & Fajardo 2014).

Understorey micro-habitats may not necessarily always represent the most ideal abiotic conditions for annuals to germinate because of low light conditions inducing low photosynthetic rates (Forseth *et al.* 2001; Jensen *et al.* 2011), and lower availability of water and nutrients (Callaway *et al.* 1991; Holzapfel & Mahall 1999). Certainly, the presence of annuals in the understorey is the product of a net positive effect that is the outcome of both positive (e.g. stress amelioration) and negative effects (e.g. reduced resources such as light or water as tested in our experiment) (Holzapfel & Mahall 1999; Callaway 2007); hence the result that seed germination seems to be reduced in understorey conditions might not be entirely unexpected. The seeds of arid annual plant species, or stress tolerant species in general, may also be adapted to higher-stress conditions (Körner 2003) and more rapid or higher germination rates in the context of overarching facilitation is not a signal that they have or can respond. Our results provide a useful insight into the widely assumed view that nurse-plants are clear examples of positive interactions in stressful environments (Maestre *et al.* 2003; Gomez-Aparicio *et al.* 2004) because evolutionary processes and the trait set in question can be important considerations. We suggest that these novel avenues for research, the seed life-stage and the impact of facilitation on evolutionary processes, be used to structure future facilitation studies in arid ecosystems.

Collectively, we found that nurse-plant positive effects do not necessarily translate into divergent seed characteristics for the understorey plant species growing in both canopies and more open micro-habitats. Apparent competition in nurse-plant canopies may not be sufficiently intense to generate selective processes that overcome

facilitation and impact the seed biology and germination of these understory species. A major implication is that local adaptation and plasticity of beneficiary species are necessary research topics to expand facilitation research. Further ecological studies should also extend the distribution ranges tested and explore density gradients to pinpoint the micro-evolutionary effects of nurse-plants on other species.

ACKNOWLEDGEMENTS

This research was funded by an NSERC DG to CJL and York University FGS salary support to DAS and LJL. We also want to thank Italo Revilla, Briggeth Flores, Paola Medina, and Jessica Turpo for seeds collection; and the community of Atiquipa for allowing us the entrance to their private reserve.

REFERENCES

- Adondakis S. & Venable D. L. (2004) Dormancy and germination in a guild of Sonoran Desert annuals. *Ecology* **85**, 2582-90.
- Armas C., Ordiales R. & Pugnaire F. I. (2004) Measuring plant interactions: a new comparative index. *Ecology* **85**, 2682-6.
- Baskin C. C. & Baskin J. M. (2001) *Ecology, biogeography, and evolution of dormancy and germination*. Academic Press, San Diego.
- Benjamini Y. & Hochberg Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. Roy. Statist. Soc. Ser. B* **57**, 289-300.
- Bertness M. D. & Callaway R. M. (1994) Positive interactions in communities. *Trends Ecol. Evol.* **9**, 191-3.
- Brako, L. & Zarucchi J. L. (1993) *Catalogue of the flowering plants and gymnosperms of Peru. Monographs in Systematic Botany Vol. 45*. Missouri Botanical Garden, St. Louis.
- Breen A. N. & Richards J. H. (2008) Irrigation and fertilization effects on seed number, size, germination and seedling growth: implications for desert shrub establishment. *Oecologia* **157**, 13-9.
- Brooker R. W., Maestre F. T., Callaway R. M. *et al.* (2008) Facilitation in plant communities: the past, the present, and the future. *J. Ecol.* **96**, 18-34.
- Bruno J. F., Stachowicz J. J. & Bertness M. D. (2003) Inclusion of facilitation into ecological theory. *Trends Ecol. Evol.* **18**, 119-25.

- Bullock J. M. & Moy I. L. (2004) Plants as seed traps: inter-specific interference with dispersal. *Acta Oecol.* **25**, 35-41.
- Callaway R. M., Nadkarni N. M. & Mahall B. E. (1991) Facilitation and interference of *Quercus douglasii* on understory productivity in Central California. *Ecology* **72**, 1484-99.
- Callaway R. M. (2007) *Positive interactions and interdependence in plant communities*. Springer, Dordrecht.
- Chesson P. (2000) Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Syst.* **31**, 343-66.
- Chesson P., Gebauer R. L. E., Schwinning S. *et al.* (2004) Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. *Oecologia* **141**, 236-53.
- Donohue K. (2009) Completing the cycle: maternal effects as the missing link in plant life histories. *Phil. Trans. R. Soc. B* **364**, 1059-74.
- Dyer A. R., Fenech A. & Rice K. J. (2000) Accelerated seedling emergence in interspecific competitive neighbourhoods. *Ecol. Lett.* **3**, 523-9.
- Ellner S., & Shmida A. (1981) Why are adaptations for long-range seed dispersal rare in desert plants? *Oecologia* **51**, 133-44.
- Facelli J. M., Chesson P., Barnes N. (2005) Differences in seed biology of annual plants in arid lands: a key ingredient of the storage effect. *Ecology* **86**, 2998-3006.
- Finch-Savage W. E. & Leubner-Metzger G. (2006) Seed dormancy and the control of germination. *New Phytol.* **171**, 501-23.
- Flemion F., & Poole H. (1948) Seed viability tests with 2,3,5 triphenyl tetrazolium chloride. *Contrib. Boyce Thomp. Inst.* **15**, 243-58.
- Flores J. & Jurado E. (2003) Are nurse-protégé interactions more common among plants from arid environments? *J. Veg. Sci.* **14**, 911-6.
- Forseth I.N., Wait D.A. & Casper B.B. (2001) Shading by shrubs in a desert system reduces the physiological and demographic performance of an associated herbaceous perennial. *J. Ecol.* **89**, 670-80.
- Galloway L. F. (2005) Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytol.* **166**, 93-100.
- Giladi I., Segoli M. & Ungar E. D. (2013) Shrubs and herbaceous seed flow in a semi-arid landscape: dual functioning of shrubs as trap and barrier. *J. Ecol.* **101**, 97-106.
- Goldberg D. E., Turkington R., Olsvig-Whittaker L. *et al.* (2001) Density dependence in an annual plant community: variation among life history stages. *Ecol. Monogr.* **71**, 423-46.
- Gomez-Aparicio L., Zamora R., Gómez J. M. *et al.* (2004) Applying plant facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse plants. *Ecol. Appl.* **14**, 1128-38.
- Holzappel C. & Mahall B. E. (1999) Bidirectional facilitation and interference between shrubs and annuals in the Mojave Desert. *Ecology* **80**, 1747-61.
- Holzappel C., Tielbörger K., Parag H. A. *et al.* (2006) Annual plant-shrub interactions along an aridity gradient. *Basic Appl. Ecol.* **7**, 268-79.
- Hufford K. M. & Mazer S. J. (2003) Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends Ecol. Evol.* **18**, 147-55.

- Jensen A. M., Lof M. & Gardiner E. S. (2011) Effects of above- and below-ground competition from shrubs on photosynthesis, transpiration and growth in *Quercus robur* L. seedlings. *Environ. Exp. Bot.* **71**, 367-75.
- Kéfi S., van Baalen M., Rietkerk M. *et al.* (2008) Evolution of local facilitation in arid ecosystems. *Am. Nat.* **172**, E1-E17.
- Körner Ch. (2003) Limitation and stress: always or never? *J. Veg. Sci.* **14**, 141-3.
- Leishman M. R. & Westoby M. (1994) The role of seed size in seedling establishment in dry soil conditions - experimental evidence from semi-arid species. *J. Ecol.* **82**, 249-58.
- Le Bagousse-Pinguet Y., Forey E., Touzard B. *et al.* (2013) Disentangling the effects of water and nutrients for studying the outcome of plant interactions in sand dune ecosystems. *J. Veg. Sci.* **24**, 375-83.
- Liancourt P. & Tielbörger K. (2011) Ecotypic differentiation determines the outcome of positive interactions in a dryland annual plant species. *Perspect. Plant Ecol. Evol. Syst.* **13**, 259-64.
- Liancourt P., Callaway R. M. & Michalet R. (2005) Stress tolerance and competitive response ability determine the outcome of biotic interactions. *Ecology* **86**, 1611-8.
- Littell R. C., Milliken G. A., Stroup W. W. *et al.* (2006) *SAS for mixed models*. SAS Institute Inc., Cary
- Luzuriaga A. L., Escudero A. & Pérez-García F. (2006) Environmental maternal effects on seed morphology and germination in *Sinapis arvensis* (Cruciferae). *Weed Res.* **46**, 163-74.
- Maestre F. T., Bautista S. & Cortina J. (2003) Positive, negative and net effects in grass-shrub interactions in Mediterranean semiarid grasslands. *Ecology* **84**, 3186-97.
- Maestre F. T., Callaway R. M., Valladares F. *et al.* (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *J. Ecol.* **97**, 199-205.
- Maranon T. & Grubb P. J. (1993) Physiological basis and ecological significance of the seed size and relative growth rate relationship in Mediterranean annuals. *Funct. Ecol.* **7**, 591-9.
- Maron J. L., Vila M., Bommarco R. *et al.* (2004) Rapid evolution of an invasive plant. *Ecol. Monogr.* **74**, 261-80.
- McIntire E. J. B. & Fajardo A. (2014) Facilitation as a ubiquitous driver of biodiversity. *New Phytol.* **201**, 403-16.
- Moles A. T., Ackerly D. D., Webb C. O. *et al.* (2005) Factors that shape seed mass evolution. *P. Natl. Acad. Sci. USA* **102**, 10540-4.
- Noy-Meir I. (1973) Desert ecosystems: environment and producers. *Annu. Rev. Ecol. Syst.* **4**, 25-51.
- Pake C. E. & Venable D. L. (1996) Seed banks in desert annuals: implications for persistence and coexistence in variable environments. *Ecology* **77**, 1427-35.
- Pugnaire F. I. & Lázaro R. (2000) Seed bank and understory species composition in a semi-arid environment: The effect of shrub age and rainfall. *Ann. Bot.* **86**, 807-13.
- Pugnaire F. I., Haase P., Puigdefabregas J. *et al.* (1996) Facilitation and succession under the canopy of a leguminous shrub: *Retama sphaerocarpa*, in a semi-arid environment in south-east Spain. *Oikos* **76**, 455-64.
- Roach D. A. & Wulff R. D. (1987) Maternal effects in plants. *Annu. Rev. Ecol. Syst.* **18**, 209-35.

- SAS Institute Inc. (2012) *JMP 10 Modeling and multivariate methods*. SAS Institute Inc., Cary.
- Seifan M., Tielbörger K. & Kadmon R. (2010) Direct and indirect interactions among plants explain counterintuitive positive drought effects on an eastern Mediterranean shrub species. *Oikos* **119**, 1601-9.
- Soliveres S., Eldridge D. J., Maestre F. T. *et al.* (2011) Microhabitat amelioration and reduced competition among understory plants as drivers of facilitation across environmental gradients: towards a unifying framework. *Perspect. Plant Ecol. Evol. Syst.* **13**, 247-58.
- Sotomayor D. A. & Jimenez P. (2008) Condiciones meteorológicas y dinámica vegetal del ecosistema costero Lomas de Atiquipa (Caravelí-Arequipa) en el sur del Perú. *Ecología Aplicada* **7**, 1-8.
- Sprague T. A. (1931) The botanical name of "Tara". *Kew Bull. Misc. Inform.* **1931**, 91-6.
- Sultan S. E. (1996) Plasticity for offspring traits in *Polygonum persicaria*. *Ecology* **77**, 1791-807.
- Sultan S. E. & Spencer H. G. (2002) Metapopulation structure favors plasticity over local adaptation. *Am. Nat.* **160**, 271-83.
- Thorpe A. S., Aschehoug E. T., Atwater D. Z. *et al.* (2011) Interactions among plants and evolution. *J. Ecol.* **99**, 729-40.
- Tielbörger K. & Kadmon R. (2000) Indirect effects in a desert plant community: is competition among annuals more intense under shrub canopies? *Plant Ecol.* **150**, 53-63.
- Valencia-Díaz S. & Montaña C. (2005) Temporal variability in the maternal environment and its effect on seed size and seed quality in *Flourensia cernua* DC. (Asteraceae). *J. Arid Environ.* **63**, 686-95.
- Venable D. L. (2007) Bet hedging in a guild of desert annuals. *Ecology* **88**, 1086-90.
- Venable D. L., Flores-Martinez A., Muller-Landau H. C. *et al.* (2008) Seed dispersal of desert annuals. *Ecology* **89**, 2218-27.
- Venable D. L. & Lawlor L. (1980) Delayed germination and dispersal in desert annuals: escape in space and time. *Oecologia* **46**, 272-82.
- Ward D. (2009) *The Biology of Deserts*. Oxford University Press, New York.
- Whitford W. G. (2002) *Ecology of desert systems*. Academic Press, San Diego.
- Wilson M. F. (1993) Dispersal mode, seed shadows and colonization patterns. *Vegetatio* **108**, 261-80.

SYNTHESIS

Aim and over-arching hypothesis

The aim of this project was to determine the direct and indirect consequences of top-down plant-plant facilitation in arid environments. Given that indirect effects are relatively under-studied (Wootton 1994, Brooker et al. 2008, McIntire & Fajardo 2014), we started by conducting a systematic review on the topic. Then, to empirically explore the aim of this project we studied a collection of field sites in the relatively under-studied Atacama Desert (Fig. 1). We hypothesized that dominant plants have direct and indirect effects on the understory species they usually facilitate, which in turn has important consequences for community organization and coexistence in stressful environments. These effects vary along spatial and temporal environmental gradients in a predictable fashion. Moreover, these dominant plants by facilitating entire understory communities, alter the outcome of interactions amongst their members and impact their evolutionary trajectories.

The following objectives and hypotheses were examined:

- To summarize and contextualize the breadth of research on indirect interactions in terrestrial plant communities within a recently proposed framework (Chapter 1).
- That the positive effects of dominant desert plants on understory communities are spatiotemporally scale dependent, from micro- to broad-scale spatial effects, and from within-seasonal to among-year temporal effects (Chapters 2 and 3).

- That dominant plants via their different traits determine the outcome of plant-plant interactions (Chapters 2 and 3).
- That dominant plants mediate the outcome of interactions amongst understory species and that their responses are species-specific (Chapters 3 and 4).
- That facilitation by dominant plants generates sufficiently different micro-environmental conditions that lead to consistent differences in seeds traits of understory plants (Chapter 5).

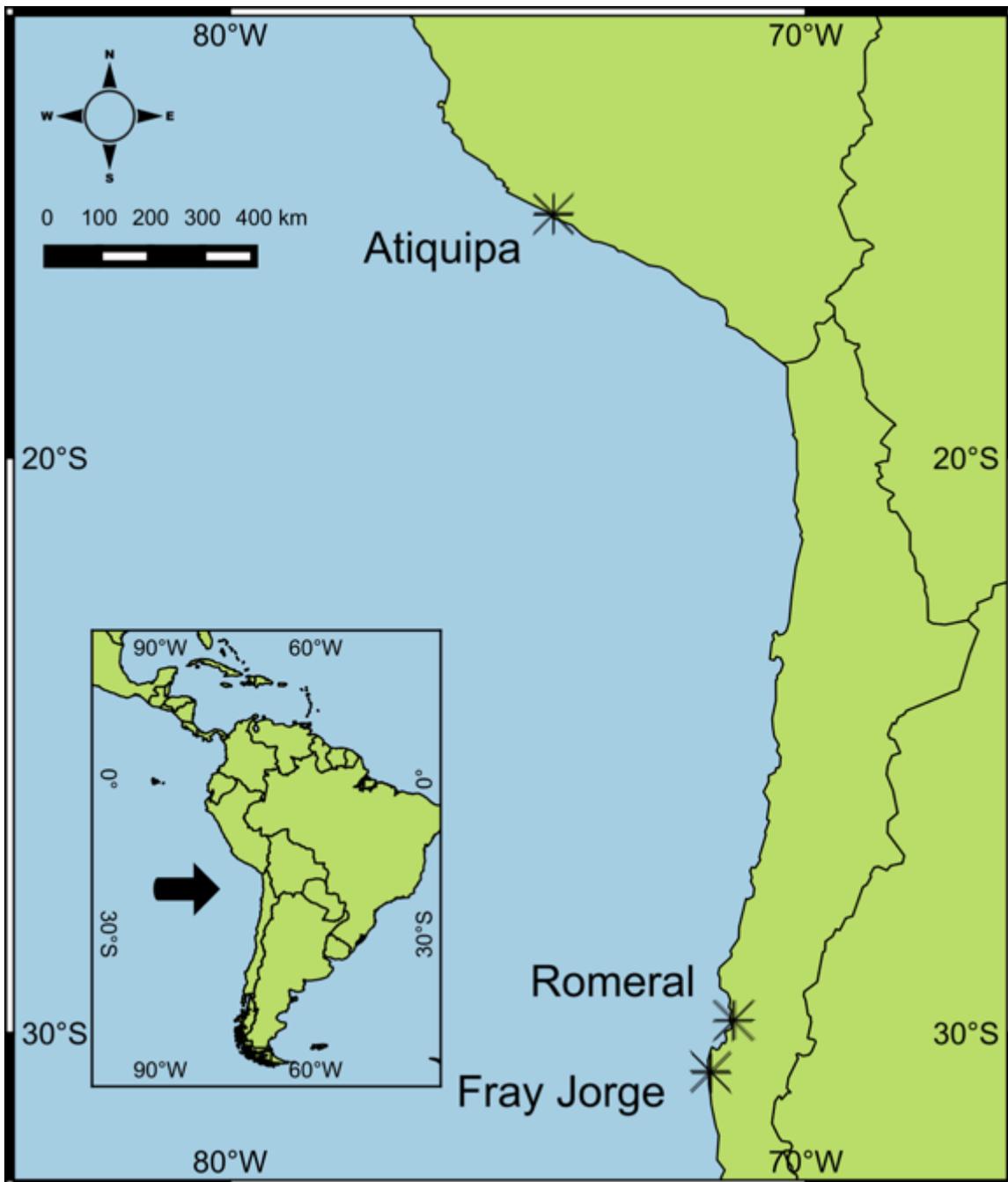


Fig. 1. Location of the study sites along the Atacama Desert.

Summary of major findings

Indirect interactions in terrestrial plant communities (Chapter 1) showed a clear geographic and ecosystem bias, with the majority of studies being conducted in North America and Europe, and in mesic ecosystems. These interactions have been reported in most geographic regions and ecosystems in the world, but have been neglected in tropical regions and in stressful ecosystems (Table 1). These biases are consistent with current trends of ecological research in general (Martin et al. 2012). The organizational framework proposed in Chapter 1 effectively contextualized the current state of research and allowed to identify specific pathways that have received considerable attention within the literature. The majority of studies have examined plant-animal feeding interactions, with studies dealing with interactions exclusively among at least three plant species, and plant-pollinator interactions relatively under-explored. We also found an important gap regarding to trait-mediated indirect effects. The proposed framework represents an important theoretical advance giving that it takes into consideration the main logical pathways and can be used to design more comprehensive studies. This review showed that studies reporting negative indirect effects were more frequent in mesic environments (Table 1), which provides partial support for an increase in the frequency of negative interactions towards less stressful/limiting environmental conditions (stress gradient hypothesis: Bertness & Callaway 1994). Overall, this review allowed us to successfully determine the mechanisms and geographical scope of indirect interactions in terrestrial communities (Fig. 2).

Table 1. Summary of findings from the study of direct and indirect consequences of plant-plant interactions.

Chapter	Purpose	Prediction/Objectives	Support/Result
Ch1. Synthesis on indirect interactions	To summarize and contextualize research on indirect interactions within a recently proposed framework	To identify geographic and ecosystem extents of indirect interactions	Majority of studies from Northern Hemisphere: North America and Europe. Indirect interactions in South America, Africa, Asia, and tropics understudied. Indirect interactions mostly examined in forests and grasslands, with less studies in stressful ecosystems (deserts, alpine ecosystems, salt marshes).
		To summarize the number of trophic levels studied when examining indirect interactions	Majority of studies focused on plant-animal interactions (70%), followed by plant-plant (20%) and plant-pollinator (10%) interactions. Majority of studies used two trophic levels. Studies included three levels in more productive environments such as agricultural ecosystems, forests, and grasslands.
		To determine whether the frequency of indirect interactions varies across large environmental gradients	Apparent competition more frequent in productive environments such as forests, grasslands, or controlled experiments. Indirect facilitation and associational resistance are not more frequent in less productive environments such as alpine ecosystems, deserts and salt marshes.
		To describe the most common experimental designs and statistical techniques used to examine indirect interactions	Single-target approach mainly used (37.7%). No trend between ecosystem productivity and number of targets, but more single-target studies used in agricultural systems, grasslands, and in controlled conditions. Mostly field studies (75%) and manipulative (73%). Mostly single-site approaches.
Ch2. Interactions at broad scales	To examine how facilitation changes within and between regional stress gradients and their temporal dynamics using dominant plants with different traits locally.	Increased frequency and intensity of facilitation with environmental severity at both regional and whole desert level, with a unimodal relationship as stress becomes extreme	Supported. Atiquipa (least stressful locality) displayed stronger positive effects than both Fray Jorge and Romeral for species richness and plant density. Romeral and Fray Jorge had neutral to negative effects that did not differ in intensity for species richness, but Romeral had more neutral effects than those of Fray Jorge. Within region (locality) effects were non significant.
		Increased frequency and intensity of facilitation as year-to-year environmental stress increases, with a similar unimodal relationship	Contradictory support. At Atiquipa, facilitative effects of 2013 were higher than those of 2011 and 2012 for species richness and plant density. At Fray Jorge, effects on species richness by 2013 were lower than those of 2011 and 2012. At Romeral, effects on plant density by 2013 were lower than those of 2012.
Ch3. Interactions at fine scales	To examine the within and between seasonal effects of	Micro-scale even within dominant plants determines the outcome of species interactions	Micro-scales did not determine the positive effects of both dominant plants for both species richness and plant density). Understory plants communities did differ by spatial scale

	facilitation, concurrently with the micro-scale effects of two dominant species	Interactions through time within- and between-seasons follow stress-gradient dynamics	Dominant plant effects changed from neutral effects at the beginning of the growing season to strong positive effects at the end in both 2011 and 2012. Facilitative effects occurred earlier in the season during 2012 and were on average stronger for both species richness and plant density.
		Dominant plants mediate indirect effects via an increase in competition under their canopies	Dominant plants did not affect intransivity estimates. These indicate lower co-occurrence, spatial segregation amongst subdominant species within all communities both from open and understory.
Ch4. Direct and indirect effects through manipulations	To examine direct and indirect effects of dominant plants on outcome of interactions for understory species with species-specific responses	Intensity of competition amongst annual plants is higher under dominants than in open non-canopied microsites	No evidence of more intense competition in the understory. In 2011, the density of <i>P. limensis</i> was positively associated with the density of all other species in understory microsites. All other relationships between species in both microsites were not statically significant.
		Neighborhood removal increases performance of target species, especially in canopied microsites	Contradictory support. <i>F. peruviana</i> plant height was reduced due to removals in open microsites. <i>P. limensis</i> after removals in open microsites had shorter plants in 2011, but taller plants in 2012; increased fruit production in understories in both years; and greater biomass in open microsites in 2012.
		Indirect effects of dominant plants are species-specific with competitive annuals experiencing greater release from competition.	Indirect effects of dominant plants were species-specific. Plant neighbors in open microsites had significant positive effects on plant height of <i>F. peruviana</i> in both years of study. Neighborhood effects on <i>P. limensis</i> performance varied according to the response variable.
Ch5. Evolutionary effects of interactions	To examine how facilitation by dominant plants generates microsites leading to consistent differences in seed traits of understory plants	Seeds associated with dominant plants' microsites are larger due to more favorable growing conditions	No differences in seed mass between understory and open micro-habitats, but there were differences among species.
		Seeds associated with dominant plants' microsite have greater viability and germination rate	Viability was not different between microsites or species. Germination was higher for seeds collected in open microsites, and in simulated open microsite conditions. Result mainly driven by <i>C. hermaphroditus</i> .
		Seeds associated with dominant plants' microsites have less variability in size and viability	No difference in coefficients of variation for seed mass and seed viability between collection sources.
		Seeds associated with dominant plants' microsites germinate faster due to apparent competition with other annuals	No difference between sources or simulated micro-habitat conditions, but there were differences among species.

We found that the positive effects of dominant desert plants on understory communities are spatiotemporally scale dependent (Table 1). Using a multi-year observational study spanning several field sites along the Atacama Desert we found that under extreme stress the positive effects on species richness and plant density provided by dominant plants became competitive effects (Chapter 2). At the regional gradient level, we did not find evidence of these gradients determining positive interactions, but at the whole desert level we found that the less arid desert location (Atiquipa) showed positive effects of canopies, while the more arid locations Romeral and Fray Jorge showed neutral and negative effects. The inter-annual effects of temporal stress were different by desert location, with the least arid location showing increased frequency and intensity of positive effects towards the most stressful year surveyed (2013), and the most arid locations showing increased frequency and intensity of negative effects towards the most stressful year surveyed. Biotic interactions are also dependent on within-seasonal changes in environmental stress, but micro-scales within dominant plants did not determine the strength of their effects, although different plant communities were facilitated at different micro-scales in comparison to open microsites (Chapter 3). Interactions between dominant plants and their understory communities changed from neutral to positive as the growing season progressed. These results show that the effects of canopies on understory communities are complex and that under extreme stress brought by environmental spatial or temporal variation the positive effects of dominant plants wane, with canopies even becoming microsites that inhibit the presence of understory plant communities. Overall, these findings underscore the

importance of exploring the impact of temporal and spatial effects on the net outcome of biotic interactions.

We found that dominant plants determined the outcome of interactions within their understory, but not in relation to their different traits (Table 1). At the regional scale we did not find differences in the strength of positive effects with understory species, even though we included dominant species differing in their thorniness (Chapter 2). This, however, also indicated that at the extreme stress sampled herbivore protection via thorns in dominant plants is not an important mechanism of plant-plant interactions (Fig. 2). At micro-scales we found consistent positive effects for both dominant plants studied, but with positive effects of similar intensity (Table 1). However, these different dominant species facilitated structurally different plant communities. This result could be attributable to microhabitat amelioration as a main mechanism of positive interactions and that beneficiary species take advantage of these microsites in a rather opportunistic fashion, a common adaptation for arid species (Noy-Meir 1973, Whitford 2002, Ward 2009). Overall, these findings indicate that the species-specific effects of dominant plants are dependent on the mechanism of plant-plant facilitation.

The effects of dominant plants on the outcome of interactions amongst understory species was contingent on the methodology used and on the traits of these understory species (Table 1). With co-occurrence analyses based on survey data we found that the intensity of interactions amongst understory plants is not strong and that spatial segregation within understory communities is not affected by the canopies of

dominant plants (Chapter 3). Using a combination of surveys and experimental manipulations we found that dominant plants have indirect interactions with their understory (Chapter 4). For one of the locally abundant beneficiary target species tested (*P. limensis*), we found a positive relationship with the abundance of other plant species in understory microsites, which provides evidence of indirect facilitation. The removal of plant neighbors of *P. limensis* resulted in its decreased plant height in open microsites, increased fruit production in understory microsites, and increased plant biomass in open microsites, which suggests increased competition in understory microsites. Relative interaction indices (RIIs) for this species indicated that canopies indirectly competed with this species. However, the removal of plants neighbors around a second target species (*F. peruviana*) did not have any effect on fruit production or final biomass, but decreased plant height in open microsites indicating that there are no significant indirect effects mediated by dominant plants for this species confirming findings of the surveys (i.e. non-significant relationship amongst *F. peruviana*'s density and other species density). The different life-history strategies of the target species can be an explanation or possible tool to examine these differences (Pages and Michalet 2006, Rolhauser and Pucheta 2015), giving that *F. peruviana* could be acting as a ruderal species (*sensu* Grime 1979), and *P. limensis* as a more competitive species. Overall, these results supported our predictions of increased intensity of competition under dominant species canopies, and that these indirect effects are species-specific (Fig. 2).

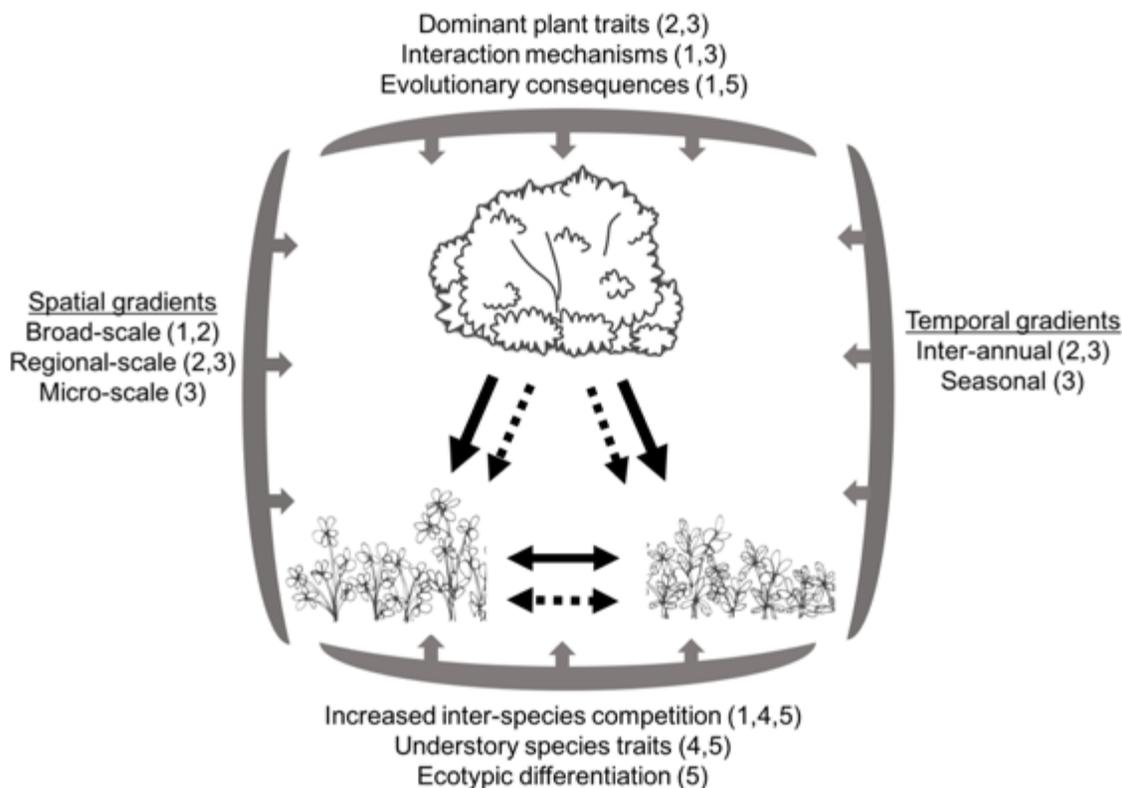


Fig. 2. Synthetic framework of the plant-plant interactions concepts examined in this project. Solid lines denote direct effects, dashed lines indirect effects, and the numbers between brackets indicate the chapter that included such concepts.

Finally, contrary to our predictions, we found that facilitation by dominant plants did not generate sufficiently different micro-environmental conditions conducive to ecotypic differentiation of understory species (Table 1). Using seed biology analyses and germination trials in growth chambers (Chapter 5) we found no differences in seed traits and that germination was significantly higher for seeds collected in open microsites and germinating in simulated open micro-habitats. However, these general differences were mainly driven by the annual species most strongly facilitated at the

plant life-stage, *Cyperus hermaphroditus*. There was no evidence for reduced variability in seed size or viability, and germination was not accelerated for seeds from under dominant plants (Fig. 2). These results indicate that the effects of the improved micro-habitat conditions generated by dominant plants do not have consistent effects on the seed biology and germinability of understory species. The protocol developed for this experiment, however, represents a novel contribution to the study of the evolutionary effects of positive interactions and should be used to further explore this hypothesis in other contexts.

Implications for ecological and biogeographical theory

Here, we developed a new framework of indirect effects within a trophic structure and explicitly considered their logical pathways of interaction. By classifying indirect interactions based on this novel conceptual framework we successfully identified research gaps in the field and provided recommendations for future studies. Additionally, previous syntheses on the topic were relatively dated (i.e. Strauss 1991, Wootton 1994, Callaway 2007), and our review updated theory and presented the strength of evidence of a topic that has seen considerable growth in the number of studies over the last two decades. We believe that new research efforts should address reported gaps and focus on indirect effects exclusively among plants, as studied in this project, and on non-feeding interactions such as plant-pollinator effects taking into consideration the main pathways identified within the conceptual framework in order to design more comprehensive studies. We are confident that this framework represents an important tool for theoretical and practical purposes.

By explicitly including a biogeographical approach to study plant-plant interactions we successfully demonstrated the scope of the spatiotemporal dependence of positive interactions. This provides a framework for future studies using a more comprehensive depiction of the conditionality of biotic effects. Our results supported specially the SGH as proposed by Bertness and Callaway (1994) for within-seasonal and inter-annual temporal gradients, and for broad spatial environmental gradients, but also the humped-back model of diversity (Michalet et al. 2006) by showing a collapse of positive interactions towards extreme stress. In this sense, our results expand the scope of these hypotheses by including temporal gradient effects, which have been relatively under-studied to date (but see Biswas & Wagner 2014, Sthultz et al. 2007, Tielborger & Kadmon 2000a, Schiffers & Tielborger 2006), especially concurrently with spatial effects. Moreover, by including novel field sites along the Atacama Desert, this project also extended the geographical scope of these hypotheses. The finding of a collapse of positive interactions at the most arid sites adds to an ongoing discussion on the limits of facilitation (Michalet et al. 2006, Michalet et al. 2015, Pugnaire et al. 2015), that has important implications under predicted climate change and for restoration purposes. Additional experimental studies manipulating water under extreme stress would be required to further this discussion. At micro-scales, even considering that benefactor species could generate micro-scale stress-related dynamics (Koyama et al. 2015, Pescador et al. 2014), we did not find evidence of different effects of dominant plants. This, however, requires further examination at larger micro-scales or different field sites as the dynamics within canopies can drastically change arid systems structure (Cipriotti

& Aguiar 2015, Xu et al. 2015) and evolutionary dynamics (Kefi et al. 2008). Overall, this project expands biogeographical and ecological theory by combining concepts of both disciplines in order to understand the context dependency of biotic interactions within plant communities.

As dominant plants usually facilitate increased densities of understory species within their canopies, multiple studies have hypothesized that this direct effect should increase the intensity of interspecific competition in those microsites (Tielbörger & Kadmon 200b, Schöb et al. 2013, Soliveres et al. 2011, Michalet et al. 2015). The results of this project provide contrasting evidence to support this hypothesis. Importantly, the contrasting results can be associated with the methodology used to determine them. Increased competition was found via manipulations of target species, while no indirect effects of canopies were showed via co-occurrence models on survey data of understory communities. This provides an interesting theoretical perspective on the nature of these effects in stressful environments, as these findings indicate that each species experience differently the direct effects of dominant plants and translate these effects to their interactions with other species within the canopy (i.e. a network of interactions). Hence, direct effects of canopies are more important than indirect effects within these microsites. Overall, our study contributes to the ongoing debate on the importance of within-canopy indirect effects, and our findings clearly illustrate that indirect effects mediated by dominant plants on understory species are species-specific. Future studies should examine this hypothesis using manipulative experiments, as interactions within understory communities can determine coexistence, diversity

maintenance and recruitment of other dominant plants (Chesson et al. 2004, Schöb et al. 2013), and hence could potentially be as important as direct effects.

The extent of species-specificity in determining the outcome of biotic interactions has also been a topic of debate within the facilitation literature (Brooker et al. 2008, Callaway 2007, Maestre et al. 2009). This project contributes to this debate by demonstrating that species-specific effects manifest according to the response variable and possible facilitation mechanism. For instance, we included thorny and non-thorny dominant plants in our surveys, but found no evidence of this trait determining the outcome of the effects provided by the dominant plants studied. When analyzing community structure, we did find differences between dominant plants. These findings suggest that dominant plants represent biologically similar microsites for understory species to survive within the arid ecosystem, and that herbivore protection is not necessarily an active mechanism of facilitation in this arid environment. Not only do dominant plants can influence the outcome of interactions, but also beneficiary species within understories can respond differently as demonstrated here via field manipulations and growth chamber trials. Thus, species-specific effects are a consequence of the mechanistic pathways of facilitation (Filazzola & Lortie 2014). This has important consequences for plant community structure and coexistence in stressful environments. Another key component in determining outcomes can be the life-history strategy of the subordinate plant species, and this can be used as a predictive tool for further studies on the effects of canopies on interactions (Grime 1979, Pages & Michalet 2006, Rolhauser & Pucheta 2015). Including the traits of the intervening species into

experimental design advances the understanding of ecological dynamics and helps developing better predictive models.

The evolutionary effects of positive interactions represents a topic that has received considerable less attention within the facilitation literature (Callaway 2007, Liancourt & Tielbörger 2011), even though facilitation in arid environments could be promoting ecotypic differentiation and ultimately speciation (Kéfi et al. 2008). This project provided evidence that understory species would germinate preferably in open simulated conditions and that there is no ecotypic differentiation. Arguably, the traits examined in this project could be very conserved (Moles et al. 2005) and hence facilitation would not represent a strong selective pressure, or alternatively the microsites under dominant plants are not the most ideal for germination because of low light conditions (Forseth *et al.* 2001; Jensen *et al.* 2011), and lower availability of water and nutrients (Callaway *et al.* 1991; Holzapfel & Mahall 1999). Nevertheless, considering the demonstrated microhabitat differences between understories and open microsites, and that theoretical models predict ecotypic differentiation (Kéfi et al. 2008), this topic represents an interesting avenue for research on the effects of dominant plants on evolutionary trajectories. Future studies, for instance, can test the importance of density gradients within understories on ecotypic differentiation, or explore further generations of understory species.

Implications for methodology

We successfully demonstrated the context dependence of plant-plant interactions using a combination of observational and manipulative approaches. All of these approaches help to construct a comprehensive understanding of the system.

Mensurative experiments often generate lower estimates of interaction strengths than manipulations (Kikvidze & Armas 2010), and usually manipulations provide a more accurate depiction of the effects among species (Díaz et al. 2003). Differences between methodologies can be due to the fact that each approach measures a different aspect of the interaction between plants (Dunne et al. 2004, Schöb et al. 2012). Survey data, for instance, would be providing a snapshot of the physiological status of different species at the moment of the survey, while measurements on final production, and reproductive output would be measuring integrated plant responses to stress. Measurements directly related to plant fitness, such as fruit production, would yield different results than those reflecting demographic processes, such as plant density (Tielbörger and Kadmon 2000b). This methodological distinction is important as some variables and approaches are more amenable and resource-consuming than others, and the reported outcomes from different methods can vary significantly (Kikvidze and Armas 2010, Gomez-Aparicio 2012, Schöb et al. 2012). Importantly, by providing a wide range of response variables and their associated underlying mechanisms, future studies can focus on the most important aspect according to the underlying question and optimize their experimental design. A comprehensive depiction of dominant plant effects, or any other biotic interaction, should certainly include both mensurative and manipulative

approaches as this will ultimately inform ecological theory and its applications more realistically.

Another major contribution of this project is the development of an experimental protocol for the assessment of ecotypic differentiation due to facilitation. Although we did not find evidence supporting our hypothesis, we believe that this protocol represents a great opportunity to deepen the understanding on the evolutionary effects of facilitation under stressful conditions. Future studies should incorporate this protocol and even translate its design to field conditions. Additional response variables and sampling protocols should also be explored as other factors could easily be added to this design. These variables can include the following: density gradients within understory microsites, species-specific effects and functional classifications, spatial environmental gradients, among others. Facilitation research would certainly benefit from understanding the transition from ecological patterns into evolutionary trajectories using the two-phase system (i.e. canopy and open microsites) that is associated with dominant plants in deserts.

Implications for coexistence in stressful environments

Indirect interactions are very frequent mechanisms and can play an important role in the coexistence of species and in promoting species diversity (Brooker et al. 2008, McIntire and Fajardo 2014). In arid environments, coexistence can be promoted via temporal differences in competitive abilities and through persistence during unfavorable periods, a mechanism termed the storage effect (Chesson 2000, Chesson

et al. 2004). This project provides evidence of this coexistence mechanism by showing less intransitivity in understory microsites, and that the effects of dominant plants are species-specific on the beneficiary plants. These effects, additionally, support the resource partitioning hypothesis as a mechanism of coexistence (Tilman 1982, Scheffer & van Nes 2006). Moreover, increased competition within understories could likely act as a mechanism promoting two-phase vegetation mosaics (i.e. understory and open microsites), because these microsites regulate the emergence of dominant plants in combination with other spatiotemporally variable mechanisms such as seed trapping or rainfall (Cipriotti & Aguiar 2015). Overall, the findings of this project showcase several coexistence mechanisms that have important implications for diversity maintenance under stressful conditions.

Another largely studied mechanism of diversity maintenance in arid environments is the nurse-plant syndrome (Drezner 2014, Franco & Nobel 1989, Filazzola & Lortie 2014, Whitford 2002, Ward 2009). When dominant plants provide ameliorated microhabitat conditions (Franco & Nobel 1989, Drezner 2007) they facilitate entire communities of understory plants, and hence contribute to diversity maintenance in arid systems. This project expands the scope of this mechanism by demonstrating its multiple determinants and how these vary along environmental gradients. We showed that dominant plants provide ameliorated microsites, and that within these microsites different plant communities coexist. We also showed overall positive effects of dominant plants on their understory, but that these effects tend to wane under extreme stress. These results support recent refinements of the nature of biotic interactions at high

stress (Michalet et al. 2006, Michalet et al. 2013, Pugnaire et al. 2015), and have important implications for coexistence and also for applied purposes.

Implications for applied ecology, global change and desertification

The canopies of dominant plants might play important roles in applied purposes. For example, they may determine the successful establishment and spread of invasive plants or the resistance of native plant communities to plant invasions (Badano et al. 2015, White et al. 2006). Moreover, following the removal treatments utilized in this project, a manager could recommend the removal of herbs to increase recruitment due to a release from competition from other species (Jensen et al. 2012, Caldeira et al. 2014). Dominant plants can also be used for restoration practices of degraded arid lands (Padilla & Pugnaire 2006), however the success of this technique has to be planned in relation to the stress level at the proposed location of the intervention. The results of this project suggest that there is a limit for the positive effects of dominant plants on understory communities, and hence this should be considered. The effects of climate change on the net outcome of biotic interactions are still largely under-explored (Brooker 2005, McIntire and Fajardo 2014). This represents a critical gap as new climate regimes will change the physiology and fitness of plants (Kirschbaum 2004, Brooker 2005), which in turn will change the intensity of interactions as they propagate through trophic structures (Woodward et al. 2010). The results of this project point to a threshold where dominant plants will not be able to facilitate understory communities, but also that this change is not necessarily irreversible as water supplementation can aid to restore the positive effects that these plants provide.

As dominant plants usually represent “hotspots” of interactions in deserts (Lortie et al. 2016), their use in restoration practices should be favored. Moreover, their conservation should be promoted as they not only maintain species diversity, but through their interactions with other trophic levels, they maintain functional diversity. The findings of this project clearly demonstrate the pivotal role of dominant woody plants in arid environments, and hence any plan to combat desertification or land use change and conserve arid lands should start with assessing the cover and population dynamics of woody species with potential facilitative effects. Additional steps should be taken to classify potential understory species according to their functional traits in order to promote ecologically functional communities. Dominant plants have direct and indirect effects on understory species that should translate to other trophic and these should be exploited to promote healthy arid ecosystems.

Conclusion

Using a series of novel field sites distributed along the Atacama Desert we found that multiple factors determine the outcome of plant-plant interactions. These factors impact both the direct and indirect effects of dominant woody plants on their understory communities and include species-specific traits of both the dominant and understory species, and the spatial and temporal environmental gradients that manifest their effects at different scales. Dominant plants usually facilitate increased richness and density of species in their understory, that in turn mediates effects amongst these species. However, these direct effects seem to have a limit given that at extremely stressful

environmental conditions they tend to change to neutral and even competitive effects of canopies on their understories. Although we did not find evidence of evolutionary effects of top-down facilitation, the methodology proposed here represents a contribution to test the conditions under which these results hold. Overall, this project illustrates the importance of understanding the multiple drivers that determine the outcome of biotic interactions.

REFERENCES

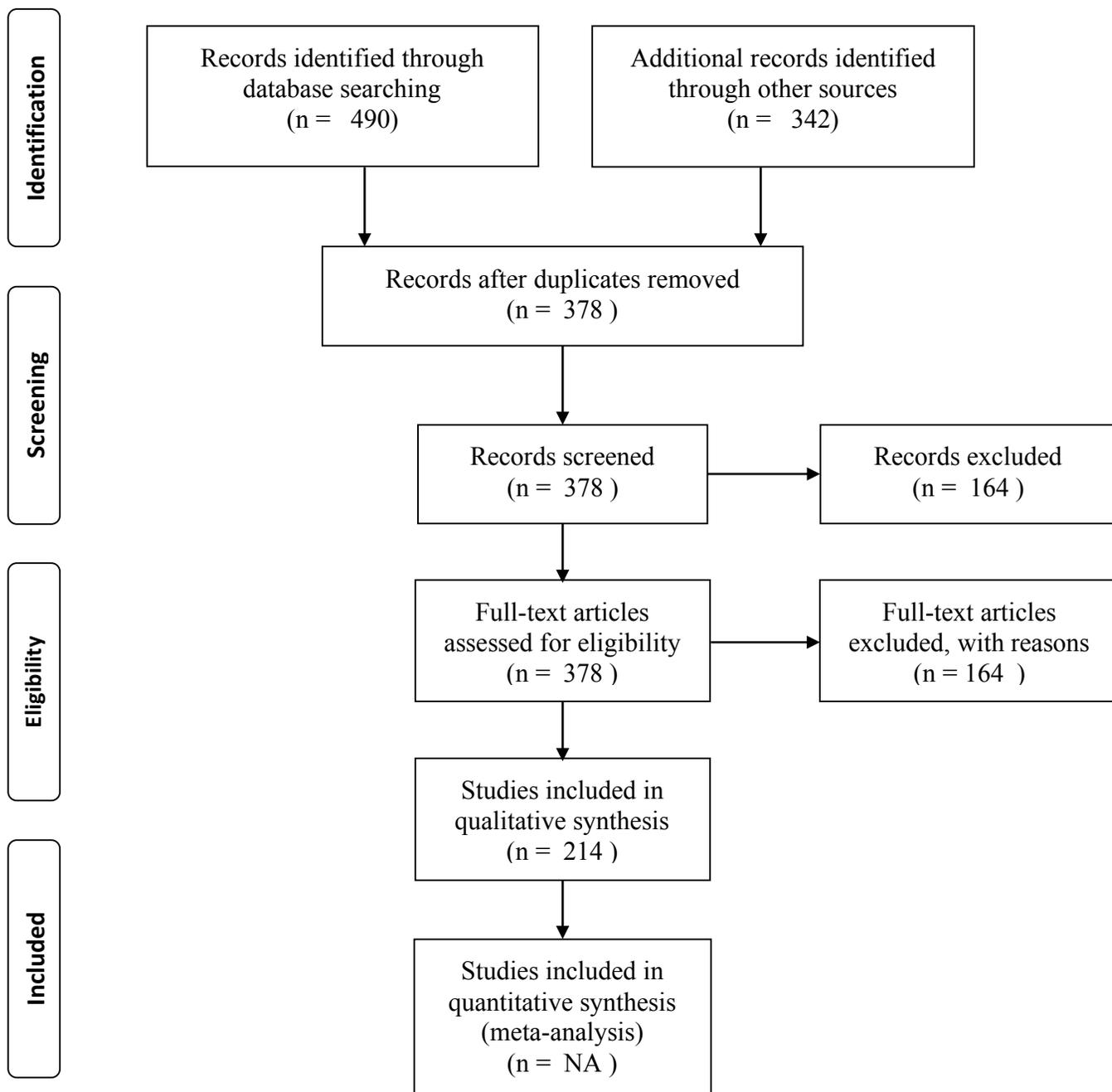
- Adondakis S. & Venable D. L. (2004) Dormancy and germination in a guild of Sonoran Desert annuals. *Ecology* **85**, 2582-90.
- Badano EI, Bustamante RO, Villarreal E, Marquet PA, Cavieres LA (2015) Facilitation by nurse plants regulates community invasibility in harsh environments. *J Veg Sci* **26**:756-767
- Bertness, M. D. & Callaway, R. M. 1994. Positive interactions in communities. – *Trends in Ecology and Evolution* **9**: 187-191.
- Biswas, S. R. & Wagner, H. H. 2014. A temporal dimension to the stress gradient hypothesis for intraspecific interactions. *Oikos* **123**: 1323-1330.
- Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. J., Cavieres, L. A., Kunstler, G., Liancourt, P., Tielborger, K., (...) & Michalet, R. 2008. Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology* **96**: 18-34.
- Bruno, J. F., Stachowicz, J. J., & Bertness, M. D. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* **18**: 119-125.
- Callaway, R. M. 2007. Positive Interactions and Interdependence in Plant Communities. Springer, New York.
- Caldeira MC, Ibáñez I, Nogueira C, Bugalho MN, Lecomte X, Moreira A, Pereira JS (2014) Direct and indirect effects of tree canopy facilitation in the recruitment of Mediterranean oaks. *J Appl Ecol* **51**:349-358
- Chamberlain, S. A., Bronstein, J. L. & Rudgers J. A. 2014. How context dependent are species interactions? *Ecology Letters* **17**: 881-890.
- Chesson, P. 2000. Mechanisms of maintenance of species diversity. *Annual Review in Ecology and Systematics* **31**:343-366.
- Chesson P, Gebauer RLE, Schwinning S, Huntly N, Wiegand K, Ernest MSK, Sher A, Novoplansky A, Weltzin JF (2004) Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. *Oecologia* **141**:236-253
- Cipriotti, P. A. & Aguiar, M. A. 2015. Is the balance between competition and facilitation a driver of the patch dynamics in arid vegetation mosaics? *Oikos* **124**: 139-149.

- Drezner, T.D. 2014. The keystone saguaro (*Carnegiea gigantea*, Cactaceae): a review of its ecology, associations, reproduction, limits, and demographics. Braun-Blanquet invited review. *Plant Ecology*. 215: 581-595.
- Drezner, T.D. 2007. An analysis of winter temperature and dew point under the canopy of a common Sonoran Desert nurse and the implications for positive plant interactions. *Journal of Arid Environments*. 69: 554-568.
- Dunne JA, Saleska SR, Fischer ML, Harte J (2004) Integrating experimental and gradient methods in ecological climate change research. *Ecology* 85:904-916
- Kéfi S., van Baalen M., Rietkerk M. et al. (2008) Evolution of local facilitation in arid ecosystems. *Am. Nat.* **172**, E1-E17.
- Flores, J. & Jurado, E. 2003. Are nurse-protégé interactions more common among plants from arid environments? *Journal of Vegetation Science* 14: 911-916.
- Franco, A. C. & Nobel, P. S. 1989. Effect of nurse plants on the microhabitat and growth of cacti. *Journal of Ecology* 77: 870-886.
- Grime JP (1979) *Plant Strategies and Vegetation Processes*. John Wiley & Sons, Chichester
- He, Q. Bertness, M. D. & Altieri, A. H. 2013. Global shifts towards positive species interactions with increasing environmental stress. *Ecology Letters* 16: 695-706.
- Kéfi S., van Baalen M., Rietkerk M. et al. 2008. Evolution of local facilitation in arid ecosystems. *American Naturalist* 172, E1-E17.
- Kéfi, S., et al. 2012. More than a meal... integrating non-feeding interactions into food webs. *Ecology Letters* 15:291-300.
- Kikvidze, Z. & Armas, C. 2010. Plant interaction indices based on experimental plant performance data. In Pugnaire, F. I. (ed.) *Positive plant interactions and community dynamics*. Pp 17-38. CRC Press, Boca Raton, USA.
- Liancourt P. & Tielbörger K. (2011) Ecotypic differentiation determines the outcome of positive interactions in a dryland annual plant species. *Perspect. Plant Ecol. Evol. Syst.* **13**, 259-64.
- Lortie, C. J. 2010. Synthetic analysis of the stress-gradient hypothesis. In Pugnaire, F. I. (ed.) *Positive Plant Interactions and Community Dynamics*. Pp 125-148. CRC Press, Boca Ratón, USA.
- Lortie, C. J., Brooker, R. W., Choler, P., Kikvidze, Z., Michalet, R., Pugnaire, F. I. & Callaway, R. M. 2004. Rethinking plant community theory. *Oikos* 107: 433-438.
- Lortie, C.J. & Callaway, R.M. 2006. Re-analysis of meta-analysis: support for the stress-gradient hypothesis. *Journal of Ecology* 94: 7-16.
- Lortie, C. J., Filazzola, A. & Sotomayor, D. A. 2016. Functional assessment of animal interactions with shrub-facilitation complexes: a formal synthesis and conceptual framework. *Functional Ecology* 30: 41-51.
- Maestre, F.T., Valladares, F. & Reynolds, J.F. 2005. Is the change of plant-plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. *Journal of Ecology* 93: 748-757.
- Maestre, F.T., Callaway, R.M., Valladares, F. & Lortie, C.J. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology* 97: 199-205.
- McIntire, E. J. B. & Fajardo, A. 2014. Facilitation as a ubiquitous driver of biodiversity. *New Phytologist* 201: 403-416.

- Michalet, R., Brooker, R. W., Cavieres, L. A., Kikvidze, Z., Lortie, C. J., Pugnaire, F. I., Valiente-Banuet, A. & Callaway, R. M. 2006. Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? *Ecology Letters* 9: 767-773.
- Michalet, R., Le Bagousse-Pinguet, Y., Maalouf, J.P. & Lortie, C.J. 2013. Two alternatives to the stress-gradient hypothesis at the edge of life: the collapse of facilitation and the switch from facilitation to competition. *Journal of Vegetation Science* 25: 609–613.
- Michalet R, Brooker RW, Lortie CJ, Pugnaire FI (2015) Disentangling direct and indirect canopy and soil effects of a legume shrub on its understorey forb community. *Oikos* 124:1251-1262
- Miriti, M. N. 2006. Ontogenetic shift from facilitation to competition in a desert shrub. *Journal of Ecology* 94: 973-979.
- Noy-Meir I. (1973) Desert ecosystems: environment and producers. *Annu. Rev. Ecol. Syst.* 4, 25-51.
- Padilla, F.M. & Pugnaire, F. I. 2006. The role of nurse plants in the restoration of degraded environments. *Frontiers in Ecology and the Environment* 4:196-202
- Pescador, D. S., Chacón-Labela, J., de la Cruz, M. & Escudero, A. 2014. Maintaining distances with the engineer: patterns of coexistence in plant communities beyond the patch-bare dichotomy. *New Phytologist* 204: 140-148.
- Pugnaire, F. I., Hasse, P., Puigdefábregas J., Cueto, M., Clark, S. C. & Incoll, L. D. 1996. Facilitation and succession under the canopy of the leguminous shrub, *Retama sphaerocarpa*, in a semi-arid environment in south-east Spain. *Oikos* 76: 455-464.
- Pugnaire, F. I., Zhang, L., Li R. & Luo, T. 2015. No evidence of facilitation collapse in the Tibetan plateau. *Journal of Vegetation Science* 26: 233-242.
- Rolhauser AG, Pucheta E (2015) Annual plant functional traits explain shrub facilitation in a desert community. *J Veg Sci* in press doi:10.1111/jvs.12335
- Schöb, C., Armas, C. & Pugnaire, F. I. 2013. Direct and indirect interactions co-determine species composition in nurse plant systems. *Oikos* 122: 1371-1379.
- Soliveres S., Eldridge D. J., Maestre F. T. *et al.* (2011) Microhabitat amelioration and reduced competition among understory plants as drivers of facilitation across environmental gradients: towards a unifying framework. *Perspect. Plant Ecol. Evol. Syst.* 13, 247-58.
- Sthultz, C. M., Gehring, C. A. & Whitham, T. G. 2007. Shifts from competition to facilitation between a foundation tree and a pioneer shrub across spatial and temporal scales in a semiarid woodland. *New Phytologist* 173: 135-145.
- Tewksbury, J. J. & Lloyd, J. D. 2001. Positive interactions under nurse-plants: spatial scale, stress gradients and benefactor size. *Oecologia* 127: 425-434.
- Tielbörger, K. & Kadmon, R. 2000a. Temporal environmental variation tips the balance between facilitation and interference in desert plants. *Ecology* 81:1544-1553.
- Tielbörger, K. & Kadmon, R. 2000b. Indirect effects in a desert plant community: is competition among annuals more intense under shrub canopies? *Plant Ecol.* 150, 53-63.
- Ward D. (2009) *The Biology of Deserts*. Oxford University Press, New York.
- White, E. M., J. C. Wilson, and A. R. Clarke. 2006. Biotic indirect effects: a neglected concept in invasion biology. *Diversity and Distributions* 12:443-455.

- Whitford W. G. (2002) *Ecology of desert systems*. Academic Press, San Diego.
- Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. *Annual Review in Ecology and Systematics* 25:443-466.
- Xu, C., Van Nes, E. H., Holmgren, M., Kefi, S. & Scheffer, M. 2015. Local facilitation may cause tipping points on a landscape level preceded by early-warning indicators. *American Naturalist* 186: E81-E90.

Appendices

Appendix 1. PRISMA Flow Diagram for the identification of studies included in the systematic review.

Appendix 2. List of research articles considered in the systematic review.

- Abdala-Roberts, L., J. C. Berny-Miery Teran, K. A. Mooney, Y. B. Moguel-Ordóñez, and F. Tut-Pech. 2014. Plant traits mediate effects of predators across pepper (*Capsicum annuum*) varieties. *Ecological Entomology* 39:361-370.
- Agrawal, A. A. 2004. Resistance and susceptibility of milkweed: Competition, root herbivory, and plant genetic variation. *Ecology* 85:2118-2133.
- Agrawal, A. A. and P. A. Van Zandt. 2003. Ecological play in the coevolutionary theatre: genetic and environmental determinants of attack by a specialist weevil on milkweed. *Journal of Ecology* 91:1049-1059.
- Alba-Lynn, C. and J. K. Detling. 2008. Interactive disturbance effects of two disparate ecosystem engineers in North American shortgrass steppe. *Oecologia* 157:269-278.
- Alcantara, S., R. H. Ree, F. R. Martins, and L. G. Lohmann. 2014. The Effect of Phylogeny, Environment and Morphology on Communities of a Lianescent Clade (Bignoniaceae-Bignoniaceae) in Neotropical Biomes. *PLoS ONE* 9(3): e90177. doi:10.1371/journal.pone.0090177
- Allison, V. J., T. K. Rajaniemi, D. E. Goldberg, and D. R. Zak. 2007. Quantifying direct and indirect effects of fungicide on an old-field plant community: an experimental null-community approach. *Plant Ecology* 190:53-69.
- Alofs, K. M., and N. L. Fowler. 2013. Loss of native herbaceous species due to woody plant encroachment facilitates the establishment of an invasive grass. *Ecology* 94:751-760.
- Ammunet, T., A. Heisswolf, N. Klemola, and T. Klemola. 2010. Expansion of the winter moth outbreak range: no restrictive effects of competition with the resident autumnal moth. *Ecological Entomology* 35:45-52.
- Anderson, P. M. M. Sadek, and F. L. Waeckers. 2011. Root herbivory affects oviposition and feeding behavior of a foliar herbivore. *Behavioral Ecology* 22:1272-1277.
- Ando, Y. and T. Ohgushi. 2008. Ant- and plant-mediated indirect effects induced by aphid colonization on herbivorous insects on tall goldenrod. *Population Ecology* 50:181-189.
- Anthelme, F. and R. Michalet. 2009. Grass-to-tree facilitation in an arid grazed environment (Air Mountains, Sahara). *Basic and Applied Ecology* 10:437-446.
- Atwater, D. Z., C. M. Bauer, and R. M. Callaway. 2011. Indirect positive effects ameliorate strong negative effects of *Euphorbia esula* on a native plant. *Plant Ecology* 212:1655-1662.
- Auer, S. K. and T. E. Martin. 2013. Climate change has indirect effects on resource use and overlap among coexisting bird species with negative consequences for their reproductive success. *Global Change Biology* 19:411-419.
- Austrheim, G., A. Mysterud, K. Hassel, M. Evju, and R. H. Okland. 2007. Interactions between sheep, rodents, graminoids, and bryophytes in an oceanic alpine ecosystem of low productivity. *Ecoscience* 14:178-187.
- Barrio, I. C., D. S. Hik, K. Peck, and C. G. Bueno. 2013. After the frass: foraging pikas select patches previously grazed by caterpillars. *Biology Letters* 9: 20130090. <http://dx.doi.org/10.1098/rsbl.2013.0090>
- Barton, B. T. and A. R. Ives. 2014. Species interactions and a chain of indirect effects driven by reduced precipitation. *Ecology* 95:486-494.

- Baskett, C. A., S. M. Emery, and J. A. Rudgers. 2011. Pollinator visits to threatened species are restored following invasive plant removal. *International Journal of Plant Sciences* 172:411-422.
- Bass K. A., E. A. John, N. C. Ewald, and S. E. Hartley. 2010. Insect herbivore mortality is increased by competition with a hemiparasitic plant. *Functional Ecology* 24:1228-1233.
- Beard, K. H., C. A. Faulhaber, F. P. Howe, and T. C. Edwards Jr. 2013. Rodent-mediated interactions among seed species of differing quality in a shrubsteppe ecosystem. *Western North American Naturalist* 73:426-441.
- Becklin, K. M., M. L. Pallo, and C. Galen. 2012. Willows indirectly reduce arbuscular mycorrhizal fungal colonization in understory communities. *Journal of Ecology* 100:343-351.
- Beguín J., D. Pothier, and S. D. Cote. 2011. Deer browsing and soil disturbance induce cascading effects on plant communities: a multilevel path analysis. *Ecological Applications* 21: 439-451
- Bennett, A. E., M. Thomsen and S. Y. Strauss. 2011. Multiple mechanisms enable invasive species to suppress native species. *American Journal of Botany* 98:1086-1094.
- Bokdam, J. 2001. Effects of browsing and grazing on cyclic succession in nutrient-limited ecosystems. *Journal of Vegetation Science* 12:875-886.
- Boughton, E. H., P. F. Quintana-Ascencio, P. J. Bohlen, and D. Nickerson. 2011. Differential facilitative and competitive effects of a dominant macrophyte in grazed subtropical wetlands. *Journal of Ecology* 99:1263-1271.
- Boulant, N., M. L. Navas, E. Corcket, and J. Lepart. 2008. Habitat amelioration and associational defence as main facilitative mechanisms in Mediterranean grasslands grazed by domestic livestock. *Ecoscience* 15:407-415.
- Bowers, M.A. and C. F. Sacchi. 1991. Fungal mediation of a plant herbivore interaction in an early successional plant community. *Ecology* 72:1032-1037.
- Branson, D. H. and G. A. Sword. 2009. Grasshopper Herbivory Affects Native Plant Diversity and Abundance in a Grassland Dominated by the Exotic Grass *Agropyron cristatum*. *Restoration Ecology* 17:89-96.
- Brody, A. K., T. M. Palmer, K. Fox-Dobbs, and D. F. Doak. 2010. Termites, vertebrate herbivores, and the fruiting success of *Acacia drepanolobium*. *Ecology* 91:399-407.
- Burger, J. C. and S. M. Louda. 1994. Indirect versus direct effects of grasses on growth of a cactus (*Opuntia fragilis*) - insect herbivory versus competition. *Oecologia* 99:79-87.
- Caccia F. D., E. J. Chaneton, and T. Kitzberger. 2009. Direct and indirect effects of understory bamboo shape tree regeneration niches in a mixed temperate forest. *Oecologia* 161:771-780.
- Caccia, F. D., E. J. Chaneton, and T. Kitzberger. 2006. Trophic and non-trophic pathways mediate apparent competition through post-dispersal seed predation in a Patagonian mixed forest. *Oikos* 113:469-480.
- Cahill, J. F., E. Elle, G. R. Smith, and B. H. Shore. 2008. Disruption of a belowground mutualism alters interactions between plants and their floral visitors. *Ecology* 89:1791-1801.

- Caldeira, M. C., I. Ibanez, C. Nogueira, M. N. Bugalho, X. Lecomte, A. Moreira, and J. S. Pereira. 2014. Direct and indirect effects of tree canopy facilitation in the recruitment of Mediterranean oaks. *Journal of Applied Ecology* 51:349-358.
- Callaway, R. M., T. H. DeLuca, and W. M. Belliveau. 1999. Biological control herbivores may increase competitive ability of the noxious weed *Centaurea maculosa*. *Ecology* 80:1196-1201.
- Callaway, R. M. and S. C. Pennings. 2000. Facilitation may buffer competitive effects: Indirect and diffuse interactions among salt marsh plants. *American Naturalist* 156:416-424.
- Callaway, R. M. and S. C. Pennings. 1998. Impact of a parasitic plant on the zonation of two salt marsh perennials. *Oecologia* 114:100-105.
- Callaway, R. M., B. E. Mahall, C. Wicks, J. Pankey, and C. Zabinski. 2003. Soil fungi and the effects of an invasive forb on grasses: Neighbor identity matters. *Ecology* 84:129-135.
- Callaway, R. M., D. Kikodze, M. Chiboshvili, and L. Khetsuriani. 2005. Unpalatable plants protect neighbors from grazing and increase plant community diversity. *Ecology* 86:1856-1862.
- Canepuccia, A. D., M. S. Fanjul, E. Fanjul, F. Botto, and O. O. Iribarne. 2008. The intertidal burrowing crab *Neohelice (=Chasmagnathus) granulata* positively affects foraging of rodents in South Western Atlantic salt marshes. *Estuaries and Coasts* 31:920-930.
- Cardinale, B. J., C. T. Harvey, K. Gross, and A. R. Ives. 2003. Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. *Ecology Letters* 6:857-865.
- Carlo, T. A. 2005. Interspecific neighbors change seed dispersal pattern of an avian-dispersed plant. *Ecology* 86:2440-2449
- Cedola, C. V., M. F. Gugole Ottaviano, M. E. Brentassi, M. F. Cingolani, and N. M. Greco. 2013. Negative interaction between twospotted spider mites and aphids mediated by feeding damage and honeydew. *Bulletin of Entomological Research* 103:233-240.
- Cereghino, R., C. Leroy, J. Carrias, L. Pelozuelo, C. Segura, C. Bosc, A. Dejean, and B. Corbara. 2011. Ant-plant mutualisms promote functional diversity in phytotelm communities. *Functional Ecology* 25:954-963.
- Chaneton, E. J., C. N. Mazia, and T. Kitzberger. 2010. Facilitation vs. apparent competition: insect herbivory alters tree seedling recruitment under nurse shrubs in a steppe-woodland ecotone. *Journal of Ecology* 98:488-497.
- Christensen, K. M. and T. G. Whitham. 1993. Impact of insect herbivores on competition between birds and mammals for pinyon pine seeds. *Ecology* 74:2270-2278.
- Crain, C. M. and M. D. Bertness. 2005. Community impacts of a tussock sedge: Is ecosystem engineering important in benign habitats?. *Ecology* 86:2695-2704.
- Cruz Sueiro, M., E. Schwindt, M. M. Mendez, A. Bortolus. 2013. Interactions between ecosystem engineers: A native species indirectly facilitates a non-native one. *Acta Oecologica-International Journal of Ecology* 51:11-16.
- Cuesta, B., P. Villar-Salvador, J. Puertolas, J. M. R. Benayas, and R. Michalet. 2010. Facilitation of *Quercus ilex* in Mediterranean shrubland is explained by both direct and indirect interactions mediated by herbs. *Journal of Ecology* 98:687-696.

- Dangremond, E. M., E. A. Pardini, and T. M. Knight. 2010. Apparent competition with an invasive plant hastens the extinction of an endangered lupine. *Ecology* 91:2261-2271.
- de Jager, M. L., L. L. Dreyer, and A. G. Ellis. 2011. Do pollinators influence the assembly of flower colours within plant communities? *Oecologia* 166:543-553.
- Debain, S., T. Curt, and J. Lepart. 2005. Indirect effects of grazing on the establishment of *Pinus sylvestris* and *Pinus nigra* seedlings in calcareous grasslands in relation to resource level. *Ecoscience* 12:192-201.
- Deveny, A. J. and L. R. Fox. 2006. Indirect interactions between browsers and seed predators affect the seed bank dynamics of a chaparral shrub. *Oecologia* 150:69-77.
- Dickie, I. A., R. T. Koide, and K. C. Steiner. 2002. Influences of established trees on mycorrhizas, nutrition, and growth of *Quercus rubra* seedlings. *Ecological Monographs* 72:505-521.
- Dyer, L. A., D. K. Letourneau, G. Vega Chavarria, and D. Salazar Amoretti. 2010. Herbivores on a dominant understory shrub increase local plant diversity in rain forest communities. *Ecology* 91:3707-3718.
- Ehlers, B. K., A. Charpentier, and E. Grondahl. 2014. An allelopathic plant facilitates species richness in the Mediterranean garrigue. *Journal of Ecology* 102:176-185.
- Elmes, G. W., R. T. Clarke, J. A. Thomas, and M. E. Hochberg. 1996. Empirical tests of specific predictions made from a spatial model of the population dynamics of *Maculinea rebeli*, a parasitic butterfly of red ant colonies. *Acta Oecologica-International Journal of Ecology* 17:61-80.
- Emery, S. M. and J. A. Rudgers. 2012. Impact of Competition and Mycorrhizal Fungi on Growth of *Centaurea stoebe*, an Invasive Plant of Sand Dunes. *American Midland Naturalist* 167:213-222.
- Englishloeb, G. M., R. Karban and D. Hougeneitzman. 1993. Direct and Indirect Competition Between Spider-Mites Feeding On Grapes. *Ecological Applications* 3:699-707.
- Erb, M., C. A. M. Robert, B. E. Hibbard, and T. C. J. Turlings. 2011. Sequence of arrival determines plant-mediated interactions between herbivores. *Journal of Ecology* 99:7-15.
- Evju M., R. Halvorsen, K. Rydgren, G. Austrheim, and A. Mysterud. 2010. Interactions between local climate and grazing determine the population dynamics of the small herb *Viola biflora*. *Oecologia* 163:921-933.
- Ewald N. C., E. A. John, and S. E. Hartley. 2011. Responses of insect herbivores to sharing a host plant with a hemiparasite: impacts on preference and performance differ with feeding guild. *Ecological Entomology* 36:596-604.
- Facelli E., S. E. Smith, J. M. Facelli, H. M. Christophersen, and F. A. Smith. 2010. Underground friends or enemies: model plants help to unravel direct and indirect effects of arbuscular mycorrhizal fungi on plant competition. *New Phytologist* 185:1050-1061.
- Fisher A. E. I., S. E. Hartley, and M. Young. 2000. Direct and indirect competitive effects of foliage feeding guilds on the performance of the birch leaf-miner *Eriocrania*. *Journal of Animal Ecology* 69:165-176.
- Flory, S. L. and J. T. Bauer. 2014. Experimental evidence for indirect facilitation among invasive plants. *Journal of Ecology* 102:12-18.

- Fritz, R. S. 1995. Direct and indirect effects of plant genetic-variation on enemy impact. *Ecological Entomology* 20:18-26.
- Fuentes-Contreras, E. and H. M. Niemeyer. 2002. Direct and indirect effects of wheat cultivars with different levels of resistance on parasitoids and entomopathogenic fungi of cereal aphids. *Ecoscience* 9:37-43.
- Gibson, M. R., D. M. Richardson, and A. Pauw. 2012. Can floral traits predict an invasive plant's impact on native plant-pollinator communities? *Journal of Ecology* 102:1216-1223.
- Giffard, B., L. Barbaro, H. Jactel, and E. Corcket. 2013. Plant neighbours mediate bird predation effects on arthropod abundance and herbivory. *Ecological Entomology* 38:448-455.
- Gillespie, S. D., and L. S. Adler. 2013. Indirect effects on mutualisms: parasitism of bumble bees and pollination service to plants. *Ecology* 94:454-464.
- Gonzalvez, F. G., L. Santamaria, R. T. Corlett, and M. A. Rodriguez-Girones. 2013. Flowers attract weaver ants that deter less effective pollinators. *Journal of Ecology* 101:78-85.
- Graff, P., M. R. Aguiar, and E. J. Chaneton. 2007. Shifts in positive and negative plant interactions along a grazing intensity gradient. *Ecology* 88:188-199.
- Graff, P. and M. R. Aguiar. 2011. Testing the role of biotic stress in the stress gradient hypothesis. *Processes and patterns in arid rangelands. Oikos* 120:1023-1030.
- Graff, P., F. Rositano, and M. R. Aguiar. 2013. Changes in sex ratios of a dioecious grass with grazing intensity: the interplay between gender traits, neighbour interactions and spatial patterns. *Journal of Ecology* 101:1146-1157.
- Grewell, B. J. 2008. Hemiparasites generate environmental heterogeneity and enhance species coexistence in salt marshes. *Ecological Applications* 18:1297-1306.
- Grewell, B. J. 2008. Parasite facilitates plant species coexistence in a coastal wetland. *Ecology* 89:1481-1488.
- Haag, J. J., M. D. Coupe, and J. F. Cahill. 2004. Antagonistic interactions between competition and insect herbivory on plant growth. *Journal of Ecology* 92:156-167.
- Hambäck, P. A. and P. Ekerholm. 1997. Mechanisms of apparent competition in seasonal environments: an example with vole herbivory. *Oikos* 80:276-288.
- Hansen, D. M., H. C. Kiesbuy, C. G. Jones, and C. B. Muller. 2007. Positive indirect interactions between neighboring plant species via a lizard pollinator. *American Naturalist* 169:534-542.
- Harmon, J. P., A. R. Ives, J. E. Losey, A. C. Olson, and K. S. Rauwald. 2000. *Colemegilla maculata* (Coleoptera : Coccinellidae) predation on pea aphids promoted by proximity to dandelions. *Oecologia* 125:543-548.
- Harri, S. A., J. Krauss, and C. B. Muller. 2008. Trophic cascades initiated by fungal plant endosymbionts impair reproductive performance of parasitoids in the second generation. *Oecologia* 157:399-407.
- Hartnett, D. C., B. A. D. Hetrick, G. W. T. Wilson, and D. J. Gibson. 1993. Mycorrhizal influence on intra- and interspecific neighbour interactions among co-occurring prairie grasses. *Journal of Ecology* 81:787-795.
- Heil, M., A. Hilpert, R. Kruger, and K. E. Linsenmair. 2004. Competition among visitors to extrafloral nectaries as a source of ecological costs of an indirect defence. *Journal of Tropical Ecology* 20:201-208.

- Hierro, J. L. and M. C. Cock. 2013. Herbivore-mediated facilitation alters composition and increases richness and diversity in ruderal communities. *Plant Ecology* 214:1287-1297.
- Hill, B. H. C. and J. Silvertown. 1997. Higher-order interaction between molluscs and sheep affecting seedling numbers in grassland. *Acta Oecologica-International Journal of Ecology* 18:587-596.
- Holzschuh, A., C. F. Dormann, T. Tscharntke, and I. Steffan-Dewenter. 2011. Expansion of mass-flowering crops leads to transient pollinator dilution and reduced wild plant pollination. *Proceedings of the Royal Society B* 278:3444-3451.
- Huntzinger, M., R. Karban, and H. Cushman. 2008. Negative effects of vertebrate herbivores on invertebrates in a coastal dune community. *Ecology* 89:1972-1980.
- Ilse, L. A. and E. C. Hellgren. 2007. Indirect interactions among dendrophages: Porcupines predispose pinyon pines to bark beetle attack. *Forest Ecology and Management* 242:217-226.
- Ishii, H. S. 2013. Community-dependent foraging habits of flower visitors: cascading indirect interactions among five bumble bee species. *Ecological Research* 28:603-613.
- Jensen, A. M., M. Lof, and J. Witzell. 2012. Effects of competition and indirect facilitation by shrubs on *Quercus robur* saplings. *Plant Ecology* 213:535-543.
- Johnson, S. D., C. I. Peter, L. A. Nilsson, and J. Ågren. 2003. Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. *Ecology* 84:2919-2927.
- Johnson, S. N., A. E. Douglas, S. Woodward, and S. E. Hartley. 2003. Microbial impacts on plant-herbivore interactions: the indirect effects of a birch pathogen on a birch aphid. *Oecologia* 134:388-396.
- Jones, T. S., H. C. J. Godfray, and F. J. F. Van Veen. 2009. Resource competition and shared natural enemies in experimental insect communities. *Oecologia* 159:627-635.
- Kaiser-Bunbury, C. N., T. Valentin, J. Mougai, D. Matatiken, and J. Ghazoul. 2011. The tolerance of island plant-pollinator networks to alien plants. *Journal of Ecology* 99:202-213.
- Kaplan, I., R. Halitschke, A. Kessler, B. J. Rehill, S. Sardanelli, and R. F. Denno. 2008. Physiological integration of roots and shoots in plant defense strategies links above- and belowground herbivory. *Ecology Letters* 11:841-851.
- Kaplan, I., S. Sardanelli, B. J. Rehill, and R. F. Denno. 2011. Toward a mechanistic understanding of competition in vascular-feeding herbivores: an empirical test of the sink competition hypothesis. *Oecologia* 166:627-636.
- Karban, R., P. Grof-Tisza, and M. Holyoak. 2012. Facilitation of tiger moths by outbreeding tussock moths that share the same host plants. *Journal of Animal Ecology* 81:1095-1102.
- Katayama, N. and N. Suzuki. 2010. Extrafloral nectaries indirectly protect small aphid colonies via ant-mediated interactions. *Applied Entomology and Zoology* 45:505-511.
- Lara, C., V. Martinez-Garcia, R. Ortiz-Pulido, J. Bravo-Cadena, S. Loranca, and A. Córdoba-Aguilar. 2011. Temporal-spatial segregation among hummingbirds foraging on honeydew in a temperate forest in Mexico. *Current Zoology* 57:56-62.
- Lau, J. A. and S. Y. Strauss. 2005. Insect herbivores drive important indirect effects of exotic plants on native communities. *Ecology* 86:2990-2997.

- Lau, J. A. 2013. Trophic consequences of a biological invasion: do plant invasions increase predator abundance? *Oikos* 122:474-480.
- Laws, A. N. and A. Joern. 2013. Predator-prey interactions in a grassland food chain vary with temperature and food quality. *Oikos* 122:977-986.
- Leege, L. M. 2006. The relationship between psyllid leaf galls and redbay (*Persea borbonia*) fitness traits in sun and shade. *Plant Ecology* 184:203-212.
- Leigh, J., A. H. Fitter, and A. Hodge. 2011. Growth and symbiotic effectiveness of an arbuscular mycorrhizal fungus in organic matter in competition with soil bacteria. *Fems Microbiology Ecology* 76:428-438.
- Lescano, M. N., and A. G. Farji-Brener. 2011. Exotic thistles increase native ant abundance through the maintenance of enhanced aphid populations. *Ecological Research* 26:827-834.
- Lescano, M. N., A. G. Farji-Brener, and E. Gianoli. 2014. Nocturnal resource defence in aphid-tending ants of northern Patagonia. *Ecological Entomology* 39:203-209.
- Levine, J. M. 1999. Indirect facilitation: Evidence and predictions from a riparian community. *Ecology* 80:1762-1769.
- Levine, J. M., S. D. Hacker, C. D. G. Harley, and M. D. Bertness. 1998. Nitrogen effects on an interaction chain in a salt marsh community. *Oecologia* 117:266-272.
- Lewis, O. T., J. Memmott, J. Lasalle, C. H. C. Lyal, C. Whiteford, and H. C. J. Godfray. 2002. Structure of a diverse tropical forest insect-parasitoid community. *Journal of Animal Ecology* 71:855-873.
- Lobo, N. and J. S. Millar. 2013. Indirect and mitigated effects of pulsed resources on the population dynamics of a northern rodent. *Journal of Animal Ecology* 82:814-825.
- Lopezaraiza-Mikel, M. E., R. B. Hayes, M. R. Whalley, and J. Memmott. 2007. The impact of an alien plant on a native plant-pollinator network: an experimental approach. *Ecology Letters* 10:539-550.
- Lynch, M. E., I. Kaplan, G. P. Dively, and R. F. Denno. 2006. Host-plant-mediated competition via induced resistance: Interactions between pest herbivores on potatoes. *Ecological Applications* 16:855-864.
- Madrigal, J., D. A. Kelt, P. L. Meserve, J. R. Gutiérrez, and F. A. Squeo. 2011. Bottom-up control of consumers leads to top-down indirect facilitation of invasive annual herbs in semiarid Chile. *Ecology* 92:282-288.
- Madrigal-Gonzalez, J., A. P. Cea, L. A. Sanchez-Fernandez, K. P. Martinez-Tilleria, J. E. Calderon, and J. R. Gutierrez. 2013. Facilitation of the non-native annual plant *Mesembryanthemum crystallinum* (Aizoaceae) by the endemic cactus *Eulychnia acida* (Cactaceae) in the Atacama Desert. *Biological Invasions* 15:1439-1447.
- Maestre, F. T., J. Cortina, and S. Bautista. 2004. Mechanisms underlying the interaction between *Pinus halepensis* and the native late-successional shrub *Pistacia lentiscus* in a semi-arid plantation. *Ecography* 27:776-786.
- Malmstrom, C. M., A. J. McCullough, H. A. Johnson, L. A. Newton, and E. T. Borer. 2005. Invasive annual grasses indirectly increase virus incidence in California native perennial bunchgrasses. *Oecologia* 145:153-164.
- Marler, M. J., C. A. Zabinski, and R. M. Callaway. 1999. Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. *Ecology* 80:1180-1186.

- Martinez, D., D. Garcia, J. M. Herrera. 2014. Consistency and reciprocity of indirect interactions between tree species mediated by frugivorous birds. *Oikos* 123:414-422.
- Martinsen, G. D., E. M. Driebe, and T. G. Whitham. 1998. Indirect interactions mediated by changing plant chemistry: Beaver browsing benefits beetles. *Ecology* 79:192-200.
- Meier, C. L. and W. D. Bowman. 2008. Phenolic-rich leaf carbon fractions differentially influence microbial respiration and plant growth. *Oecologia* 158:95-107.
- Meisner, M., J. P. Harmon, and A. R. Ives. 2007. Presence of an unsuitable host diminishes the competitive superiority of an insect parasitoid: a distraction effect. *Population Ecology* 49:347-355.
- Metlen, K. L., E. T. Aschehoug, and R. M. Callaway. 2013. Competitive outcomes between two exotic invaders are modified by direct and indirect effects of a native conifer. *Oikos* 122:632-640.
- Miller, T. E. 1994. Direct and indirect species interactions in an early old-field plant community. *American Naturalist* 143:1007-1025.
- Moeller, D. A. 2004. Facilitative interactions among plants via shared pollinators. *Ecology* 85:3289-3301.
- Moeller, D. A. 2005. Pollinator community structure and sources of spatial variation in plant-pollinator interactions in *Clarkia xantiana* ssp *xantiana*. *Oecologia* 142:28-37.
- Molina-Montenegro, M. A., E. I. Badano, and L. A. Cavieres. 2008. Positive interactions among plant species for pollinator service: assessing the 'magnet species' concept with invasive species. *Oikos* 117:1833-1839.
- Morris, R. J., O. T. Lewis, and H. C. J. Godfray. 2005. Apparent competition and insect community structure: towards a spatial perspective. *Annales Zoologici Fennici* 42:449-462.
- Morvillo C. M., E. B. de la Fuente, A. Gil, M. A. Martínez-Ghersa, and J. L. González-Andújar. 2011. Competitive and allelopathic interference between soybean crop and annual wormwood (*Artemisia annua* L.) under field conditions. *European Journal of Agronomy* 34:211-221.
- Muhamed, H., B. Touzard, Y. Le Bagousse-Pinguet, and R. Michalet. 2013. The role of biotic interactions for the early establishment of oak seedlings in coastal dune forest communities. *Forest Ecology and Management* 297:67-74.
- Mulder, C. P. H. and R. W. Ruess. 1998. Effects of herbivory on arrowgrass: Interactions between geese, neighboring plants, and abiotic factors. *Ecological Monographs* 68:275-293.
- Munoz, A. and R. Bonal. 2007. Rodents change acorn dispersal behaviour in response to ungulate presence. *Oikos* 116:1631-1638.
- Nakamura, M., Y. Miyamoto, and T. Ohgushi. 2003. Gall initiation enhances the availability of food resources for herbivorous insects. *Functional Ecology* 17:851-857.
- Niemela, M., A. Markkola, and P. Mutikainen. 2008. Modification of competition between two grass species by a hemiparasitic plant and simulated grazing. *Basic and Applied Ecology* 9:117-125.
- Nishida, T., N. Katayama, N. Izumi, and T. Ohgushi. 2010. Arbuscular mycorrhizal fungi species-specifically affect induced plant responses to a spider mite. *Population Ecology* 52:507-515.

- Offenberg, J., D. J. Macintosh, and M. G. Nielsen. 2006. Indirect ant-protection against crab herbivory: damage-induced susceptibility to crab grazing may lead to its reduction on ant-colonized trees. *Functional Ecology* 20:52-57.
- Oliver, T. H., J. M. Cook, and S. R. Leather. 2007. When are ant-attractant devices a worthwhile investment? *Vicia faba* extrafloral nectaries and *Lasius niger* ants. *Population Ecology* 49:265-273.
- Omacini, M., T. Eggers, M. Bonkowski, A. C. Gange, and T. H. Jones. 2006. Leaf endophytes affect mycorrhizal status and growth of co-infected and neighbouring plants. *Functional Ecology* 20:226-232.
- Orrock, J. L., M. S. Witter, and O. J. Reichman. 2008. Apparent competition with an exotic plant reduces native plant establishment. *Ecology* 89:1168-1174.
- Orrock, J. L. and M. Witter. 2010. Multiple drivers of apparent competition reduce re-establishment of a native plant in invaded habitats. *Oikos* 119:101-108.
- Pages, J. P. and R. Michalet. 2006. Contrasted responses of two understorey species to direct and indirect effects of a canopy gap. *Plant Ecology* 187:179-187.
- Pages, J. P., G. Pache, D. Joud, N. Magnan, and R. Michalet. 2003. Direct and indirect effects of shade on four forest tree seedlings in the French Alps. *Ecology* 84:2741-2750.
- Palladini, J. D., and J. L. Maron. 2013. Indirect competition for pollinators is weak compared to direct resource competition: pollination and performance in the face of an invader. *Oecologia* 172:1061-1069.
- Pearse, I. S. 2010. Bird rookeries have different effects on different feeding guilds of herbivores and alter the feeding behavior of a common caterpillar. *Arthropod-Plant Interactions* 4:189-195.
- Pellegrino, G., F. Bellusci, and A. Musacchio. 2008. Double floral mimicry and the magnet species effect in dimorphic co-flowering species, the deceptive orchid *Dactylorhiza sambucina* and rewarding *Viola aethnensis*. *Preslia* 80:411-422.
- Pellissier, L., K. A. Brathen, J. Pottier, C. F. Randin, P. Vittoz, A. Dubuis, N. G. Yoccoz, T. Alm, N. E. Zimmermann, and A. Guisan. 2010. Species distribution models reveal apparent competitive and facilitative effects of a dominant species on the distribution of tundra plants. *Ecography* 33:1004-1014.
- Poelman, E. H., R. Gols, T. A. L. Snoeren, D. Muru, H. M. Smid, and M. Dicke. 2011. Indirect plant-mediated interactions among parasitoid larvae. *Ecology Letters* 14:670-676.
- Power, A. G. 1996. Competition between viruses in a complex plant-pathogen. *Ecology* 77:1004-1010.
- Preisser, E. L. and J. S. Elkinton. 2008. Exploitative competition between invasive herbivores benefits a native host plant. *Ecology* 89:2671-2677.
- Preus, L. E. and P. A. Morrow. 1999. Direct and indirect effects of two herbivore species on resource allocation in their shared host plant: the rhizome galler *Eurosta comma*, the folivore *Trirhabda canadensis* and *Solidago missouriensis*. *Oecologia* 119:219-226.
- Prevosto, B., Y. Monnier, C. Ripert, and C. Fernandez. 2012. To what extent do time, species identity and selected plant response variables influence woody plant interactions? *Journal of Applied Ecology* 49:1344-1355.

- Pugnaire, F. I., P. Haase, J. Puigdefabregas, M. Cueto, S. C. Clark, and L. D. Incoll. 1996. Facilitation and succession under the canopy of a leguminous shrub, *Retama sphaerocarpa*, in a semi-arid environment in south-east Spain. *Oikos* 76:455-464.
- Puustinen, S. and P. Mutikainen. 2001. Host-parasite-herbivore interactions: Implications of host cyanogenesis. *Ecology* 82:2059-2071.
- Rand, T. A. 2003. Herbivore-mediated apparent competition between two salt marsh forbs. *Ecology* 84:1517-1526.
- Rand, T. A. 1999. Effects of environmental context on the susceptibility of *Atriplex patula* to attack by herbivorous beetles. *Oecologia* 121:39-46.
- Recart, W., J. D. Ackerman, and A. A. Cuevas. 2013. There goes the neighborhood: apparent competition between invasive and native orchids mediated by a specialist florivorous weevil. *Biological Invasions* 15:283-293.
- Redman, A. M. and J. M. Scriber. 2000. Competition between the gypsy moth, *Lymantria dispar*, and the northern tiger swallowtail, *Papilio canadensis*: interactions mediated by host plant chemistry, pathogens, and parasitoids. *Oecologia* 125:218-228.
- Ridenour, W. L. and R. M. Callaway. 2003. Root herbivores, pathogenic fungi, and competition between *Centaurea maculosa* and *Festuca idahoensis*. *Plant Ecology* 169:161-170.
- Riginos, C. and T. P. Young. 2007. Positive and negative effects of grass, cattle, and wild herbivores on *Acacia* saplings in an East African savanna. *Oecologia* 153:985-995.
- Ritchie, M. E. and D. Tilman. 1993. Predictions of species interactions from consumer-resource theory - experimental tests with grasshoppers and plants. *Oecologia* 94:516-527.
- Roder, G., M. Rahier, and R. E. Naisbit. 2007. Coping with an antagonist: the impact of a phytopathogenic fungus on the development and behaviour of two species of alpine leaf beetle. *Oikos* 116:1514-1523.
- Rolhauser, A. G., E. J. Chaneton, and W. B. Batista. 2011. Influence of conspecific and heterospecific adults on riparian tree species establishment during encroachment of a humid palm savanna. *Oecologia* 167:141-148.
- Rott, A. S. and H. C. J. Godfray. 2000. The structure of a leafminer-parasitoid community. *Journal of Animal Ecology* 69:274-289.
- Rousset, O. and J. Lepart. 2000. Positive and negative interactions at different life stages of a colonizing species (*Quercus humilis*). *Journal of Ecology* 88:401-412.
- Russell, F. L., S. M. Louda, T. A. Rand, and S. D. Kachman. 2007. Variation in herbivore-mediated indirect effects of an invasive plant on a native plant. *Ecology* 88:413-423.
- Saccone, P., J-P. Pages, J. Girel, J-J. Brun, and R. Michalet. 2010. *Acer negundo* invasion along a successional gradient: early direct facilitation by native pioneers and late indirect facilitation by conspecifics. *New Phytologist* 187:831-842.
- Sakata, H. and Y. Hashimoto. 2000. Should aphids attract or repel ants? Effect of rival aphids and extrafloral nectaries on ant-aphid interactions. *Population Ecology* 42:171-178.
- Samson, D. A., T. E. Philippi, and D. W. Davidson. 1992. Granivory and competition as determinants of annual plant diversity in the Chihuahuan Desert. *Oikos* 65:61-80.

- Savage, A. M., and J. A. Rudgers. 2013. Non-additive benefit or cost? Disentangling the indirect effects that occur when plants bearing extrafloral nectaries and honeydew-producing insects share exotic ant mutualists. *Annals of Botany* 111:1295-1307.
- Schellhorn, N. A., T. R. Kuhman, A. C. Olson, and A. R. Ives. 2002. Competition between native and introduced parasitoids of aphids: Nontarget effects and biological control. *Ecology* 83:2745-2757.
- Schoeb, C., C. Armas, and F. I. Pugnaire. 2013. Direct and indirect interactions co-determine species composition in nurse plant systems. *Oikos* 122:1371-1379.
- Schoeman, C. S. and M. J. Samways. 2011. Synergisms between alien trees and the Argentine ant on indigenous ant species in the Cape Floristic Region, South Africa. *African Entomology* 19:96-105.
- Seifan, M. and R. Kadmon. 2006. Indirect effects of cattle grazing on shrub spatial pattern in a mediterranean scrub community. *Basic and Applied Ecology* 7:496-506.
- Seifan, M., K. Tielboerger, and R. Kadmon. 2010. Direct and indirect interactions among plants explain counterintuitive positive drought effects on an eastern Mediterranean shrub species. *Oikos* 119:1601-1609.
- Seifan, M., E-M. Hoch, S. Hanoteaux, and K. Tielboerger. 2014. The outcome of shared pollination services is affected by the density and spatial pattern of an attractive neighbour. *Journal of Ecology* 102:953-962.
- Sessions, L. and D. Kelly. 2002. Predator-mediated apparent competition between an introduced grass, *Agrostis capillaris*, and a native fern, *Botrychium australe* (Ophioglossaceae), in New Zealand. *Oikos* 96:102-109.
- Shiojiri, K., J. Takabayashi, S. Yano, and A. Takafuji. 2001. Infochemically mediated tritrophic interaction webs on cabbage plants. *Population Ecology* 43:23-29.
- Snell, R. S. and J. F. Addicott. 2008. Direct and indirect effects of ants on seed predation in moth/yucca mutualisms. *Ecoscience* 15:305-314.
- Soliveres, S., D. J. Eldridge, F. T. Maestre, M. A. Bowker, M. Tighe, and A. Escudero. 2011. Microhabitat amelioration and reduced competition among understory plants as drivers of facilitation across environmental gradients: towards an unifying framework. *Perspectives in Plant Ecology, Evolution and Systematics* 13:247-258.
- Soliveres, S., P. Garcia-Palacios, A. P. Castillo-Monroy, F. T. Maestre, A. Escudero, and F. Valladares. 2011. Temporal dynamics of herbivory and water availability interactively modulate the outcome of a grass-shrub interaction in a semi-arid ecosystem. *Oikos* 120:710-719.
- Stahl, J., A. J. Van Der Graaf, R. H. Drent, and J. P. Bakker. 2006. Subtle interplay of competition and facilitation among small herbivores in coastal grasslands. *Functional Ecology* 20:908-915.
- Staley, J. T., D. B. Stafford, E. R. Green, S. R. Leather, J. T. Rossiter, G. M. Poppy, and D. J. Wright. 2011. Plant nutrient supply determines competition between phytophagous insects. *Proceedings of the Royal Society B-Biological Sciences* 278:718-724.
- Staley, J. T., S. R. Mortimer, M. D. Morecroft, V. K. Brown, and G. J. Masters. 2007. Summer drought alters plant-mediated competition between foliar- and root-feeding insects. *Global Change Biology* 13:866-877.
- Steffan, S. A. and W. E. Snyder. 2010. Cascading diversity effects transmitted exclusively by behavioral interactions. *Ecology* 91:2242-2252.

- Stein, C., C. Rissmann, S. Hempel, C. Renker, F. Buscot, D. Prati, and H. Auge. 2009. Interactive effects of mycorrhizae and a root hemiparasite on plant community productivity and diversity. *Oecologia* 159:191-205.
- Stephens, A. E. A. and J. H. Myers. 2014. Testing biological control agent compatibility: *Cyphocleonus achates* and *Larinus minutus* on diffuse knapweed. *Biological Control* 70:48-53.
- Stinson, K. A., S. A. Campbell, J. R. Powell, B. E. Wolfe, R. M. Callaway, G. C. Thelen, S. G. Hallet, D. Prati, and J. N. Klironomos. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *Plos Biology* 4: e140. DOI: 10.1371/journal.pbio.0040140.
- Strauss, S. Y. 1991. Direct, indirect, and cumulative effects of 3 native herbivores on a shared host plant. *Ecology* 72:543-558.
- Strong, D. R., A. V. Whipple, A. L. Child, and B. Dennis. 1999. Model selection for a subterranean trophic cascade: Root-feeding caterpillars and entomopathogenic nematodes. *Ecology* 80:2750-2761.
- Swope, S. M. and I. M. Parker. 2010. Trait-mediated interactions and lifetime fitness of the invasive plant *Centaurea solstitialis*. *Ecology* 91:2284-2293.
- Tack, A. J. M., O. Ovaskainen, P. J. Harrison, and T. Roslin. 2009. Competition as a structuring force in leaf miner communities. *Oikos* 118:809-818.
- Tadey, M. and A. G. Farji-Brener. 2007. Discriminating direct and indirect effects of exotic grazers on native plant cover in the Monte desert of Argentina. *Journal of Arid Environments* 69:526-536.
- Tan, X-L., S. Wang, J. Ridsdill-Smith, and T-X. Liu. 2014. Direct and Indirect Impacts of Infestation of Tomato Plant by *Myzus persicae* (Hemiptera: Aphididae) on *Bemisia tabaci* (Hemiptera: Aleyrodidae). *PLoS ONE* 9: e94310. doi:10.1371/journal.pone.0094310.
- Tielborger, K. and R. Kadmon. 2000. Indirect effects in a desert plant community: is competition among annuals more intense under shrub canopies? *Plant Ecology* 150:53-63.
- Van Hovel, M. D., B. A. Evans, and V. A. Borowicz. 2011. Hemiparasite - host plant interactions and the impact of herbivory: a field experiment. *Botany-Botanique* 89:537-544.
- Van Rijn, P. C. J., Y. M. Van Houten, and M. W. Sabelis. 2002. How plants benefit from providing food to predators even when it is also edible to herbivores. *Ecology* 83:2664-2679.
- van Veen, F. J. F., C. E. Brandon, and H. C. J. Godfray. 2009. A positive trait-mediated indirect effect involving the natural enemies of competing herbivores. *Oecologia* 160:195-205.
- van Veen, F. J. F. and D. Sanders. 2013. Herbivore identity mediates the strength of trophic cascades on individual plants. *Ecosphere* 4:64. <http://dx.doi.org/10.1890/ES13-00067.1>.
- Van Zandt, P. A. and A. A. Agrawal. 2004. Community-wide impacts of herbivore-induced plant responses in milkweed (*Asclepias syriaca*). *Ecology* 85:2616-2629.
- Vandenberghe, C., C. Smit, M. Pohl, A. Buttler, and F. Frelechoux. 2009. Does the strength of facilitation by nurse shrubs depend on grazing resistance of tree saplings?. *Basic and Applied Ecology* 10:427-436.

- Veblen, K. E. and T. P. Young. 2010. Contrasting effects of cattle and wildlife on the vegetation development of a savanna landscape mosaic. *Journal of Ecology* 98:993-1001.
- Veblen, K. E. 2008. Season- and herbivore-dependent competition and facilitation in a semiarid savanna. *Ecology* 89:1532-1540.
- Veech, J. A. 2000. Predator-mediated interactions among the seeds of desert plants. *Oecologia* 124:402-407.
- Vesterlund, S-R., O. Suominen, R. Bergstrom, K. Danell, and I-I. Persson. 2012. The impact of simulated moose densities on conifer aphids along a productivity gradient. *Ecography* 35:105-112.
- White, E. M., J. C. Wilson, and A. R. Clarke. 2008. Plant-pollinator interactions in sympatric exotic and native *Senecio* species: is facilitation or competition for pollinators occurring?. *Plant Protection Quarterly* 23:120-126.
- White, E., and G. Vivian-Smith. 2011. Contagious dispersal of seeds of synchronously fruiting species beneath invasive and native fleshy-fruited trees. *Austral Ecology* 36:195-202.
- White, J. A. and D. A. Andow. 2006. Habitat modification contributes to associational resistance between herbivores. *Oecologia* 148:482-490.
- Wielgoss, A., Y. Clough, B. Fiala, A. Rumedé, and T. Tschardtke. 2012. A minor pest reduces yield losses by a major pest: plant-mediated herbivore interactions in Indonesian cacao. *Journal of Applied Ecology* 49:465-473.
- Wielgoss, A., T. Tschardtke, A. Rumedé, B. Fiala, H. Seidel, S. Shahabuddin, and Y. Clough. 2014. Interaction complexity matters: disentangling services and disservices of ant communities driving yield in tropical agroecosystems. *Proceedings of the Royal Society B-Biological Sciences* 281: 20132144.
<http://dx.doi.org/10.1098/rspb.2013.2144>
- Wu, X., C. Zhang, J. N. Griffin, and S. Sun. 2014. The brown-world role of insectivores: Frogs reduce plant growth by suppressing detritivores in an alpine meadow. *Basic and Applied Ecology* 15:66-74.
- Zarnetske, P. L., T. C. Gouhier, S. D. Hacker, E. W. Seabloom, V. A. Bokil. 2013. Indirect effects and facilitation among native and non-native species promote invasion success along an environmental stress gradient. *Journal of Ecology* 101:905-915.
- Zhang, M-J., M. Liu, Y. Li, C. Xu, and S. An. 2011. The combined positive effects of two dominant species in an arid shrub-herbaceous community: implications from the performance of two associate species. *Plant Ecology* 212:1419-1428.
- Zhang, Q., Q. Sun, R. T. Koide, Z. Peng, J. Zhou, X. Gu, W. Gao, and M. Yu. 2014. Arbuscular mycorrhizal fungal mediation of plant-plant interactions in a marshland plant community. *Scientific World Journal* 2014:923610.
<http://dx.doi.org/10.1155/2014/923610>.
- Zhang, X-D., X. Jia, Y-Y. Chen, J-J. Shao, X-R. Wu, L. Shang, and B. Li. 2013. Crabs mediate interactions between native and invasive salt marsh plants: a mesocosm study. *PLoS ONE* 8: e74095. doi:10.1371/journal.pone.0074095.
- Zhong, Z., D. Wang, H. Zhu, L. Wang, C. Feng, and Z. Wang. 2014. Positive interactions between large herbivores and grasshoppers, and their consequences for grassland plant diversity. *Ecology* 95:1055-1064.

Appendix 3. Summary of findings for the indirect plant interactions literature, including hypotheses tested and information about experimental procedures, target species and field sites.

Hypotheses tested†	N (%)	Experimental approach (%) ‡	Experimental setting (%) §	Number of target species	Geographical region ¶	Ecosystem type #
Apparent competition	89 (41.6)	MN (78.7), MS (15.7), B (5.6)	FL (59.6), GL (25.8), B (14.6)	1-248 (several)	AF, AS, CA, EU, NA, OC, SA	AG, AL, CO, DE, FO, GR, GL, OF, SM, SH
Indirect facilitation	76 (35.5)	MN (65.8), MS (23.7), B (10.5)	FL (85.5), GL (7.9), B (6.6)	1-21 (several)	AF, AS, CA, EU, NA, OC, SA	AG, AL, CO, DE, FO, GR, GL, OF, RP, SM, SH
Exploitative competition and facilitation	20 (9.3)	MN (75), MS (15), B (10)	FL (75), GL (10), B (15)	1-4 (several)	AS, EU, NA, SA	AG, AL, CO, FO, GR, GL, OF, SH
Associational resistance	19 (8.9)	MN(73.7), MS (15.8), B (10.5)	FL (100)	1-34 (several)	AF, EU, NA, SA	AL, DE, FO, GR, RP, SM, SH
Trophic cascades	9 (4.2)	MN (66.7), MS (22.2), B (11.1)	FL (77.8), GL (22.2)	1-4 (several)	EU, NA, SA	AG, FO, GR, GL, OF
Shared defenses	1 (0.5)	MN (100)	FL (100)	4	EU	FO

† See text for more details on hypotheses tested.

‡ Experimental approach: manipulative (MN), mensurative (MS), both (B).

§ Experimental setting: Field (FL), Greenhouse/Laboratory (GL), both (B).

¶ Geographical region: North-America (NA), South-America (SA), Central-America (CA), Europe (EU), Asia (AS), Oceania (OC), Africa (AF).

Ecosystem type: agricultural (AG), alpine (AL), coastal (CO), desert (DE), forest (FO), grassland (GR), greenhouse/laboratory (GL), old field (OF), riparian (RP), salt marsh (SM), shrubland (SH).

Appendix 4. Site selection in each of the three desert localities included in this project.

In order to select five sites within each desert locality that represented an environmental gradient, we conducted a survey along their range. This survey systematically sampled 1-km grids using one 20 x 20 m quadrat per grid to determine plant cover and species composition within each of these grids. At Atiquipa we sampled 15 of these quadrats, 19 at Romeral, and 13 at Fray Jorge. Cover data of perennial species were then combined with climate information from the database WorldClim (Hijmans et al. 2005) and used to quantitatively determine the plots to be used for this project. Climate information included: annual mean temperature (AnnTemp), mean diurnal range (DayRng), temperature seasonality (TempSeas), annual precipitation (AnnPrec), and precipitation seasonality (PrecSeas). Elevation (Elev) was also included as a predictor in these models. These two datasets (i.e. plant cover and climate) were then combined via Canonical Correspondence Analysis (CCA) (i.e., direct ordination analysis with *vegan* (Oksanen et al. 2016)), in order to properly determine the complex environmental gradients within each locality (Lortie 2010). Ordination diagrams were inspected in order to select sites to sample dominant plants. At Atiquipa (Fig. 1) sites 1, 4, 8, 12 and 14 were selected. At Romeral (Fig. 2), sites 1, 5, 9, 16 and 18 were selected. At Fray Jorge (Fig. 3), sites 3, 4, 9, 12 and 13 were selected. Selected sites within each locality were separated by at least 2 km from one another, but no more than 5 km.

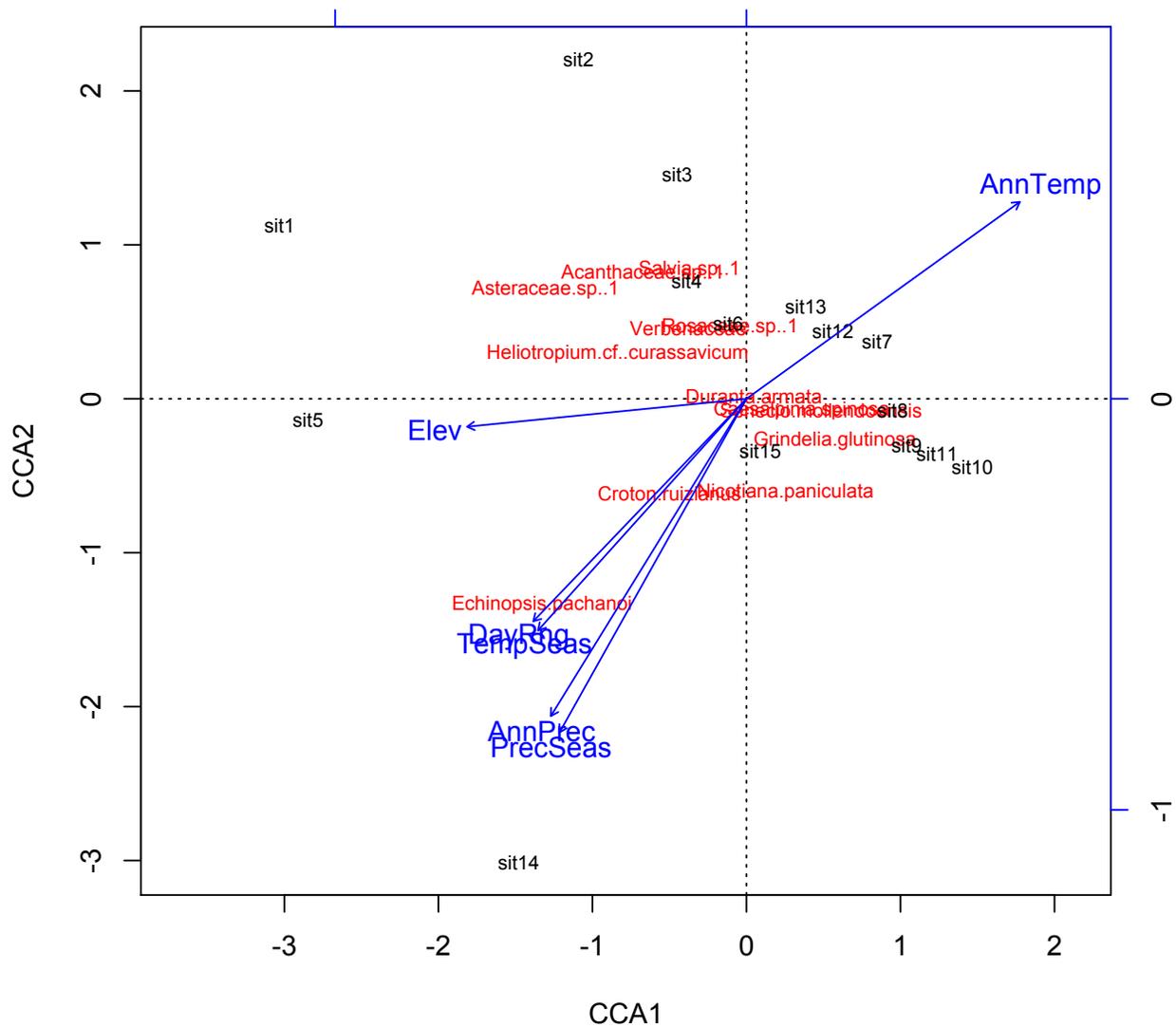


Fig 1. Canonical Correspondence Analysis (CCA) of 15 sites sampled at Atiquipa, Southern Peru. Plant cover data sampled locally was combined with climate data and elevation of each site to produce this ordination diagram.

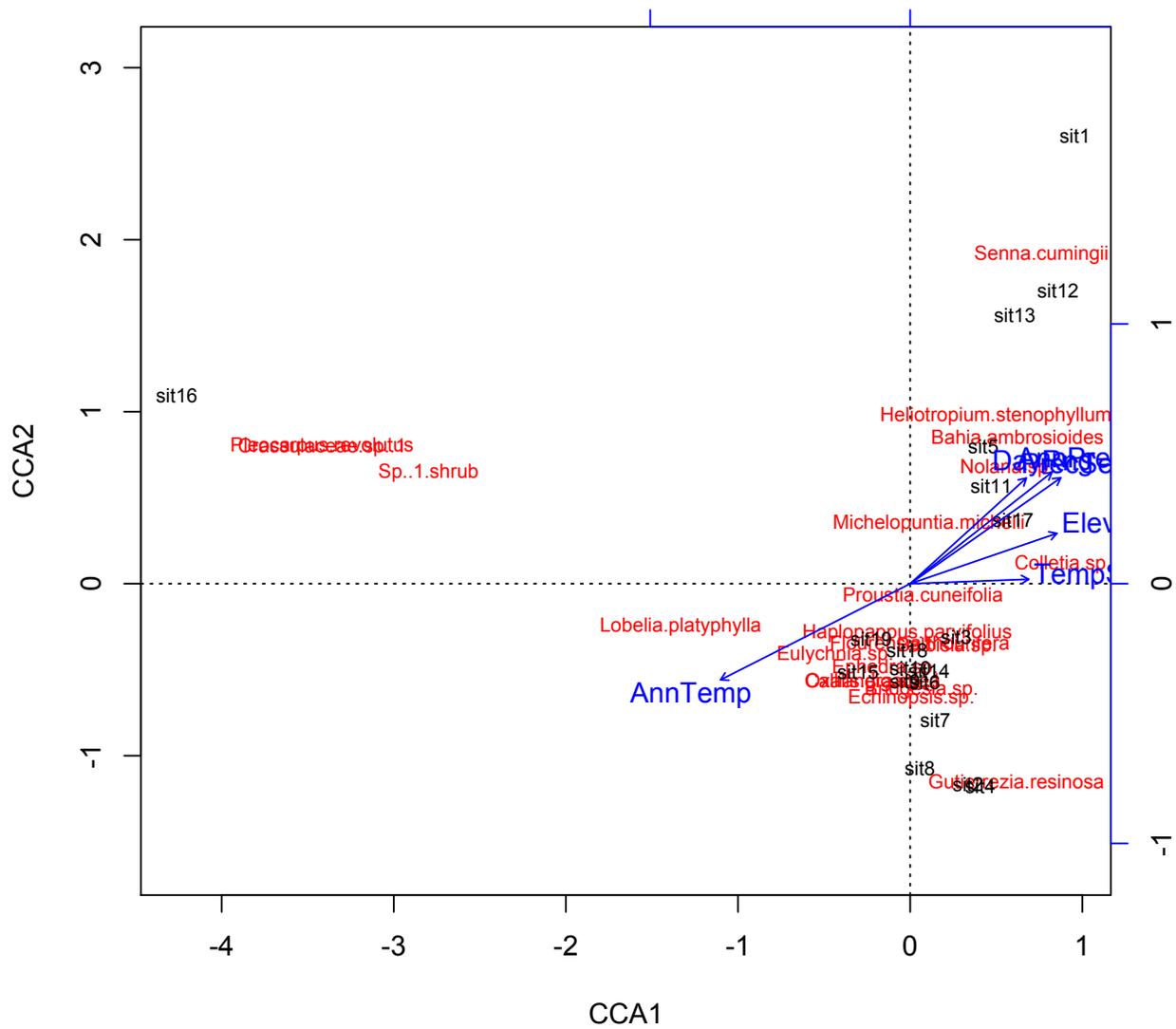


Fig 2. Canonical Correspondence Analysis (CCA) of 19 sites sampled at Romeral, North-Central Chile. Plant cover data sampled locally was combined with climate data and elevation of each site to produce this ordination diagram.

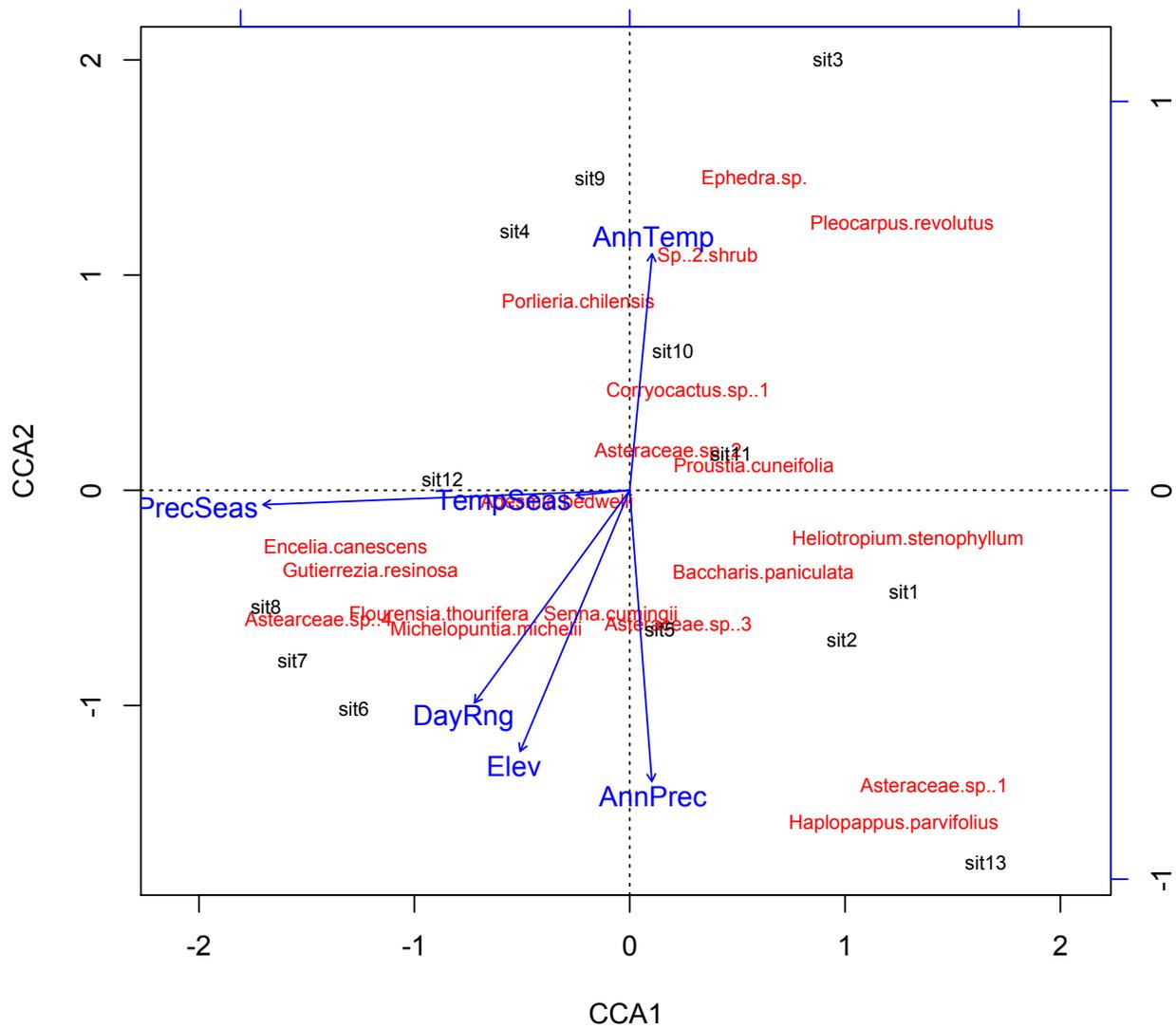


Fig 3. Canonical Correspondence Analysis (CCA) of 13 sites sampled at Fray Jorge, North-Central Chile. Plant cover data sampled locally was combined with climate data and elevation of each site to produce this ordination diagram.

References

- Hijmans, R. J., Cameron, S. E. Parra, J. L., Jones, P. G. & Jarvis, A. 2005. Very High Resolution Interpolated Climate Surfaces for Global Land Areas. *International Journal of Climatology* 25: 1965-1978.
- Lortie, C. J. 2010. Synthetic analysis of the stress-gradient hypothesis. In Pugnaire, F. I. (ed.) *Positive Plant Interactions and Community Dynamics*. Pp 125-148. CRC Press, Boca Ratón, USA.
- Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson G. L., Solymos, P., Stevens, M. H. H. & Wagner, H. 2016. Community Ecology Package, vegan, V 2.3-5. <https://github.com/vegandevs/vegan>.

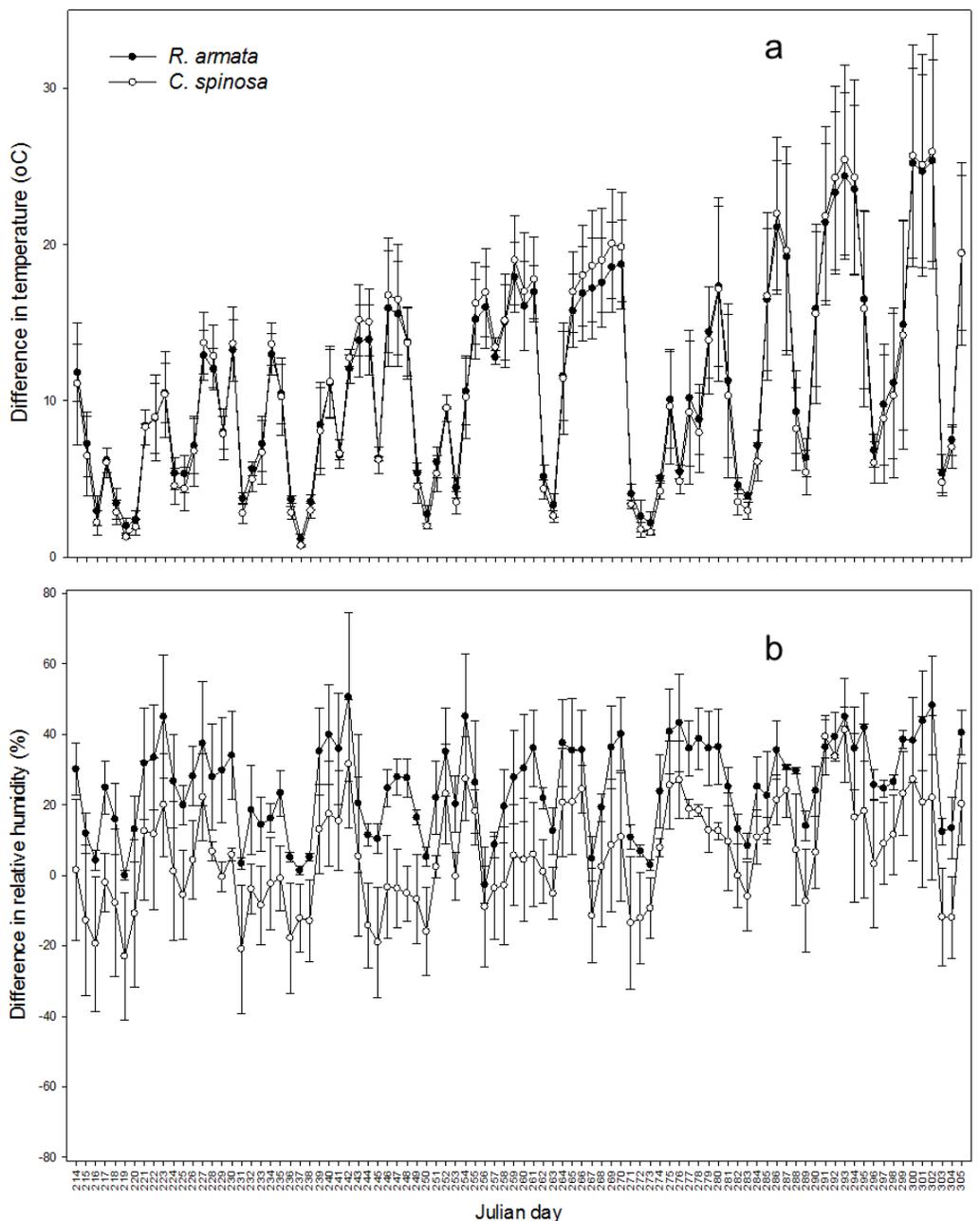
Appendix 5. Summary of GLMMs contrasting species richness among microsites and gradients in three different years along the Atacama Desert. *P*-values <0.05 are bolded and indicate significant differences.

Source	Atiquipa		Romeral		Fray Jorge		
	Chi-Square	P-value	Chi-Square	P-value	Chi-Square	P-value	
Microsite	2	346.28	<.0001	0.69	0.7059	4.64	0.0981
Gradient	4	324.22	<.0001	5.55	0.2350	4.17	0.3839
Nurse*Gradient	8	334.25	<.0001	2.60	0.9567	4.27	0.8324
Year	2	438.04	<.0001	38.41	<.0001	45.40	<.0001
Nurse*Year	4	341.02	<.0001	1.11	0.8928	3.82	0.4306
Gradient*Year	8	329.39	<.0001	3.17	0.9235	1.49	0.9929
Nurse*Gradient*							
Year	16	322.95	<.0001	2.69	0.9999	3.95	0.9990

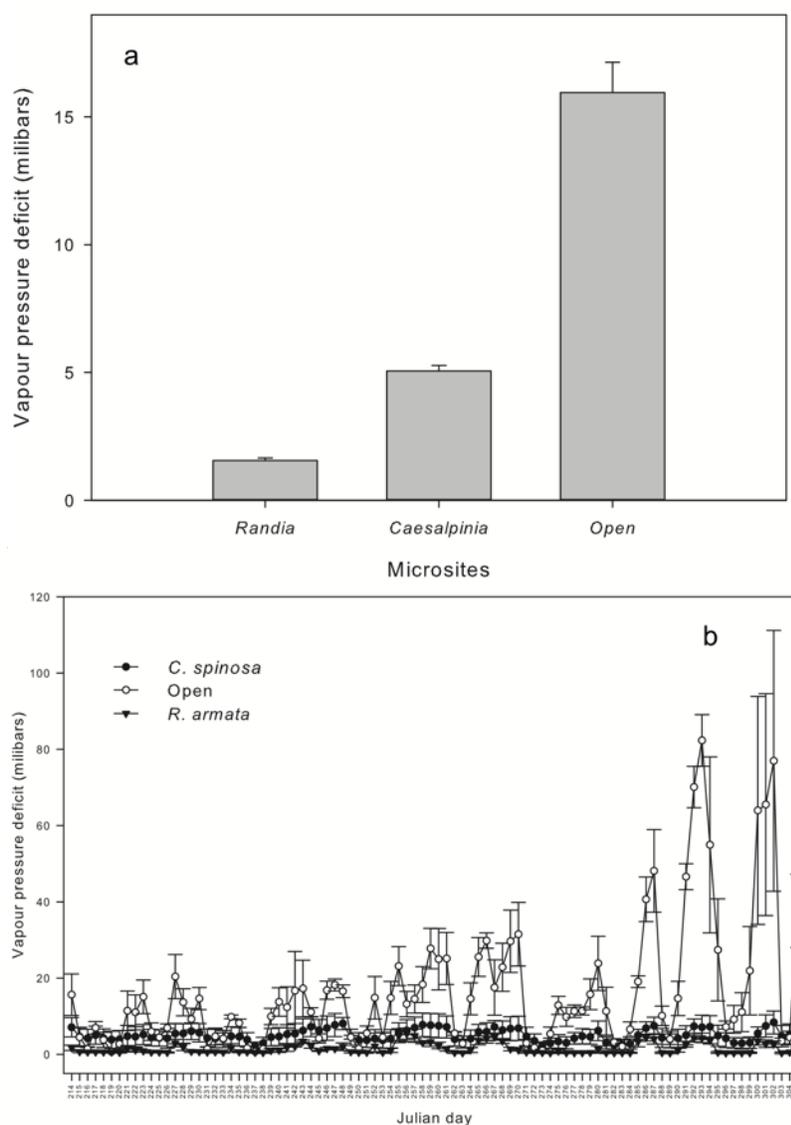
Appendix 6. Summary of GLMMs contrasting plant density among microsites and gradients in three different years along the Atacama Desert. *P*-values <0.05 are bolded and indicate significant differences.

Source	DF	Atiquipa		Romeral		Fray Jorge	
		Chi-Square	P-value	Chi-Square	P-value	Chi-Square	P-value
Microsite	2	396.07	<.0001	4.05	0.1321	18.14	0.0001
Gradient	4	331.14	<.0001	3.11	0.5389	12.16	0.0162
Microsite*Gradient	8	338.29	<.0001	7.62	0.4718	7.84	0.4493
Year	2	598.75	<.0001	161.69	<.0001	42.03	<.0001
Microsite*Year	4	387.48	<.0001	9.34	0.0531	5.21	0.2660
Gradient*Year	8	328.75	<.0001	10.48	0.2332	13.34	0.1005
Microsite*Gradient*							
Year	16	329.08	<.0001	19.24	0.2564	12.46	0.7120

Appendix 7. Absolute differences along with standard errors between microhabitat conditions for two dominant plant microsites in relation to open microsites at noon (12 hrs.) during the growing season of 2012 (August-October) in Atiquipa, Southern Peru. a. Differences in temperature ($^{\circ}\text{C}$). b. Differences in relative humidity (%).



Appendix 8. Vapor pressure deficit (VPD) along with standard errors for three microsites associated with two dominant plants and nearby open microsites at noon (12 hrs.) during the growing season of 2012 (August-October) in Atiquipa, Southern Peru. The three microsites correspond to the understory of *C. spinosa* and *R. armata*, and open nearby microsites. a. Overall averages per microsite; b. Seasonal trend according to Julian Day. VPD was calculated using Hartman (1994)'s equation based on Temperature ($^{\circ}\text{C}$), and Relative Humidity (%).



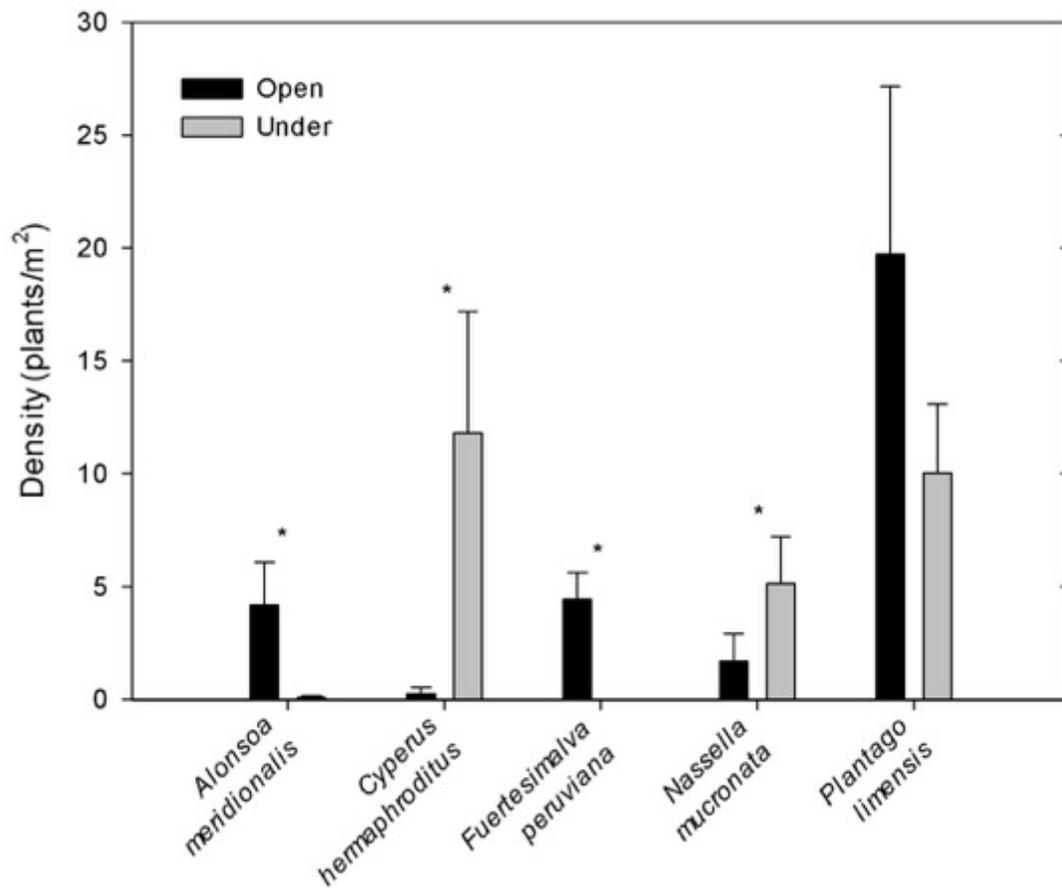
Appendix 9. Study sites along with their climatic characteristics (Chapter 4). De Martonne AI was calculated using mean annual temperature and annual precipitation extracted from WorldClim (Hijmans et al. 2005) for each site.

Site	Geographical location (LS, LW)		Elevation (m)	DeMartonne AI
1	15.763	74.369	741	0.177
2	15.785	74.392	921	0.141
3	15.774	74.385	1042	0.179
4	15.732	74.372	1092	0.403
5	15.748	74.388	1124	0.288

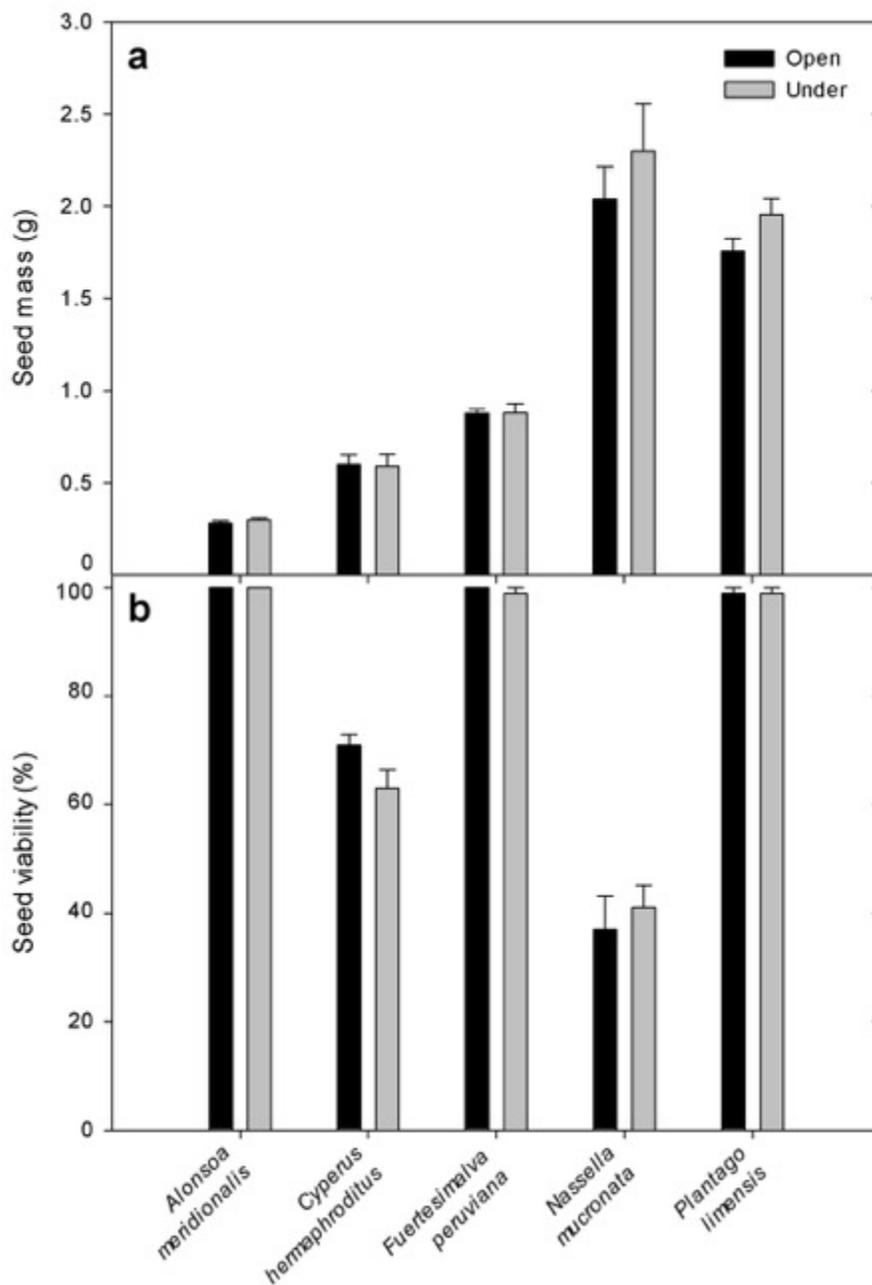
Appendix 10. Summary of Akaike Information Criterion (AIC) values for probability distribution functions fitted to the response variables analyzed in different models of Chapter 4. Lower AIC values indicate a better fit.

Variable	Normal	Exponential
Plant height	3225.61	3199.31
Fruit production	4361.54	3996.63
Biomass	1359.09	929.74
Total density	1974.26	1941.99
<i>F. peruviana</i> density	1552.59	1048.42
<i>P. limensis</i> density	1622.78	1202.77
Neighbors density	1914.87	1883.35

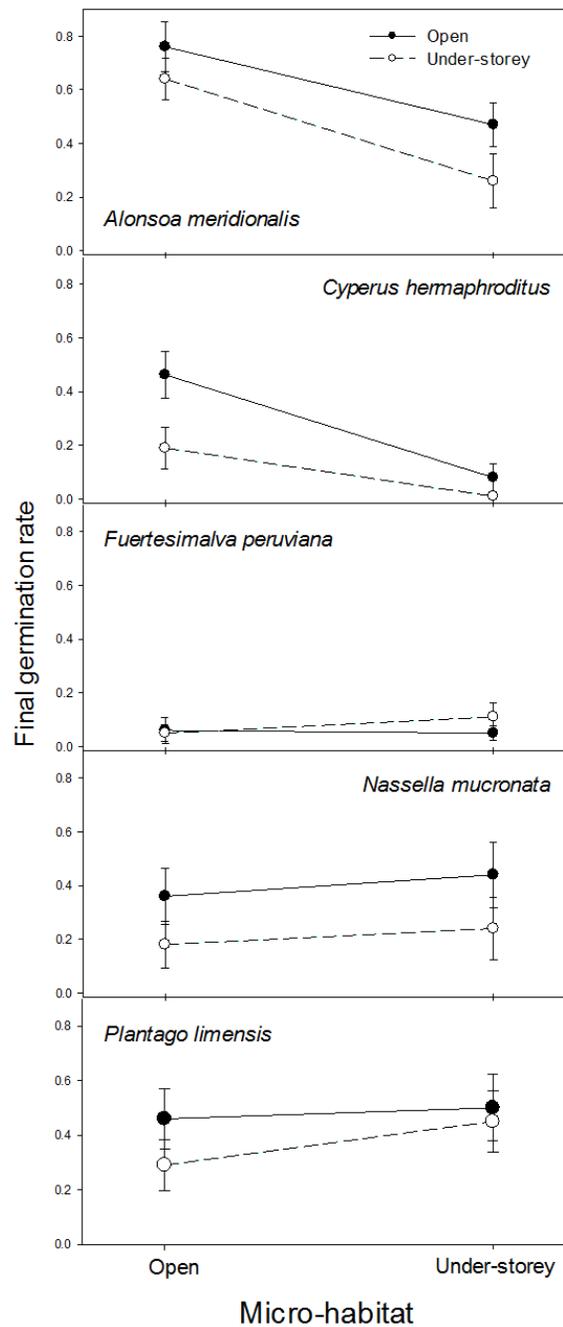
Appendix 11. Density (mean \pm SE) of five annual species in open and understorey micro-habitats at Atiquipa, southern Peru. Asterisks denote statistically significant differences.



Appendix 12. Seed attributes of the five studied species. (a) Seed mass (\pm SE) in open and understorey micro-habitats. (b) Seed viability (\pm SE) using Tetrazolium tests for seeds collected in open and understorey micro-habitats. Asterisks denote statistically significant differences.



Appendix 13. Final germination rates (\pm SE) for seeds of five annual species that were collected in two different micro-habitats (i.e. source: open and understorey, different line patterns) and germinated under two different simulated micro-habitat conditions (i.e. micro-habitat: open and understorey).



Appendix 14. Numbers of days required to 50% germination (\pm SE) for seeds of five annual species that were collected in two different micro-habitats (i.e. source: open and understorey, different line patterns) and germinated under two different simulated micro-habitat conditions (i.e. micro-habitat: open and understorey).

