

University of Nevada, Reno

Global Change and trophic interaction diversity: complex local and regional processes

A dissertation submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Ecology, Evolution, and Conservation Biology

by

Nicholas A. Pardikes

Dr. Lee A. Dyer/Dissertation Advisor

May 2017



THE GRADUATE SCHOOL

We recommend that the dissertation
prepared under our supervision by

NICHOLAS PARDIKES

Entitled

**Global Change And Trophic Interaction Diversity: Complex Local And Regional
Processes**

be accepted in partial fulfillment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

Lee Dyer, Ph.D, Advisor

Matt Forister, Ph.D, Committee Member

David Wagner, Ph.D, Committee Member

Peter Weisberg, Ph.D, Committee Member

Chris Jeffrey, Ph.D, Graduate School Representative

David W. Zeh, Ph. D., Dean, Graduate School

May, 2017

ABSTRACT

The structure and functioning of ecosystems across the globe are rapidly changing due to several components of global environmental change (GEC). My dissertation aims to illustrate how regional and local aspects of GEC impact diverse assemblages of species and species interactions. All organisms are embedded in complex networks of species interactions, and future efforts to predict and mitigate the impacts of GEC on ecological communities will be facilitated by such studies that incorporate a suite of species and species interactions. This study advances our understanding of how GEC will impact ecological communities by investigating two questions about GEC: 1) How will shifts in global climate cycles (e.g., El Nino Southern Oscillation), as a consequence of global warming, impact a diverse assemblage of butterflies that exist across a heterogeneous landscape? 2) What are the consequences of woody plant encroachment on complex, specialized interactions between plants, insect herbivores, and natural enemies (e.g., insect parasitoids)? Furthermore, I helped develop a tool to identify characteristics of ecological communities that are essential for promoting the diversity of trophic interactions. While the loss of species diversity is well recognized, interactions among species are vanishing at an astonishing rate, yet we know little about factors that determine the diversity of interactions within a community. Using data from a long-term butterfly monitoring dataset, I was able to demonstrate the utility of large-scale climate indices (e.g., ENSO) for modeling biotic/abiotic relationships for migratory butterfly species. Next, I used encroaching juniper woodlands in the Intermountain West to uncover that population age structure of dominant trees, such as juniper, can affect plant-insect dynamics and have implications for future control efforts in the expanding

woodlands. Additionally, reductions of understory plant diversity, as a consequence of juniper expansion, resulted in significantly lower parasitism rates and parasitoid species diversity. Finally, simulated food webs revealed that species diversity and, to a lesser degree, consumer diet breadth, promote the diversity of trophic interactions. As ecosystems across the globe experience changes and the loss of species diversity continues, these findings offer insight into how GEC will impact species and species interactions.

DEDICATION

This dissertation is dedicated to my partner, Moria. Without your support and love, this work wouldn't have been possible. Thank you for always being there when I needed it most. To the many adventures we have waiting for us and to all the great memories that made us who we are today.

“May your trails be crooked, winding, lonesome, dangerous, leading to the most amazing view.”

Edward Abbey

ACKNOWLEDGMENTS

I wish to thank my family for all of the support throughout this process. It is because of you that I developed my interest and love of science and nature. I also want to thank the Earthwatch Institute for providing me with an opportunity to work with a diverse group of citizen scientists. Teachers, students, and bankers all helped collect data used in this dissertation and it would not have been possible without their help. I also want to thank my advisor, Lee Dyer, for providing me with the opportunity to contribute to research in your lab. You have been instrumental in shaping me into the scientist I am today and introducing me to the magnificent world of plants and insects. Finally, I want to thank my partner, Moria, and our wonderful dog, Peanut, for bringing joy and happiness to my life.

Life is so much fun with you two around.

TABLE OF CONTENTS

ABSTRACT.....	i
DEDICATION.....	iii
ACKNOWLEDGMENTS.....	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES.....	vii
LIST OF FIGURES.....	ix
INTRODUCTION.....	1
CHAPTER 1 – Global weather and local butterflies: variable responses to a large-scale climate pattern along an elevational gradient.....	6
Abstract.....	7
Introduction.....	8
Methods.....	10
Results.....	14
Discussion.....	19
Acknowledgments.....	25
Tables.....	26
Figure Legends.....	28
Figures.....	30
Supplementary material.....	34
Appendix A.....	35
Appendix B.....	51
CHAPTER 2 - Preferences and performance of Juniper caterpillar assemblages in expanding juniper woodlands of the Intermountain West.....	57
Abstract.....	58
Introduction.....	60
Methods.....	63
Results.....	70

Discussion.....	75
Acknowledgments.....	82
Tables.....	83
Figure Legends.....	90
Figures.....	94
Supplementary material	101
CHAPTER 3 - Testing the <i>enemies hypotheses</i> in expanding juniper woodlands	107
Abstract.....	108
Introduction.....	110
Methods.....	114
Results.....	123
Discussion.....	125
Acknowledgments.....	132
Tables.....	133
Figure Legends.....	135
Figures.....	136
Supplementary material	138
CHAPTER 4 - Trophic interaction diversity: simulated networks generate relevant hypotheses	149
Abstract.....	150
Introduction.....	152
Methods.....	155
Results.....	161
Discussion.....	166
Acknowledgments.....	176
Figure Legends.....	177
Figures.....	179
Supplementary material	185
CONCLUSION.....	189
REFERENCES	193

List of Tables

Table 1-1. Guiding questions and general structures of the four models used in analyses of relationships between butterfly abundance and the El Niño-Southern Oscillation.

Table 1-2. Summary of results from analyses of deviance for each model tested in Table 1-1.

Table 2-1. Summary of results from a logistic regression to model frequency of survival across four caterpillar species feeding on foliage from young and old *J. osteosperma*.

Table 2-2. Summary of results from a logistic regression to model frequency of survival across two juniper-feeding caterpillar species feeding on foliage from young and old *J. osteosperma* and *J. occidentalis*.

Table 2-3. Summary of results from generalized linear models investigating several measurements of caterpillar performance across four juniper-feeding caterpillar species feeding on foliage from young and old *J. osteosperma*.

Table 2-4. Summary of results from generalized linear models investigating several measurements of caterpillar performance across two juniper-feeding caterpillar species feeding on foliage from young and old *J. osteosperma* and *J. occidentalis*.

Table 2-5. Summary of results from generalized linear mixed models investigating how plant phytochemical diversity (Alpha) influences several measurements of caterpillar performance across two juniper-feeding caterpillar species feeding on foliage from young and old *J. osteosperma* and *J. occidentalis*. Caterpillar species and juniper species were modeled as random effects.

Table 2-6. Results from several linear models investigating whether the diversity, richness, and evenness of plant secondary compounds differed significantly between juniper age and juniper species.

Table 2-7. Results from a negative binomial generalized linear model to investigate whether caterpillar abundances in the field differed across juniper tree ages.

Table 3-1. Summary of results from generalized linear mixed models investigating determinants of parasitoid rates and parasitoid species richness across a gradient of understory plant richness in expanding juniper woodlands.

Table 3-2. Summary of results from generalized linear mixed models investigating determinants of caterpillar abundance and species richness in expanding juniper woodlands.

List of Figures

Figure 1-1. A time series of the Fraction of Day Positives (FDP) (Solid Line) and ENSO derived Sea-Surface Temperature anomaly (SSTA) (Dashed Line) from 1988-2010 at two sites and for two species used in this analysis.

Figure 1-2. Odds ratios and standard errors for predictor variables SSTA and precipitation on butterfly abundance included in Models 1-3 from Table 1.

Figure 1-3. Odds ratios and standard errors associated with the SSTA variable from the logistic regression are displayed for six of the 28 species investigated.

Figure 1-4. A path diagram displaying standardized path coefficients across all sites and species from a path analysis. Lines ending with an arrow represent positive coefficients, while lines ending with a circle represent negative coefficients. The dashed line represents the total indirect effects of SSTA on the abundance of butterflies.

Figure 2-1. A) Proportion of survival for four juniper-feeding caterpillar species on foliage from young and old *J. osteosperma* trees and B) results from the logistic regression on survival. C) Displays proportion survival for two juniper-feeding caterpillar species feeding on foliage from young and old *J. osteosperma* and *J. occidentalis* trees

and the D) results from a logistic regression of caterpillar survival.

Figure 2-2. Shows the mean and standard error of A) Development time (days to pupa), B) pupal mass (g), C) frass produced per day, and D) growth per day (g/day) for four juniper-feeding caterpillars on foliage from young and old *J. osteosperma* trees.

Figure 2-3. Shows the mean and standard error of A) Development time (days to pupa), B) pupal mass (g), C) frass produced per day, and D) growth per day (g/day) for two juniper-feeding caterpillars on foliage from young and old *J. osteosperma* and *J. occidentalis* trees.

Figure 2-4. Displays the linear relationships between the diversity of secondary compounds across the four treatments and A) development time (days to pupa), B) pupal mass (g), C) frass produced per day, and D) growth per day (g/day) for two juniper-feeding caterpillars on foliage from young and old *J. osteosperma* and *J. occidentalis* trees.

Figure 2-5. A) Raw counts of oviposited eggs during preference assays by 17 *C. grynueus* females on foliage from young and old *J. osteosperma* trees. B) The results from a Bayesian preference model to investigate preference for foliage from old or young *J. osteosperma* trees.

Figure 2-6. Non-metric multidimensional scaling (NMDS) of foliage from young and old *J. osteosperma* and *J. occidentalis* trees. Old *J. occidentalis* samples (occ_o) are represented by the color green, while young *J. occidentalis* samples (occ_y) are shown in blue. Old *J. osteosperma* (ost_o) samples are displayed in red, while young *J. osteosperma* (ost_y) samples are shown in purple.

Figure 2-7. Shows A) the abundance and B) density of caterpillars collected on old and young *J. osteosperma* and *J. occidentalis* trees in the field. A significant difference is denoted with an asterisk (* $P < 0.05$).

Figure 3-1. Displays the results from the best performing generalized linear mixed model (GLMM), based on AICc, to investigate drivers of parasitism rates in expanding juniper woodlands. Each plot is from variables included in the best performing model with A) revealing the relationship between parasitism rates and understory plant richness, while B) shows the relationship between parasitism rates and caterpillar density.

Figure 3-2. Displays the results from the best performing generalized linear mixed model (GLMM), based on AICc, to investigate determinants of parasitoid species richness in expanding juniper woodlands. Variables included in each figure were found in the best performing model with A) revealing the relationship between parasitoid species richness and understory plant richness, while B) shows the relationship between parasitoid species richness and caterpillar abundance.

Figure 4-1. Displays a sample tri-trophic ecological network that was generated using the food web simulation model. This network was built using the “bipartite” package in program R. Each bar represents an individual species; with the width corresponding to its abundance within the community. Lines connecting each bar represent trophic interaction and the width is a measure of frequency of that resource-consumer interaction. Species bars with green shading represent individuals within that species that were not consumed by a higher trophic level. Numbers represent individual species identifications.

Figure 4-2. Shows rarefaction curves for interactions (red) and species (blue) for the 100 separate communities. Rarefaction curves are further broken up into plant-herbivore (PH), herbivore-enemy (HE), and plant-herbivore-enemy (PHE) networks.

Figure 4-3. Bar plots displaying the mean posterior probabilities and standard deviation (σ) of the slope of each rarefaction curve and Chao1 estimates of richness generated from the package ‘BEST’ in R. Interactions are displayed in grey, while species are in white. The lines represent the standard deviation. The mean slope was acquired by calculating the slope of each rarefaction curve when half of the species or interactions were sampled.

Figure 4-4. Scatterplots displaying the semi-partial correlations between the residuals of a linear model regressing species diversity and interaction diversity with mean consumer diet

breadth, species richness, and abundance. We investigated this relationship for all three networks (e.g. PH, HE, PHE). Solid lines indicate results from linear regressions.

Figure 4-5. Results from a path analysis display the standardized path coefficients across 5000 local communities. Lines ending with an arrow represent positive coefficients, while lines ending with a circle represent negative coefficients. The width of the arrow indicates the intensity of the coefficient.

Figure 4-6: Plots exhibiting how standardized path coefficients from Figure 5 change as the number of sampled interactions included in the path analysis increases. The strength of the path coefficient is shown on the y-axis and number of samples included in the model is shown on the x-axis. The dashed line represents outcome of linear or polynomial regressions. Not all path coefficients are shown (see supplemental material for remaining path coefficients).

INTRODUCTION

Across the globe, ecosystems are experiencing rapid and dramatic changes (MEA 2005). Human-caused global environmental changes (GEC) such as increased levels of atmospheric CO₂, climate change, deposition of anthropogenically fixed Nitrogen (N), habitat modification (e.g., fragmentation and loss), and invasive species are all interacting to alter the structure and function of Earth's ecosystems (Vitousek 1994, Vitousek et al. 1997, Tylianakis et al. 2008). Future efforts to predict and mitigate the effects of GEC on biotic communities will be a challenge for all ecologists as biotic communities are exposed to multiple GEC factor simultaneously and these factors likely act on the ecology of organisms in a synergistic manner. The effect of GEC on biotic communities has primarily focused on determining the impacts on population abundances (dynamics), community composition, and organismal physiology. However, all organisms are embedded in a complex network of interactions among species and our understanding of how GEC is likely to alter interactions among species is limited. Given that species interactions, such as pollination, predation, herbivory, and parasitism, are dependent on the phenology, abundance, and identity of multiple species, biotic interactions are likely to be even more sensitive to GEC than individual species (Tylianakis et al. 2008). For example, while two species may co-occur, an interaction can be driven to extinction if the phenologies of two interacting organisms become asynchronous (Stireman et al. 2005b, Schweiger et al. 2008). While the effects of GEC on biotic interactions may be less obvious, biotic interactions play a critical role in ecosystems such as helping maintain biodiversity within a community, contribute to community stabilization (resilience and resistance), and can mitigate ecosystem responses to GEC. Thus, determining the

combined effects of GEC on species and species interactions will be a key challenge for community ecologists as the effects of GEC on biotic communities accumulate.

My dissertation attempts to identify the ecological consequences of several aspects of GEC on both populations and species interactions. In my first chapter, I utilize a long-term butterfly monitoring dataset to investigate the impacts of a large-scale climate pattern on the spatial population dynamics of a diverse butterfly assemblage. Besides influencing weather patterns on land, global climate change is currently increasing sea-surface temperatures across the globe. Sea-surface temperatures are the primary source of weather patterns across the globe and are the main drivers of natural climatic variation. The El Niño Southern Oscillation (ENSO) cycle of alternating warm and cold sea-surface temperatures in the tropical Pacific Ocean, is one of the most important drivers of year-to-year climatic fluctuations across the globe (McPhaden et al. 2006, Cai et al. 2014). However, global warming is predicted to increase the frequency and strength of this large-scale climate pattern, and our understanding of how biotic communities respond to ENSO is limited. Using this long-term butterfly monitoring dataset, we investigated relationships between butterfly abundance and ENSO cycles, and asked whether the strength of that relationship is consistent across butterfly species and space.

In my second and third chapter, I utilized the current expansion of juniper in the Intermountain West to investigate how juniper expansion influences trophic interactions between juniper, its associated caterpillar community, and the parasitoid natural enemies of juniper-feeding caterpillars. Woody plants in arid and semi-arid ecosystems have been increasing in density and distribution globally over the past 150 years (Knapp et al. 2008,

Eldridge et al. 2011). Increased grazing intensity, shifting fire regimes, climate change, and increased CO₂ are several presumed causes of the increase in woody plant abundance. While the particular cause of encroachment varies across sites, this shift in dominance to woody plants is typically viewed as a consequence of human activities (Van Auken 2000, Knapp et al. 2008). Furthermore, the continuous nature of woody plant expansion suggests that GEC may be driving many of these ecosystems to new alternative stable states (Scheffer et al. 2001, Briggs et al. 2005, D'odorico et al. 2012). However, few studies have investigated the consequences of woody plant expansion on the complex network of species interactions.

Juniper woodland expansion in the Intermountain West has resulted in an influx of young, immature juniper trees on the landscape. Given that all plants undergo significant morphological and physiological changes across their ontogeny, the invasion of immature juniper trees is sure to alter the chemical and nutritional landscape that herbivores interact with (Hunter 2016). Juniper is the sole host for a specialized group of caterpillars (larval Lepidoptera) and other arthropods and no study has investigated whether a shift forest age structure, as a consequence of juniper expansion, influences any aspect of these arthropod communities. Using assays in the lab and observations in the field, I attempted to investigate whether survival and performance of the specialized caterpillars on juniper differs across juniper ontogeny and whether the effects are consistent across multiple expanding juniper species. This study provides important information about the substantial role plant ontogeny can play in determining preference/performance in a widespread, specialized herbivore community.

In addition to altering juniper woodland age structure, the expansion of juniper woodlands has significantly reduced understory plant diversity. Declines in the diversity of primary producers is likely to cascade to higher trophic levels and alter the associated multi-trophic structure of an entire ecosystem. While several studies have investigated the impacts of juniper expansion on individual organisms (e.g., birds, butterflies, and plants), no study has investigated the impacts of juniper encroachment on species interactions such as herbivory or rates of parasitism (Rosenstock and Charles Van Riper 2001, Coultrap et al. 2008, McIver and Macke 2014). I tested the *enemies hypothesis* which proposed that predators and parasitoids are more effective at controlling their prey in more biodiverse communities. Utilizing the reduction in understory plant diversity in encroaching juniper woodlands, we investigated whether parasitism rates and species richness of parasitoids that attack juniper-feeding caterpillars are positively associated with understory (non-host) plant diversity. This study is one of the first to examine consequences of juniper encroachment on multi-species interactions and provides novel insight into the factors that determine host-parasitoid interactions in juniper woodlands.

My final chapter attempts to expand on what we learned in juniper woodlands to investigate basic determinants of trophic interaction diversity. One of the most striking effects of GEC has been a massive decline in global levels of biodiversity, especially species diversity (Sala et al. 2000, Mokany et al. 2012). However, a more subtle and damaging type of extinction is the extinction of species interactions (Janzen 1974, Tylianakis et al. 2008, Hughes 2012, Valiente-Banuet et al. 2015). Species interactions are an important component of diversity because they affect multiple community attributes, including evolutionary diversification and community structure (Dyer et al.

2010). Furthermore, the complexity of interactions within a community has been shown to be important for maintaining and organizing species diversity (Paine 1966, Hagen et al. 2012, Rzanny and Voigt 2012). Despite the realization of the importance of interactions among species, many theories on biodiversity have ignored interactions or assumed them to be homogeneously distributed across species (Bascompte and Stouffer 2009). Interaction diversity, which is defined as the number of links that connect species in a dynamic biotic community, is a measure of biodiversity that has been recognized as being important and a hidden consequence of species extinction, but rarely quantified or used as a metric of biodiversity. Current losses of species diversity are likely to have large impacts on the diversity and complexity of ecological networks, yet our understanding of what determines the diversity of interactions in a community is limited even though the exclusion of species interactions may conceal essential patterns and processes of community organization (Dyer et al. 2010). We developed a simulation model to investigate how three fundamental characteristics of biotic communities, consumer diet-breadth, relative abundances, and species richness, jointly determine interaction diversity within a community. We quantified interaction diversity within a given community, similarly to species diversity, and tested specific hypotheses about the influences of diet-breadth, abundance, and species richness on structuring these complex ecological networks.

Chapter 1

Global weather and local butterflies: variable responses to a large-scale climate pattern along an elevational gradient

Nicholas A. Pardikes¹, Arthur M. Shapiro², Lee A. Dyer¹, and Matthew L. Forister¹

¹ *Department of Biology, Program in Ecology, Evolution, and Conservation Biology,
University of Nevada, Reno, NV 89557*

² *Center for Population Biology, University of California, Davis*

Key Words: *butterfly, elevational gradient, El Niño Southern Oscillation (ENSO)*

ABSTRACT

Understanding the spatial and temporal scales at which environmental variation affects populations of plants and animals is an important goal for modern population biology, especially in the context of shifting climatic conditions. The El Niño-Southern Oscillation (ENSO) generates climatic extremes of inter-annual variation, and has been shown to have significant effects on the diversity and abundance of a variety of terrestrial taxa. However, studies that have investigated the influence of such large-scale climate phenomena have often been limited in spatial and taxonomic scope. We used 23 years (1988-2010) of a long-term butterfly monitoring dataset to explore associations between variation in population abundance of twenty-eight butterfly species and variation in ENSO-derived Sea Surface Temperature Anomalies (SSTA) across ten sites that encompass an elevational range of 2750 meters in the Sierra Nevada mountain range of California. Our analysis detected a positive, regional effect of increased SSTA on butterfly abundance (wetter and warmer years predict more butterfly observations), yet the influence of SSTA on butterfly abundances varied along the elevational gradient, and also differed greatly among the twenty-eight species. Migratory species revealed the strongest relationships with ENSO-derived SSTA, suggesting that large-scale climate indices are particularly valuable for understanding biotic-abiotic relationships of the most mobile species. In general, however, the ecological effects of large-scale climatic factors are context dependent between sites and species. Our results illustrate the power of long-term datasets for revealing pervasive yet subtle climatic effects, but also caution against expectations derived from exemplar species or single locations in the study of biotic-abiotic interactions.

INTRODUCTION

Large-scale climatic phenomena, such as the El Niño Southern Oscillation (ENSO) and North Atlantic Oscillation (NAO), influence weather patterns around the world and provide an opportunity to evaluate the effects of climatic variation on natural populations (Holmgren et al. 2001, Jaksic 2001, Stenseth et al. 2003, Hallett et al. 2004, Halkka et al. 2006). Such large-scale climatic phenomena are of particular interest because they have the potential to homogenize or synchronize biotic patterns of phenology or population abundance across large areas (Post and Forchhammer 2002, Stenseth et al. 2003, Hallett et al. 2004, Halkka et al. 2006). Given that some climate change models predict an increase in the frequency and intensity of these large-scale climatic cycles, the study of heterogeneity in population response is of applied and basic interest because it is important to know if observations can be generalized among species, sites or time periods. This is particularly important when making predictions about long-term effects of global climate change on biotic communities (Parmesan and Yohe 2003, Latif and Keenlyside 2009, Blois et al. 2013, Nice et al. 2014, Srygley et al. 2014, Cai et al. 2014).

Previous studies suggest a strong association between insect populations and ENSO. For example, the abundance of a migratory sulfur butterfly (Pieridae) in Panama and a migratory brush-footed butterfly (Nymphalidae) in the United States were closely coupled to ENSO-derived Sea-Surface Temperature Anomalies (SSTA) (Vandenbosch 2003, Srygley et al. 2010, 2014). Both authors proposed that these relationships were largely due to a tight association between SSTA, precipitation, and host plant

productivity. However, these studies were limited to a few species or a single location and it remains to be seen how the effects of ENSO may vary with taxa or habitat. When considering a species rich insect community that exists across a diverse range of habitats, we expected significant variability in the strength of climatic-biotic associations among species and sites (as documented among species at a single one of our study sites by Nice et al. 2014).

In this study, we investigated the relationship between ENSO-derived SSTA and the abundance of 28 co-occurring butterfly species along an elevational gradient (0-2750m) in the Sierra Nevada Mountains of California in the western United States. ENSO has been shown to influence regional weather patterns in California; in particular strong ENSO events commonly result in more precipitation across the region (Shang et al. 2011). We wanted to understand if the relationship between butterfly abundance and the global climate cycle, ENSO, is detectable across a diverse assemblage of species and sites, and if so, identify how its influence varied between species and sites along an elevational gradient (see FIG. 1). Using 23 years (1988-2010) of a long-term butterfly monitoring dataset we specifically addressed the following questions: 1) Is there a detectable, regional effect of a global climate cycle (ENSO) on butterfly populations across all study sites and species? 2) To what extent does the response to a global climate cycle (ENSO) vary along the elevational gradient and between species? 3) Are ENSO driven patterns across sites different depending on the species (i.e. is there a species by site interaction)? Ultimately, the goal of this study was to explore taxonomic and spatial variability in responses to a large-scale climate pattern, ENSO, using a species rich butterfly assemblage that occurs across a variety of habitats.

METHODS

Butterfly Data

One of us (A.M.S) has been monitoring butterflies in California since 1972 by collecting presence/absence data on a biweekly basis for all butterflies at ten sites across Northern California, including the western slope of the Sierra Nevada Mountains (for a total elevation gain across sites of 0 - 2750m). Sites were originally chosen to maximize habitat diversity, butterfly diversity, and proximity to local weather stations (Table S1). Sampling at all sites followed the “Pollard walk” method, with a fixed route being walked and the presence of all butterfly species noted. The analyses reported here include data collected between 1988 and 2010, and a subset of species that were observed at all 10 sites at least once during the 23 years, which included 28 species from 5 butterfly families (Table S2). Butterfly populations were also identified as being resident or non-resident; where a butterfly population is considered a resident if it maintains a breeding population year around. The number of confirmed presences over the 23-year monitoring period for each species at each site is shown in Table S3.

Local Weather Data

Monthly values of total precipitation, average maximum temperature and average minimum temperature were collected from weather stations near each site for years matching the butterfly data (Table S1; also see FIG. 1 Forister et al. 2010). Gates Canyon contained several missing monthly data points, so Probabilistic Principle Coordinates Analysis (PPCA), in the R-package “pcaMethods”, was utilized to interpolate missing

values (Table S1). This method uses an Expectation-Maximization (EM) approach for PCA with a probabilistic model to calculate the likelihood of a reconstructed value (Tipping and Bishop 1999, Stacklies et al. 2007). Three weather stations (Castle Peak, Rancho Cordova, and Lang Crossing) contained missing values that exceeded 10% of the total data for those stations, so PRISM (<http://www.prism.oregonstate.edu/>) weather data were used as a replacement (Table S1). The acquired PRISM data were compared to a subset of the local weather station data to evaluate accuracy, and Pearson correlation coefficients between PRISM and available local weather data exceeded 95% in all cases. Yearly averages of total monthly precipitation, maximum monthly temperature, and minimum monthly temperature were used in the analyses presented here. The annual time frame corresponds to the precipitation cycle (the “water year”) that is common in Mediterranean climates, which begins in September of the previous year and ends with the following August (e.g. 1988 = September 1987 through August 1988) (Forister et al. 2010). All weather data were z-standardized prior to analyses. These data were also used to examine relationships between ENSO-derived SSTA and local weather across the elevational gradient.

El Niño Southern Oscillation (ENSO) data

As an indicator of ENSO, we used the sea-surface temperature anomaly (SSTA) from 1988-2010 in the Niño 3.4 region published by the Climate Prediction Center of the National Oceanographic and Atmospheric Administration (<http://www.cpc.ncep.noaa.gov/data/indices>). The Niño 3.4 SSTA is defined as a departure from the long-term SST mean in the Niño 3.4 region of the eastern tropical

Pacific Ocean. According to the Climate Prediction Center (NOAA), sea-surface temperatures in the Niño 3.4 region of the Pacific Ocean have been found to be effective in characterizing ENSO patterns (Srygley et al. 2010). They capture sea surface temperatures near the equator, but are also indicative of temperatures along the coast (Vandenbosch 2003). The mean SSTA of December and January from the current “water-year” were used in analyses; these two months were chosen because they correspond to the peak of ENSO (Vandenbosch 2003).

GLMM and Generalized Linear Model statistical analyses

The fraction of day positives (FDP) (i.e. the number of days with positive sightings, weighted by the total number of times that a site was visited during a year) served as our response variable and a proxy for butterfly abundance (Casner et al. 2014) in Generalized Linear Mixed Models (GLMM) and Generalized Linear Models (summarized in Table 1). GLMMs were utilized to account for random variation among replicate units (sites and species), which might influence population abundance, and to identify robust relationships with ENSO-derived SSTA across all elevations and species (Hebblewhite and Merrill 2008, Bolker et al. 2009). A logit link function specifying a binomial error distribution was used in all subsequent models and all GLMM results were fit by Laplace approximation. Prior to building each model, correlations among the variables were investigated (Table S4). To investigate differences in the strength and variance of butterfly responses to SSTA and local weather, odds ratios (OR) and standard errors were compared within each model. Models reported here do not include interactions among variables because preliminary investigations during model

development revealed that main effects did not differ significantly when interactions were included, but biological interpretation was considerably more difficult.

Additionally, our goal was to compare the strength and variance of responses to SSTA and local weather, which was not facilitated by the inclusion of interactions among climatic factors in the models. A type III Wald χ^2 analysis of deviance identified significant variables and interactions within each model (Fox et al. 2015). We quantified the coefficient of determination (pseudo- R^2) for GLMM using methods developed by Nakagawa and Schielzeth (2013). All statistical analyses were conducted in R (version 3.1.1) using the packages “lme4” for mixed-effects logistic regressions, “car” for analyses of deviance and “pscl” for GLM pseudo- R^2 calculations. (Bates et al. 2014, R Core Team 2014, Fox et al. 2015).

Path Analysis

Considering the potential complexity of regional and local weather effects on our focal species, path analysis was utilized to investigate specific mechanistic hypotheses focused on direct and indirect associations between butterfly populations, ENSO-derived SSTA, and local weather. In particular, we wanted to test hypotheses that the effects of SSTA detected in linear models described above could be explained solely through the influence of the regional climate cycle on local weather, or if effects of SSTA on butterflies might be detectable regardless of any effects mediated through local weather. Any SSTA effects beyond the influence on local weather could be strongest for species with wide-ranging populations or migratory habits, and the regional climatic index of SSTA could be the most useful climatic predictor for those species. The endogenous

variable for all models was the number of positive sightings, and models included the number of visits per year as a covariate to account for sampling effort. All variables from the previous models were used in these analyses, with year as an additional variable to account for trends in butterfly populations not explained by weather. Data from all sites and species were used to generate a full path model, then the same path model was performed for each individual species and each individual site to examine how the model support (χ^2) and path coefficients change depending on the species or site. Direct and total indirect effects of SSTA on butterfly abundance were estimated for all species and sites to address hypotheses that SSTA influences butterfly abundance both directly and indirectly via local weather. Path Analysis was performed with PROC CALIS in SAS 9.4.

RESULTS

Model 1: The effect of ENSO is detectable across all butterflies and sites

Our analysis contained 6440 observations that spanned 23 (1988-2010) years, and included 28 species across 10 sites (0-2750m elevation). All variables included in the model significantly influenced butterfly abundance in Northern California (FIG. 2). We detected a positive, regional effect of ENSO-derived SSTA on the butterfly populations (FIG. 2; also supported by the type III χ^2 analysis of deviance, Table 2, Table S5). When all sites and species are considered, the probability of obtaining additional positive sightings increased on average by 3% with each unit increase in the SSTA. Across all sites, resident and non-resident populations exhibited dissimilar relationships with ENSO-derived SSTA (FIG. 2). Based on the odds ratios, the probability of obtaining an

additional positive sighting for non-resident taxa increased on average by 6% with each unit increase in SSTA, while resident taxa revealed only a 2% increase. Differences between mountain and valley sites were less substantial, but valley sites (OR = 1.12, 95% CI = \pm 0.018) exhibited a stronger and less variable response to SSTA than mountain sites (OR = 1.07, 95% CI = \pm 0.023).

It has been recognized that SSTA and precipitation are strongly correlated in California, therefore, butterfly associations with precipitation and SSTA were examined to determine if the observed relationships between SSTA and butterfly abundance were parallel to those with precipitation (FIG. 2). Unlike SSTA, precipitation displayed a negative relationship with butterfly abundances for all datasets except the non-residents. Besides maximum temperature, SSTA was the only variable that significantly influenced butterfly populations across all five datasets analyzed and it was a consistent, positive effect (FIG. 2, FIG. S1; type III χ^2 analysis of deviance for each of the four separate analyses are reported in Tables S6-S9).

Model 2: The effects of ENSO on butterfly abundance differ between elevations

Butterfly responses to ENSO differed significantly between sites (SSTA by site interaction; Table 2, Table S5, S10). Increased SSTA had a positive effect on butterfly abundance at all ten elevations (FIG. 2), and all were significant except the easternmost site, Sierra Valley. Castle Peak, the highest elevation site, exhibited the strongest positive effect of ENSO on butterfly abundance. Linear regressions revealed that odds ratios associated with ENSO-derived SSTA did not significantly increase with elevation (β =

7.93e-06, SE= 1.21e-05, $P = 0.531$). However, variance increased significantly with elevation ($\beta = 4.79e-06$, SE= 1.39e-06, $P = 0.009$), which indicates that butterfly populations respond more variably to ENSO at higher elevations. Responses to local weather variables, including precipitation, were more erratic across the elevational gradient, and unlike SSTA, no single, local weather variable displayed a consistently positive or negative response across the entire gradient (FIG. 2, FIG. S1).

Model 3: The effects of ENSO on butterfly abundance varies among species

Responses to ENSO differed significantly between the 28 butterfly species (SSTA by species interaction, Table 2, Table S11). Species displayed considerable variability in both magnitude and variance in their response to ENSO-derived SSTA (FIG. 2). The abundances of eleven species increased significantly in response to increased SSTA, while significantly negative responses were not detected for any species. Approximately 10 of the 28 species showed little or no relationship to ENSO across all ten sites and only three of these species displayed a negative relationship to increased ENSO-derived SSTA. *Vanessa cardui* (Nymphalidae) exhibited the strongest, positive response to ENSO cycles, which is consistent with results reported by Vandenbosch (2003) for the same species. The abundance of *Adelpha bredowii californica* (Nymphalidae) revealed the most negative relationship to increases in ENSO-derived SSTA. The positive, but insignificant association by the nymphalid, *Nymphalis milbertii* and the hesperiid, *Euchloe ausonides* might be attributed to low abundances across several sites. Species that displayed significant relationships with SSTA did not match with those that responded significantly to increased precipitation. One of the species most influenced by

increases in SSTA, *Pontia protodice*, displayed a strong, negative relationship to increased precipitation across the gradient. This suggests that the different climatic indices (local weather and SSTA) contain different information with respect to understanding butterfly populations. Butterfly relationships with maximum and minimum temperature were also dissimilar from SSTA (FIG. S1).

Model 4: Responses to ENSO are typically not site and species-specific

The SSTA by species by site interaction was not a significant predictor of butterfly abundance (Table 2; Table S12). These results indicate that butterfly populations have not responded to ENSO-derived SSTA in a site and species-specific manner. We selected several species that displayed either positive or negative relationships to SSTA in the previous model to understand how site-specific responses to ENSO-derived SSTA relate to the outcome of that model (FIG. 3; site-specific responses to SSTA for the remaining 22 species are displayed in FIG. S2), and some of these species are discussed further here to explore the possibility of species-specific responses to different sites.

Adelpha bredowii displayed the most negative response to SSTA across the elevational gradient, but was not significantly affected at any individual site. Negative relationships to ENSO-derived SSTA were more pronounced at the highest elevations (CP, DP, and LC), but this species is rarely observed at North Sacramento (NS) and West Sacramento (WS), which may explain the extreme odds ratios and standard errors. Site-specific responses of *Nymphalis californica* indicate that the butterfly's negative relationship to increased SSTA is primarily restricted to the five mountain sites, with the

three highest sites (CP, DP, and LC) showing significant negative responses. The significant positive response at WS for *Nymphalis californica* may help explain the lack of relationship in the previous model for this species. *Vanessa cardui* and *Junonia coenia* both displayed strong positive associations to ENSO-derived SSTA in the previous model. *Vanessa cardui* exhibited significant positive responses across all ten sites, except the easternmost site (SV), while *Junonia coenia* responded positively to increases in SSTA at three mountain sites and the lowest elevation site (SM).

Path Analysis reveals direct and indirect effects of SSTA on butterfly abundance

Path analysis, across all sites and species, revealed both significant direct and indirect effects of SSTA on butterfly abundance (FIG. 4; Table S13). The χ^2 model fit for the full path model was weakly supported ($\text{Pr} > \chi^2 = 0.07$, $\chi^2 \text{ DF} = 1$). Several paths included in the model are not summarized within the path diagram (FIG. 4; path coefficients and associated standard errors reported in Table S13). The total indirect effects of SSTA on butterfly abundance were negative, while the direct effect was significantly positive. SSTA significantly influences all three local weather variables used in this analysis, but across all sites and species, only maximum temperature has a significant positive effect on butterfly abundance. The negative path coefficient from “Year” to “# Positive Sightings”, indicates that butterfly abundances have significantly declined over the last 23-years, consistent with other work on this long-term data (Forister et al. 2010, 2011).

Path analyses for all 28 species were well supported ($\text{Pr} > \chi^2 = 0.72$, $\chi^2 \text{ DF} = 1$). Significant direct effects of SSTA on butterfly abundance were found for seven

individual species, and all were positive (Table S14). Species models that included a direct positive path coefficient from SSTA to butterfly abundance were the same species that showed that relationship in our previous analyses. Significant indirect effects of SSTA were revealed in seven species, all but one (i.e. *Adelpha bredowii californica*) being negative. Two species had significant direct plus indirect effects (*Atalopedes campestris* and *Colias eurytheme*). Similar to our previous models, the abundances of *Vanessa cardui* and *Pontia protodice* were characterized by the most positive direct effect of increases in SSTA. In contrast, most path models for individual sites were not well supported (Table S15), which is consistent with our finding that butterfly responses to climate are heterogeneous, even within sites. Of the site models that were supported, only Gates Canyon (GC), revealed a significant positive effect of increases in SSTA on the butterfly populations present at that site.

DISCUSSION

Previous research has shown that at least some butterfly species fluctuate in association with climatic phenomena that are tied to ENSO indices, and the results presented here support these previous efforts (Vandenbosch 2003, Cleary and Genner 2004, Srygley et al. 2010, 2014). However, these studies have often encompassed small numbers of species or sites, thus the generality of those findings to larger spatial scales and for entire communities were unclear. At the regional scale (i.e. across all sites and species), our analysis detected a significant positive association between butterfly populations and ENSO-derived SSTA. Specifically, butterfly abundances showed a significant, positive ($53\% \pm 1.7\%$) response to increased SSTA. However, non-resident

populations exhibited a stronger response to increased SSTA than residents. This suggests that resident populations, which maintain breeding populations at a site, are less responsive to the global climate phenomenon, ENSO, than non-residents. It is possible that resident and non-resident populations respond to climatic forces that act at different time scales. Another possibility is that non-resident species are less affected by local weather at individual sites, and thus their dynamics are simply better captured by the regional weather variable (SSTA) that encompasses climatic dynamics at other locations outside of our study sites (presumably including those where they maintain breeding populations).

Consistent with the possibility of differential impacts of local and regional weather on resident and non-resident species, the path analysis detected both positive (direct) and negative (indirect) effects of SSTA on butterfly abundance. Indirect effects are represented by the total sum of effects of SSTA on butterfly abundance via the local weather variables. The negative indirect effect is driven by the contrasting relationships between SSTA, maximum temperature, and butterfly abundance. Increases in yearly maximum temperature typically result in an increase in butterfly abundance, however increased SSTA characteristically results in lower maximum temperatures for the year (FIG. S3), which has an accompanying negative effect.

It is unclear which particular aspect of ENSO-derived SSTA results in an increase in the abundance of butterflies, but increases in SSTA are often associated with increases in precipitation across Northern California. In arid regions like the Mediterranean climate of California, increased precipitation has been shown to lead to increases in primary

productivity, and consumers subsequently respond positively to increased availability of resources (Rosenzweig 1968, Markham and McLain 1977). However, we did not find that local precipitation causes an increase in butterfly abundance when all sites and species are considered (FIG. 2). This suggests that the positive relationship between SSTA and butterfly abundance is complex and involves more than a simple connection between SSTA and precipitation.

While a regional effect of ENSO-derived SSTA on butterfly abundance was detected, we also found that the impact of ENSO varied significantly among elevations and species (FIG. 2). SSTA and butterfly abundance displayed a variable, but positive relationship at all ten elevations. SSTA was the only climatic variable that displayed a positive effect on butterfly abundance across the entire elevational gradient. Local weather variables displayed an unpredictable relationship with butterflies, which provides support that using large-scale climate indices offer a less complex view of biotic-abiotic relationships of butterflies in Northern California and may predict ecological processes more accurately than local weather (Stenseth et al. 2003, Hallett et al. 2004). The effect of SSTA on butterfly abundance did not significantly strengthen along the elevational gradient, but the associated variance did. The type of precipitation along the elevational gradient may explain the increased variance. Most precipitation in this region of California arrives during the winter, so valley sites receive rain while mountain sites get snow. Thus precipitation could have more variable effects on the mountain populations than valley populations because the flight period and host-plant availability in the mountains depends on the snow depth and timing of snow melt (Boggs and Inouye 2012, Roland and Matter 2012). Years with high snowpack reduce flight periods, while years

with low snowpack could reduce plant growth and important nectar and food resources. Conversely, the low elevation sites receive moisture from local precipitation events and additional water from the yearly snowmelt in the mountains, and that combination of rain and runoff from mountain snow could be an inherently less variable process. It will be interesting to learn if this pattern persists in the future as the elevation of snow levels for winter storms in many mountain ranges in the west is getting higher and more precipitation is falling as rain instead of snow (Knowles et al. 2006, Mote 2006, Stoelinga et al. 2010, Svoma 2011).

As with heterogeneity among sites, associations with ENSO parameters differed significantly among the twenty-eight butterfly species. Site was modeled as a random effect, so that we could identify species that show robust relationships to ENSO-derived SSTA across all sites (and generalize to their entire geographic distributions). The abundance of most species displayed positive trends with increased SSTA, but only eleven of those were statistically significant. Of the butterfly species that showed significant positive associations with SSTA across all sites, six are within the family Nymphalidae, which tend to have relatively large wingspans, can travel long distances, and frequently undertake seasonal migrations (across elevations and latitudes). *Vanessa cardui*, *Nymphalis californica*, and *Danaus plexippus* all undergo long distance migrations and reveal strong, but dissimilar relationships with SSTA. Several other species that displayed strong responses to SSTA (e.g. the nymphalids, *Nymphalis californica*, *Nymphalis milberti*, *Vanessa atalanta*, *Vanessa virginiensis*, and the pierid, *Pontia protodice*) undergo elevational migrations and travel up the slope of the Sierra Nevada as the snowline retreats. *Junonia coenia* is a non-resident at sites over 1600 m,

but can sometimes reach high densities due to waves of immigrants in the early summer. Both forms of migration appear to have strong influences on the relationship between butterfly abundance and ENSO-derived SSTA in California and support our previous finding that the abundances of non-resident populations are more closely coupled to ENSO-derived SSTA than resident populations (Vandenbosch 2003, Srygley et al. 2010).

Although the SSTA by site by species interaction was not identified as being significant, populations of the same species can respond differently to ENSO parameters at different elevations (FIG. 3; FIGURE S2). For example, most of the selected species did not reveal significant relationships with ENSO across their entire elevational range. Instead, significant relationships with ENSO were often restricted to sites located in either the mountains or valleys. With the exception of *Vanessa cardui*, species that showed consistent relationships to SSTA along the elevational gradient were typically those that exhibited little or no relationship with ENSO. These results support previous findings that associations with ENSO-derived SSTA are not consistent throughout a species' distribution and that parallel responses among species typically occur in similar environments (Vandenbosch 2003). However, our results indicate that corresponding responses to ENSO in similar environments is highly variable between species. The lack of synchrony with ENSO parameters across all sites is potentially due to differences in host plant availability or phenology, natural enemy intensity, or microclimate differences that vary between different habitats, all of which can influence characteristics of population dynamics such as birth, growth, and death rates (Liebhold et al. 2004, 2006, Preisser and Strong 2004). However, we can only raise these possibilities at the current time as avenues for future work.

In summary, the influences of ENSO-derived SSTA on butterfly abundances are regionally detectable; however, these effects vary between elevations and taxa. These results add to a growing literature on the impacts of climate and weather on butterfly populations, and understanding these responses will be important for future predictions on the effects of climate change on natural ecosystems. Some current models predict that ENSO events will become more frequent and more intense and that weather patterns may shift as a result (Latif and Keenlyside 2009, Srygley et al. 2014, Cai et al. 2014). Furthermore, as global levels of SSTA are predicted to increase, these results suggest that migratory butterfly species will benefit from these increases more than others, but that these benefits are likely to vary across the landscape. The fact that most populations investigated did not show synchronous responses to ENSO-derived SSTA across the elevational gradient, raises the possibility that the extreme habitat heterogeneity provided by the elevational gradient in the Sierra Nevada may increase persistence of Lepidopteran species during climate change (Oliver et al. 2010). However, given the variability of response reported here, our results should ultimately raise a note of caution in extrapolating biotic-abiotic relations from studies conducted with single species or over limited spatial extent (Garcia et al. 2014).

ACKNOWLEDGEMENTS

This research was funded by National Science Foundation Grant DEB-9306721 to A.M.S, and the Forister lab has been supported by NSF DEB-1050726 and DEB-1145609. Thanks to Jim Thorne and Dave Waetjen for data management. This manuscript was improved by two anonymous reviewers. During the preparation of this manuscript, the Earthwatch Institute supported N.A.P.

Tables:**Table 1.** Guiding questions and general structures of the four models used in analyses.

<i>Model</i>	<i>Fixed-Effects</i>	<i>Random-Effects</i>
1) Can we detect effects of ENSO on the abundance of butterflies across all sites and species?	SSTA + $N_{(t-1)}$ + Precip + MaxT + MinT	$\gamma_{1jk}^{(species)} + \gamma_k^{(site)}$
2) Do the effects of ENSO on the abundance of butterflies vary among sites along an elevational gradient in California?	(SSTA × Site) + $N_{(t-1)}$ + Precip + MaxT + MinT	$\gamma_{1jk}^{(species)}$
3) Do the effects of ENSO on the abundance of butterflies vary among species?	(SSTA × Species) + $N_{(t-1)}$ + Precip + MaxT + MinT	$\gamma_k^{(site)}$
4) Are the effects of ENSO on the abundance of the butterflies site and species specific?	(SSTA × Site × Species) + $N_{(t-1)}$ + Precip + MaxT + MinT	None

Notes: The notation for random effects follows Hebblewhite and Merrill (2008). The random-effects model structures are: $\gamma_{jk}^{(species)}$, random intercept for effect of species, $\gamma_k^{(site)}$, random intercept for effect of site.

Table 2. Results from analyses of deviance for each model from Table 1.

<i>Model</i>	<i>Random-effects</i>	χ^2	<i>DF</i>	$Pr(>\chi^2)$	R^2
1) SSTA	$\gamma_{1jk}^{(\text{species})} + \gamma_k^{(\text{site})}$	237.57	1	< 0.0001 *	0.2443
2) SSTA \times Site	$\gamma_{1jk}^{(\text{species})}$	33.79	9	< 0.0001 *	0.25
3) SSTA \times Species	$\gamma_k^{(\text{Site})}$	627.15	27	< 0.0001 *	0.321
4) SSTA \times Species \times Site	None	268.4	$\frac{24}{3}$	0.1259	0.6222

Notes: Each analysis of deviance was performed in the R package “car” (Fox et al. 2015). All analyses of deviance are Type III. The models correspond to Table 1 and the specific hypothesis that were being tested. χ^2 and $Pr(>\chi^2)$ represent only the variable or interaction of interest and not the entire model. Conditional R^2 for the first three models (GLMM) were calculated using the methods from Nakagawa and Schielzeth (2013). McFadden’s pseudo- R^2 was used for Model 4 from the “pscl” package in R (Jackman et al. 2015). * indicates significance at $P < 0.05$

Figure Legends:

FIG. 1: A time series of the Fraction of Day Positives (FDP) (Solid Line) and ENSO derived Sea-Surface Temperature anomaly (SSTA) (Dashed Line) from 1988-2010 at two sites and for two species used in this analysis. Castle Peak (CP) and Suisun Marsh (SM) were chosen to compare the highest (CP) and lowest (SM) elevation sites in this study.

FIG. 2: Odds ratios and standard errors for SSTA and precipitation variables included in Models 1-3 from Table 1. Numbers represent the results from each corresponding model. Odds ratios greater than one signify an increase in the odds of attaining an additional positive sighting with each unit increase in SSTA and z-standardized annual precipitation. A significant effect on the abundance of butterflies is indicated with an asterisk (*= $P < 0.05$). (1) Each analysis contained identical fixed and random effects, but were performed with different subsets of the data. All Data represents the outcomes from the entire dataset ($n=6440$). The resident analysis contained 4646 observations, while non-resident data contained 1794 observations. Mountains and Valley analyses had the same number of observations ($n=3220$). (2) Results for each site along the elevational gradient. Sites on the y-axis are oriented from lowest (Suisun Marsh) to highest (Castle Peak) elevation. (3) To more easily compare how species responses to SSTA differ from annual precipitation, species in both figures are ordered from highest to lowest SSTA odds ratio. Species marked with "(M)" represent migratory species (i.e. long-distance and elevational). Several other species (e.g. *Pieris rapae* and *Strymon melinus*) also display some degree of seasonal elevational migration, but as individuals and not en masse.

FIG. 3: Odds ratios and standard errors associated with the SSTA variable from the logistic regression are displayed for six of the 28 species investigated. These species were chosen to show site-specific responses of species that displayed either negative or positive responses to ENSO-derived SSTA in Model 3 (the other 22 species are shown FIGURE S2). Sites on the y-axis are oriented from low to high elevation. Significant effects of SSTA on the abundance of butterflies at each site are indicated with an asterisk (* = $P < 0.05$).

FIG. 4: A path diagram displaying the standardized path coefficients across all sites and species. Lines ending with an arrow represent positive coefficients, while lines ending with a circle represent negative coefficients. The dashed line represents the total indirect effects of SSTA on the abundance of butterflies. To improve coherency of the path diagram, not all paths were included in the figure (See Table S13 for the results for all paths included in the model). Significant path coefficients are indicated with an asterisk (* = $P < 0.05$).

Figures

FIG. 1

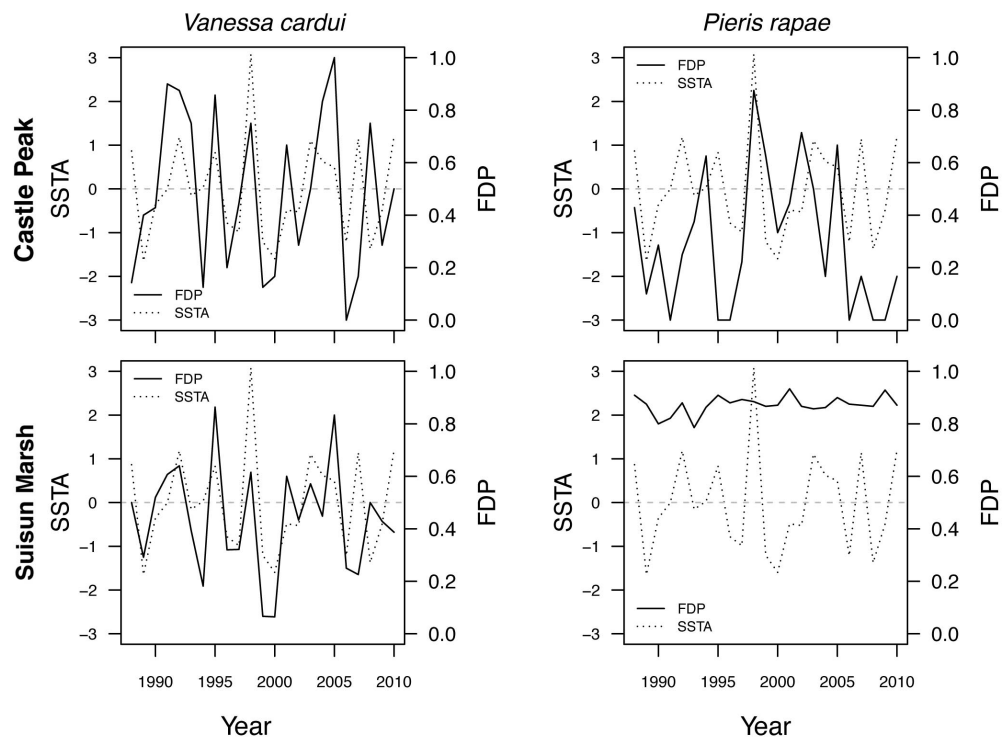


FIG. 2

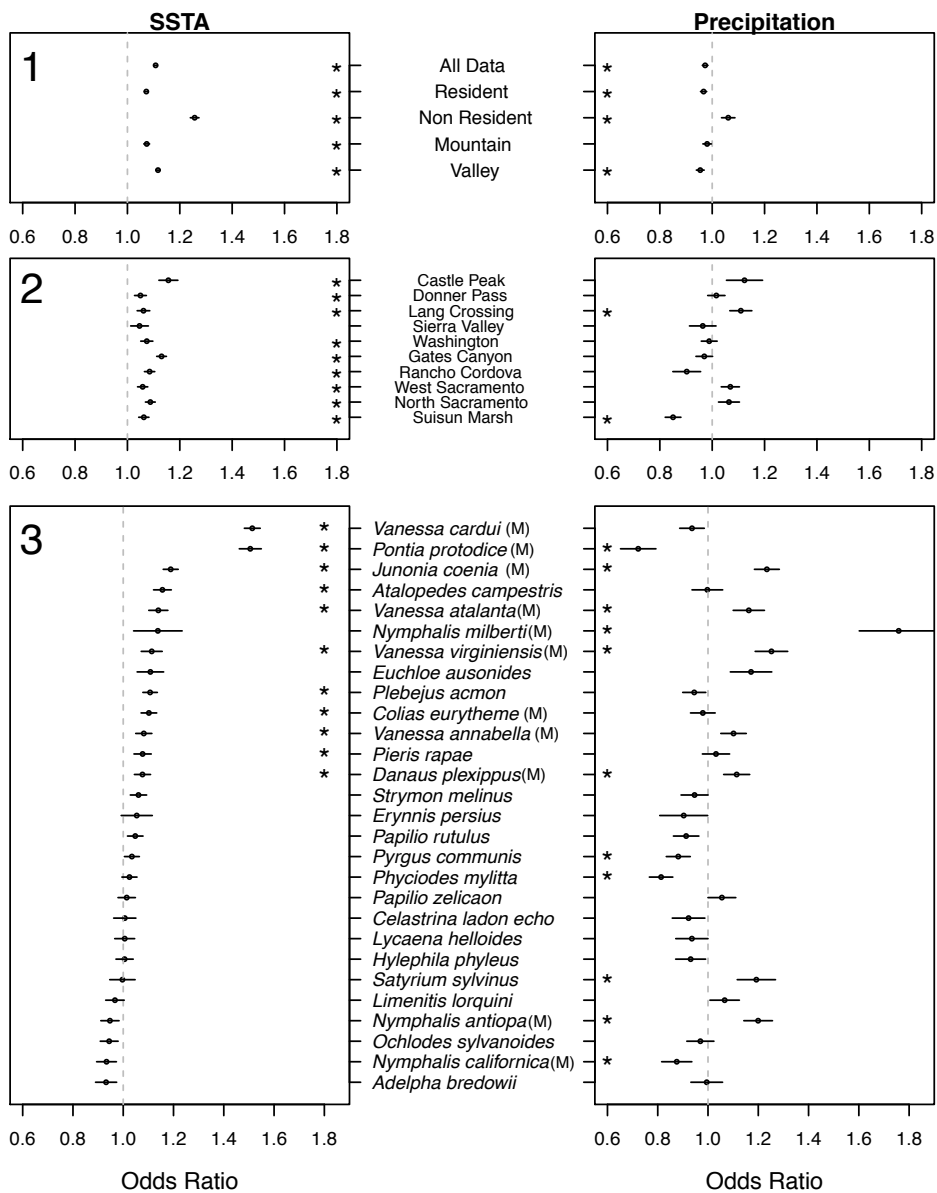


FIG. 3

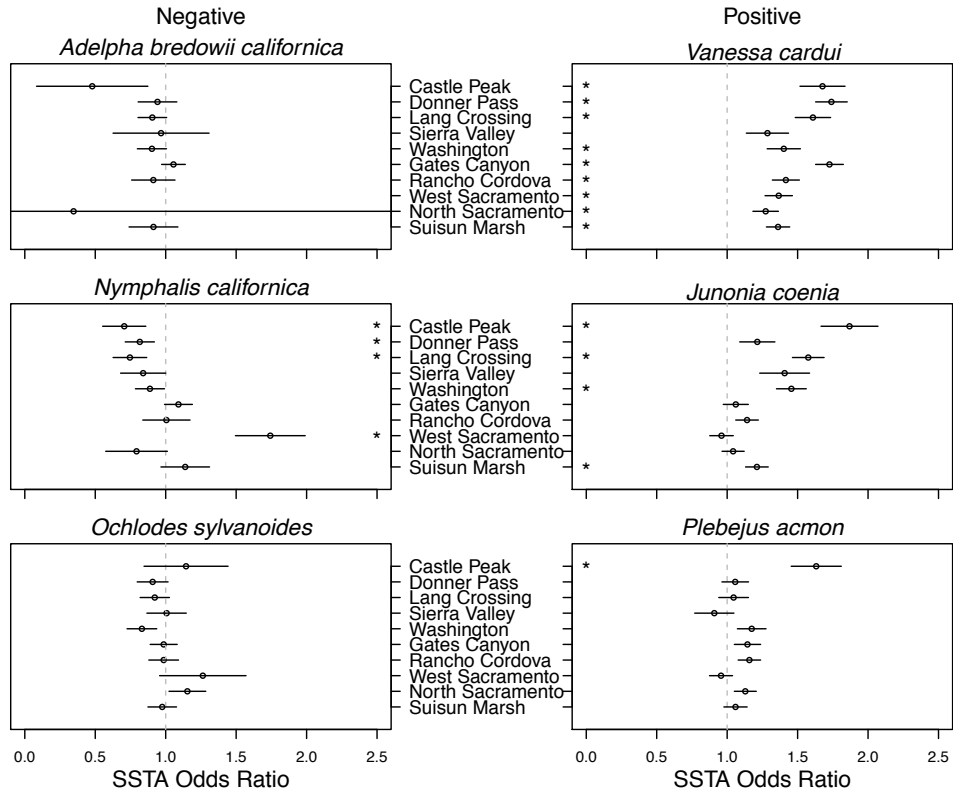
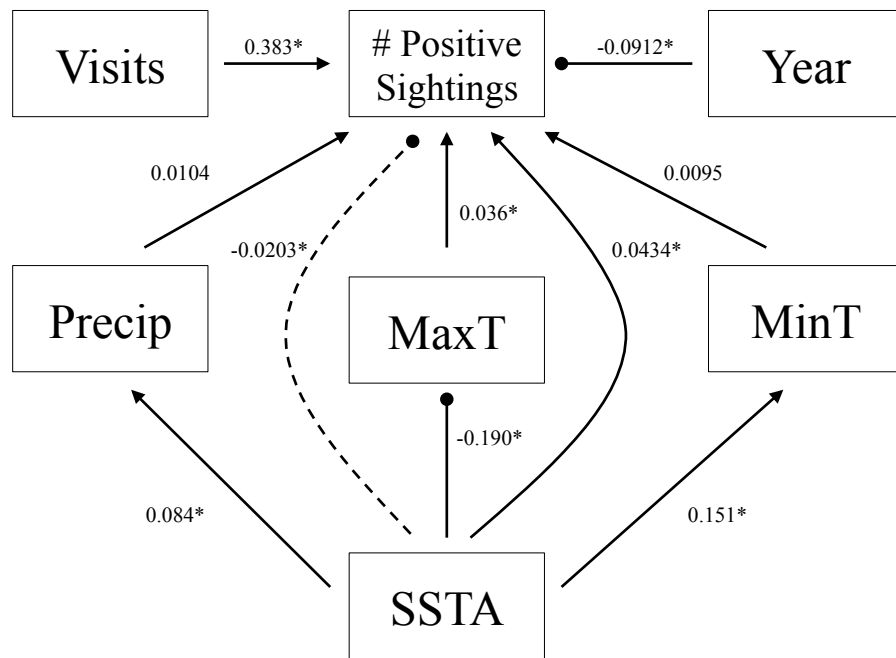


FIG. 4



Supplementary Material

Chapter 1

Global weather and local butterflies: variable responses to a large-scale climate pattern along an elevational gradient

Nicholas A. Pardikes¹, Arthur M. Shapiro², Lee A. Dyer¹, and Matthew L. Forister¹

¹ *Department of Biology, Program in Ecology, Evolution, and Conservation Biology, University of Nevada, Reno, NV 89557*

² *Center for Population Biology, University of California, Davis*

Appendix A. Supplementary information, including detailed monitoring site information, species used in this analysis, presence data over the 23-year monitoring period, correlation matrix, additional ANOVA tables, and results from additional path analyses.

Table S1: A table revealing the sources for local weather values and the months in which data was missing.

<i>Site</i>	<i>Elevation (m)</i>	<i>Weather Station</i>	<i>Missing Data filled in with PPCA</i>
Suisun Marsh	0-1	Fairfield, 042934 (38.2667, -122.06667)	N/A
North Sacramento	8	Sac. FAA Airport, 047630 (38.5069, -121.5)	N/A
West Sacramento	9	Sac. 5 ESE, 047633 (38.55556, -121.95)	N/A
Rancho Cordova	18	PRISM (39.6241, -121.2777)	N/A
Gates Canyon	190-600	Vacaville, 049200 (38.416667, -121.95)	1988 (April, May, June), 1989 (Nov.), 1990 (March, Dec.), 1994 (Oct.), 1998 (Aug.)
Washington	850-1,200	Nevada City, 6316 (39.26, -121.02)	N/A
Sierra Valley	1,500	Sierraville Ranger Station, 048218 (39.58333, -120.36667)	N/A
Lang Crossing	1,500-1,700	PRISM (39.315, -120.662)	N/A
Donner Pass	2,000-2,200	Sierra Snow Lab, 049998 (39.326, -120.367)	N/A
Castle Peak	2,400-2,775	PRISM (39.3395, -120.3474)	N/A

Notes: Sites are ordered from low to high elevation. Latitude and longitude are provided in parentheses for each weather station. Missing values were filled in using a Probabilistic Principle Coordinates Analysis (PPCA) in the “pcaMethods” package in R (Stacklies et al. 2012). N/A values represent sites that did not have any missing values.

Table S2: A list of the 28 butterfly species and their family used in this analysis.

Species	Family
<i>Adelpha bredowii californica</i>	Nymphalidae
<i>Atalopedes campestris</i>	Hesperiidae
<i>Celastrina ladon echo</i>	Lycaenidae
<i>Colias eurytheme</i>	Pieridae
<i>Danaus plexippus</i>	Nymphalidae
<i>Erynnis persius</i>	Hesperiidae
<i>Euchloe ausonides</i>	Pieridae
<i>Hylephila phyleus</i>	Hesperiidae
<i>Junonia coenia</i>	Nymphalidae
<i>Limenitis lorquini</i>	Nymphalidae
<i>Lycaena helloides</i>	Lycaenidae
<i>Nymphalis antiopa</i>	Nymphalidae
<i>Nymphalis californica</i>	Nymphalidae
<i>Nymphalis milberti</i>	Nymphalidae
<i>Ochlodes sylvanoides</i>	Hesperiidae
<i>Papilio rutulus</i>	Papilionidae
<i>Papilio zelicaon</i>	Papilionidae
<i>Phyciodes mylitta</i>	Nymphalidae
<i>Pieris rapae</i>	Pieridae
<i>Plebejus acmon</i>	Lycaenidae
<i>Pontia protodice</i>	Pieridae
<i>Pyrgus communis</i>	Hesperiidae
<i>Satyrium sylvinus</i>	Lycaenidae
<i>Strymon melinus</i>	Lycaenidae
<i>Vanessa annabella</i>	Nymphalidae
<i>Vanessa atalanta</i>	Nymphalidae
<i>Vanessa cardui</i>	Nymphalidae
<i>Vanessa virginiensis</i>	Nymphalidae

Table S3: Number of years that each species was seen over the 23-year monitoring period.

Species	SM	NS	WS	RC	GC	WA	SV	LC	DP	CP
<i>A. bredowii californica</i>	14	1	2	14	20	17	9	18	18	8
<i>Atalopedes campestris</i>	19	21	20	20	19	14	8	3	1	1
<i>Celastrina ladon echo</i>	3	4	1	3	16	15	7	17	18	11
<i>Colias eurytheme</i>	21	18	20	19	19	19	16	15	14	6
<i>Danaus plexippus</i>	19	21	21	19	19	18	19	20	16	11
<i>Erynnis persius</i>	2	4	2	4	15	17	3	17	8	2
<i>Euchloe ausonides</i>	19	16	15	11	17	7	17	4	1	7
<i>Hylephila phyleus</i>	19	20	17	18	21	14	3	3	4	7
<i>Junonia coenia</i>	21	19	17	19	22	20	12	19	15	9
<i>Limenitis lorquini</i>	2	13	22	15	17	17	13	19	15	9
<i>Lycaena helloides</i>	20	19	21	10	15	6	16	14	8	3
<i>Nymphalis antiopa</i>	16	18	19	21	17	21	14	19	21	12
<i>Nymphalis californica</i>	13	11	9	18	18	19	17	19	20	13
<i>Nymphalis milberti</i>	2	8	1	3	1	2	5	2	15	13
<i>Ochlodes sylvanoides</i>	16	16	8	19	15	18	16	14	16	10
<i>Papilio rutulus</i>	18	17	18	17	19	19	16	16	19	10
<i>Papilio zelicaon</i>	19	18	19	19	18	18	11	10	18	11
<i>Phyciodes mylitta</i>	18	18	21	21	19	18	18	20	18	7
<i>Pieris rapae</i>	15	18	20	21	17	17	15	16	18	14
<i>Plebejus acmon</i>	19	21	21	19	19	16	16	18	16	11
<i>Pontia protodice</i>	13	14	17	18	11	9	20	11	18	8
<i>Pyrgus communis</i>	18	19	16	21	16	20	19	19	14	13
<i>Satyrium sylvinus</i>	1	17	15	6	18	14	9	14	15	3
<i>Strymon melinus</i>	19	17	19	17	19	17	14	10	13	6
<i>Vanessa annabella</i>	18	21	20	20	21	17	17	16	19	15
<i>Vanessa atalanta</i>	19	19	21	20	20	12	6	9	8	3
<i>Vanessa cardui</i>	21	20	21	22	20	16	20	15	18	16
<i>Vanessa virginiensis</i>	16	19	20	19	16	18	16	16	18	12

Notes: A.M.S. visited each site multiple times throughout the year; therefore years that the butterfly was absent from a particular site are meaningful absences.

Table S4: Pearson's Correlation Coefficients for the variables used in these analyses.

	<i>Visits</i>	<i>N</i>	<i>N(t-1)</i>	<i>MinT</i>	<i>MaxT</i>	<i>Precip</i>	<i>SSTA</i>
<i>Visits</i>	1	0.0489	0.066	-0.11	-0.009	0.0143	0.001
<i>N</i>	-	1	0.812	0.020	0.026	0.0038	0.034
<i>N(t-1)</i>	-	-	1	0.0007	-0.015	0.045	-0.024
<i>MinT</i>	-	-	-	1	0.439	0.245	0.155
<i>MaxT</i>	-	-	-	-	1	-0.408	-0.216
<i>Precip</i>	-	-	-	-	-	1	0.129
<i>SSTA</i>	-	-	-	-	-	-	1

Notes: SSTA and local weather variables are z-standardized.

Table S5: Results from χ^2 Type III analyses of deviance for the GLMM, Table 1, Model 1

<i>Fixed-Effect</i>	χ^2	<i>DF</i>	$Pr(>\chi^2)$	
(Intercept)	668.5707	1	<0.0001	***
N (t-1)	8557.3922	1	<0.0001	***
SSTA	237.5695	1	<0.0001	***
MaxT	84.1841	1	<0.0001	***
Precip	6.6673	1	0.010	**
MinT	3.9659	1	0.05	*

Notes: The analysis of deviance was performed in the R package “car” (Fox et al. 2015). Variables are ordered from highest to lowest χ^2 values. The main effect of interest (SSTA) is shown in bold. *indicates significance at $P<0.05$

Table S6: Results from χ^2 Type III analyses of deviance for the GLMM, Table 1, Model 1 (Resident Data)

<i>Fixed-Effect</i>	χ^2	<i>DF</i>	<i>Pr(>χ^2)</i>	
(Intercept)	364.6199	1	<0.0001	***
N (t-1)	5181.5489	1	<0.0001	***
SSTA	91.2648	1	<0.0001	***
MaxT	71.7935	1	<0.0001	***
Precip	8.1446	1	0.004	**
MinT	0.5231	1	0.47	

Notes: The analysis of deviance was performed in the R package “car” (Fox et al. 2015). This model corresponds to Model 1, Table 1, but only uses resident data. Variables are ordered from highest to lowest χ^2 values. The main effect of interest (SSTA) is shown in bold. *indicates significance at $P < 0.05$

Table S7: Results from χ^2 Type III analyses of deviance for the GLMM, Table 1, Model 1 (Non-Resident Data)

<i>Fixed-Effect</i>	χ^2	<i>DF</i>	$Pr(>\chi^2)$	
(Intercept)	49.6655	1	<0.0001	***
SSTA	191.7437	1	<0.0001	***
N (t-1)	126.6247	1	<0.0001	***
MaxT	7.891	1	0.005	**
Precip	5.3939	1	0.020	*
MinT	0.9715	1	0.324	

Notes: The analysis of deviance was performed in the R package “car” (Fox et al. 2015). This model corresponds to Model 1, Table 1, but only uses non-resident data. Variables are ordered from highest to lowest χ^2 values. The main effect of interest (SSTA) is shown in bold. *indicates significance at $P < 0.05$

Table S8: Results from χ^2 Type III analyses of deviance for the GLMM, Table 1, Model 1 (Valley Data)

<i>Fixed-Effect</i>	χ^2	<i>DF</i>	$Pr(>\chi^2)$	
(Intercept)	204.7118	1	<0.0001	***
N (t-1)	2697.164	1	<0.0001	***
SSTA	172.0705	1	<0.0001	***
MaxT	61.315	1	<0.0001	***
Precip	9.8796	1	0.002	**
MinT	0.0999	1	0.752	

Notes: The analysis of deviance was performed in the R package “car” (Fox et al. 2015). This model corresponds to Model 1, Table 1, but only uses data from the five valley sites. Variables are ordered from highest to lowest χ^2 values. The main effect of interest (SSTA) is shown in bold. *indicates significance at $P < 0.05$

Table S9: Results from χ^2 Type III analyses of deviance for the GLMM, Table 1, Model 1 (Mountain Data)

<i>Fixed-Effect</i>	χ^2	<i>DF</i>	$Pr(>\chi^2)$	
(Intercept)	283.8398	1	<0.0001	***
N (t-1)	1956.8224	1	<0.0001	***
SSTA	42.3341	1	<0.0001	***
MaxT	10.7295	1	0.0011	**
MinT	9.5517	1	0.0020	**
Precip	1.4842	1	0.2231	

Notes: The analysis of deviance was performed in the R package “car” (Fox et al. 2015). This model corresponds to Model 1 Table 1, but only uses data from the five mountain sites. Variables are ordered from highest to lowest χ^2 values. The main effect of interest (SSTA) is shown in bold. *indicates significance at $P < 0.05$

Table S10: Results from χ^2 Type III analyses of deviance for the GLMM, Table 1, Model 2

<i>Fixed-Effect</i>	χ^2	<i>DF</i>	$Pr(>\chi^2)$	
(Intercept)	582.2475	1	<0.0001	***
N (t-1)	8539.4405	1	<0.0001	***
MaxT	81.617	1	<0.0001	***
Site	70.0246	9	<0.0001	***
SSTA × Site	33.7933	9	<0.0001	***
SSTA	10.9411	1	0.0009	***
Precip	6.9186	1	0.009	**
MinT	4.0445	1	0.0443	*

Notes: The analysis of deviance was performed in the R package “car” (Fox et al. 2015). Variables are ordered from highest to lowest χ^2 values. The interaction of interest (SSTA × Site) is shown in bold. *indicates significance at $P < 0.05$

Table S11: Results from χ^2 Type III analyses of deviance for the GLMM, Table 1, Model 3

<i>Fixed-Effect</i>	χ^2	<i>DF</i>	<i>Pr(>χ^2)</i>	
(Intercept)	2954.7533	1	<0.0001	***
N (t-1)	8723.557	1	<0.0001	***
Species	1245.2295	27	<0.0001	***
SSTA × Species	627.1538	27	<0.0001	***
MaxT	82.9208	1	<0.0001	***
Precip	6.9124	1	0.00856	**
MinT	4.2628	1	0.03896	*
SSTA	1.465	1	0.22613	

Notes: The analysis of deviance was performed in the R package “car” (Fox et al. 2015). Variables are ordered from highest to lowest χ^2 values. The interaction of interest (SSTA × Species) is shown in bold. *indicates significance at P<0.05

Table S12: Results from Type III analyses of deviance for the GLMM Table 1, Model 4

<i>Fixed-Effect</i>	<i>LR χ^2</i>	<i>DF</i>	<i>Pr(>χ^2)</i>	
Species \times Site	5237.3	243	<0.0001	***
Site	909.9	9	<0.0001	***
Species	720.1	27	<0.0001	***
N (t-1)	436.6	1	<0.0001	***
SSTA \times Species \times Site	268.4	243	0.1259	
SSTA \times Species	74.3	27	2.71E-06	***
MaxT	55.3	1	1.03E-13	***
SSTA \times Site	11.9	9	0.2186	
SSTA	2.6	1	0.1067	
MinT	0.5	1	0.4847	
Precip	0.2	1	0.6765	

Notes: The analysis of deviance was performed in the R package “car” (Fox et al. 2015). Variables are ordered from highest to lowest χ^2 values. The interaction of interest (SSTA \times Species \times Site) is shown in bold. *indicates significance at P<0.05

Table S13: Results from the path analysis in FIG. 4

<i>Path</i>	<i>Estimate</i>	<i>SE</i>	<i>t-value</i>	<i>P-value</i>
Visits → #Pos. Sightings	0.38	0.01	34.70	< .0001 *
Year → Visits	0.18	0.01	14.91	< .0001 *
SSTA → MintT	0.15	0.01	12.36	< .0001 *
Precip → Visits	0.12	0.02	8.17	< .0001 *
MaxT → Visits	0.11	0.02	6.74	< .0001 *
SSTA → Precip	0.08	0.01	6.75	< .0001 *
<i>SSTA</i> → <i>#Pos. Sightings</i>	<i>0.04</i>	<i>0.01</i>	<i>3.52</i>	<i>0.0004 *</i>
Year → Precip	0.04	0.01	2.93	0.0034 *
MaxT → Pos. Sightings	0.04	0.02	2.12	0.0342 *
Year → MinT	0.03	0.01	2.37	0.0180 *
Precip → #Pos. Sightings	0.01	0.01	0.70	0.4824
MinT → #Pos. Sightings	0.01	0.02	0.60	0.5506
<i>SSTA</i> → <i>#Pos. Sightings (indirect)</i>	<i>-0.02</i>	<i>0.00</i>	<i>-4.27</i>	<i>< .0001 *</i>
Year → MaxT	-0.07	0.01	-5.81	< .0001 *
Year → #Pos. Sightings	-0.09	0.01	-7.75	< .0001 *
SSTA → MaxT	-0.19	0.01	-15.90	< .0001 *
MinT → Visits	-0.20	0.02	-12.99	< .0001 *

Notes: Displays model paths and their associated coefficients from Figure 4, including paths that were omitted from the figure for simplicity sake. Paths omitted from Figure 4 are shown in bold. Direct (*SSTA* → *#Pos. Sightings*) and indirect (*SSTA* → *#Pos. Sightings (indirect)*) effects of SSTA on the abundance of butterflies are shown in italics. Paths are ordered from most positive to most negative path coefficients. *indicates significance at $P < 0.05$.

Table S14: Results from the path analyses in FIG. 4 for each individual species.

Species	Direct SSTA Est.	P-value Direct SSTA	Total Indirect SSTA Est.	P-value Indirect SSTA
<i>V. cardui</i>	0.41	< .0001 *	0.02	0.5248
<i>P. protodice</i>	0.27	< .0001 *	-0.04	0.1611
<i>V. virginensis</i>	0.14	0.03 *	-0.03	0.2215
<i>J. coenia</i>	0.13	0.001 *	-0.05	0.056
<i>A. campestris</i>	0.12	0.003 *	-0.09	0.0014 *
<i>V. atalanta</i>	0.11	0.0122 *	-0.03	0.2995
<i>C. eurytheme</i>	0.10	0.0033 *	-0.07	0.0123 *
<i>P. acmon</i>	0.10	0.06	-0.04	0.1044
<i>N. milberti</i>	0.08	0.17	-0.04	0.1588
<i>E. ausonides</i>	0.07	0.25	-0.04	0.0719
<i>P. rutulus</i>	0.06	0.27	-0.05	0.0607
<i>D. plexippus</i>	0.06	0.32	-0.01	0.6748
<i>V. annabella</i>	0.05	0.35	0.01	0.5815
<i>S. melinus</i>	0.05	0.21	-0.05	0.0436 *
<i>P. rapae</i>	0.04	0.08	-0.05	0.0557
<i>L. helloides</i>	0.04	0.53	-0.05	0.0604
<i>P. communis</i>	0.04	0.40	-0.07	0.0093 *
<i>S. sylvinus</i>	0.03	0.65	-0.09	0.0024 *
<i>H. phyleus</i>	0.02	0.61	-0.06	0.0153 *
<i>P. myllitta</i>	0.01	0.90	-0.04	0.1417
<i>L. lorquini</i>	-0.01	0.89	-0.01	0.6342
<i>P. zelicaon</i>	-0.03	0.60	0.01	0.7054
<i>E. persius</i>	-0.03	0.67	0.04	0.0952
<i>N. antiopa</i>	-0.05	0.42	-0.04	0.1639
<i>C. ladon echo</i>	-0.06	0.39	0.05	0.0559
<i>N. californica</i>	-0.09	0.18	0.01	0.7184
<i>A. bredowii</i>	-0.10	0.17	0.08	0.008 *
<i>O. sylvanoides</i>	-0.13	0.06	0.04	0.1006

Notes: Species are ordered from highest to lowest direct SSTA estimate. The total indirect SSTA estimates coincide with the dashed line from FIG. 4. χ^2 values of model fit were all the same for each species (Pr (> $\chi^2=0.7195$)). *indicates significance at P<0.05.

Table S15: Results from the path analyses in FIG. 4 when performed for each individual site

Site	$Pr(>\chi^2)$	Direct SSTA Est.	P-value Direct Est	Total Indirect SSTA Est.	P-value Indirect Est.
CP	< .0001	0.096	0.017 *	0.0004	0.9767
DP	< .0001	0.032	0.4279	0.0203	0.0533 *
LC	0.0321	0.043	0.3065	-0.0238	0.1324
SV	0.1494	0.035	0.5475	-0.0022	0.9604
WA	0.3216	0.040	0.3745	-0.007	0.7637
GC	0.0049	0.090	0.0417 *	-0.005	0.8163
RC	< .0001	0.046	0.2951	0.0137	0.4679
WS	< .0001	0.021	0.6227	-0.002	0.8979
NS	0.0001	0.043	0.321	-0.006	0.7307
SM	0.1201	0.043	0.3597	-0.0126	0.6183

Notes: Sites are ordered from highest to lowest elevation. Higher p-values represent greater support for the path structure. *indicates significance at $P < 0.05$.

Appendix B. Supplementary figures, including relationships between butterfly abundance and maximum and minimum temperatures, site and species-specific responses to ENSO-derived SSTA for the remaining 22-species not shown in Fig. 3, and relationships between SSTA and local weather (i.e. precipitation, maximum and minimum temperature).

Figure Legends:

FIG. S1: Odds ratios and standard errors for Maximum Temperature and Minimum Temperature variables included in Models 1-3 from Table 1. Numbers represent the results from each corresponding model. Odds ratios greater than one signify an increase in the probability of attaining an additional positive sighting with each unit increase in z-standardized annual maximum and minimum temperatures. A significant effect on the abundance of butterflies is indicated with an asterisk ($*=P<0.05$). (1) Each analysis contained identical fixed and random effects, but were performed with different subsets of the data. All Data represents the outcomes from the entire dataset ($n=6440$). The resident analysis contained 4646 observations, while non-resident data contained 1794 observations. Mountains and Valley analyses had the same number of observations ($n=3220$). (2) Results for each site along the elevational gradient. Sites on the y-axis are oriented from lowest (Suisun Marsh) to highest (Castle Peak) elevation. (3) Similar to Figure 2, species in both figures are ordered from highest to lowest SSTA odds ratio.

FIG. S2: Odds ratios and standard errors from the fixed-effects logistic regression are displayed for the remaining twenty-two species investigated (Table 1, Model 4). Each species/site combination ($n=280$) contained twenty-three years of observational data. Sites on the y-axis are oriented from the lowest (SM) to highest elevation (CP) and site abbreviations are as follows: SM (Suisun Marsh), WS (West Sacramento), NS (North Sacramento), RC (Rancho Cordova), GC (Gates Canyon), WA (Washington), SV (Sierra Valley), LC (Lang Crossing), DP (Donner Pass), and CP (Castle Peak). Some site-specific responses may not be depicted for each species, given that there were several

extreme responses to SSTA. Significant effects of ENSO on the abundance of butterflies at each site are indicated with an asterisk (* = $P < 0.05$).

FIG. S3: Reveals the estimates and standard errors of linear regressions between local weather variables (e.g. Precipitation, Maximum Temperature, Minimum Temperature) and SSTA at each of the ten sites used in this analysis. It is important to recognize that the effects of SSTA on local weather vary along the elevational gradient and these relationships differ between the three local weather variables. Sites follow the same abbreviations as FIGURE S2.

FIG. S1:

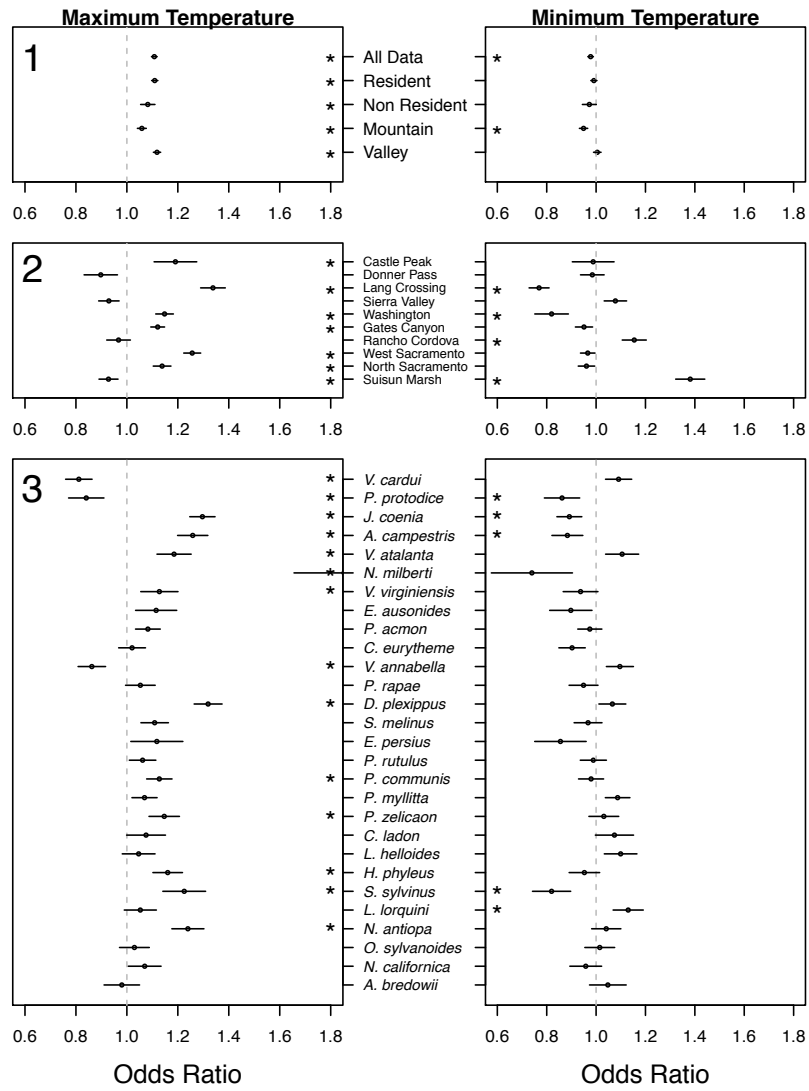


FIG. S2:

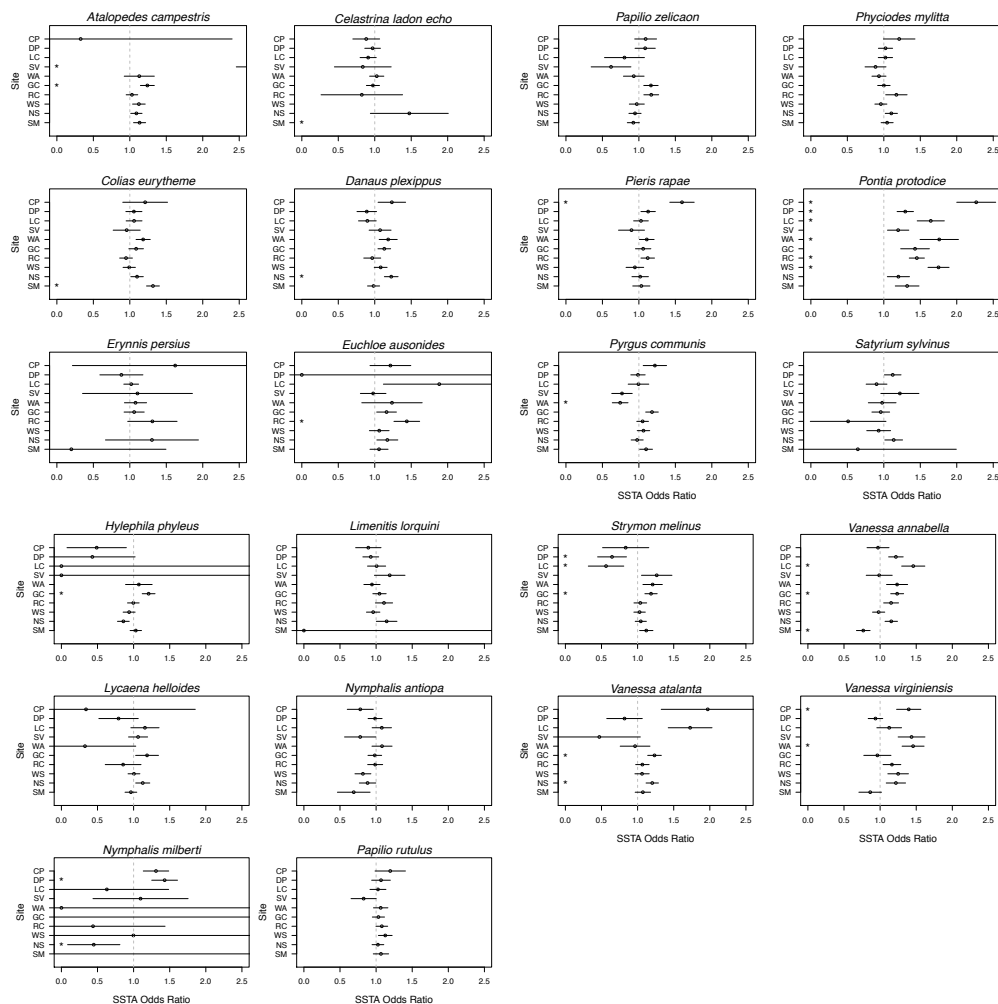
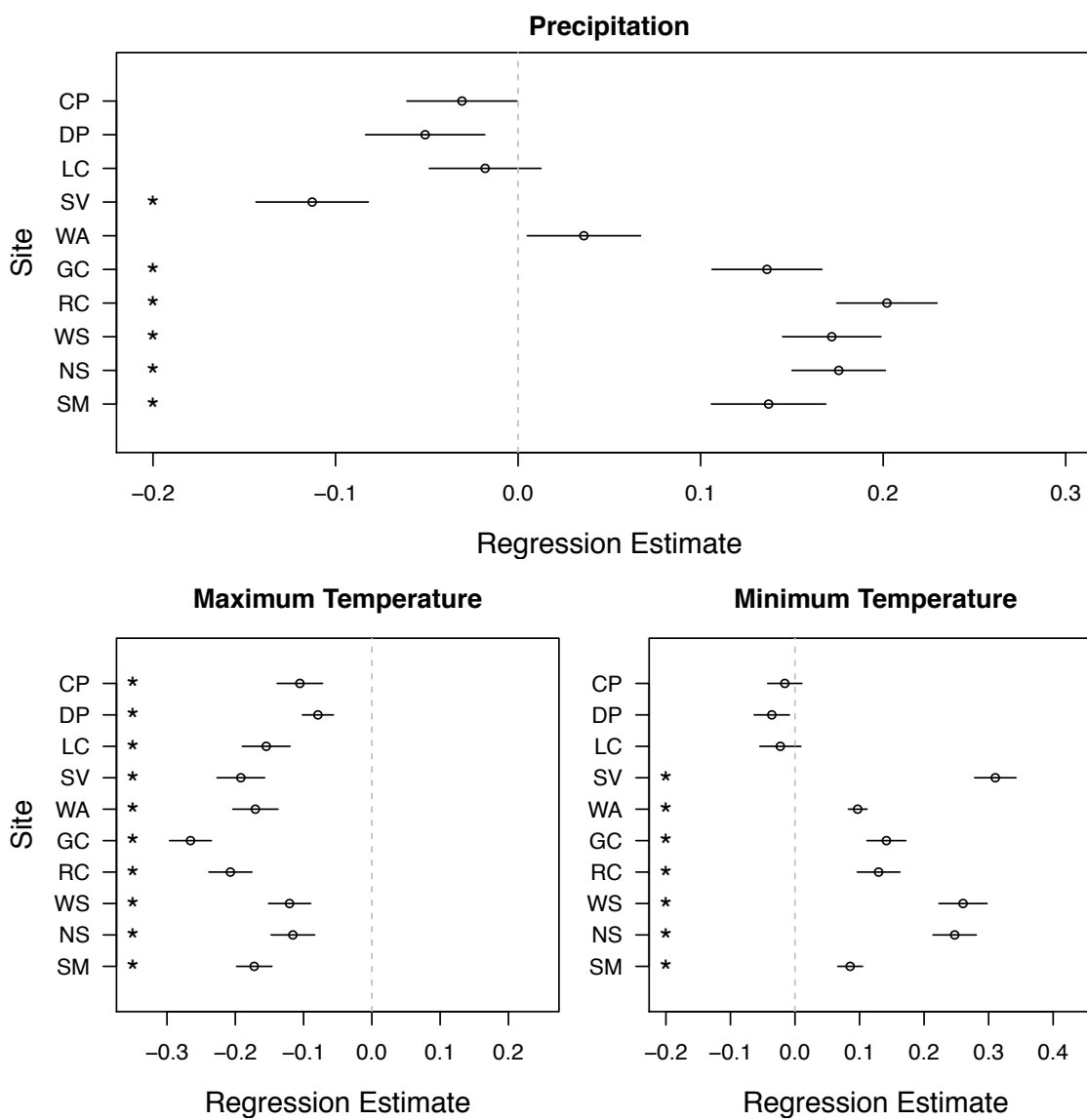


FIG. S3:



Chapter 2

Preferences and performance of Juniper caterpillar assemblages in expanding juniper woodlands of the Intermountain West

Nicholas A. Pardikes, Matt L. Forister, and Lee A. Dyer

*Department of Biology, Program in Ecology, Evolution, and Conservation Biology,
University of Nevada, Reno, NV 89557*

Key words. Tree ontogeny, preference, performance, *Juniperus*, *Callophrys gryneus*
specialist herbivores

ABSTRACT

As trees age, they undergo significant physiological and morphological changes. However, tree ontogeny and its impacts on interactions with their herbivores are often overlooked as determinants of plant-herbivore population dynamics and the strength of plant-herbivore interactions. *Juniperus* (Cupressaceae) is a dominant, long-lived (individual trees can live longer than 2000 years) conifer that serves as the sole host to dietarily specialized assemblages of caterpillars. *Juniperus* has more than 60 species worldwide, and over 15 species can be found within North America. Over the past 150 years, several juniper species have expanded their distribution in Western North America, which has resulted in an increase of young, immature trees on the landscape. However, the consequences of this expansion for the specialized herbivores that feed on juniper are unclear. Using assays in the laboratory and observations in field, we examined the effects of tree ontogeny on oviposition preference, caterpillar performance, and caterpillar abundance in the field of multiple insect herbivore species. We also investigated whether observed differences in preference or performance can be explained by differences in major secondary metabolites among foliage from different-aged juniper trees. We specifically wanted to investigate whether responses to tree ontogeny were consistent across several dietarily specialized caterpillar species, and whether the effects differed between juniper host species. We found that tree age was a reliable predictor of caterpillar performance, however the strength of its influence differed across caterpillar and juniper species. Most caterpillar species developed more quickly and grew larger when fed foliage from young trees, but the relative amount of that shift was dependent on

the juniper species. Interestingly, the specialist Lycaenidae butterfly, *Callophrys gryneus* (Lycaenidae), displayed an oviposition preference for foliage from old-growth *J. osteosperma* trees, despite the fact that larvae of this species performed poorly on older trees. Finally, differences in chemical diversity among foliage from old-growth and young juniper trees are significant predictors of performance in the lab, but do not appear to be primary factors driving observed differences in larval abundance in the field. We conclude that young juniper trees are an important resource for the specialized insect herbivore community. Juniper-feeding caterpillars are most likely to persist in juniper woodlands with a blend of tree ages, and these woodlands may support higher diversity assemblages of juniper specialists. These herbivore preference and performance consequences of intraspecific variation in tree ontogeny are likely to cascade throughout multitrophic communities and influence ecosystem-level processes.

INTRODUCTION

Plants undergo significant morphological and physiological changes across their ontogeny, including substantive changes in metabolism and genetic expression (Poethig 1990, 2003). For example, during a plant's development growth rates and metabolic activity typically decrease, while root-shoot ratios and partitioning of tissue to different plant organs increase (Bryant et al. 1991, Kramer and Kozlowski 2012). The re-allocation of resources across the ontogeny of a plant is likely to have significant impacts on the herbivores that feed on it, and several authors have argued for examining changes across plant ontogeny in plant-associated arthropod communities, and for the consideration of ontogenetic changes in ecological theory of plant-herbivore interactions (Boege et al. 2011, Barton and Boege 2017)

Several studies have found that plant ontogeny can have significant effects on rates of herbivory and can alter the abundances of herbivore populations as the suite of herbivores and the expression of distinctive defensive traits change during the course of a plant's lifetime (Karban 1990, Lawrence et al. 2003, Donaldson and Lindroth 2004, Boege and Marquis 2006, Shiojiri and Karban 2006, Donaldson et al. 2006, Elger et al. 2009, Boege et al. 2011). Mammals appear to prefer foliage from older plants, while insect herbivores prefer foliage from young plants (reviewed by Boege and Marquis 2005). However, plants are host to a diverse array of insect herbivores and ontogenetic changes are unlikely to influence entire insect assemblages in the same way (Bowers and Stamp 1993, Forister 2004). For example, generalist and specialist herbivores are known to respond differently to plant defense strategies, and it is possible that specialist herbivores might show stronger responses than generalists to ontogenetic differences

among the same host plant species (Bowers and Stamp 1993, Bernays 2001, Forister 2004, Ali and Agrawal 2012). Studies of ontogenetic effects on herbivores that incorporate a diversity of herbivore species across several hosts will provide more generalizable results and an improved understanding of how plant ontogeny contributes to the structure of plant-herbivore communities.

Across the Intermountain West, juniper (*Juniperus*, Cupressaceae) and piñon-juniper (*Pinus edulis* and *P. monophylla*, Pinaceae) woodlands have been experiencing a dramatic increase in both density and distribution (Belsky 1996, Miller et al. 2007, 2008, Weisberg et al. 2007). While the cause is unclear (e.g., shift in fire regime, over-grazing, climate change), this expansion is characterized by a dramatic influx of young, immature trees across the landscape (Belsky 1996, Miller et al. 2007, 2008, Bradley and Fleishman 2008). Juniper trees can survive for more than 2000 years, and an invasion of young immature trees is sure to alter the chemical, nutritional, and physical landscape with which insect herbivores interact (Boege and Marquis 2005, 2006, Boege et al. 2011, Hunter 2016). It is difficult to predict the effects such changes will have on herbivore assemblages and entire arthropod communities because there have not been any empirical studies investigating the effects juniper tree ontogeny has on juniper-associated herbivore communities (but see Lucas et al. 2014).

Juniperus is host to a highly specialized assemblage of caterpillars (larval Lepidoptera), which serve as an important resource for higher trophic levels, such as resident and migratory insectivorous birds, insect predators, and insect parasitoids (Belsky 1996). While the consequences of juniper expansion on plants and birds has been investigated, impacts on herbivore communities, or even individual herbivore populations

are undetermined (Bates et al. 1998, Bunting et al. 1999). Here, we investigate variation in the preference and performance of several juniper caterpillar species in response to different ontogenetic stages of two expanding juniper species (*J. osteosperma* and *J. occidentalis*) with the goal of understanding whether tree ontogeny influences multiple specialized caterpillar species similarly and if those differences are consistent across hosts. Specifically, we address the following questions; (1) does herbivore performance differ across juniper tree ontogeny and, if so, how consistent are effects across multiple insect herbivores? (2) Are performance differences due to tree ontogeny consistent across multiple juniper species? (3) Does oviposition preference of *J. osteosperma* differ across juniper ontogeny? and (4) Do differences in secondary metabolites between foliage from immature and old-growth juniper trees explain observed differences in herbivore performance? Finally, we explore whether observed abundances in the field match what we predict from the results of the performance assays. Based on patterns reported for two closely related caterpillar and tree taxa (*Callophrys gryneus thornei* (Lycaenidae) and *Hesperocyparis forbesii* (Cupressaceae)) (Barton and Koricheva 2010, Lucas et al. 2014), we hypothesized that caterpillars should prefer and perform better on foliage from young, immature juniper trees.

The broad goal of this research is to determine the community level consequences of juniper expansion via changes in forest stand structure. Furthermore, juniper encroachment has significant economic implications, as it reduces grass and forb cover, which to those with livestock interest equates to a significant loss of rangeland. It is likely that there are significant impacts of tree ontogeny on plant-animal communities, and

these effects are more important when the communities are widespread and dominant, like juniper woodlands.

METHODS

Study system

Two closely related species of juniper, within the serrated-leaf junipers in North America, *J. occidentalis* and *J. osteosperma*, were used in this analysis (Adams and Schwartzbach 2013; Mao et al. 2010). Both species are found in the Intermountain West and have been expanding in their density and distribution for the past 150 years resulting in a significant regionwide recruitment of young, immature trees. Additionally, both tree species are subject to control efforts to limit their expansion via chaining, herbicide, fire, or selected removal of mostly young, immature trees.

The experiments described here use *J. osteosperma* samples collected from Lemmon Valley, Nevada (39.669376, -119.803727). Most trees within Lemmon Valley are less than 150-years old, but several old-growth trees are found on the ridges above the valley. Old-growth trees were identified using morphological characteristics that help classify tree age (Tausch et al. 2009) instead of cores because center often rots in old-growth juniper trees making it difficult to core. Old-growth (>200 years old) *J. osteosperma* were distinguished by their thick and fibrous bark, dead branches, and branches covered with lichen (Tausch et al. 1999). Young trees (<50- years old) were restricted to those that are less than two-meters tall, to minimize error in the categorization of young and old.

Juniperus occidentalis samples were collected from Sierra Valley, California (39.646757, -120.370448), which is located on the east slope of the Sierra Nevada at 1500m, northeast of Reno. Most juniper woodlands in Sierra Valley are located on private property, and old trees were difficult to find. Samples were collected from several individual trees located near Highway CA 49 and between Loyalton and Sierraville, California. Tree age was determined similarly to *J. osteosperma*, but with a specific guide to *J. occidentalis* (Miller et al. 2007). Young trees were abundant near the road, while “old-growth” trees found along the road were no more than 200 years old. Samples were used from at least 5 individuals for each treatment (e.g., young and old-growth) and randomly assigned to a trial. Foliage from both young and old-growth were collected from the same general area to remove the possible effects of environmental heterogeneity on leaf quality. Furthermore, to reduce any impacts of new growth tissue on herbivore performance, foliage was collected from juniper branches closer to the trunk (which are older needles on juniper).

Juniperus osteosperma and *J. occidentalis* share recent evolutionary histories and chloroplast and nuclear DNA have confirmed hybridization between these two species where their distributions overlap (Vasek 1966, Terry et al. 2000, Adams 2013). Samples from *J. occidentalis* and *J. osteosperma* used in this analysis likely contain genetic material from both lineages because obtaining pure genetic populations was not feasible because one would have to travel to southern Oregon for pure *J. occidentalis* and eastern Utah for pure *J. osteosperma* lineages (Terry et al. 2010).

Our study utilized a subset of the caterpillar species that feed on juniper. *Callophrys gryneus* (Juniper Hairstreak Family Lycaenidae) was utilized for preference

and performance trials for each individual treatment. Three geometrids were utilized (*Digrammia atrofasciata*, *G. quinquelinearia*, and an unidentified *Glena sp.* (near *G. kirkwoodaria*)) for the performance trials. Oviposition preference trials were not conducted for the moths because geometrids frequently oviposit off host plant foliage. All four species occur on plants in relatively low densities, and have larval parasitism levels of 10% +/- 0.02 SE (N. Pardikes, unpublished data). All females were collected from Texas and none had been previously exposed to either juniper species used in this assay.

Performance assays

We used performance assays to address whether tree ontogeny influenced performance of several juniper-feeding caterpillars. We first investigated performance of four juniper specialist herbivores on foliage from different aged *J. osteosperma* trees. Females from *C. gryneus* ($N=23$), *D. atrofasciata* ($N=1$), *G. quinquelinearia* ($N=2$), and *Glena sp.* ($N=1$) were collected in the wild and maintained in small oviposition vials to acquire eggs for each assay (Table S1). Eggs or first instars were removed from the oviposition vials and randomly assigned to either foliage from young or old-growth *J. osteosperma* foliage treatments. Performance assays were performed for two caterpillar species (e.g., *C. gryneus* and *G. quinquelinearia*) on both juniper species (*J. osteosperma* and *J. occidentalis*), to investigate whether the effects of tree ontogeny on herbivore performance were consistent across caterpillar and juniper species.

Plant material was stored in a refrigerator to keep cuttings fresh and samples were replaced every three weeks to minimize the chemical and physical changes that occur once the foliage is removed from its parent plant. Larvae were fed fresh cuttings of

juniper foliage at least every 5 days. Each larva was reared individually in 150 mm diameter Petri dishes and assays were performed in Percival Scientific growth chambers that were set at a 16-hour day and 8-hour night cycle. Daytime temperatures were set at 25 °C, while nighttime temps dropped to 20 °C. Development time (days to pupa), pupal mass (pupal mass after 10 days of being a pupa), adult mass (mass of eclosed adult), and frass mass (mass of frass produced throughout larval development) were recorded and served as measures of performance. All masses were measured to the nearest 0.001 g using a Sartorius LA 310s microbalance.

All statistical analyses were performed in program R (3.3.2) (R Core Team 2014). Logistic regression was used to model survival as a consequence of tree age, caterpillar species, and tree species. Starting from a saturated generalized linear model with a binomial error distribution, backward model selection using Akaike's Information Criterion (AIC) was used to determine the most parsimonious model that best fit the data. The deviance and χ^2 of full and reduced models were used to examine conditional dependence among variables. A Fisher's exact test identified odds ratios of the number of caterpillars that survived or died for each treatment (e.g., young and old) and species combination. A likelihood ratio test ("car" package, Fox et al. 2015), was performed to identify significant variables and interactions within the model. Standardized parameter estimates were used to identify relative strengths of each predictor variable (Fletcher 2012).

Factorial analysis of variance (ANOVA) was used to model the effects of tree age on caterpillar performance across several caterpillar and juniper species. One-way ANOVA identified differences in performance between four caterpillar species feeding

on *J. osteosperma*, while a two-way ANOVA investigated whether effects of performance are consistent across caterpillar and juniper species. Both main and interactive effects of feeding on foliage from different-aged juniper trees were examined and sum-to-zero contrasts were used in all type III analysis of deviance models. Development time (days to pupa), pupal mass, adult mass, frass production per day, and growth efficiency (pupal mass/development time) were used as response variables in each distinct model. Generalized linear mixed models (GLMM), from the “lme4” package, were also used to identify whether the effects of tree age were still apparent when caterpillar and juniper species were modeled as random effects (Bates et al. 2014).

Preference assays of the Juniper Hairstreak butterfly

Two-way choice assays were used to investigate whether preferences of *C. gryneus* female butterflies differed among juniper trees from different ages. *Callophrys gryneus* females were sent from Austin (N=10) and San Marcos, Texas (N=23) (Table S1). The two hosts included in preference assays consisted of cuttings from young and old-growth *J. osteosperma*. Cuttings were collected the same day that preference assays were conducted. By collecting *J. osteosperma* cuttings from a single location, potential variation in phytochemistry and other leaf quality parameters among juniper populations was reduced.

Assays were performed in plastic cups that contained sprigs of each of the two age-classes of *J. osteosperma*. At the bottom of each cup were several holes that each cutting could fit through and extend into water in a second, smaller cup. The sprigs and the butterfly were contained in the cup by fine mesh that was periodically sprayed with

fruit punch Gatorade[®], which served as a food (nectar) source to the adult females throughout the experiment. Experiments were set up the same day the butterflies arrived in Reno, NV, which was usually 1-day after collection in Texas. The preference experiments were conducted in Percival Scientific growth chambers that were set to same conditions mentioned above. Each female butterfly was left to oviposit for 48h, after which the butterfly was removed from the array and eggs on each foliage type were counted (we did not count eggs that were oviposited away from the host plant).

The “BayesPref” package in R was used to analyze preference among juniper trees of different ages (Gompert and Fordyce 2012). This package utilizes a hierarchical Bayesian model to analyze ecological count data (Fordyce et al. 2011). Significant differences in estimates of preference for juniper trees of different ages were detected using the mean posterior probability and 95% credible interval. Posterior probabilities and credible intervals that did not overlap were interpreted as having different preferences.

Chemical analysis of juniper foliage

To investigate whether ontogenetic variation in secondary metabolites could be correlated with any observed differences in caterpillar performance, we collected foliage from the same *J. osteosperma* and *J. occidentalis* trees that were used throughout the performance assays and allowed them to dry at room temperature for several weeks. Once dried, extracts from 0.100g of dried plant material were produced using a protocol developed by Giavalisco et al. (2011) to acquire polar, semi-polar, and hydrophobic fractions of juniper metabolites. A diluted methyl-tert-butyl-ether (MTBE) fraction was

injected directly onto an Agilent 7890A gas chromatograph coupled to a 5975C quadrupole mass spectrometer (GCMS) and chromatograms were used for metabolomics analyses to characterize each sample with distinct chromatographic profiles. Molecular masses, retention times, and peak integrations were quantified and aligned using the “metaMS” package (Wehrens et al. 2014). o-xylene served as an internal standard and allowed for the acquisition of relative concentrations for each peak. The “vegan” package was used to calculate the richness and diversity of compounds within each separate chromatogram (e.g., juniper tree sample) (Oksanen et al. 2015). Bray-Curtis dissimilarity and non-metric multidimensional scaling (NMDS) were used to identify unique clusters among the multivariate chemical data. Multiple Response Permutation Procedure (MRPP) was used to identify significant differences between treatments. Linear models were used to identify whether diversity and richness of compounds were significantly different among juniper species and the different age classes of juniper. We also quantified chemical diversity of secondary compounds using the R package “hierDiversity” (Marion et al. 2015), which quantifies chemical diversity for each set of samples in a given treatment using hierarchical group-wise partitioning. These values were then used to perform linear mixed-effects modeling of alpha chemical diversity and several measures of caterpillar performance (Bates et al. 2014). Caterpillar species was modeled as a random effect to investigate the relationship between caterpillar performance and chemical diversity across caterpillar species.

Larval surveys

Larval surveys were conducted to document the abundance of caterpillars on juniper trees of different ages. Each survey was performed in *J. osteosperma* stands (Lemmon Valley) and *J. occidentalis* stands (Shinn Mountain, CA 40.695284, -120.276585). Circular plots of 25-m diameter were set up and each juniper tree within the plot was sampled for caterpillars using beating sheets. Trees within the plot were categorized by age-class (e.g., young, old-growth). Larvae were collected, identified, and reared in the lab for parasitoids. Due to many trees without caterpillars, abundance at the individual tree level was overdispersed (i.e. variance was greater than the mean), therefore a negative binomial (“MASS”; Venables and Ripley 2013) generalized linear model was used to identify differences in abundance of caterpillars across trees of different ages. Standardized values of total number of leaves (e.g., leaf count) and number of leaves sampled were included as covariates in each model. Type III analysis of deviance identified whether abundances of caterpillars differed significantly across ontogenetic stages of juniper trees.

RESULTS

Survival

Survival was significantly greater for two of the four caterpillar species when fed foliage from young *J. osteosperma* trees (Fig. 1A & 1B). Fisher exact tests identified *C. gryneus* ($\beta=1.19$, $P=0.006$) and *G. quinquelinearia* ($\beta=-1.23$, $P=0.013$) as having odds ratios significantly different from 1.0. *C. gryneus* survival was significantly greater on foliage from young *J. osteosperma* trees, while *G. quinquelinearia* survival was greater on old-growth *J. osteosperma* trees. Type III analysis of deviance (likelihood ratio test)

identified a significant interaction between treatment and species (Table 1), revealing that the effect of tree age on survival depends on the Lepidopteran species. Differences among species were the strongest predictors of survival, though tree age was still a significant predictor of survival when all four caterpillar species were treated as a random effect (Table S2). Survival across multiple species of juniper was dependent on tree age, caterpillar species, and juniper species (Table 2). Tree age significantly reduced survival when fed foliage from *J. osteosperma*, but not for *J. occidentalis*. The proportions of caterpillars that survived were significantly different for two out of four combinations of juniper and caterpillar species (Figs. 1C & 1D) and a Fisher exact test revealed that survival of *C. gryneus* ($\beta=0.41$, $P=0.25$) and *G. quinquelinearia* ($\beta=1.45$, $P=0.10$) on *J. occidentalis* were not different between foliage from young and old growth trees. Treating caterpillar species as a random effect did not reveal a significant interaction between tree age and juniper species, suggesting that survival, as a consequence of tree age, does not differ between the two juniper species (Table S3).

Performance assays

Feeding on foliage from different aged *J. osteosperma* trees had significant impacts on several aspects of performance of the four juniper specialist herbivores. Development times of those that survived to the pupal stage, (*C. gryneus* ($n=58$); *Digrammia atrofasciata* ($n=87$); *Glena quinquelinearia* ($n=40$); *Glena sp.* ($n=24$)), were significantly reduced when feeding on foliage from young *J. osteosperma* trees (Fig. 2A). The significant interaction between tree age and species for development time suggests that caterpillar species responded differently to being fed foliage from different aged *J.*

osteosperma trees (Table 3). *Glena sp.* was the only caterpillar species to spend significantly more days as a larvae when fed foliage from young *J. osteosperma* trees and its removal resulted in a non-significant interaction between species and treatment. However, when caterpillar species were modeled as a random effect, that significant interaction was conserved (Table S4). While significant effects of tree age on pupal mass ($F_{1, 160} = 19.84$, $P < 0.005$; Table 3) and growth efficiency ($F_{1, 160} = 25.95$, $P < 0.005$; Table 3) were detected, the responses of pupal mass and growth efficiency from feeding on foliage from different aged *J. osteosperma* trees was consistent across the four herbivore species (Figs. 2B-D). Across all species, pupal masses and growth efficiency decreased when fed foliage from old-growth *J. osteosperma* trees. Although it was predicted that frass production would be reduced when feeding on foliage from old-growth *J. osteosperma*, our results do not support this prediction (Fig 2C). The significant effect of tree age on pupal mass and growth/day is conserved when considering caterpillar species as a random effect (Table S4).

The performances of two caterpillar species (*C. gryneus* and *G. quinquelinearia*) were compared when fed foliage from different aged *J. osteosperma* and *J. occidentalis* trees. Although patterns were similar to the four specialist herbivore species on *J. osteosperma*, several performance measures showed significant differences across both caterpillar and juniper species. For example, the effect of tree age on development time differed significantly between caterpillar species and the strength of that effect varied across the two juniper species (Table 4 & Fig. 3A). Caterpillars developed significantly faster when fed foliage from young juniper trees, but the reduction of development time is different between the two juniper species. This may be driven primarily by *C. gryneus*,

for which the statistical model revealed a significant interaction between tree age and juniper species ($F_{1, 125} = 4.20$, $P=0.04$). Following AIC model selection (“step”), the three-way interaction (tree age by juniper species by caterpillar species) was not included in the best model for pupal mass (Table 4). Pupal mass and growth efficiency were significantly reduced when fed foliage from old-growth juniper trees, and both effects were not significantly different among juniper species (Fig. 3B-D). Similar to the previous analysis on *J. osteosperma*, differences in frass production per day due to tree age were minimal (Fig 3C). GLMM, with caterpillar species modeled as a random effect, did not reveal a significant interaction between tree age and juniper species in any response variable of interest (Table S5). This suggests that the responses to tree age are consistent across multiple juniper species.

Preference of the Juniper Hairstreak butterfly on J. osteosperma

Female *C. gryneus* ($n=17$) were presented with a choice between foliage from young and old growth *J. osteosperma* trees. Females showed high variability in preference (Fig 4A), but overall there was a greater preference for foliage from old-growth *J. osteosperma* trees (0.34 ± 0.06 , mean young and credible interval; 0.66 ± 0.10 , mean old and 95% credible interval) (Figure 4B). This pattern was consistent across both populations of *C. gryneus* (Travis, $n=6$; San Marcos, $n=11$). Although these populations of *C. gryneus* are naïve to *J. osteosperma*, the results presented here support the potential for preference to differ across *J. osteosperma* tree ontogeny.

Chemical differences between young and old J. osteosperma and J. occidentalis

A NMDS analysis of foliage from different aged *J. osteosperma* and *J. occidentalis* trees revealed a 2-axis solution, and found that chemical differences between juniper species were greater than that among young and old-growth juniper trees (Fig 6). MRPP revealed significant differences among juniper species (Delta =0.04), but significant difference were not observed between the four treatments (Delta = 0.07) or tree age (Delta = 0.18). However, the variances among old-growth samples appear to be greater than young trees for both juniper species. Furthermore, significant differences in diversity, richness, and evenness were not identified across age class, species, or their interaction (Table 6).

Linear mixed-effects models revealed significant associations among several measures of caterpillar performance and chemical diversity (Fig. 4; Table 5). Foliage from old-growth juniper trees revealed higher alpha chemical diversity values than foliage from young juniper trees. Increases in chemical diversity resulted in significantly slower development times (Fig. 4A), a reduction in pupal mass (Fig. 4B), and decreased growth efficiency (Fig. 4D). However, the production of frass was not significantly altered by changes in alpha chemical diversity (Fig. 4C).

Larval Surveys on J. osteosperma and J. occidentalis

A total of 96 juniper trees (*J. osteosperma* (n=61) and *J. occidentalis* (n=35)) were sampled at Lemmon Valley, NV and Shinn Mountain, CA across two age classes (n=61 old-growth trees and n = 35 young trees), and a total of 151 (n=124 *J. osteosperma* and 27 on *J. occidentalis*) caterpillars were collected. When both juniper species are considered, a type III analysis of deviance (Wald test) revealed a significant interaction

between tree age and juniper species, which suggests that the effects of tree age on caterpillar abundance differ between juniper species (Table 7). Splitting the data into each juniper species separately showed that the relationship between caterpillar abundance and tree age differed between species. Even with juniper biomass included as a cofactor, old-growth *J. osteosperma* trees had significantly more caterpillars than young trees (Figure 7A) and although not a significant effect, the relationship between tree age and caterpillar abundance for *J. occidentalis* is in the opposite direction, i.e., younger trees have greater densities of caterpillars (Fig. 7B). However, this second relationship should be taken with caution given the small number of *J. occidentalis* trees and caterpillars that were collected. The negative binomial GLM was only able to explain 16% of variation associated with caterpillar abundance (null deviance – residual deviance/null deviance).

DISCUSSION

Tree ontogeny is a pervasive form of intraspecific variation on the landscape, yet it has not been frequently considered in traditional studies of plant-herbivore interactions (Boege and Marquis 2005, Bolnick et al. 2011, Violle et al. 2012). While several studies have investigated how tree ontogeny alters insect herbivores, researchers have not asked if the influence of tree ontogeny is consistent across multiple herbivore and host plant species (Lawrence et al. 2003, Boege 2005, Boege and Marquis 2006, Shiojiri and Karban 2006). Understanding how specialist herbivores are impacted across tree ontogeny will be useful for predicting how diverse plant-herbivore communities might be affected by different disturbance regimes (e.g., deforestation, wildfire, drought). The

results reported here demonstrate that tree ontogeny has complex effects on the preference and performance of several species of a dietarily specialized caterpillar assemblage on a widespread, foundational steppe and savannah tree genus (*Juniperus*, Cupressaceae). The preference of a specialized juniper associated butterfly differed across juniper ontogeny, and reduced numerous measures of performance of several dietarily specialized caterpillar species that feed exclusively on juniper. Importantly, the effects of feeding on foliage from different aged juniper trees differed among caterpillar species and this effect sometimes varied across species of juniper. These results emphasize the importance of incorporating interspecific (among herbivores) and intraspecific variation (among different host age classes) when determining the influences of tree ontogeny of diverse plant-herbivore communities (Bolnick et al. 2011, Violle et al. 2012).

Laboratory-rearing experiments revealed the majority of caterpillar species examined performed significantly better on foliage from young juniper trees, suggesting that young juniper trees are an important resource on the landscape. Trees undergo significant changes in their chemical and physical properties as they develop from seedling to mature individuals, and these changes can have substantial influences on the insects that interact with them (Boege et al. 2011). Our work supports previous work with a subspecies of the Juniper Hairstreak, *Callophrys gryneus thornei*, which found that caterpillars grew larger when fed foliage from young Tecate cypress (*Hesperocyparis forbesii*, Cupressaceae) trees (Lucas et al. 2014), which is the sister genus to *Juniperus*. Our study adds to the work of Lucas et al. (2014), by investigating multiple caterpillar species and attempts to investigate whether the observed responses of feeding on foliage

from different-aged juniper trees are consistent across multiple juniper species. Caterpillars reared on foliage from old-growth juniper trees developed more slowly and grew to be smaller pupa. Unexpectedly, *C. gryneus* was the only species that did not reveal significant differences in pupal mass when fed foliage from different aged juniper trees, and this was consistent across both juniper species. However, other performance metrics corroborated results from Lucas et al. (2014), as *C. gryneus* performed significantly better on foliage from young juniper trees. Insect herbivores often co-occur with multiple host plant species and the effects of tree ontogeny on insect performance are likely to differ across their geographic and host-plant range. Of the two juniper species investigated in this study, herbivores on *J. osteosperma* seems to show stronger differences in performance between old and young juniper trees than *J. occidentalis*. This suggests immature *J. osteosperma* trees are ecologically valuable on the landscape-level to caterpillar-dependent predators and parasitoids, compared to *J. occidentalis*. However, other factors, such as microclimatic conditions and predation risk are also likely to differ between different stand structures with primarily young or old juniper trees.

Significant variation in the oviposition preference was observed among the individual females of *C. gryneus*, and based on egg counts, there was a meaningful preference for foliage from old-growth *J. osteosperma* trees. This is in contrast to what we predicted given the increased performance of caterpillars on foliage from young *J. osteosperma* trees. The absence of a positive association between preference and performance is not uncommon in these assays (Thompson 1988, Gripenberg et al. 2010). Given that performance assays were completed in the lab, it is difficult to know whether eggs oviposited on old-growth juniper trees truly perform worse than those on immature

trees. Given that old-growth trees are generally larger and are more structurally complex, caterpillars may be harder to locate for predators (Finke and Denno 2002). However, other studies have found that predation risk by birds, predatory wasps, and parasitoids are higher in trees of reproductive stages relative to saplings (Domínguez et al. 1989, Van Bael et al. 2003, Boege and Marquis 2006). Furthermore, predators and parasitoids of these small, cryptic caterpillars are rather specialized and not negatively affected by complexity of habitat, due to adaptations to efficiently finding small, cryptic prey items. It is also possible that the complex structure of old-growth *J. osteosperma* could provide additional protection from natural enemies for early instars and larvae move onto young juniper trees as that mature to take advantage of the higher quality foliage.

The preference for foliage from old-growth *J. osteosperma* trees may also be due to chemical similarities to the sympatric juniper species that are present in central Texas (*J. ashei*, *J. virginiana*, or *J. pinchotii*). Downey and Nice (2011) revealed that this same butterfly species displayed significant preference for local species of juniper and showed evidence of ongoing host race formation for this butterfly species on the different juniper species. Moreover, it is uncertain if and how tree ontogeny among these juniper species alters the preference of ovipositing juniper hairstreak females across these three juniper species. Future studies should attempt to test for effects of tree ontogeny on preference when considering sympatric and allopatric plant-herbivore communities (see Forister 2004).

We observed significant differences in the preference and performance of specialist Lepidoptera on two different species of juniper and phytochemical differences among the different-aged trees are minimal, but appear to be correlated with these

dissimilarities. The effects of phytochemistry on plant-herbivore interactions are often subtle and it can be difficult to identify effects of phytochemistry in driving the herbivore performance (Barton and Koricheva 2010, Rosenthal and Berenbaum 2012). Most studies investigate the effects of single compounds on herbivore performance, but herbivores are exposed to a rich mixture of phytochemicals that likely act in synergy to influence preference and performance (Dyer et al. 2003, Richards et al. 2010). While phytochemical differences were more pronounced between the two juniper species, and very little chemical separation between different-aged trees was observed in either species, chemical diversity did significantly influence herbivore performance. We predicted that old-growth juniper trees would be more chemically defended, and our results revealed that foliage from old-growth juniper trees contain greater diversity of compounds than foliage from young trees (Miller et al. 1999, Fritz et al. 2001, Val and Dirzo 2003, Boege and Marquis 2006, Stewart et al. 2015). Other factors potentially responsible for the differences in preference and performance could be nutritional or physical differences (Awmack and Leather 2002, Barton and Koricheva 2010). Old-growth trees are characterized by slow growth, and the rate of production of new biomass can approach zero (Miller et al. 1999), which likely results in foliage that is physically tougher and more difficult to digest on old-growth juniper trees. A recent study by Stewart et al. (2015) revealed significant difference in digestibility and nutritional composition between different stages of maturity for several juniper species. Immature juniper trees had great concentrations of crude protein and lower levels of neutral detergent fiber (NDF). Associations between secondary defensive compounds and plant nutrition likely vary across tree ontogeny (Stewart et al. 2015), however few studies have

considered how these two fundamental components of a host plant, interact to alter the preference and performance of specialist herbivores in changing landscapes (Quintero and Bowers 2012).

The results that younger trees in our experiment increased larval performance across several juniper herbivores and juniper species were somewhat contradictory to what we observed in our larval surveys in the field. Larval abundance was significantly greater on old-growth juniper trees when compared to young trees, but only for *J. osteosperma*. However, our preference assays may offer a unique insight into these contradictory results, in that female *C. gryneus* butterflies preferred foliage from old-growth juniper trees. Although, we were not able to perform preference assays with the other juniper herbivores, it would be interesting to ask if the preference for foliage from old-growth juniper trees is consistent across many members of the juniper caterpillar assemblage. It is unknown whether patterns of preference for different-aged trees is consistent across the host range or is only present where juniper expansion is occurring.

Regardless, the increased abundance of caterpillars on old-growth *J. osteosperma* trees could simply be a consequence of the fact that old-growth trees are larger and attract more adults. *C. gryneus* not only use juniper as the host for larval development, but host trees also support territorial males and mating events (Forister 2004, Downey and Nice 2011). There are likely other factors, besides oviposition preference, such as predation that are leading to the observed distribution of caterpillars in the wild. Behavior of foraging parasitoids likely differs between mature and immature trees, and this could be due to ontogenetic changes in volatile organic compounds (VOCs) during a plants development (Cole 1980, Boege and Marquis 2006, Boege et al. 2011).

CONCLUSION

In summary, we found that tree ontogeny can have dramatic consequences on the oviposition preference and larval performance of a dietarily specialized Lepidopteran assemblage. Though the strength of these effects are contingent on the caterpillar and juniper species investigated, young juniper trees appear to be an important resource. Not all insect herbivores respond similarly to tree ontogeny, thus management of juniper woodlands in the Intermountain West should attempt to maintain a mixture of tree ages across the landscape. Juniper woodlands of a single age (e.g., following control efforts, plantations, or after a fire) are less likely to benefit all members of the community. Juniper is a foundational (dominant) species in the Intermountain West and the effects of tree ontogeny are likely to cascade to other members of the community, such as birds and parasitoids (Kearsley and Whitham 1989). Furthermore, in widespread, species poor communities, such as the community on juniper, tree ontogeny is a significant form of intraspecific variation that influences plant-herbivore dynamics and eventually entire ecosystem processes (Crutsinger et al. 2006, Bolnick et al. 2011, Violle et al. 2012, Barbour et al. 2016). It will be important to incorporate tree ontogeny into ecological theory of plant-herbivore communities, and recognize that not all species within the community respond similarly to intraspecific variation. Whether natural or human-induced, many woodlands and forests around the world are experiencing a homogenization in tree age-class structure (Didion et al. 2007). Woodlands composed of a single age-class of trees via stand displacing disturbances or widespread management activities can be more susceptible to pest outbreaks and plant pathogens (Raffa et al.

2008). Variation in tree ontogeny is likely to have consequences that cascade throughout the community and an improved understanding of how plant ontogeny alters defensive strategies and the risk of attack is likely to inform more sustainable forestry and agricultural practices (Boege and Marquis 2005, Bybee et al. 2016).

ACKNOWLEDGMENTS

We thank Chris Nice, John Lane, Dave Wagner, and Bill and Pam Dempwolf for help collecting moths and butterflies used in all preference and performance assays. This research was funded by the University of Nevada, Reno Graduate Student Association (GSA). Volunteers from the Earthwatch Institute helped collect caterpillars in the field. Victoria Cernoch, Quinn Campbell, and Dan Urruty helped throughout the rearing experiments. LAD and MLF were supported by NSF DEB 1442103.

Tables

Table 1

<i>Variables</i>	β	$LR-\chi^2$	<i>df</i>	<i>P</i>	
Tree Age	0.90	8.177	1	0.004	*
Lep. Species		119.066	3	< 0.005	*
Tree Age \times Lep. Species		15.718	3	0.001	*

Type III Analysis of deviance with the “car” package in R (Fox et al. 2009). Logistic generalized linear model with a binomial error distribution to model frequency of survival across four species of caterpillars (C. gryneus, G. quinquelinearia, D. atrofasciata, and Glena sp.) feeding on foliage from young and old J. osteosperma trees. β represents the standardized beta coefficient. A significant effect on the observed frequency is indicated with an asterisk ($P < 0.05$).*

Table 2

<i>Variables</i>	β	$LR-\chi^2$	<i>df</i>	<i>P</i>	
Tree Age	0.40	1.566	1	0.211	
Lep. Species		10.069	1	0.002	*
Tree Species		4.918	1	0.027	*
Tree Age \times Lep. Species		1.997	1	0.158	
Tree Age \times Tree Species		1.566	1	0.211	
Lep. Species \times Tree Species		4.182	1	0.041	*
Tree Age \times Lep. Species \times Tree Species		11.611	1	0.001	*

Results from a type III analysis of deviance with the “car” package in R (Fox et al. 2009). Logistic generalized linear model with a binomial error distribution to model frequency of survival for C. gryneus and G. quinquelinearia on young and old J. osteosperma and J. occidentalis trees. β represents the standardized beta coefficient. A significant predictor of the frequency is indicated with an asterisk (P , 0.05).*

Table 3

<i>Variable</i>	<i>Development Time</i>			<i>Pupal Mass</i>			<i>Growth/Day</i>		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
Tree Age (TA)	1	4.88	0.028	1	19.84	<0.005	1	25.9	<0.005
Lep Species (LS)	3	3.70	0.012	3	130.18	<0.005	3	24.9	<0.005
TA × LS	3	3.60	0.014	3	2.38	0.072	3	1.6	0.20
Residuals	201			160			160		

Type III Analysis of deviance from the “car” package in R for several measures of caterpillar performance for four caterpillar species feeding on foliage from young and old J. osteosperma trees. A significant predictor of the frequency is indicated in bold ($P < 0.05$).

Table 4

<i>Variable</i>	<i>Development Time</i>			<i>Pupal Mass</i>			<i>Growth/Day</i>		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
Tree Age (TA)	1	3.286	0.071	1	3.92	0.05	1	21.96	<0.005
LepSpecies (LS)	1	6.820	0.010	1	192.22	<0.005	1	69.05	<0.005
TreeSpecies (TS)	1	2.200	0.140	1	5.09	0.03	1	12.68	0.001
TA × LS	1	2.087	0.150	1	4.78	0.03	1	0.12	0.73
TA × TS	1	3.675	0.057	1	-	-	1	0.12	0.74
TA × LS × TS	1	4.493	0.035	1	-	-	1	2.00	0.16
Residuals	187			144			144		

Type III analysis of deviance from the “car” package in R for several measures of caterpillar performance for C. gryneus and G. quinquelinearia on young and old J. osteosperma and J. occidentalis trees. A significant predictor of the frequency is indicated in bold ($P < 0.05$).

Table 5

Variable	Development Time				Pupal Mass				Growth/Day			
	β	χ^2	df	P	β	χ^2	df	P	β	χ^2	df	P
Intercept		2.45	1	0.12		24.1	1	0.005		54.1	1	0.005
Alpha	2.93	45.04	1	0.005	-0.003	7.75	1	0.005	-0.0003	31.3	1	0.005
R ²	0.25				0.76				0.58			

*Results from a linear mixed effects model to investigate the relationship between alpha chemical diversity across the four treatments and several measurements of caterpillar performance. Caterpillar species was treated as a random effect. Type III analysis of deviance from the “car” package in R for several measures of caterpillar performance for *C. gryneus* and *G. quinquelinearia*. A significant predictor of the frequency is indicated in bold ($P < 0.05$). R² is a pseudo variance explained for mixed-effects models from (Nakagawa and Schielzeth 2013). Alpha is measure of within-group (alpha) chemical diversity from the “hierDiversity” package in R (Marion et al. 2015).*

Table 6

<i>Response Variable</i>	<i>Predictor</i>	<i>df</i>	<i>F</i>	<i>P</i>
Diversity	Tree Age	1	0.2706	0.61
Richness	Tree Age	1	0.2627	0.62
Evenness	Tree Age	1	0.4985	0.49

Type III analysis of deviance from the “car” package in R when modeling chemical properties of J. osteosperma and J. occidentalis. These come from three separate linear regressions with an interaction between tree age × tree species were modeled. Only the results from ‘Tree Age’ as the predictor are show here.

Table 7

<i>Variable</i>	<i>df</i>	χ^2	<i>P</i>
(Intercept)	1	0.338	0.561
Tree Age	1	0.062	0.803
Tree Species	1	4.170	0.041 *
Leaves Sampled	1	5.040	0.025 *
Total Leaf Count	1	2.831	0.092
Tree Age \times Tree Species	1	4.959	0.026 *

A GLM with a negative binomial error distribution (abundance data was overdispersed) was used to model observed caterpillar abundances in the field. Results shown here are from a type III analysis of deviance from the “car” package in R. A significant predictor of caterpillar abundance is indicated with an asterisk ($P < 0.05$).*

Figure legends

Figure 1: A) Displays proportion of caterpillars that survived on foliage from young and old-growth *J. osteosperma* trees across four juniper-feeding caterpillar species. B) Results from a logistic regression of survival for four herbivore species on *J. osteosperma*. Odds-ratios are displayed and reveal how the odds of survival when moving from foliage from old-growth to young *J. osteosperma* trees. Species with odds-ratios greater than 1.0 reveal improved odds of survival on foliage from young *J. osteosperma* trees, while odds-ratios less than 1.0 have greater odds of survival on foliage from old-growth *J. osteosperma* trees. The y-axis is on a log scale. C) Displays proportion of caterpillars that survived on foliage from young and old-growth *J. osteosperma* and *J. occidentalis* trees across *Callophrys gryneus* and *Glena quinquelinearia* caterpillars. D) Results from a logistic regression of survival for *C. gryneus* and *G. quinquelinearia* herbivore species on foliage from young and old-growth *J. osteosperma* and *J. occidentalis*. Odds-ratios and 95% confidence intervals are displayed and reveal odds of survival when moving from foliage from old-growth to young *J. osteosperma* and *J. occidentalis* trees. Species with odds-ratios greater than 1.0, reveal improved odds of survival on foliage from young juniper trees, while odds-ratios less than 1.0 have greater odds of survival on foliage from old-growth juniper trees. The y-axis is on a log scale.

Figure 2: A) Displays the mean and standard error development time (days to pupa) for four species feeding on foliage from young and old-growth *J. osteosperma* trees. B) Displays the mean and standard error of pupal mass (g) for four species feeding on foliage from young and old-growth *J. osteosperma* trees. C) Displays the mean and

standard error of frass produced per day for four species feeding on foliage from young and old-growth *J. osteosperma* trees. D) Displays the mean and standard error of growth per day (g/day) for four species feeding on foliage from young and old-growth *J. osteosperma* trees. A significant difference in development time between treatments within each species is indicated with an asterisk (* $P < 0.05$; ** $P < 0.01$).

Figure 3: A) Displays the mean and standard error development time (days to pupa) for *C. gryneus* and *G. quinquelinearia* species feeding on foliage from young and old-growth *J. osteosperma* and *J. occidentalis* trees. B) Displays the mean and standard error for pupal mass (g) for *C. gryneus* and *G. quinquelinearia* species feeding on foliage from young and old-growth *J. osteosperma* and *J. occidentalis* trees. C) Displays the mean and standard error for frass produced per day (g/days as larva) for *C. gryneus* and *G. quinquelinearia* species feeding on foliage from young and old-growth *J. osteosperma* and *J. occidentalis*. D) Displays the mean and standard error for growth per day (g/days as larva) for *C. gryneus* and *G. quinquelinearia* species feeding on foliage from young and old-growth *J. osteosperma* and *J. occidentalis*. A significant difference in development time between treatments within each species is indicated with an asterisk (* $P < 0.05$; ** $P < 0.01$).

Figure 4: Linear regressions of several measures of caterpillar performance to alpha chemical diversity. A) Development time, B) Pupal mass, C) frass produced per day (g), and D) growth per day (g). Alpha chemical diversity was calculated with “hierDiversity” R package (Marion et al. 2015). “lme4” was used for a linear mixed-effects model, with caterpillar species as a random effect (Bates et al. 2014). Open circles (°) represent

foliage from old-growth juniper trees, while closed circles (●) represent foliage from young juniper trees. The 95% confidence interval is plotted in grey.

Figure 5: A) Displays counts of oviposited eggs for each individual female *C. gryneus* (n=17) butterfly that participated in the preference assay. Black bars represent eggs counted on foliage from old-growth *J. osteosperma*, while white bars represent egg counts on foliage from young *J. osteosperma* trees. B) Mean posterior probability and 95% credible interval for the Bayesian preference model from the R package “bayespref” (Fordyce et al. 2011). Estimates of preference are shown for the San Marcos (n=11), Austin (n=6), and combined (n=17) populations separately. Posterior probabilities greater than 0.5 identify a preference for foliage from old-growth *J. osteosperma* trees, while values less than 0.5 reveal a preference for foliage from young *J. osteosperma* trees.

Figure 6: Non-metric multidimensional scaling (NMDS) of foliage from young and old-growth *J. osteosperma* and *J. occidentalis* trees. NMDS was performed using the “vegan” R package (Oksanen et al. 2015). Old *J. occidentalis* samples (occ_o) are represented by the color green, while young *J. occidentalis* samples (occ_y) are shown in blue. Old *J. osteosperma* (ost_o) samples are displayed in red, while young *J. osteosperma* (ost_y) samples are shown in purple.

Figure 7: A) Displays the median, 25% and 75% quantiles, and outlier observations for caterpillar abundance on young and old-growth *J. osteosperma* and *J. occidentalis* trees. Observed abundances on each individual sampled tree are shown as grey points. A significant difference in caterpillar abundance between tree ages within each species is indicated with an asterisk (* $P < 0.05$; ** $P < 0.01$). B) Displays the median, 25% and

75% quantiles, and outlier observations for caterpillar density on young and old-growth *J. osteosperma* and *J. occidentalis* trees. Observed abundances on each individual sampled tree are shown as grey points.

Figures

Figure 1

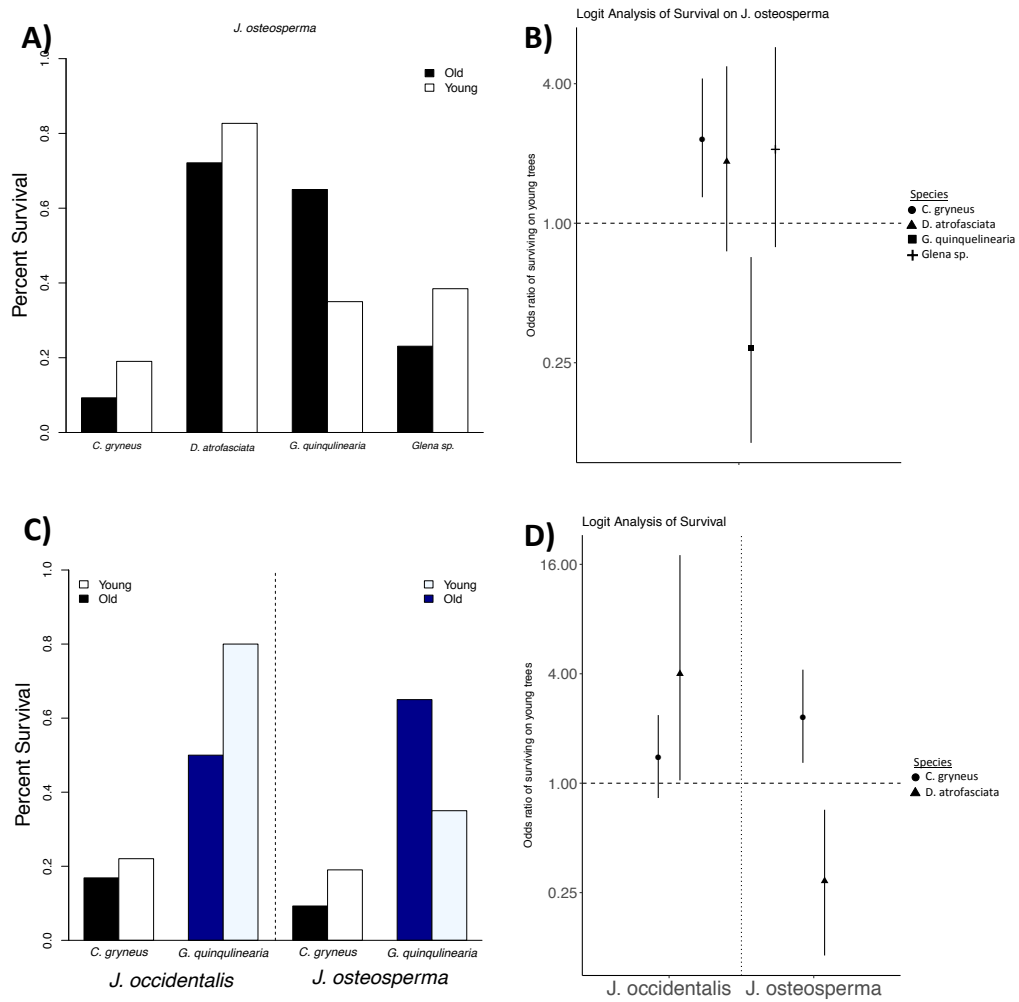


Figure 2

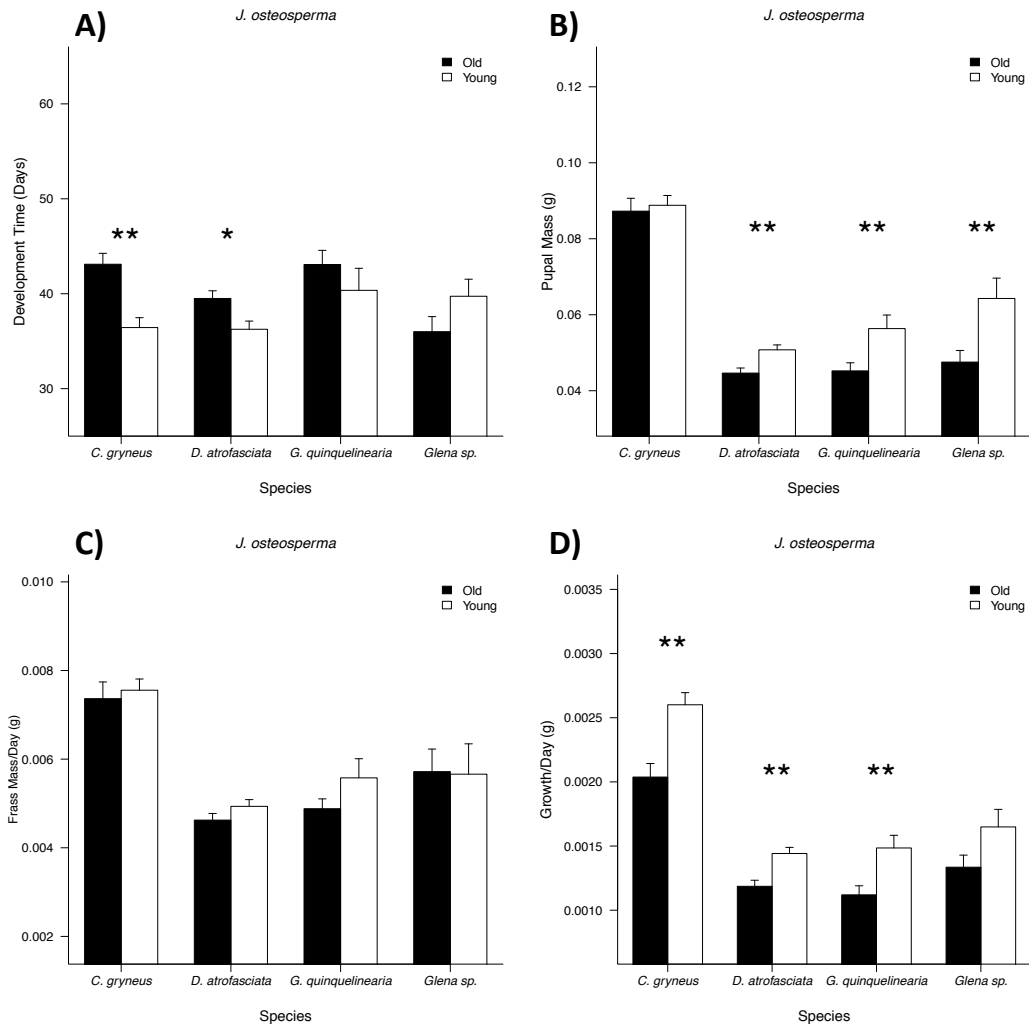


Figure 3

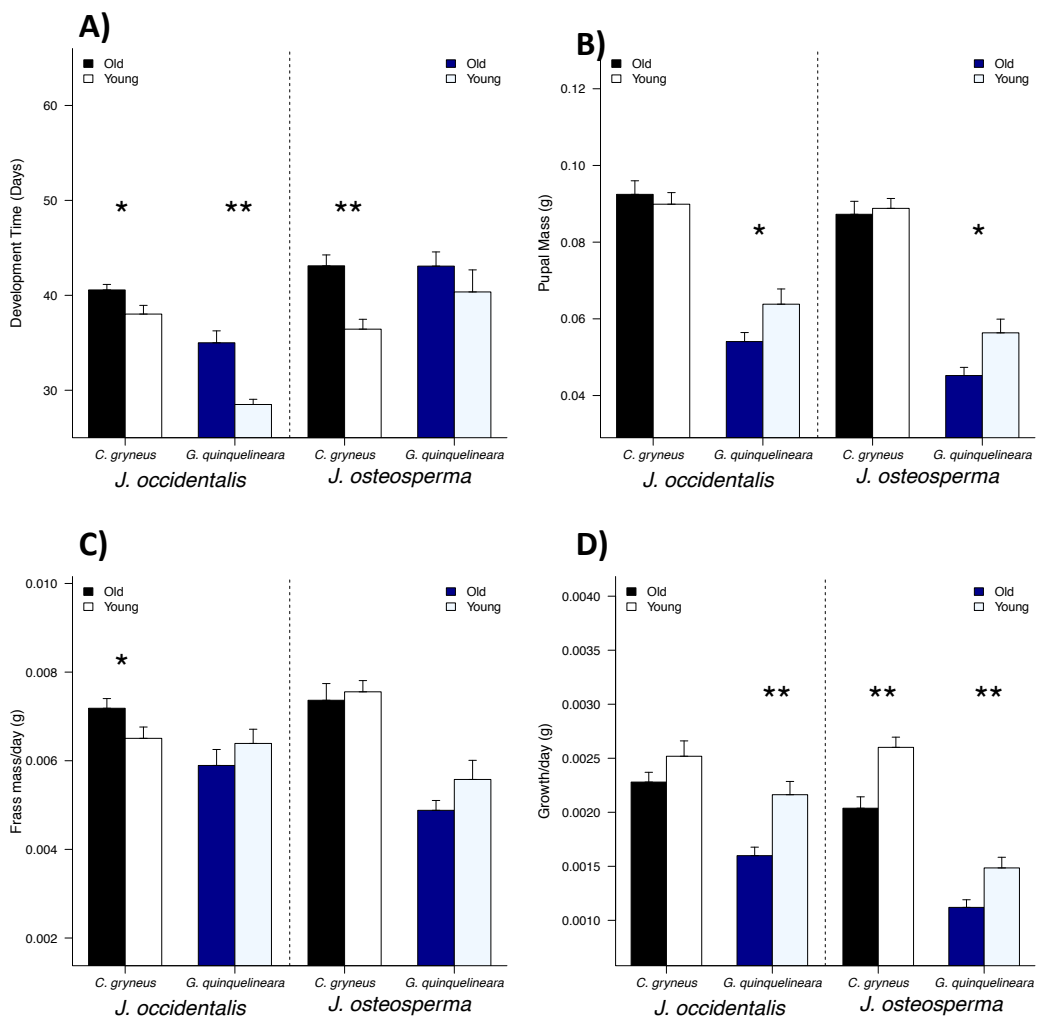


Figure 4

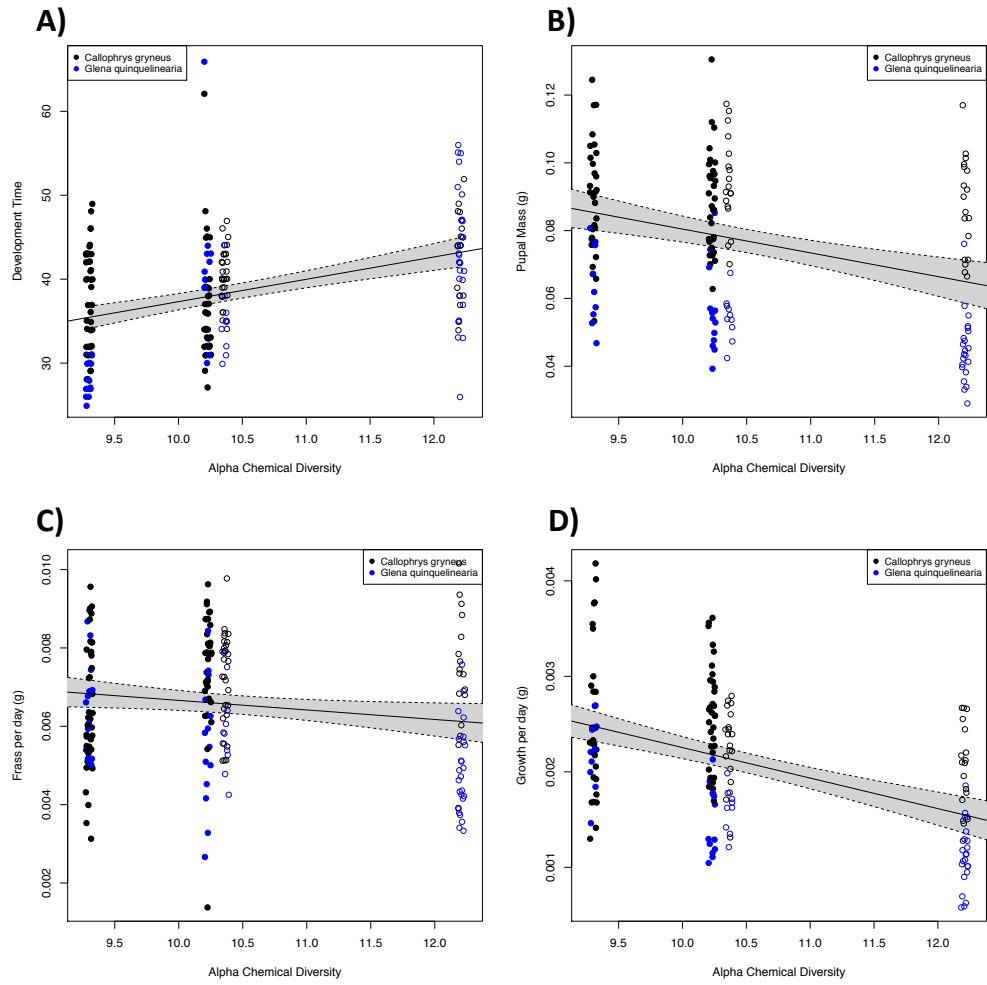
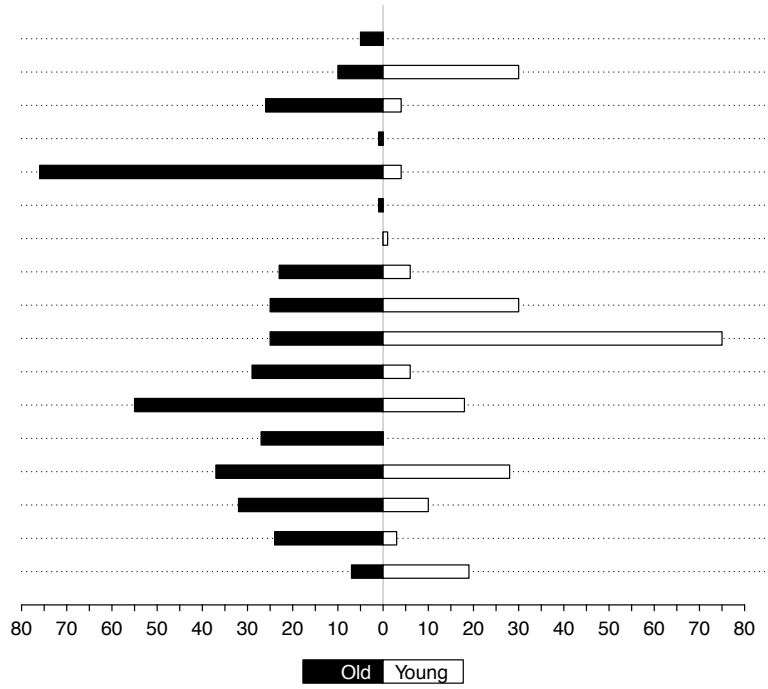


Figure 5

A) Preference of *C. gryneus* on *J. osteosperma*



B) Preference

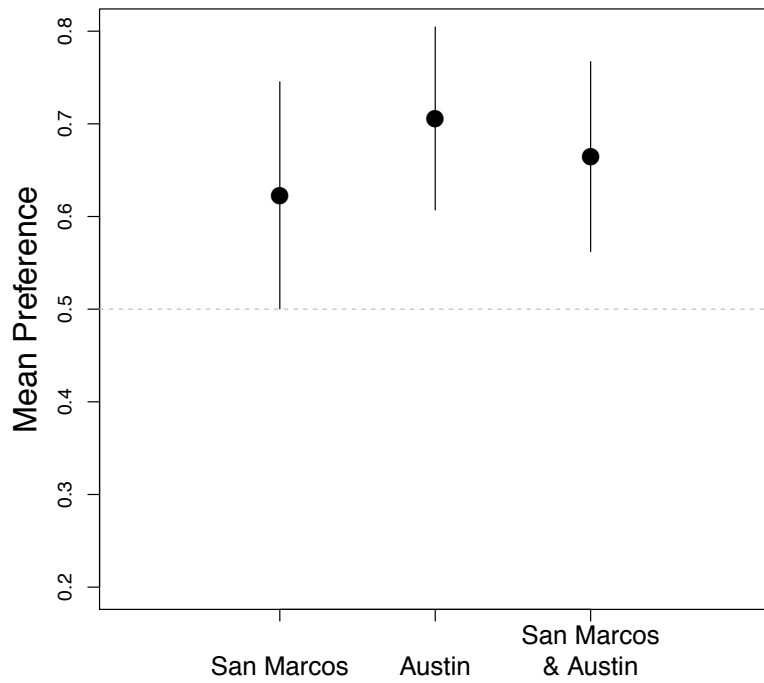


Figure 6

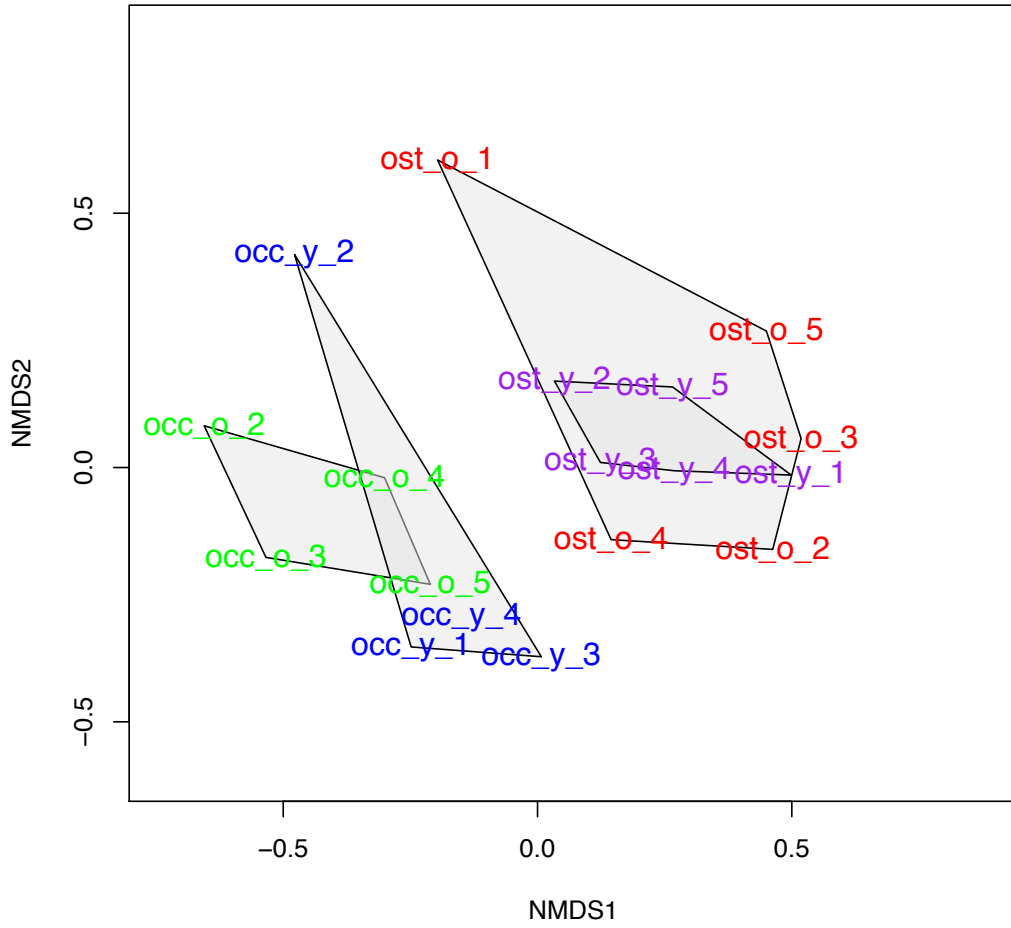
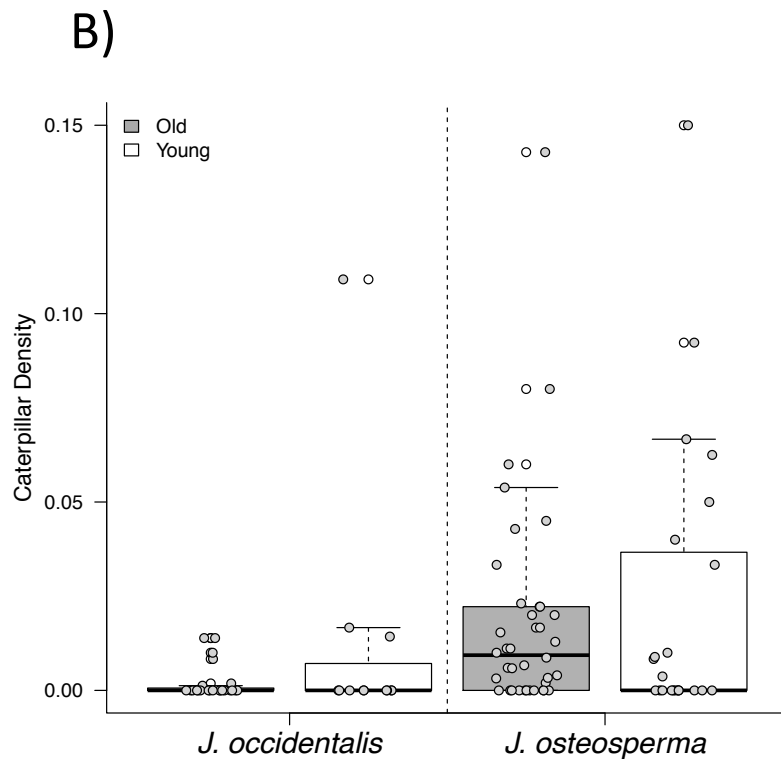
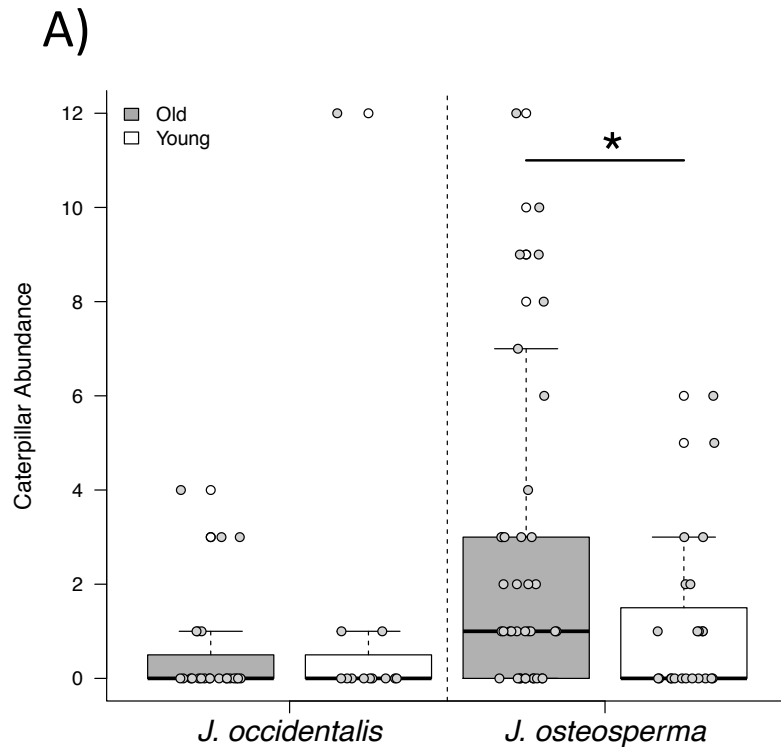


Figure 7



Supplementary Material

Chapter 2

Preferences and performance of Juniper caterpillar assemblages in expanding juniper woodlands of the Intermountain West

Nicholas A. Pardikes, Matt L. Forister, and Lee A. Dyer

*Department of Biology, Program in Ecology, Evolution, and Conservation Biology,
University of Nevada, Reno, NV 89557*

Table S1: Locations and numbers of each species used in this analysis.

<i>Species</i>	<i>N</i>	<i>Location</i>	<i>Date</i>	<i>Collector</i>
<i>D. atrofasciata</i>	1	TX: Boerne, Delmar Cain home, Clear Creek Circle, 29°52'51", - 98°36'50"	7/29/14	David Wagner and James McDermott
<i>G. quinquelinearia</i>	1	TX: Boerne, Delmar Cain home, Clear Creek Circle, 29°52'51", - 98°36'50"	7/29/14	David Wagner and James McDermott
<i>Glena sp. (nr kirkwoodaria)</i>	1	TX: Boerne, Delmar Cain home, Clear Creek Circle, 29°52'51", - 98°36'50"	7/29/14	David Wagner and James McDermott
<i>G. quinquelinearia</i>	1	Fort Davis, TX	4/21/15	David Wagner
<i>C. gryneus</i>	10	Travis Co, TX Waters Park	4/2/14	Bill and Pam Dempwolf
<i>C. gryneus</i>	23	San Marcos, TX	4/23/14	Chris Nice

Table S2

<i>Variables</i>	χ^2	<i>DF</i>	<i>P</i>
Intercept	0.9032	1	0.34191
Tree Age	3.6615	1	0.05568

Type III Analysis of deviance from the “car” package in R. GLMM with a binomial distribution of survival and caterpillar species as a random effect. Looking at four species on J. osteosperma.

Table S3

<i>Variable</i>	χ^2	<i>df</i>	<i>p-value</i>
Intercept	1.0585	1	0.30355
Tree Age	3.4996	1	0.06138
Tree Species	1.3486	1	0.24553
Tree Age \times Tree Species	0.4421	1	0.50609

Type III Analysis of deviance from the “car” package in R. GLMM with a binomial distribution of survival and caterpillar species as random effects. Looking at C. gryneus and G. quinquelinearia on J. osteosperma and J. occidentalis.

Table S4

<i>Variable</i>	Development Time			Pupal Mass			Growth/Day		
	χ^2	<i>DF</i>	<i>P</i>	χ^2	<i>DF</i>	<i>P</i>	χ^2	<i>DF</i>	<i>P</i>
Intercept	2279.626	1	<0.005	42.815	1	<0.005	32.4	1	<0.005
Tree Age	13.846	1	<0.005	15.323	1	<0.005	39.7	1	<0.005

Type III Analysis of deviance from the “car” package in R. GLMM with caterpillar species as a random effect. Looking at four species on J. osteosperma.

Table S5

Variable	<i>Development Time</i>			<i>Pupal Mass</i>			<i>Growth/Day</i>		
	χ^2	<i>df</i>	<i>p-value</i>	χ^2	<i>df</i>	<i>p-value</i>	χ^2	<i>df</i>	<i>p-value</i>
Intercept	550.56	1	<0.005	17.33	1	<0.005	21.45	1	<0.005
Tree Age	8.37	1	0.004	0.027	1	0.88	5.42	1	0.02
Tree Species	11.31	1	<0.005	5.17	1	0.023	6.95	1	0.008
Tree Age×Tree Sp.	2.0204	1	0.1552	1.433	1	0.23	1.82	1	0.178

Type III Analysis of deviance from the “car” package in R. GLMM with caterpillar species as a random effect. Looking at C. gryneus and G. quinquelinearia on J. osteosperma and J. occidentalis.

Chapter 3

Testing the enemies hypothesis in expanding juniper woodlands

Nicholas A. Pardikes and Lee A. Dyer

Department of Biology, Program in Ecology, Evolution, and Conservation Biology,

University of Nevada, Reno, NV 89557

Keywords: *enemies hypothesis, parasitism rates, juniper, encroachment*

ABSTRACT

The positive effect of plant diversity on natural enemy abundance and diversity is well established in agricultural systems, but relationship between plant diversity and natural enemies in natural systems is more complex. The *enemies hypothesis*, which posits that predator and parasitoid control of prey communities is more effective in habitats with more plant diversity, has been supported primarily in agricultural systems, but it is less certain whether it is supported in more species rich natural systems. Juniper woodlands in the Intermountain West have been encroaching onto grass and rangelands, resulting in a reduction of understory plant diversity. While several studies have investigated the consequences of this expansion on plants and ecosystem level processes, it is uncertain how the reduction of plant diversity impacts interactions among insect herbivores and parasitoid natural enemies. We studied rates of parasitism and parasitoid species richness across a gradient of understory plant diversity to test whether predictions from the *enemies hypothesis* are supported in expanding juniper woodlands of the Intermountain West. We found that overall rates of parasitism and parasitoid species richness decreased with increasing understory plant diversity. Parasitism rates and parasitoid species richness were instead responding to the density and abundance of their caterpillar hosts. Our results do not support predictions from the *enemies hypothesis*. Our findings suggest that for highly specialized communities of herbivores and parasitoids, the concentration of hosts is the strongest predictor of parasitism rates in expanding juniper woodlands. The *enemies hypothesis* appears to be more applicable to generalist predators and parasitoid assemblages, but as plant diversity is declining across the globe

it will be essential to understand how species diversity can alter the effectiveness of localized resource-consumer interactions.

Keywords: *enemies hypothesis, parasitism rates, juniper, encroachment*

INTRODUCTION

All biological communities are composed of complex webs of interactions and ecological theory suggests that the diversity of these interactions enhance stability and functioning in ecosystems (Goodman 1975, Loreau et al. 2002, Ings et al. 2009). The consequences of current global declines of species diversity are unknown, especially in the context of trophic interactions (Vitousek et al. 1997, Chapin III et al. 2000, Sala et al. 2000). Less diverse communities are thought to be more unstable and less efficient at capturing limited resources, which decreases rates of primary and secondary production and negatively impacts ecosystem functioning (Naeem et al. 2002, Aquilino et al. 2005). Thus the effectiveness of consumers in capturing their prey and transferring energy throughout the food web is critical for the health of both natural and agricultural systems (Chapin et al. 1998). Consequently, understanding how species diversity within a trophic level affects rates of consumer-resource interactions at higher or lower trophic levels is an important goal in community ecology (Aquilino et al. 2005).

The diversity of plants in a given habitat can have profound impacts on the diversity of herbivores and their natural enemies, and several competing theories have been developed to predict the effects of plant diversity on resource-consumer interactions at higher trophic levels (MacArthur 1955, Hutchinson 1959, Root 1973, Elton 2000, McCann 2000, Moreira et al. 2016). For example, the *enemies hypothesis* predicts that predators and parasitoids should be more effective in controlling their prey in diverse plant systems because additional plant species should provide natural enemies with alternative prey species, direct food sources, and appropriate microhabitat conditions (Root 1973, Letourneau 1987, Russell 1989, Björkman et al. 2010). The *enemies*

hypothesis has been corroborated by data from a diverse array of agricultural systems, but support for the *enemies hypothesis* in natural systems has been more variable (Letourneau 1987, Russell 1989, Riihimäki et al. 2005, Schuldt et al. 2011). Several studies in natural systems found that increased plant diversity decreased the attack rate of predators and parasitoids and did not consistently result in the reduction of herbivore abundance (Risch et al. 1983, Sheehan 1986, Letourneau 1987, Russell 1989, Andow 1991, Bommarco and Banks 2003, Stireman et al. 2005a, Haddad et al. 2009, Scherber et al. 2010). Natural enemies may not respond to increased plant diversity due to several potential mechanisms. Unlike generalist predators and parasitoids, specialized parasitoids do not benefit from the presence of alternative prey that additional plant species provide; based on this, specialist parasitoids should be most abundant in monocultures, where their host is likely to attain higher densities (Sheehan 1986). Second, with additional plant and herbivore species, the ability of specialist predators to successfully locate their host is inhibited due to a greater mix of visual and chemical cues (Sheehan 1986). However, some have found that the presence of other plant species provides complementary food sources (e.g., nectar and honeydew from aphids) for adult parasitoids, including specialist parasitoids, which is an important food resource (Harmon et al. 2000, Tylianakis et al. 2004, Lavandero et al. 2005, Stireman et al. 2005a, Blaauw and Isaacs 2012). Adult parasitoids with available nectar resources can live longer and parasitize hosts at higher rates, than those without direct food resources (Tylianakis et al. 2004)

An important question that puts the *enemies hypothesis* into the perspective of current global environmental change is: How do community changes across a landscape, such as many of the changes in diversity or community assemblages that are part of

global environmental change, affect natural enemies? Woody plants (e.g., shrubs and trees) in many arid and semiarid biomes have been increasing in density and distribution (Cabral et al. 2003, Van Auken 2009, Eldridge et al. 2011), and this encroachment is converting many grasslands, savannas and rangelands into shrublands and woodlands. Among the many consequences of woody plant encroachment, this change is accompanied by a shift from mostly belowground biomass to aboveground plant biomass and a reduction in plant species diversity (Van Auken 2009, Ratajczak et al. 2012). While many studies have investigated the consequences of woody plant encroachment on the plant community and ecosystem function, few studies have examined the consequences for higher trophic levels such as insect herbivores and their natural enemies, despite the great value of such data for understanding relationships between perturbation of communities, plant diversity, insect diversity, and trophic interaction diversity. Furthermore, this information would be useful for future management of these changing ecosystems (Koch et al. 2015). Given the severe reduction in plant diversity, as a consequence of woody plant encroachment, it is an ideal mensurative experiment for examining how biodiversity at different trophic levels influences the effectiveness of localized trophic interactions and food web dynamics, both of which influence ecosystem functioning (Holt and Loreau 2001, Raffaelli et al. 2002, Aquilino et al. 2005, Riihimäki et al. 2005, Vehviläinen et al. 2007, Duffy et al. 2007, Schuldt et al. 2011).

Juniperus (Cupressaceae) woodlands across the Intermountain West have seen drastic increases in density and distribution during the last 140 years (Knapp et al. 1998, Weisberg et al. 2007, Auken and Smeins 2008). Regional changes in the disruption of fire regimes, amplified grazing pressures, natural range expansion, and a changing

climate, are thought to be the main drivers of this expansion (Miller and Rose 1995, Miller 2005, Johnson and Miller 2008, Romme et al. 2009, Tausch et al. 2009, Dyer and Letourneau 2013). Juniper expansion has resulted in a significant increase of canopy cover across the landscape, the replacement of shrub-steppe and grassland communities, and overall reduction of understory plant diversity (Weisberg et al. 2007, Miller et al. 2008, Coultrap et al. 2008, Huffman et al. 2013). While conversion of these shrub and grassland plant communities to juniper woodlands are both detrimental and beneficial depending on the focal species of a given observer (Belsky 1996, Rosenstock and Charles Van Riper 2001, Wenninger and Inouye 2008, Weisberg et al. 2014, McIver and Macke 2014), little is known about the consequences for the specialized juniper caterpillar (Lepidoptera) community and the parasitoids that feed on them. Species interactions, such as plant-herbivore and herbivore-parasitoid interactions, play an important role in maintaining biodiversity and help stabilize many ecosystem services and functions (Janzen 1974, Dyer and Letourneau 1999, Bascompte and Jordano 2006, Ives and Carpenter 2007, Dyer et al. 2010, Dyer and Letourneau 2013). However, research has not investigated the effects of juniper expansion on trophic interactions, even though they are likely to be more sensitive to this expansion due to their high degree of dietary specialization, and a dependence on the phenology, behavior, and abundances of multiple other species (McCann 2007, Suttle et al. 2007, Tylianakis et al. 2007).

In this study, we examined predictions of the *enemies hypothesis* in expanding juniper woodlands by quantifying parasitism rates and parasitoid species richness across a gradient of canopy cover (a proxy for plant diversity). We specifically tested whether non-host plant diversity increased parasitism rates between juniper caterpillars and their

parasitoid natural enemies. We utilized a multi-trophic, interaction-based approach to explore the relationships between understory vegetation, canopy cover, parasitism rates, and parasitoid species richness. We addressed the following questions: 1) Does an increase in understory plant richness increase parasitism rates? 2) Is the richness of parasitoids highest when non-host plant richness is highest? The *enemies hypothesis* predicts that rates of attack should be greatest in more diverse plant systems, however insect herbivores in juniper woodlands may be primarily affected by inherent characteristics of their host plants, such as architecture and phytochemistry, while the parasitoid communities may be more influenced by habitat characteristics – such as diversity of plant species or nectar plant resources (Root 1973, Hawkins 2005, Blaauw and Isaacs 2012). Finally, we extended existing tests of the *enemies hypothesis* by exploring whether phytochemical diversity of host plants (e.g., *Juniperus*) could help explain parasitism rates and parasitoid species richness. Chemical diversity could be an obvious mechanism by which plant diversity affects natural enemies – either via toxicity of hosts that are mixing foods, via volatiles, kairomones, or other chemically mediated effects (Smilanich et al. 2009b, Richards et al. 2015). In addition to generating important empirical data and hypothesis tests for understanding relationships between diversity at different trophic levels, this project provided a strong applied element focused on community-level impacts of current and future juniper control efforts.

METHODS

Study system

This study was conducted across four sites that have been experiencing juniper expansion (Herrick et al. 2010) and included several species of juniper: Lemmon Valley, Nevada (LV) (39.66968, -119.803292), Shinn Mountain, California (SM) (40.69670, -120.274311), Santa Rita Experimental Range, Arizona (SRER) (31.728961, -110.878908), and Paradise, Arizona (PA) (31.934134, -109.209449). LV is located in Washoe County, Nevada (USA) at an elevation of 1300-1500 m on Bureau of Land Management (BLM) land and is dominated by *Juniperus osteosperma*. SM is located in Lassen County, California (USA) at an elevation of 1500-1600 m. It also is on BLM and is dominated by *J. occidentalis*. Both LV and SM are located in the western Great Basin Desert, and are characterized by sagebrush-steppe vegetation and Basin-Range topography, where juniper dominated woodlands are restricted to higher elevations and excluded from most of the lowland desert. The western portion of Great Basin Desert, where the rain shadow effect from the Sierra Nevada is more pronounced receives an average of 230 mm precipitation a year. It is characterized by hot, dry summers and cold, snowy winters. Juniper dominated woodlands in the Great Basin are typical of wooded shrub-steppe habitats which is primarily composed of shrubs (e.g. *Artemisia*, *Purshia tridentata*, *Chrysothamnus viscidiflorus*, *Ericameria nauseosus*), forbs (e.g. *Lupinus*, *Phlox*, *Eriogonum*), and several species of bunch grass.

SRER is an active rangeland research facility ran by the University of Arizona located in Pima County Arizona (USA) and its elevation ranges from 1000-1700 m. Paradise, AZ (USA) is located in Cochise County Arizona at elevations ranging from 1400-1700 m and is within the Coronado National Forest. Both Arizona locations are in the transition between the Sonoran and Chihuahuan Deserts in southeastern Arizona and

contain both *Juniperus deppeana* and *J. arizonica*. The Chihuahuan Desert receives a yearly average of 235 mm of precipitation, but there is a high degree of yearly variation and some years can accumulate over 400 mm of precipitation. Most precipitation falls in the summer, during the North American Monsoon (July-October). Sampling locations in the Chihuahuan Desert were primarily oak-juniper savanna, with an understory dominated by grasses, shrubs (*Prosopis velutinus*, *Garrya wrightii*, *Senegalia constricta*, *Mimosa biuncifera* and *M. dysocarpa*), and forbs e.g., *Viguiera dentata* and *V. multiflora*). All sites either currently allow grazing or allowed it in the recent past.

The caterpillar community associated with juniper is composed of several leaf chewing families: Geometridae (e.g., *Digrammia*, *Glena*, *Holochroa dissociarius*, *Stannoctenis*, *Eupithecia*, *Pityeja ornata*, *Carphoides inconspicuaris*), Noctuidae (e.g., *Lithophane*, *Abagrotis glenni*), Lycaenidae (e.g., *Callophrys gryneus*), Sphingidae (e.g., *Sphinx dollii*, *S. sequoiae*), Gelechiidae (e.g., *Gelechia*), Lasiocampidae (e.g., *Gloveria arizonensis*), and Erebidae (e.g., *Lophocampa argentata*). Most caterpillars that feed on juniper are dietary specialists on the genus, with the exception of *G. arizonensis* and *L. argentata* and perhaps some of the unknown species. The parasitoid assemblage that attacks juniper-feeding caterpillars is composed of hymenopteran and dipteran parasitoids. Several hymenopteran families attack juniper feeding Lepidoptera: Braconidae (e.g., Rogadinae, Meterorinae, Microgastrinae), Ichneumonidae (e.g. Campopleginae, Cremastinae, Anomaloninae), Eulophidae, and Torymidae. Dipteran parasitoids are limited to the family Tachinidae (e.g., Exoristinae, Dexiinae).

Sampling methods

From 2012-2015, we established circular plots 25-meters in diameter at locations that were chosen haphazardly, but stratified across a gradient of canopy cover (e.g., a proxy for plant diversity) at each site. New plots were generated each year and no two plots were sampled twice within the same year. All plots were located at least 50-meters apart. Each year intensive sampling was performed for a two-week period that matched peak abundances for juniper-feeding caterpillars. The Julian dates varied little across years, but fell within the same 2-week period. The Great Basin juniper species were sampled from June 14-July 4. The Chihuahuan Desert juniper species were sampled twice a year, during the monsoon from July 18-August 8, and following the monsoon from October 20-November 7.

Each plot was centered on a juniper tree and extended radially 12.5m from the center tree. Individual juniper trees greater than one meter tall were marked and identified to species. Estimates of leaf biomass were generated for each tree using leaf counts, with an individual juniper needle being approximately two centimeters in length. Additionally, diameter root collar (DRC) was acquired for single-trunked juniper trees to generate correlations with biomass estimates. For trees with multiple stems that forked near ground level, a DRC was measured for each stem to obtain an equivalent DRC (EDRC) by taking the square root of the sum of squared values for each individual stem (Natural Resources Conservation Service and Grazing Lands Technology Institute 1997, Thompson and Toone 2012). Individual juniper trees were also classified as either young, mature, or climax based on previously described morphological characteristics (Natural Resources Conservation Service and Grazing Lands Technology Institute 1997).

Within the 25-meter circular plot, a 10-m concentric circular plot was outlined to acquire understory plant diversity measurements; the abundance and richness of all forbs and shrubs were counted within that 10m plot. Grasses and cacti were omitted from this analysis. If a plant was unknown, a morphospecies description was given to the specimen and a sample was obtained to try to properly identify it in the lab. If proper and accurate identification was not possible (e.g., no flowers were available), the morphospecies was maintained for calculations of richness within each plot.

Once the plot was set, we sampled caterpillars from each marked juniper using a beating sheet. Juniper trees were sampled as completely as possible and we standardized our sampling effort by estimating the proportion sampled for each juniper tree. This provided the number of juniper needles sampled within each plot, which was used to standardize all metrics of abundance, richness, and diversity within a plot. All caterpillars were brought back to the lab and reared to acquire adult moths for identification. If a parasitoid instead issued from a larval collection, the adult parasitoids were collected and stored in 95% ethanol for future identification. Many juniper-feeding Lepidoptera have converged to a similar phenotype, and differentiating species as caterpillars is challenging. Therefore, DNA barcoding the COI mitochondrial sequence was used to identify 33 specimens that pupated, but did not successfully eclose (Ratnasingham and Hebert 2007). Specimen samples were sent to the Canadian Centre for DNA Barcoding at the University of Guelph and sequences were contributed to the Barcode of Life database (BOLD). Metrics of abundance, richness, and diversity were calculated at the plot level and standardized by the total number of leaves sampled within each plot (Jost 2006).

Parasitism rates at the plot level were quantified as the number of caterpillars that were parasitized divided by the sum of caterpillars collected in that plot.

Canopy cover

We calculated canopy cover using remote sensing methods with an object-oriented classification in ArcMap 10.3 (ArcMap 2010). Digital ortho quarter quad tiles (DOQQs) from National Agricultural Imagery Program (NAIP) were acquired for each location (NRCS 2008). All images were taken in 2010, with a few exceptions: images from 2013 were used for 2015 plots in Arizona because 2010 data was no longer available through the AZGEO Imagery Server on ArcGIS. All DOQQs have a 1-m spatial resolution and have four spectral bands: red, green, blue, and infrared. The difference in years between ground measurements, collected insects, and images for remote sensing was considered to be negligible for tree canopy cover. Furthermore, we avoided areas with any signs of recent tree mortality or other major disturbances. Arizona NAIP data was only available through an online server and generated NAIP imagery for the entire state. In order to utilize our object-oriented classification method, 350-m radius circular cuttings, centered on a plot, from the state NAIP map were cut out. These 350-m circular clipped images from the Arizona map were used to calculate canopy cover at the plot level.

For each DOQQ or 350-m circular clipped image, NAP manually selected several classifications within the image: tree, shrub, grass, bare-ground, and shadow. These training points were then used to generate a signature file of the classifications and eventually generated a maximum likelihood classification of the image. The statistical

probability for each class is computed to decide the inclusion of each cell to a class. NAP manually examined each image to identify the most accurate supervised classification for each plot. We did not differentiate between species of trees (*Juniperus*, *Quercus*, *Pinus*) that were contributing to canopy cover within each plot. Percent canopy cover was calculated by dividing the total number of pixels that were classified as a tree by the total number of pixels within the plot. Utilizing this method, we were able to expand our estimates of canopy cover beyond the plot level and canopy cover was calculated at several larger spatial scales (30-m radius, 150-m, 300-m) to investigate whether the influences of canopy cover on parasitism rates are dependent on the spatial extent of canopy cover.

Chemical Extraction

To investigate whether variation in juniper phytochemistry contributes to observed parasitism rates and species richness, juniper leaf samples were obtained from each individual tree within a plot. Samples were labeled and placed in paper bags for long-term storage and left to dry at room temperature. Although these samples were not stored in a freezer, samples were located in extremely dry conditions and no samples were contaminated with mold. In September 2015, 1-mg of dried leaf material was added to 2-ml lock top Eppendorf tubes. Leaf samples were ground using the Qiagen TissueLyser II by adding two tungsten carbide beads and shaking the tubes for four minutes at 30 Hz (1800 oscillations/minute). New and old leaf tissues were always combined in each tube to incorporate differences in leaf chemistry due to leaf age.

We utilized an extraction method developed by the Max Plank Institute of Molecular Plant Physiology (Giavalisco et al. 2011), which iteratively increases hydrophobicity of the extract buffer. This method generates several layers which contain distinctive metabolic classes that are separated via liquid:liquid separation. The aqueous (polar) phase contains the polar primary- and semi-polar secondary metabolites, while the organic (MTBE) phase contains most of the lipid compounds. Both phases can be analyzed by GC- and LC-MS-based metabolomics.

The metabolites were extracted by adding 1 ml of a homogenous mixture of HPLC grade methanol, methyl-tert-butyl-ether (MTBE), and deionized water in a 1:3:1 ratio respectively. In order to achieve a homogenous mixture, N.A.P. added an additional 1 ml of methanol to the 1:3:1 ratio. Additionally, *o*-xylene served as an internal standard and 40 μ l was added to the 200 ml homogenous mixture. The addition of an internal standard allowed us to determine concentrations of other analytes relative to the known concentration of *o*-xylene. 200 μ l were isolated from the upper organic phase and transferred to a 2 ml GC glass vial. 800 μ l of MTBE was directly added to each vial to produce 1.0 ml of the isolated organic phase. GC-MS was immediately performed on the organic (MTBE) layer the same day of extraction. The aqueous layer and protein pellet were stored at -20 °C for future analyses.

Statistical Analysis

All analyses were performed using R 3.3.2 (R Core Team 2014). We investigated the relationships between parasitism rates, canopy cover, understory plant richness, caterpillar abundance, and caterpillar density within each 25m diameter circular plot. To

satisfy assumptions of normal residuals and help with interpretation of relative strengths of each effect, all independent variables were standardized to z-scores. Given that samples were acquired across multiple years and locations, a mixed-effects logistic regression, using the R package “lme4” (Bates et al. 2014), was used to model proportion of caterpillars parasitized within each plot. To account for random variation across time and space, we modeled year as a random effect with month nested within year and state as a random effect, with location nested within state. Standardized values of juniper biomass sampled and total biomass were included as covariates within each model. Mixed-effects Poisson regression was used to investigate relationships between parasitoid species richness and understory plant richness. The same predictor variables used to model parasitism rates were also applied to model species richness of parasitoids. Variance inflation factors were used to identify multicollinearity within all models. Several potential interactions were examined by inclusion in candidate models, but no interactions among fixed effects were included in final models.

Model selection was performed using an information-theoretic approach. The most parsimonious model, based on Akaike Information Criterion (AICc), was selected using the ‘dredge’ function in the R package ‘MuMIn’ (Barton 2013). Predictor variables that reduced AICc values were removed, until a best-fit model with the lowest global AICc was found. All predictor variables used in these analyses were based on ecological meaningful predictions. If more than one model was selected based on AICc values ≤ 2 , the model with the fewest number of predictors was preferred (Burnham and Anderson 2002). To identify whether the selected model performed significantly better than the global model, χ^2 anova model comparison was performed. Residuals were investigated

for assumptions of normality and heteroscedasticity. Given unequal sample sizes across years and locations, a type III analysis of deviance, from the ‘car’ package in R (Fox et al. 2015), was performed to identify significant predictors within the model. Conditional pseudo- R^2 , which describes the proportion of variance explained by both the fixed and the random factors was calculated for each GLMM (Nakagawa and Schielzeth 2013).

Chemical analysis

GC chromatograms were processed using “metaMS” package in R (Wehrens et al. 2014). This package integrates the area under each peak and aligns numerous chromatograms to generate a matrix of chemical abundances for each aligned peak. The peak area of the internal standard, σ -xylene, was used to normalize all peak integrations, which provided abundances of each peak, relative the known concentration of the internal standard. The “hierDiversity” R package (Marion et al. 2015) was used to quantify chemical diversity within each plot. The within-group (alpha) and among-group (beta) chemical diversity within each plot was calculated. Both alpha and beta phytochemical diversities were used as predictor variables of parasitism rates and parasitoid species richness. Further, we investigated phytochemical diversity as a determinant of caterpillar abundance and richness. In both cases, we used the same AICc model selection procedure previously discussed.

RESULTS

Across all locations, we had complete data for 77 plots (Table S1-S4). Within the sampled plots we collected 517 caterpillars from 642 sampled juniper trees. 57

caterpillars were parasitized, belonging to 28 parasitoid species across 6 families. 517 caterpillars were composed of 10 families of Lepidoptera, belonging to 25 caterpillar species (Figs S1-S6). Extrapolated estimates of species and interaction richness revealed that the caterpillar community is relatively well sampled, while parasitoid species richness and caterpillar-parasitoid interactions are well below estimated richness values (Figs S1-S6). The relationship between canopy cover and understory plant richness was negative, though non-significant across all sampled sites ($\beta = -0.36$, $SE = 0.23$, $P=0.13$; Fig. S7). The species richness of understory plants decreased marginally from an average of 6 plant species in plots with the lowest levels of canopy cover to an average of 4 plant species in plots with the highest levels of canopy cover. The highest levels of understory plant diversity (10-14 species) primarily occurred in lower levels (<20%) of canopy cover (Fig. S7).

Following AICc model selection, understory plant richness and caterpillar density best explained the proportion of caterpillars parasitized in each plot (Table 1; Fig. 1). Significantly fewer proportions of caterpillars were parasitized as understory plant richness increased (Log odds = -0.49, $SE = \pm 0.16$, $P= 0.003$), while the density of caterpillars within each plot resulted in significantly greater proportion of caterpillars parasitized (Log odds=0.56, $SE = \pm 0.25$, $P= 0.02$). Parasitoid richness and proportion of caterpillars parasitized were positively correlated across all plots (Pearson's correlation = 0.62) and similarly to parasitism rates, the richness of parasitoids significantly decreased as understory plant richness increased (Table 1; Fig 2A). Based on the parameters measured and included in the full model, AICc model selection identified understory plant richness and caterpillar abundance as the best predictors of parasitoid species

richness across all plots. Based on standardized coefficients, caterpillar abundance had the strongest positive effect on parasitoid richness (Fig 2B), followed by a negative influence of understory plant richness.

Numerous plots had incomplete phytochemical data, so our investigations into relationships between parasitism rates and phytochemical diversity were limited to 31 observations. Alpha (within-group) and beta (among-group) measures of phytochemical diversity within a plot were not significant predictors of species richness of parasitoids or rates of parasitism. A significant, positive effect of phytochemical beta diversity on caterpillar abundance and species richness was observed, but this effect was driven primarily by the diversity of different-aged juniper trees within the plot (Table 2). Plots with higher diversity of different-aged juniper trees, had higher values of phytochemical beta diversity.

DISCUSSION

While numerous studies have investigated the *enemies hypothesis*, results have been dissimilar across ecosystems and among organisms (Letourneau 1987, Russell 1989, Riihimäki et al. 2005, Schuldt et al. 2011). Most studies have investigated the relationship between plant and natural enemy diversity in agricultural or species-poor systems, and our understanding of these relationships in more complex forest systems is limited (Riihimäki et al. 2005, Schuldt et al. 2011). For juniper woodlands, which are globally important, no study has investigated how declines in plant diversity, as a consequence of juniper encroachment, affect the diversity and abundance host-parasitoid interactions. Understanding how reductions in plant species diversity, due to woody plant

encroachment, influences interactions between predators and their prey will be important to help maintain the complex networks of interactions among species in these shifting ecosystems (Tylianakis et al. 2007, 2008). Our results contribute to others, which suggest that the *enemies hypothesis* does not apply to more diverse and specialized insect assemblages associated with natural systems. Our findings also provide noteworthy insights into drivers of host-parasitoid interactions in highly specialized assemblages of caterpillars and parasitoids in expanding juniper woodlands.

Contrary to what the *enemies hypothesis* predicts, we observed a significant decrease in parasitism rates in plots with greater understory plant diversity. This suggests that parasitoids of juniper-feeding caterpillars are affected by the diversity of non-host plants, but opposite of what the *enemies hypothesis* predicts. The *enemies hypothesis* may appear to be inappropriate for explaining patterns of host-parasitoid interactions in ecosystems characterized by high degrees of dietary specialization. We instead found that parasitism rates of the juniper caterpillar assemblage were positively associated with the density of juniper feeding caterpillars within the plot, which suggests a density-dependent relationship (Vargas et al. 1993, Stireman and Singer 2003). Such a response by parasitoids fits within the *resource concentration hypothesis* (Root 1973), which predicts that herbivores are more likely to locate and remain on their host plant in less diverse systems because their host is more concentrated (Andow 1991, Hambäck and Englund 2005). While the *resource concentration hypothesis* was initially developed to explain interactions between plants and herbivores, this same framework should extend to interactions between parasitoids and their host, in that parasitoids should be more likely to locate and stay on their host when their host is at higher concentrations (Sheehan and

Shelton 1989). Even though the relative densities of other potential Lepidopteran hosts were not measured in this system, increased juniper cover reduced plant richness and therefore host plant availability for other possible hosts. To help disentangle the combined effects of plant diversity and host densities on rates of parasitism, future studies should investigate relationships between plant diversity, relative densities of caterpillars on other plants, and their associated parasitism rates.

Our finding that parasitism rates declined with increased understory plant richness is consistent with numerous studies showing that the efficacy of natural enemies finding their host decreases in more diverse habitats (Evans 1976, Kaiser 1983, Andow and Risch 1985, Russell 1989, Weisser 1995, Gingras and Boivin 2002, Hoddle 2003, Aquilino et al. 2005). Many natural enemies, including parasitoids, have developed host location cues to help locate and identify potential prey items, and more diverse systems are thought to be more structurally complex, which can inhibit search efficiency of predators (Kareiva 1987). However, juniper is already a structurally complex host plant (habitat), and the addition of other, less structurally complex plants is unlikely to inhibit the parasitoid's ability to find its host (Andow and Prokrym 1990, Gingras et al. 2002). A more likely explanation for the reduction of parasitism in more diverse plant systems is the obstruction of chemical signals that parasitoids use to locate prey (Turlings and Wackers 2004). Many parasitoids use volatile organic compounds (VOC), which are released by herbivore-damaged leaves, as host location cues (Bukovinszky et al. 2005, D'Alessandro et al. 2006, Girling et al. 2011). Not only can parasitoids use VOCs to locate hosts, studies have shown that parasitoids can differentiate VOC profiles among plants with higher densities of herbivores, from that of plants with equal levels of mechanical

damage (Girling et al. 2011). Though it is unknown whether parasitoids are able to differentiate among highly aromatic conifer plants, as they were not used in this study. With additional plant species, the mixture of chemical signals that a parasitoid must examine increases and it can inhibit the searching efficiency of parasitoids (Van Dam and Poppy 2007). *Juniperus* is characterized by a complex mixture of terpenes, which are one of the most common classes of organic compounds associated with VOCs (Holopainen 2004, Adams 2014). While no study has investigated whether juniper VOCs provide reliable host location cues for parasitoids of juniper caterpillars, it is known that terpenes contribute to host location for parasitoids (Vinson 1976, Vogler et al. 2009), and it is certainly possible that *Juniperus* terpenes act similarly. Given that many plant species which coexist with juniper also release high concentrations of VOCs (e.g., *Artemisia tridentata*, *Ericameria nauseosa*), it is likely that their presence can obscure chemical signals that specialist parasitoids cue in on (Karlik et al. 2002, Shiojiri and Karban 2006, Jaeger et al. 2016). It would be interesting to investigate whether certain plant species obscure the host location cue for parasitoids more so than others (Wilson et al. 2015, Wilson and Woods 2016).

If plots with higher plant diversity had more insect herbivore species, and therefore more parasitoid species, these additional parasitoids did not parasitize juniper-feeding caterpillars. Given the extreme cryptic morphology of juniper feeding caterpillars and the complex mixtures of VOCs in juniper woodlands, it's possible that these additional parasitoids were unable to locate juniper-feeding caterpillars. Without knowing the appropriate search image, or signal (chemical), the likelihood of a naïve generalist parasitoid finding a caterpillar on juniper is extremely low (Wang and Keller 2002, Ishii

and Shimada 2010, Bukovinszky et al. 2012). It is also conceivable that generalist parasitoids were able to locate and parasitize juniper-feeding caterpillars, but the caterpillar's immune response was able to target the foreign body (e.g., egg or larva) in the haemocoel and successfully kill the parasitoid (Smilanich et al. 2009b). The immune response of insects, including caterpillars, perform encapsulation and melanization to enclose, asphyxiate, and poison foreign bodies (Strand 2008). While the immune system can be compromised, either through the injection of polydnaviruses or the secondary chemistry of the herbivore's host plant, research has shown that immune responses are not equivalent across caterpillar species (Godfray 2004, Smilanich et al. 2009b, 2009a). The high degree of host-parasitoid specialization in this system, suggests that specialist parasitoids are able to circumvent juniper-feeding caterpillar immune responses, but it is unknown whether generalist parasitoids are able to do the same.

Other predators such as gleaning birds, spiders, and other arthropod predators also consume juniper-feeding caterpillars, but it is uncertain how these additional natural enemies within juniper woodlands respond to changes in canopy cover and understory plant diversity. Given the exceptionally low densities of juniper-feeding caterpillars, the removal of caterpillars by other predators is likely to have large impacts on observed parasitism rates. For example, if a caterpillar is parasitized, but is then eaten by a competing natural enemy, we never observe that parasitism event. Some juniper-woodland inhabiting birds, such as the Gray Vireo (*Vireo vicinior*), prefer transition zones (lower values of canopy cover) between sagebrush (grassland) and juniper woodlands, and could preferentially consume certain caterpillars in more open woodlands, thus reducing prey abundance and observed parasitism rates (Rosenstock and

Charles Van Riper 2001, Reinkensmeyer et al. 2008). Future studies should investigate how multiple kinds of natural enemies respond to changes in canopy cover and plant diversity to understand how they influence each other and rates of consumption.

Similar to rates of parasitism, species richness of parasitoids was negatively associated with plant richness and positively associated with the abundance of caterpillars collected within each plot. We expected that decreases in plant species richness, as a consequence of juniper encroachment, would result in the losses of herbivore and parasitoid species associated with the understory plant community (Price et al. 1980, Hunter and Price 1992, Scherber et al. 2010). However, the best predictor of parasitoid species richness was caterpillar abundance. Unfortunately, parasitoid species richness values were limited to number of caterpillars collected within each plot. Therefore it makes sense that parasitoid species richness is significantly associated with caterpillar abundance, but whether these results are biologically meaningful or just a statistical sampling issue is difficult to know. Though, previous work has suggested that more productive habitats (e.g., more herbivores) should support greater diversity of secondary consumers (Haddad et al. 2009). In other words, plots with greater abundance of juniper-feeding caterpillars, should support more diverse community of parasitoids associated with juniper. The best predictors of caterpillar abundance and caterpillar species richness were understory plant richness and tree age diversity (Table 2).

While phytochemical diversity did not directly predict parasitism rates or species richness of parasitoids, it did emerge as a significant predictor of caterpillar abundance and richness. These results indicate that phytochemical diversity could be an important driver of the abundance and richness of juniper-feeding caterpillars and not to changes in

diversity at other trophic levels (Ode 2006, Gols et al. 2008, Richards et al. 2015). Though, it is difficult to disentangle other confounding habitat variables that covary with leaf chemistry such as canopy architecture, stand structure, and tree age. Regardless, these results suggest that phytochemical diversity of juniper could indirectly influence rates of parasitism via its influence on caterpillar abundance and richness. Interestingly, the effects of phytochemical diversity on caterpillars were manifested through beta phytochemical diversity and not alpha diversity. In other words, the dissimilarity of phytochemical compounds among individual juniper trees is a stronger determinant of the diversity and abundance of trophic interactions than the overall alpha diversity of compounds within a plot. Plots with a greater diversity of different-aged juniper trees typically had the highest values of phytochemical beta diversity, suggesting that having different-aged juniper trees is critical for attracting a diversity of specialized juniper-feeding caterpillars and maintaining a complex tri-trophic food web.

CONCLUSIONS

Our results do not support the *enemies hypothesis*; we found that rates of parasitism and species richness of parasitoids associated with juniper both decline as understory plant diversity increases. Parasitoids of this community responded to the density and abundance of the caterpillars feeding on juniper, and not to the additional resources (e.g., microhabitats, other caterpillar hosts, nectar) that higher levels of plant diversity provided. The significant negative influence of plant diversity on parasitism rates supports previous findings that increased levels of plant diversity reduced efficiency of capturing resources for specialized parasitoids, though this is only speculation and

numerous other potential explanations exist. Nevertheless, this is one of the first studies to investigate consequences of juniper encroachment on rates of parasitism and as woody plants continue their expansion around the globe, reducing plant diversity, it will be important to understand how declines in plant diversity influence localized multitrophic interactions.

ACKNOWLEDGEMENTS

I wish to thank the Earthwatch Institute and Earthwatch volunteers for helping collect and rear out caterpillars and parasitoids throughout this study. I also thank University of Nevada, Reno Graduate Student Association (GSA) for funding to perform chemical analysis on juniper foliage. This research was performed as part of my dissertation work at the University of Nevada, Reno. I also wish to thank S. Shaw, J. Stireman III, Andre Rangel, and David Wagner for taxonomic identification of parasitoid flies, wasps, and Lepidoptera.

Tables

Table 1

<i>Fixed Effects</i>	Parasitism rate				Parasitoid species richness			
	β	χ^2	DF	P	β	χ^2	DF	P
(Intercept)	-2.12	-	-	-	-0.43	-	-	-
Plant Richness	-0.49	8.72	1	<0.005	-0.47	5.82	1	0.016
Canopy Cover	-	-	-	-	-	-	-	-
Juniper Sampled	-	-	-	-	-	-	-	-
Cat. Abundance	-	-	-	-	2.63	33.51	1	<0.005
Cat. Density	0.56	4.85	1	0.03	0.52	-	1	-

	DF		DF	
AIC full model	11	150.45	11	152.2
AIC reduced model	7	143.93	7	148.5
Conditional-R ²	0.08		0.48	

Results from a mixed-effects logistic regression (rate) and generalized linear mixed model with a Poisson error distribution (richness). The results shown are following AIC model selection. All variables were scaled (z-scores) prior to analysis, thus beta coefficients represent standardized coefficients. In the mixed-effect logistic regression, the standardized coefficient (β) is represented as log-odds of being parasitized, where positive values reveal an increase in the odds of being parasitized. Conditional pseudo-R² was used to identify variance explained for each GLMM. Type-3 analysis of deviance was used to identify significant P-values and) χ^2 values.

Table 2

<i>Fixed Effects</i>	Caterpillar Abundance				Caterpillar species richness			
	β	χ^2	<i>DF</i>	<i>P</i>	β	χ^2	<i>DF</i>	<i>P</i>
(Intercept)	1.39	8.16	1	<0.005	0.77	32.43	1	<0.005
Plant Richness	0.42	8.47	1	<0.005	-	-	-	-
Canopy Cover	-	-	-	-	-	-	-	-
Cat. Abundance	-	-	-	-	1.75	14.5	1	<0.005
Chemical Diversity	-	-	-	-	-	-	-	-
Juniper Tree Age	0.29	12.07	1	<0.005	0.26	3.84	1	0.05
Juniper Richness	-	-	-	-	0.13	3.3	1	0.07

	<i>DF</i>		<i>DF</i>	
AIC full model	11	209.14	12	115.05
AIC reduced model	7	205.20	8	109.93
Conditional-R ²	0.88		0.47	

Results from a mixed-effects logistic regression (rate) and generalized linear mixed model with a Poisson error distribution (richness). The results shown are following AIC model selection. All variables were scaled (z-scores) prior to analysis, thus beta coefficients represent standardized coefficients. In the mixed-effect logistic regression, the standardized coefficient (β) is represented as log-odds of being parasitized, where positive values reveal an increase in the odds of being parasitized. Conditional pseudo-R² was used to identify variance explained for each GLMM. Type-3 analysis of deviance was used to identify significant P-values and) χ^2 values.

Figure Legends

Figure 1: Results from a logistic generalized linear mixed model (GLMM) to investigate determinants of parasitism rates in expanding juniper woodlands. A) Displays the proportion of caterpillars parasitized as a product of understory plant species richness within each plot (N=77). The solid line represents the predicted parasitism rates given increased plant richness with 95% confidence intervals shown as dotted lines. B) Displays the parasitism rates as a product of caterpillar density. Caterpillar density was calculated by dividing the total number of caterpillars collected by the total number of juniper needles sampled. This value was then multiplied by 1000 to give a density of caterpillars per 1000 leaves sampled. The predicted proportion of caterpillars parasitized is shown as a solid black line surrounded by 95% confidence intervals represented by black dotted lines.

Figure 2: Results from a Poisson generalized linear mixed model (GLMM) to investigate the determinants of parasitoid species richness in expanding juniper woodlands. Model results shown are from the model with the lowest AIC values. A) Displays parasitoid species richness as a product of understory plant richness. The solid black line represents predicted parasitoid richness, given increased values of plant richness. 95% confidence intervals are denoted as dotted black lines. B) Shows the association between parasitoid richness and caterpillar abundance within each plot. The predicted relationship based on the Poisson GLMM is shown as a solid black line and the 95% confidence intervals are signified as dotted black lines. Both variables were identified as being significant predictors of parasitoid richness in expanding juniper woodlands.

Figures

Fig 1.

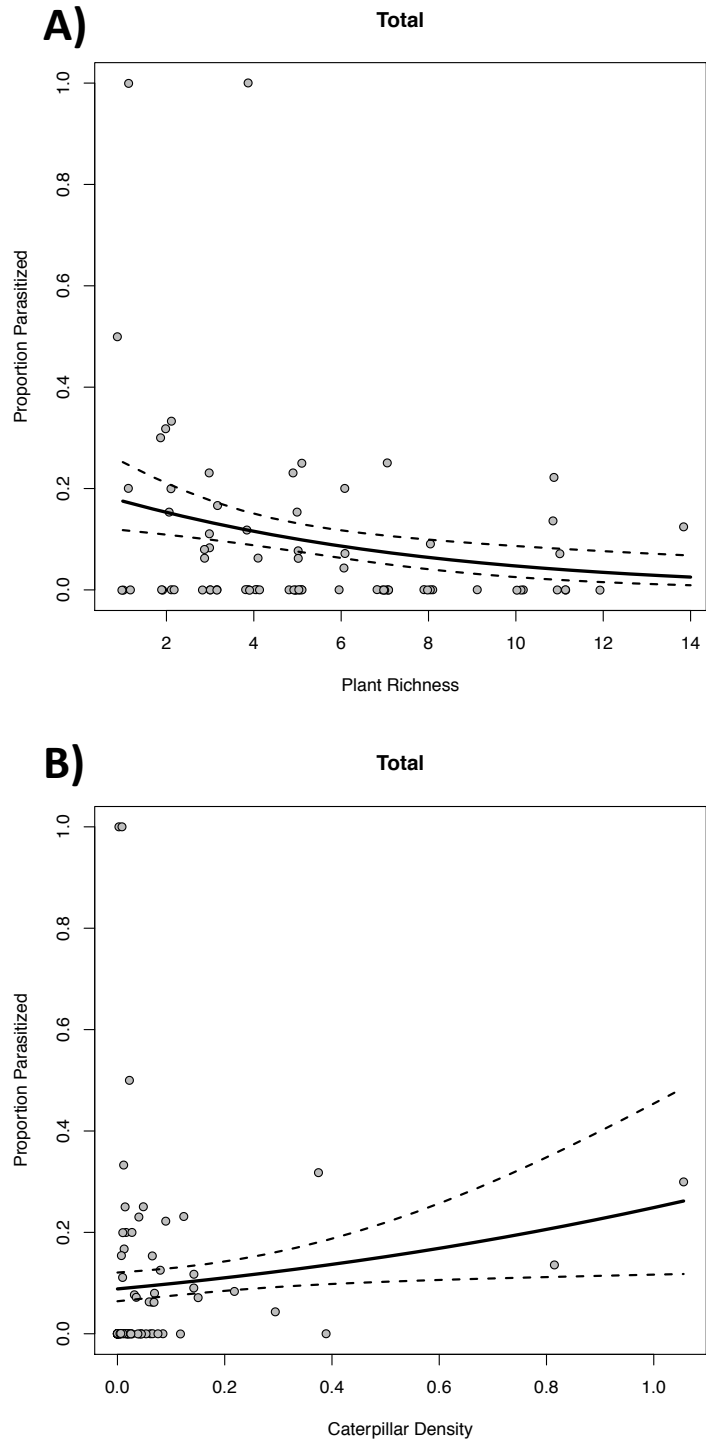
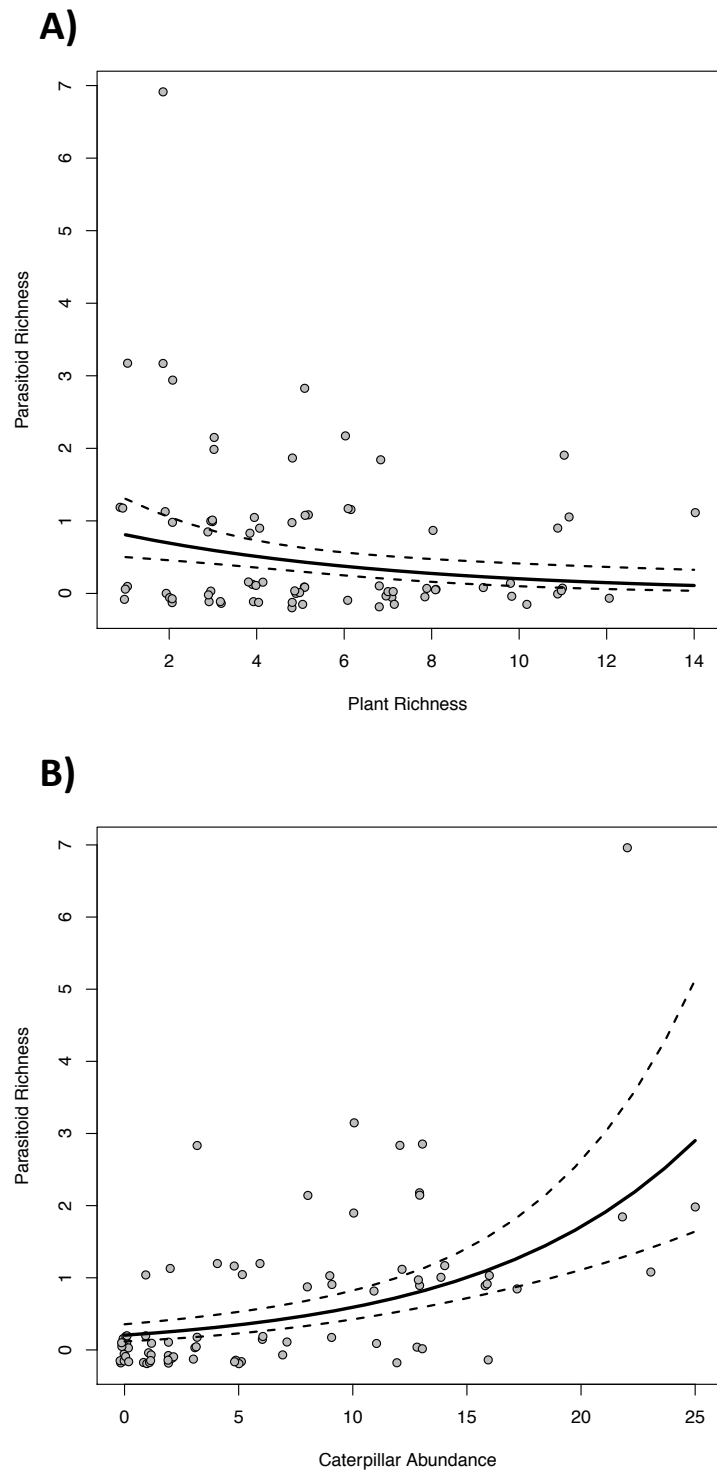


Fig 2.



Supplementary Material

Testing the enemies hypothesis in expanding juniper woodlands

Nicholas A. Pardikes and Lee A. Dyer

*Department of Biology, Program in Ecology, Evolution, and Conservation Biology,
University of Nevada, Reno, NV 89557*

Table S1

<i>Year</i>	2012	2013	2014	2015
<i># Plots</i>	26	19	17	15

Number of plots done each year of collection across all locations.

Table S2

<i>State</i>	CA	NV	AZ
<i># Plots</i>	10	8	59

Number of plots done in each state (California = CA, Nevada = NV, Arizona = AZ) over all 4 years. Numerous plots were not included from Nevada (NV) and California (CA) due to missing data.

Table S3

	2012	2013	2014	2015
AZ	25	10	12	12
CA/NV	1	9	5	3

Counts of the number of plots that were conducted during each year, in each location. Plots sampled in California and Nevada during a particular year were combined for this table (CA/NV).

Table S4

Location	State	2012	2013	2014	2015
Donner Pass Road	CA	0	3	1	1
Hidden Valley	NV	0	1	0	0
Lemmon Valley	NV	0	3	2	2
Shinn Mountain	CA	1	2	2	0
Box Canyon	AZ	2	2	1	1
Madera Canyon	AZ	4	0	2	2
Onion Saddle	AZ	1	0	0	0
Paradise	AZ	6	0	3	2
Rustler Park	AZ	4	3	4	3
SWRS	AZ	8	5	2	4

Displays the number plots that were sampled from in each location over the four-year sampling period. Several locations are shown here, but are grouped together in the analysis.

Figures

Fig S1: Displays estimates of the extrapolated species richness for caterpillars feeding on several species of juniper (*J. osteosperma*, *J. occidentalis*, *J. grandis*, *J. deppeana*, and *J. arizonica*). These values are based across 77 plots in Nevada, California, and Arizona. “S” represents estimates from Estimate S, Chao estimate of species richness, a jackknife 1 and jackknife 2 estimate of species richness, and a bootstrap estimate of species richness. All values were calculate using the “specpool” function in the “vegan” package in R (Oksanen et al. 2015).

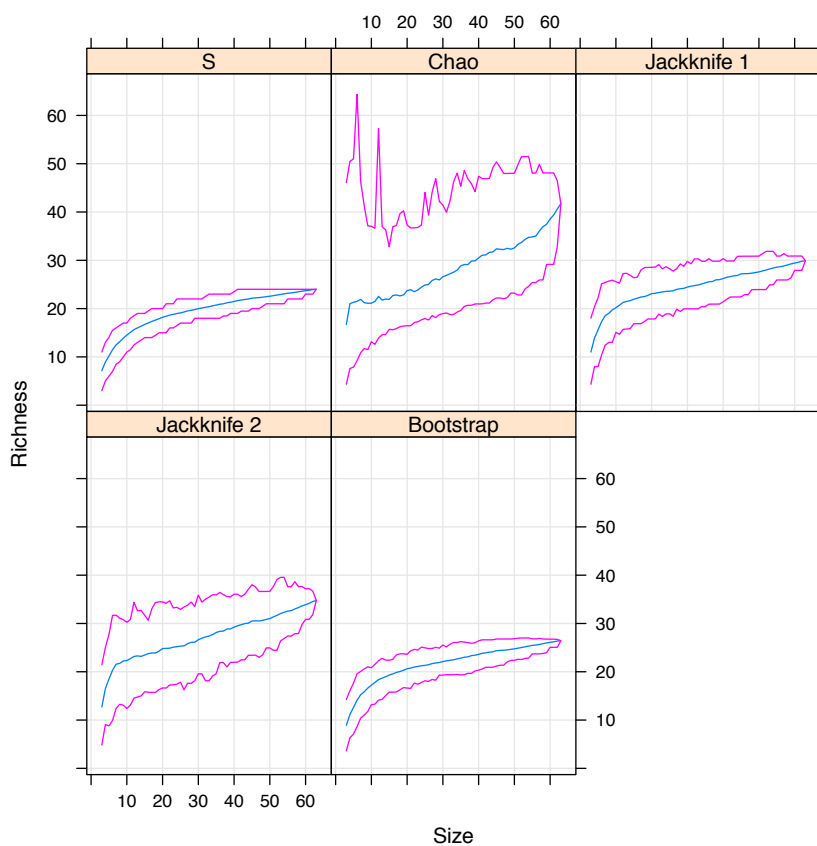


Fig S2: Displays estimates of the extrapolated species richness for parasitoids reared from juniper-feeding caterpillars across several species of juniper (*J. osteosperma*, *J. occidentalis*, *J. grandis*, *J. deppeana*, and *J. arizonica*). These values are based across 77 plots in Nevada, California, and Arizona. “S” represents estimates from Estimate S, Chao estimate of species richness, a jackknife 1 and jackknife 2 estimate of species richness, and a bootstrap estimate of species richness. All values were calculate using the “specpool” function in the “vegan” package in R (Oksanen et al. 2015).

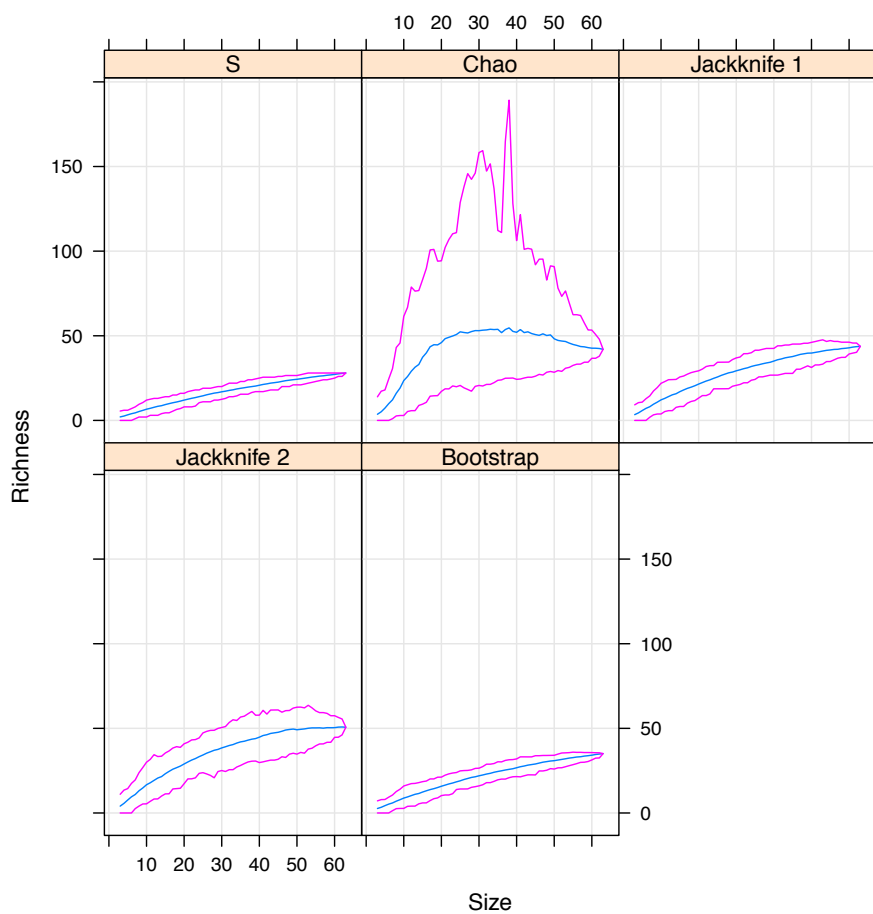


Fig S3: Displays estimates of the extrapolated interaction richness for caterpillar-parasitoid interactions associated with several species of juniper (*J. osteosperma*, *J. occidentalis*, *J. grandis*, *J. deppeana*, and *J. arizonica*). These values are based across 77 plots in Nevada, California, and Arizona. “S” represents estimates from Estimate S, Chao estimate of species richness, a jackknife 1 and jackknife 2 estimate of species richness, and a bootstrap estimate of species richness. All values were calculate using the “specpool” function in the “vegan” package in R (Oksanen et al. 2015).

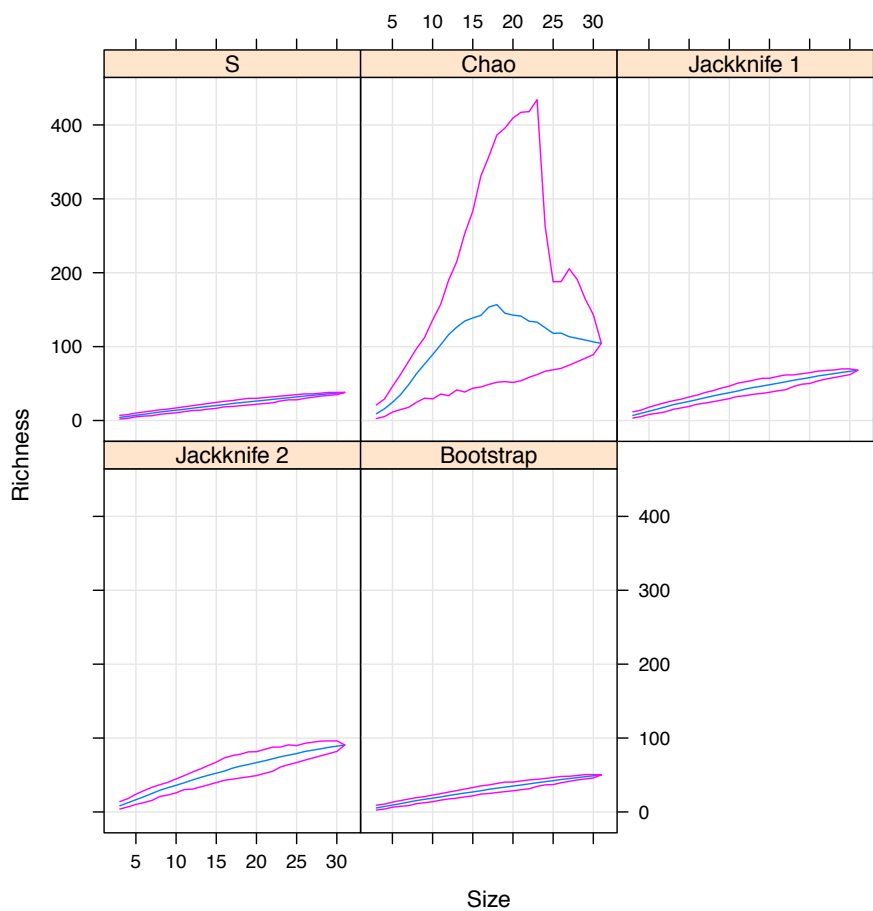


Fig S4: Displays the number of caterpillars that were collected from several families of Lepidoptera. These counts are limited to caterpillars collected in the 77 plots used for this analysis.

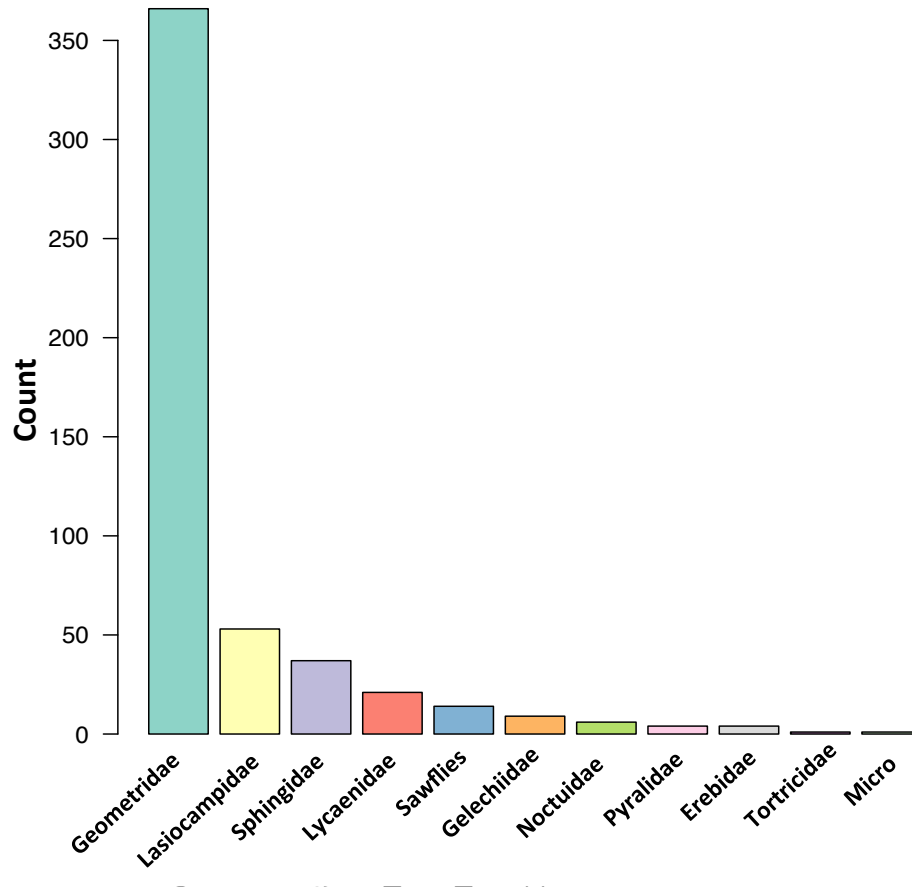


Fig S5: Displays the number of parasitoids that were collected from several families of Diptera (Tachinidae) and Hymenoptera (everything else). These counts are limited to parasitoids reared from caterpillars collected in the 77 plots used for this analysis.

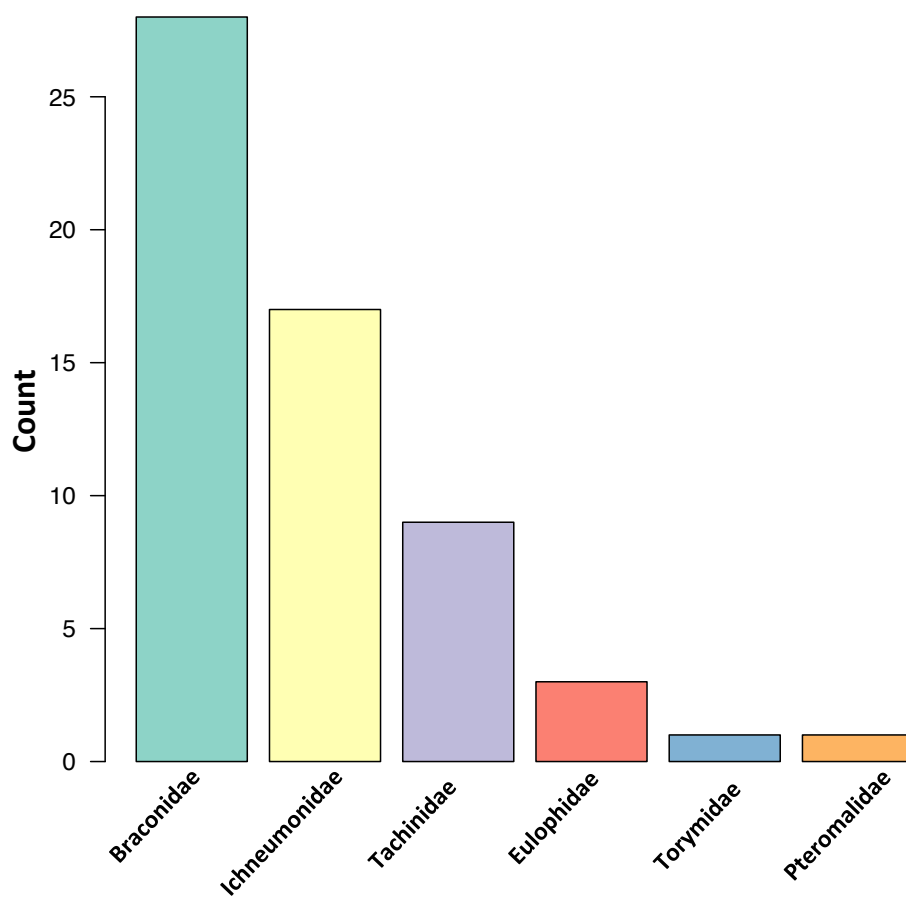
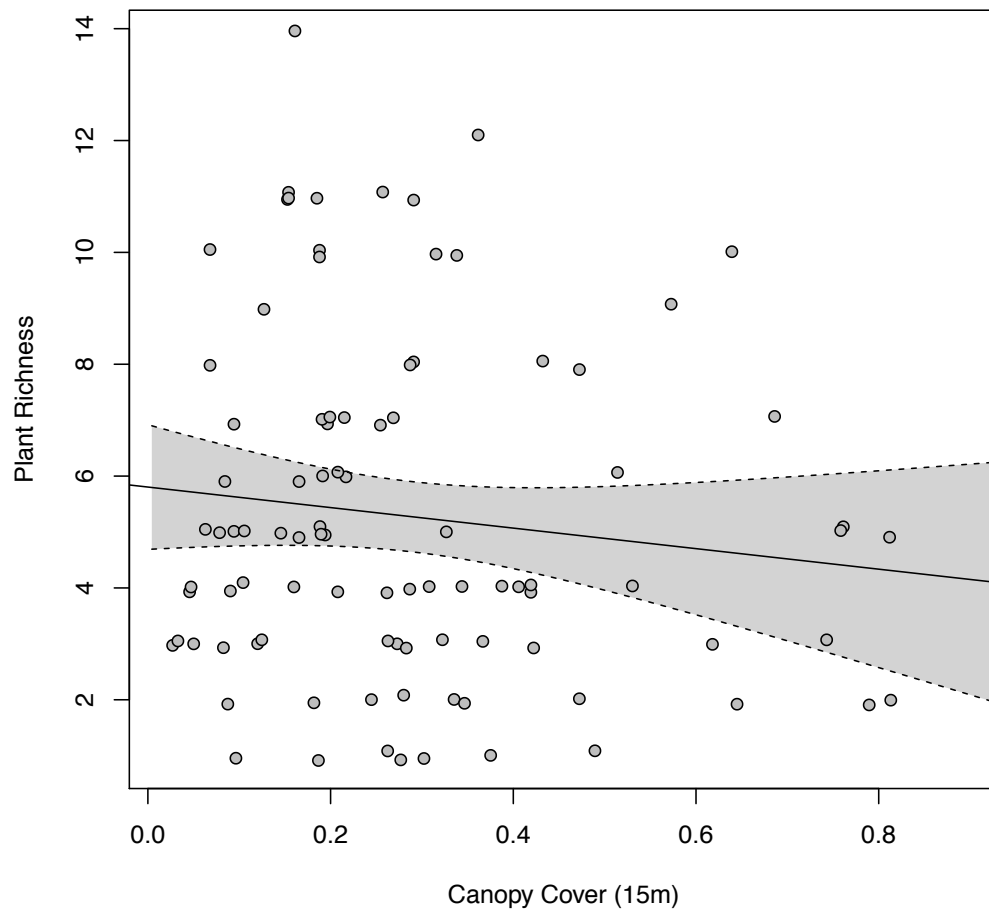


Fig. S7: Relationship between canopy cover, calculated using maximum likelihood classification method discussed in the methods, and understory plant richness. The relationship is negative, but not significant across all 77 sites ($\beta = -0.36$, $SE = 0.23$, $P=0.13$).



Chapter 4

Trophic interaction diversity: simulated networks generate relevant hypotheses

Nicholas A. Pardikes¹, Will Lumpkin¹, Paul J. Hurtado^{1,2}, and Lee A. Dyer¹

¹*Department of Biology, Program in Ecology, Evolution, and Conservation Biology,
Department of Biology, University of Nevada, Reno*

²*Department of Mathematics and Statistics, University of Nevada, Reno*

Key words: *interaction diversity, diet breadth, tri-trophic, path analysis, food web simulation*

ABSTRACT

Most of earth's diversity lies in the interactions among species, yet our understanding of the factors responsible for determining patterns of interaction diversity across space and time are lacking. Even though species interactions affect multiple ecosystem attributes, from primary productivity to population dynamics, measures of biodiversity typically disregard trophic interactions. Developing a practical index for estimating interaction diversity and generating hypotheses of how interaction diversity and other ecological network indices are impacted by taxonomic diversity and consumer diet breadth will facilitate resolving some important questions in biodiversity research. Previous studies have examined the effects of sampling bias and specialization on determining patterns of network structure, but these studies have often been to two trophic levels and did not incorporate realistic variation in taxonomic diversity and diet breadth. Here, we developed a simulated food web model to generate tri-trophic networks, and we evaluated specific hypotheses about how interaction diversity is influenced by consumer diet breadth, taxonomic abundance, and richness. Specifically, we examined how species and interaction diversity can be different by comparing species and interaction diversities derived from discrete sampling efforts from a variety of simulated multi-trophic communities. We also investigated how distributions of sampled species and interactions differ across this broad range of communities. We show that distributions of sampled interactions are not necessarily more kurtotic than species abundance distributions, as hypothesized before, but they do accumulate more quickly – thus, interactions may require less sampling effort than species. Taxonomic richness and abundance influenced the correlation between species and interaction diversity

significantly more than consumer diet breadth. Interestingly, based on a path analysis of hypothesized causal relationships, consumer (i.e. herbivore and enemy trophic levels) diet breadth had strong, positive effects on interaction diversity; however, the strength of both factors is dependent on the sample size. Our simulation model can help develop realistic predictions of interaction and species diversity of multi-trophic communities, which is needed to help improve our understanding of the drivers of interaction diversity, especially in this period of accelerated global change.

Key words: *interaction diversity, diet breadth, tri-trophic, path analysis, food web simulation*

INTRODUCTION

The devaluation of natural history and taxonomy has added to the failure of ecologists to document biodiversity and subsequently to understand the magnitude and consequences of the growing extinctions caused by global change (Tewksbury et al. 2014). Knowledge of basic natural history is especially important for quantifying biotic interaction diversity, which encompasses most of earth's diversity (Ohgushi et al. 2007) and should be tightly linked to variables such as community stability and ecosystem services (Dyer et al. 2010, Mougi and Kondoh 2012). The loss of interaction diversity is one of the least understood responses to species extinctions, partly because it has not been consistently treated as a response variable in theoretical or empirical studies of biodiversity and because getting good quantitative data on interaction diversity often requires considerable fieldwork over time. Although network approaches have provided more focus on interaction diversity, most analyses are not based on detailed natural history data that is linked with experimental evidence of interactions actually occurring together (e.g., Novotny et al. 2002, Janzen et al. 2005). In contrast, a sampling approach allows for a more rigorous and repeatable resolution of interaction networks at any appropriate scale (Dyer et al. 2010), but it is not clear how much sampling is necessary for accurate measurements nor how relevant larger scale networks are to local interaction diversities (Poisot et al. 2012, Fründ et al. 2016).

Here and elsewhere, we define interaction diversity as the relative abundance and richness of interactions linking species together into dynamic biotic communities (Janzen 1974, Thompson 1996, 1997, Dyer et al. 2010, Dáttilo and Dyer 2014). For this metric of diversity, the calculation of richness, diversity indices, and rarefaction diversity is based

on experimentally established links between interacting individuals rather than species alone, or alternatively, lists or observations of species found in the same area to determine network nodes and edges. Studies that are focused specifically on trophic interaction networks are encouraged to utilize experimental approaches to verify the nodes and edges, or some other means of validation to increase confidence that the putative herbivore, parasite, or predator is reared on the host upon which it is observed in the field. An increasing number of studies indicate that theoretical and applied research in ecology and conservation need an approach that goes beyond documenting taxonomic and genetic diversity and takes into account interaction diversity (Cohen and Briand 1984, Ohgushi et al. 2007, Tylianakis et al. 2007, 2010, Del-Claro and Torezan-Silingardi 2009). Trophic interactions, such as enemy-herbivore-plant interactions, are well studied and important because they have large effects on all ecosystem attributes (Gross et al. 2009, Jiang et al. 2009, Dyer et al. 2010). Here we focus on this interaction diversity across multiple trophic levels.

Large-scale patterns suggest that two components of interaction diversity differ substantially between different ecosystems: overall taxonomic diversity and host specificity of consumers (Darwin 1859, Wallace 1878, Novotny et al. 2006, 2007, Dyer et al. 2007, 2010, 2012, Dáttilo and Dyer 2014). Understanding how these components influence interaction diversity, and quantifying the causes and effects of variation in interaction diversity are important goals for applied issues, such as responses to disturbances at local and global scales (Stireman et al. 2005b).

Most communities can never be completely sampled, and the true community values of diversity and other network parameters are impossible to precisely quantify at

community scales larger than a hectare (Novotny et al. 2010); further, community composition and weather are dynamic and interactions are ever-changing across space and time, thus careful sampling approaches are necessary for characterizing interaction diversity. Here we simulate a standardized sampling effort that accumulates individual interactions until each interaction has been accounted for. Utilizing this sampling approach mimics existing systematic sampling protocols in the field (e.g., Forister et al. 2015) and allows the comparison of interaction diversity across a broad range of community types. Furthermore, our approach permits us to identify differences between the actual community and a subsample of the community. Certain community characteristics may be more sensitive to disparate sampling efforts than others; therefore it is important to identify the effects of sampling effort on diversity and network attributes, such as connectance and specialization (Dormann et al. 2009, Thébault and Fontaine 2010, Fründ et al. 2016). Recently, Fründ et al. (2016) investigated the effects of sampling bias on quantifying specialization in bipartite networks and found significant effects of sampling bias on selected properties, while identifying network parameters that are robust to limited sampling. However, this investigation was restricted to two-trophic levels and the range of taxonomic richness and degree of specialization of their simulated communities was narrow. To add to this existing work, our approach simulated 5000 different combinations of richness, abundance, and consumer diet-breadth, allowing for a comprehensive investigation into the determinants of interaction diversity across a wide-range of multitrophic communities.

The focus of this study is to test specific hypotheses about the relationships between community species diversity, consumer diet breadth, interaction diversity,

geographic variation, and network structure. We addressed the following questions with simulation models, statistical models, and empirical data:

- 1) If interactions are more kurtotic, do interactions asymptote more quickly from a discrete sample (area)?
- 2) What are the interactive effects of consumer diet breadth and community diversity on interaction diversity?
- 3) Are different determinants of interaction diversity affected by the number of observed interactions included in the model?

We sampled simulations of interacting communities; mimicking field sampling methods outlined in Dyer et al. (2010) and tested a specific structural equation meta model (SEMM, *sensu* Jiménez-Alfaro et al. 2016).

METHODS

Food Web Simulation

The goal of this model is to generate a random tri-trophic food web, at the scale of a single study site, based on several pre-specified properties as inputs to investigate possible contributions to interaction diversity. Specifically, these inputs are the number of species at each trophic level (i.e., richness; R_1, R_2, R_3), the overall abundance of each trophic level (i.e., abundance; A_1, A_2, A_3), and a diet breadth parameter (α_2, α_3) for the consumers that determine the diet breadth distribution for that trophic level according to a truncated discrete Pareto distribution (Forister et al. 2015).

The abundance distribution for trophic level i is constructed by taking a random sample of size R_i from a lognormal distribution with $\mu = 0$ and $\sigma = 1$, scaled to sum to the

prespecified overall abundance A_i , and then rounded to the nearest integer (Magurran 2013). We denote the abundance of species j in trophic level i as A_{ij} , where $A_i \approx \sum_{j=1}^{R_i} A_{ij}$. Diet breadth values (number of species each consumer has in their diet) are chosen to get an empirical distribution that is as close as possible to the desired discrete truncated Pareto distribution. These values are obtained by calculating density values for a (continuous) Pareto I distribution (truncated at the number of species at the lower trophic level) with survival function (aka complementary CDF) $S(y) = (1/y)^\alpha$.

The diets for each consumer species (i.e., the list of resource species they potentially can consume) are then sampled uniformly from the list of species in the lower trophic level (with replacement). In sampling real systems in the field, individual consumers are assumed to have been found by sampling their resource (i.e., herbivores are detected by inspecting host plants, and parasitoids are found by inspecting host herbivores). Therefore we assume each individual parasitoid/enemy is associated with an individual herbivore, and each individual herbivore with an individual plant. Interactions among individuals are therefore constructed as follows. Individual herbivores of species j (recall there are A_{2j} such individuals) are assigned a plant species by cycling through the list of species in their diet. Then each individual plant is assigned an individual herbivore, based on these assignments, and we assume only one herbivore per plant. This is repeated for each herbivore species, and for any individual herbivore for which there are no unoccupied plants, that individual herbivore is removed from the community. This process is repeated for enemies, assigning them to herbivores under the same one-to-one assumption, and any unassociated parasitoids are removed from the community.

To generate random food webs, we sample R_i uniformly from the set of integers $\{3, 4, \dots, 120\}$ and α_i uniformly over the interval $[1,5]$. Total abundances for each trophic level A_i are uniformly sampled from the integers $\{3, 4, \dots, 500\}$. Variable ranges with specific distributions for species richness, relative abundances, and alpha parameters were based on food web data from sites across the Americas (Dyer et al. 2007). Using this approach, we generated 100 random food webs.

Food Web Sampling

The community is sampled by subsampling the individual plants (which each have at most one herbivore and at most one enemy) and assuming perfect detection of the herbivores and their parasitoids. Randomly sampled rows from each local interaction food web were used to calculate the cumulative interaction diversity for each sample. Sampled interaction diversity was calculated using the inverse of the Simpson's entropy ($1/D$) for each cumulative plant-herbivore, herbivore-enemy, and plant-herbivore-enemy interaction. Sampling was completed once all plant individuals within each local community were sampled. Sampling within the local community occurred without replacement. In our simulation, the larger pool is the full community (of which we generated 100) and smaller-scale communities are assembled from the regional pool because each time a sample is added in the species accumulation curve, it simulates a community of that size.

Total Network Analysis

We quantified several network-level properties to identify how species richness and specialization influence the structure of entire networks as measured by connectance and linkage density (hereafter "interaction density"), two commonly used network parameters (Dormann et al. 2009, Thébault and Fontaine 2010). Connectance and interaction density allowed us to identify network-level consequences of changing diet-breadth and taxonomic diversity on the complexity of entire networks. To accomplish this, we assembled three separate, but not mutually exclusive, networks within each individual local community described above. A plant-herbivore (PH), herbivore-enemy (HE), and plant-herbivore-enemy (PHE) network were assembled separately to quantify connectance and interaction density and compare outcomes when examining two- or three-trophic-level networks.

A weighted network was constructed from each local community by generating a bipartite matrix with the abundance of interactions that occur between members of each community. Plant-herbivore and herbivore-enemy matrices were built based on each local community to calculate network-level properties concerning two trophic levels. To investigate plant-herbivore-enemy networks, we generated a matrix of producers (e.g. plants and herbivores) and consumers (e.g. herbivores and enemies) and quantified network-level properties similarly to the previously mentioned bipartite networks. For each distinct network (e.g., PH, HE, PHE), the R-package "bipartite" (version 2.05) was utilized to quantify connectance and interaction density (Dormann et al. 2008). In all subsequent network analyses, empty columns and rows were deleted before calculating network-level metrics. These values were integrated with other diversity measurements from our sampling scheme to investigate the desired relationships.

Rarefaction analyses

To compare the rate of accumulation of species and interactions in a given local community, we used rarefaction curves and the Chao estimator of richness (Chao 1984). Rarefaction curves were generated using the ‘vegan’ package (version 2.2-1) in R (Oksanen et al. 2015). To allow for comparison of communities that differed greatly in sample effort, abundance, and taxonomic richness, the slope of each rarefaction curve was calculated at the number of samples it took to sample half the total richness for each local community. These values allowed us to compare the accumulation rates between species and interactions across a wide range of local communities. The Chao estimator is a non-parametric estimator of species richness and was used to compare estimated richness for interactions and species (Chao 1984). Chao estimates of richness were calculated for plant-herbivore, herbivore-enemy, and plant-herbivore-enemy networks. Slopes and estimated Chao1 richness were compared using Bayesian estimation for two groups in the R package “BEST” (Meredith and Kruschke 2015). This method provides an alternative to classic t-tests and creates posterior estimates for group means and standard deviations. Point estimates and credible intervals were used to identify differences for Chao estimates of interactions and species for all 100 local communities. The observed differences between the means and standard deviations for interaction and species networks were used as priors. Given the large sample size, the method provides robust posterior probabilities identifying differences between sample means.

Statistical Analysis

Path analysis and linear regression were used to identify the relative importance of taxonomic diversity and diet-breadth on determining interaction diversity and other network structure metrics. To examine both direct and indirect effects, we used path analysis to test a previously hypothesized structure equation meta-model. Path coefficients for direct effects were obtained from the structural equation model, whereas indirect effects were calculated as the product of direct effects in any given pathway. For our *a priori* specified structural equation model, we identified causal relationships to formulate a simple set of paths with three exogenous variables (plant abundance, herbivore diet breadth, enemy diet breadth) predicting four endogenous variables (interaction diversity, interaction density, species diversity, connectance); no latent variables were used. Specifically, on the basis of literature, our own empirical data, and assumptions of the simulations, all exogenous variables were predicted to increase interaction diversity, species diversity, and connectance. In addition, these exogenous variables were expected to have positive effects on connectance via interaction diversity and density. In this analysis, data from 5000 unique communities was used to test each *a priori* hypothesis. We tested the fit of this model using SAS procedures (PROC CALIS) and selected the formulation of the reticular action model to define models (SAS n.d.). Starting values for the parameter estimates were determined by using a combination of three methods: observed moments of variables, the McDonald method, and two-stage least squares. The estimation method for the model was maximum likelihood, and the Levenberg-Marquardt algorithm was used to iterate solutions for optimization. The χ^2 for the absolute index was used to assess the fit of the model, with $P > 0.05$ (with 2 df) as an indication of a good fit to the data. Residuals met assumptions for multiple regressions.

This approach was utilized for the full communities generated by our simulations as well as for random samples from each community that started at 5 interactions sampled up to 500 interactions sampled and path coefficients were compared from the identical models across these sample sizes. Comparing coefficients across a range of sample sizes allowed us to investigate how predicted relationships among variables changes as the number of observed interactions increase.

We also used simple linear regression to examine how consumer diet breadth and taxonomic diversity influence the association between interaction and species diversity. Species diversity was regressed against interaction diversity (both of which were log-transformed) and the residuals from that model were used as a dependent variable in subsequent linear models. Linear regressions were performed to identify how diet breadth, abundance, and taxonomic richness altered relationships between interaction and species diversity using the aforementioned residuals. In some cases, non-linear relationships were examined using polynomial terms in the linear regression. This analysis was implemented for each distinct network (e.g., PH, HE, PHE). The mean observed diet breadth for consumers was utilized as a measure of specialization. Diet breadth was restricted to mean herbivore diet breadth for PH networks, mean enemy diet breadth for HE networks, and the mean diet breadth for herbivores and enemies for PHE networks. The sum of taxonomic richness and abundance across all trophic levels in the local network was used for measures of richness and abundance. All analyses were performed using program R (version 3.3.2) (R Core Team 2014).

RESULTS

Interaction and species rarefaction curves

100 different local communities were generated and cumulatively sampled (Fig. 1). We compared rarefaction curves between interactions and species for PH, HE, and PHE networks (Fig. 2). The shape and slope of each rarefaction curve differed significantly among local communities and between interactions and species (Fig. 3). Additionally, the patterns in accumulation curves also differed between the three networks (e.g. PH, HE, PHE) (Fig. 3). Each step in the accumulation curves can be considered a different scale of sampling for local communities.

Mean slope values at the number of samples it took to accumulate half of the total richness (a value analogous to the Michaelis constant in Michaelis-Menton enzyme dynamics) differed significantly among species and interaction rarefaction curves, and among the three networks types (Fig. 3)(i.e. PH, HE, PHE). Rarefaction slopes of PH ($HDI_{sp} = 0.37-0.45$, $HDI_{int} = 0.6-0.67$, $HDI_{diff} = -0.28 - -0.17$) and HE ($HDI_{sp} = 0.58-0.55$, $HDI_{int} = 0.71-0.79$, $HDI_{diff} = -0.19 - -0.08$) interactions were consistently higher than species. Mean slopes for interactions were less than species in PHE ($HDI_{sp} = 0.77-0.85$, $HDI_{int} = 0.37-0.45$, $HDI_{diff} = 0.34- 0.45$) networks. The variance associated with mean slopes across all networks were similar. Effect size was greatest within the PHE networks (Effect Size = 2.45).

Mean estimates of Chao2 measures of richness differed significantly among interactions and species in PH networks and the 95% high-density intervals (HDI) did not overlap one another (Fig. 3)($HDI_{sp} = 133-149$, $HDI_{int} = 89-101$, $HDI_{diff} = 35-56$). Similarly, Chao2 richness estimates differed among species and interactions for the HE network ($HDI_{sp} = 132-150$, $HDI_{int} = 85-99$, $HDI_{diff} = 37-60$) and PHE networks ($HDI_{sp} =$

198-220, $HDI_{int} = 158-178$, $HDI_{diff} = 26-56$). The effect size was greatest for the PH network (Effect Size = 0.83) and smallest for the PHE network (Effect Size = 0.50), but the effect size never overlapped with zero for any network investigated. Mean estimates of Chao2 for species richness were always greater than interactions in all three networks. However, the variances associated with estimates of richness were greatest when three trophic levels were considered.

Relationships between species and interaction diversity

The correlation between species and interaction diversity was strongest among PH networks (Pearson's Corr. = 0.97) and gradually decreased with HE (Pearson's Corr. = 0.94) and PHE networks (Pearson's Corr. = 0.40). This pattern was consistent with the slope and coefficient of determination (R^2) (Table S1).

Diet breadth, species richness, and species abundance all significantly influenced the association between interaction diversity and species diversity differently, but significant effects depended on the network being investigated (Fig. 4; Table S2). Increases in the mean consumer diet breadth (i.e. increased generalization) resulted in statistically significantly more positive residuals between species and interaction diversity in PH networks ($\beta = 2.5$, $P < 0.001$) (Fig. 3A). Positive residuals in this case signify higher interaction diversity than expected given the diversity of species. Similar, but larger effects of diet breadth on relationships between species and interaction diversity were observed in HE ($\beta = 3.28$, $P = 0.003$) and PHE ($\beta = 10.41$, $P = 0.01$) networks.

Species richness only had a significant positive influence on the relationships between species and interaction diversity for the PHE network (Table S2). Increased species richness was not significantly associated with the residual values for PH networks ($\beta = 0.003$, $P=0.83$) or HE networks ($\beta = 0.0213$, $P=0.22$). PHE network ($\beta = 0.256$, $P<0.001$) residuals displayed significantly positive linear relationship with increases in species richness. This result revealed that local communities with high species richness yielded more interactions than expected based on the number of species. Explained variance increased in higher trophic levels and the number of trophic levels included in the model (Table S2).

Abundance only revealed statistically significant linear relationships with residual values for PHE networks, but the strength of these associations were weak. Abundance in PH ($\beta= 0.0037$, $P=0.06$) and HE ($\beta= 0.004$, $P=0.09$) networks displayed weak relationships with residual values and variance explained was small in both models (Table S2). PHE abundance revealed the largest positive estimate, but was still noticeably weak ($\beta= 0.018$, $P<0.001$). In all three cases (e.g., diet, richness, abundance), explained variance was greatest when all three trophic levels were considered. Changes in consumer diet breadth resulted in the largest estimate, but models that included richness explained the most variance.

Path analysis and the effects of sampling

The path model using all samples was a reasonably good fit to the data ($\chi^2 = 4.8$, $df = 2$, $P = 0.09$; $AIC = 42.8$) and it performed better than all other models with the same degrees of freedom (mean $AIC = 52.1$). The Pareto distribution of diet breadth used in these 5000 communities was highly skewed and undervalued the importance of diet

breadth. Species diversity and herbivore diet breadth showed the strongest positive effects on PHE interaction diversity (Fig. 5). Only plant abundance within the local community was negatively associated with interaction diversity, such that communities with higher plant abundance had lower interaction diversity. However, plant abundance had a strong positive effect on species diversity.

To investigate the sensitivity of each path to the number of observations included in the path analysis, path coefficients were derived from the SEM that used random samples from simulated communities that started at 5 interactions and increased up to 500 interactions (Fig. 6; Fig. S1). These random samples are analogous either to actual sampling in a biotic community or to smaller scale communities that are derived from a regional pool of species and potential interactions. The path coefficients displayed variable strengths and patterns in response to number of interactions included in the path model. Most path coefficients displayed minimal change as the number of samples (or size of the local community) increased and the strength of the coefficient remained negligible even as the number of observations increased (Fig. S1). Other path coefficients responded strongly in a linear or non-linear fashion to increases in number of interactions sampled, several of which are shown in Figure 3. Species diversity exerted a strong positive effect on interaction diversity and that effect increased as the interactions sampled increased (Fig. 3A; $\beta = 0.0009$, $P < 0.0001$). Mean enemy and herbivore diet breadth displayed non-linear relationships with interaction diversity (Fig. 3B and 3C). The strength and direction of herbivore diet breadth on interaction diversity was typically negative, but fluctuated greatly as sampled interactions increased. However, at the highest sampled interactions, mean herbivore diet breadth exerted a strong positive effect

on interaction diversity. Mean enemy diet breadth caused a clear, non-linear increase in interaction diversity as sampled interactions increased (Fig. 3C); the path coefficients for this relationship were always greater than zero and the strength of the path coefficient increased until approximately 300 sampled interactions followed by a steady decline in the path coefficient size. The effect of plant abundance on species diversity was close to zero, until sampled interactions became abundant, at which point the effect increases (Fig. 3D). The model revealed a similar, but opposite pattern of plant abundance changing interaction diversity, such that as sampled interactions increased the effect of plant abundance on interaction diversity became more negative (Fig. S1).

DISCUSSION

The interest in interaction diversity as a network parameter has developed separately from natural history studies that attempt to rigorously document (trophic) interactions at local and regional scales (Dyer et al. 2012). Interaction diversity and related variables, such as connectance have been gleaned from loosely constructed networks (e.g., from literature searches or brief observational studies), and these parameters have been utilized as measures relevant to network structure and resilience. This view of network edge diversity is distinct from issues surrounding biodiversity, productivity, ecosystem function, and extinction. Our simulation generates hypotheses relevant to the power of sampling actual interactions and calculating the diversity of interacting individuals across a variety of ecological communities. The clearest patterns that emerged and are worth pursuing with empirical data were: 1) randomly assembled networks produce accumulation curves for interaction diversity that reach an apparent

asymptote more quickly than species diversity, so interaction diversity may be easier to estimate than species diversity in real ecosystems – this is especially true at intermediate sample sizes (or local community sizes) (Dyer et al. 2010); 2) for our simulated tri-trophic communities, local species diversity and consumer diet breadth are the best predictors of local interaction diversity, which is highest for species rich and generalized communities; 3) consumer diet breadth, defined by a Pareto distribution, is likely to have large effects on local interaction diversity, as more generalized communities will have higher interaction diversity; 4) species diversity and local plant abundance are also likely to predict other tri-trophic network parameters, such as connectance and interaction (or link) density; and 5) local network parameters are likely to be quite different from the regional networks, and this relationship changes as the networks grow in size.

The Interaction Diversity Model

Our approach to simulating tri-trophic networks provides randomly assembled quantitative communities that can be separated into discrete bipartite networks nested within a randomly assembled community. This provides an opportunity to investigate how the number and position of trophic interactions influences network-level properties from a discrete sampling procedure. It also provides insight into how sample size or scale can affect network properties. Collecting quantitative network data on complex webs of interactions is an important step beyond binary network data and can provide additional information on network dynamics and function (Blüthgen 2010, Tylianakis et al. 2010). The addition of a third trophic level separates our model and sampling approach from previous simulated network data (e.g., Thébaud and Fontaine 2010, Fründ et al. 2016),

and for both modeling and empirical approaches to ecological networks, expanding to more complex interaction networks should be a focus. Network-level properties may be highly influenced by the number and position of trophic levels that are being analyzed, especially when considering plant, herbivore, and natural enemy communities. Sampling or including higher trophic levels is completely dependent on the successful sampling of associated hosts, which can have significant impacts on the structure and diversity of a sampled network. As more trophic levels are included in a network, the dependencies of sampled (or included) interactions increase.

The simulation of tri-trophic networks developed “complete” networks that were assembled with only one assumption – networks consisted of consumers with restricted diets and included realistic numbers of species and interactions (based on empirical interaction diversity data). Our goal was to generate a network that is more consistent with standard neutral assumptions (no assembly rules) combined with niche-based assumptions (specialization), rather than following an abundance-based simulation null model (Dormann et al. 2009). The flexibility of our simulation model, which allows the manipulation of richness, abundance, and diet breadth for each trophic level included in the community, can also incorporate other assumptions, such as assembly rules (Keddy 1992, Weiher and Keddy 2001), or to omit the assumption of restricted consumer diet. Our utilization of a truncated Pareto distribution for host range is well supported in plant-arthropod networks (Forister et al. 2015) and provides a realistic measure of host specialization in multi-trophic networks that include plants, insect herbivores, and parasitoid natural enemies. The manipulation of richness, abundance, and diet breadth and their distributions, allows for a useful tool to compare observed data to simulated

data from the model. This can help with determining the importance of diet breadth distribution or degree of specialization versus other factors in sampled networks when exploring relationships between diversity, network processes, and network patterns (Fründ et al. 2016).

Species and interaction rarefaction curves

Few studies have attempted to compare rarefaction curves for species and interactions across a wide range of multitrophic communities (but see Vazquez et al. 2009, Burkle and Knight 2012, López-Carretero et al. 2014). Rarefaction is used to easily compare measures of richness between communities in which the sampling effort is different and can be useful to help identify the completeness of sampling that has occurred in a community (Gotelli and Colwell 2001). It is assumed, though never tested, that given the substantially more potential interactions than species, interactions should accumulate much more slowly than species when sampling from a discrete sample area. However, many interactions never occur (i.e. they are forbidden or not observed) and it is possible that interactions are characterized by a more kurtotic distribution than species, which should result in interactions obtaining an apparent asymptote more quickly than species (Dyer et al. 2010). In other words, similar to species distributions, interactions are typically dominated by a few, abundant connections, with many singleton or rare interactions. Therefore, the shape of rarefaction curves may be highly influenced by the abundance distributions, taxonomic richness, and host range of consumers in multitrophic communities.

The values of interaction richness yielded by this simulation may be considerably lower than species richness due to our high levels of host specialization. A truncated Pareto distribution involves few generalist and many specialist species, which reduces the number of unique interactions that occur when there are no assembly rules or differences in densities for consumers of different diet breadths. Other networks (e.g., plant-pollinator) have revealed higher numbers of interactions than species (e.g. plants and pollinators) (Gibson et al. 2011, Chacoff et al. 2012, Fang and Huang 2016), but these mutualistic communities are normally characterized by more generalized interactions, they are typically regional networks (i.e. large scale), and the networks are almost always based on all visitors rather than true pollinators (Vázquez and Aizen 2004, Petanidou et al. 2008, King et al. 2013). Using a truncated Pareto distribution of host specialization may be most useful when studying antagonistic interactions, especially those involving plants, insects, and parasitoid natural enemies. However, the simulation approach is adaptable and any distribution of host utilization is possible, and modified assumptions would be necessary for communities other than plant, insect herbivore, and parasitoid communities.

Associations between species diversity and interaction diversity

We observed a strong positive correlation between species and interaction diversity, and this relationship was more stable than anticipated across the diverse range of communities, scales, and sample sizes. We hypothesized that while sampling multitrophic communities, consumer diet breadth and other community parameters (e.g. richness and abundance) should alter the correlation among interaction and species

diversity. Specifically, more specialized communities (higher α -parameters) result in lower positive correlation coefficients (fewer links per node) due to the decrease of generalized interactions. Based on our simulations, notable changes in the correlation coefficient or slope among species and interaction diversity across a wide range of combinations of community parameters were observed (Fig. 4, Tables S1 & S2). More specialized communities displayed more negative residuals, which revealed that based on the linear regression there are fewer interactions than expected based on the number of species. Although this effect was small, it supports the hypothesis that generalized interactions are rare, but have large effects on interaction diversity locally (Dyer et al. 2010). Generally, community parameters (e.g., richness, abundance, diet breadth) had little effect on the relationship between species and interaction diversity, probably due to the lack of assembly rules and low numbers of generalists. The main parameters that altered the associations between species and interaction diversity were the number of trophic levels and species diversity within the local community, though the strength of this affect also appears minimal.

An important contribution of our simulation is that it included more than two trophic levels in an effort to understand how the position and number of trophic levels in a community can drive relationships between species and interaction diversity. Many network studies have been limited to plant-pollinator or plant-herbivore networks, yet communities are far more complex, and patterns of interaction diversity and network topology from two-trophic-level analysis are likely different from more realistic multi-trophic communities. Our results revealed that when sampling from a discrete area, the observed interactions between higher trophic levels (e.g. herbivore-enemy) are contingent

on sampling partners at lower trophic levels. In other words, the likelihood of sampling enemies is founded on the likelihood of sampling an herbivore, which results in a propagation of effects, changing the probability density functions of interactions differently from species density functions. Furthermore, differences in consumer specialization among herbivores and enemies can completely change measures of interaction diversity.

Thus, our finding that correlations between species and interaction diversity decrease when investigating more than two trophic levels magnify the impacts of consumer specialization and richness on driving food web patterns (Beaver 1985). Utilizing interaction diversity, as a metric of biodiversity, to help with conservation and management issues will be most useful when more than two trophic levels are investigated. Otherwise, species diversity should be a reasonable proxy for interaction diversity when a community is dominated by only plants and herbivores since disparities between interaction and species diversity are lowest for two trophic levels.

Effects of primary productivity, diet breadth, species diversity, and number of observations on network structure.

When we assembled thousands of tri-trophic communities with only constraints on consumer diet-breadth distributions, there was considerable variance in interaction diversity, due to random effects and partly due to the deterministic effects of the manipulated parameters. By utilizing a path analysis framework we were able to identify direct and indirect effects of multiple community parameters on interaction diversity. Under this framework, consumer diet breadth, species diversity, and to a lesser extent

initial conditions of plant abundance revealed the strongest direct effects determining interaction diversity. As expected, species diversity had a strong positive effect on interaction diversity. Surprisingly, the strongest path coefficient was the direct effect of herbivore diet breadth on interaction diversity, with a higher mean diet breadth for the community causing greater effective numbers of interactions (Simpson's effective species numbers). The effect of enemy diet breadth was similarly positive but not as strong. These results are what we originally predicted given that we expected interaction diversity to be an emergent consequence of distributions of consumer specialization and taxonomic richness (Beckerman et al. 2006). Connectance is also thought to decrease as species richness increases, but consumer traits, such as diet breadth, likely alter that relationship (Winemiller 1989, Beckerman et al. 2006).

This effect of generalists on interaction diversity would only be expected at larger scales and only in instances where specialists have lower or comparable densities as generalists. There are no good empirical studies examining the relationship between consumer diet breadth and density, but there are theoretical reasons to assume that specialists should be present at higher densities than generalists (Dyer et al. 2010). Both empirical data and more modeling that examines densities of generalists versus specialists will help determine the role of diet breadth on interaction diversity, but it is clearly an important predictor.

As discussed above, the strong effect of species diversity on local network structure is also not surprising, but it is important to note that other network parameters, in particular connectance and interaction density, are far less affected by the total number of species. In fact, diet breadth is also the best predictor of these variables, especially

connectance. In this case, as herbivores become more specialized, connectance increases, while increases in enemy diet breadth (more generalized predators) cause increases in connectance – the different direction in effects is due to the fact that enemies are less abundant and diverse in our simulated networks (Fig. 1), but this is not always the case in real networks so it warrants more examination with models and empirical data. The positive effect of local plant abundance on species diversity was somewhat counteracted by a negative direct effect on interaction diversity. Of course, if plants are not constrained in associated arthropod communities, this effect could be positive, but given equal probabilities of colonization across plant species, an increase in plant richness is more likely to increase interaction diversity than an increase in plant abundance.

Using the same path analysis, we found that the number of observations included in the model biases the strength of several path coefficients, but this could also be viewed as a scaling issue – lower numbers of observations in our model are analogous to more localized assemblages within a community. Studies investigating these sampling or scaling effects on ecological network parameters are rare, but they are important because ecological networks are especially vulnerable to sampling effects as well as scale (Nielsen and Bascompte 2007, Dormann et al. 2009, Fründ et al. 2016). The effects of diet breadth (e.g., herbivore and enemy) on interaction diversity revealed the strongest evidence of sample size on the strength of path coefficients. Specialized communities are typically less impacted by sampling bias, since fewer observations are needed to identify all of the actual interactions (Fründ et al. 2016). However, identifying the effects of specialization on interaction diversity may be difficult given the nonlinear relationship with the sample size or changes in scale.

CONCLUSION

While this model will be useful for developing basic hypotheses concerning the drivers of interaction diversity, there are details in our model that merit further work. We utilized this simulation to test hypotheses about accumulation patterns of species and interactions, but this modeling approach is appropriate for investigating spatial scaling of interactions and species. A great deal of progress has been made towards understanding species diversity, but we lack even a rudimentary understanding of the determinants and spatial or temporal dynamics of interaction diversity. The modeling approach utilized here can be developed to investigate more about the relationships between local and regional interaction diversity (Cornell and Lawton 1992, Ricklefs and Schluter 1993), which will provide insight into the utility of the preponderance of regional networks.

In conclusion, we demonstrated that in highly specialized communities, interactions accumulate more quickly than species. We showed that diet breadth and taxonomic richness both interact to influence relationships between species and interaction diversity. The position and number of trophic levels being investigated strongly impacted species and interaction diversity correlations. The separation between interaction and species diversity is greatest when investigating more than two trophic levels, so utilizing interaction diversity as a metric of biodiversity will be most useful for multi-trophic investigations for both applied and basic research questions such as spatiotemporal dynamics, a biogeographical theory of species interactions (Poisot et al. 2012), and the effects of climate change on biological networks.

ACKNOWLEDGMENTS

We would like to thank graduate students in the Ecology, Evolution, and Conservation Biology program at the University of Nevada, Reno for suggestions and discussions during the development of this model.

Figure Legends:

Figure 1: Example network produced from our simulation. The figure was generated using Package “bipartite” in R. Bars represented in green are individuals that are not involved in interactions within the community. The thickness of each edge and node in the network denotes the abundance of interactions or species. Only species that were sampled are shown in this network. Numbers above each species correspond to a species within that community.

Figure 2: Rarefaction curves are displayed for interactions and species for 100 generated communities. Rarefaction curves were generated using the “vegan” package in R. Rarefaction curves were generated for all three networks within each communities: Plant-Herbivore (PH), Herbivore-Enemy (HE), and Plant-Herbivore-Enemy (PHE). PHE networks include only PHE interactions, and exclude PH interactions that were not involved in HE interactions. These rarefaction curves were generated using a modified version of rarefaction curves in vegan and sampled with replacement 500 times.

Figure 3: Bar plots displaying the mean posterior probabilities and standard deviation (σ) of the slope of each rarefaction curve and Chao1 estimates of richness generated from the package BEST in R. Interactions are displayed in grey, while species are in white. The lines represent the standard deviation. The mean slope was acquired by calculating the slope of each rarefaction curve when half of the species or interactions were sampled. Chao1 estimates of richness were acquired using the vegan package.

Figure 4: Scatterplots displaying the semi-partial correlations between the residuals of a linear model regressing species diversity and interaction diversity with mean consumer diet breadth, species richness, and abundance. We investigated this relationship for all three networks (e.g. PH, HE, PHE). The top three panels represent changes in mean diet breadth for each consumer

trophic level. The mean herbivore and enemy diet breadth was used for the PH and HE networks respectively, while the mean diet breadth for herbivores and enemies was used for PHE networks. The middle three panels denote community richness for each respective network. Richness for each network is equal to the total number of species for each trophic level. The lower panel displays the correlation with community abundance. Abundance for each respective network equals the sum of all individuals within each trophic level. Dashed-lines indicate results from linear regressions.

Figure 5: A path diagram displaying the standardized path coefficients across all 5000 local communities. Lines ending with an arrow represent positive coefficients, while lines ending with a circle represent negative coefficients. The width of the arrow indicates the intensity of the coefficient. Only significant path coefficients are included ($P < 0.05$).

Figure 6: Plots displaying the changing intensity of each path coefficient (Fig. 5) as the number of sampled interactions included in the path analysis increases. The strength of the path coefficient is shown on the y-axis and number of samples included in the model is shown on the x-axis. The dashed line represents outcome of linear or polynomial regressions. Not all path coefficients are shown (see supplemental material for remaining path coefficients).

Figures

Figure 1

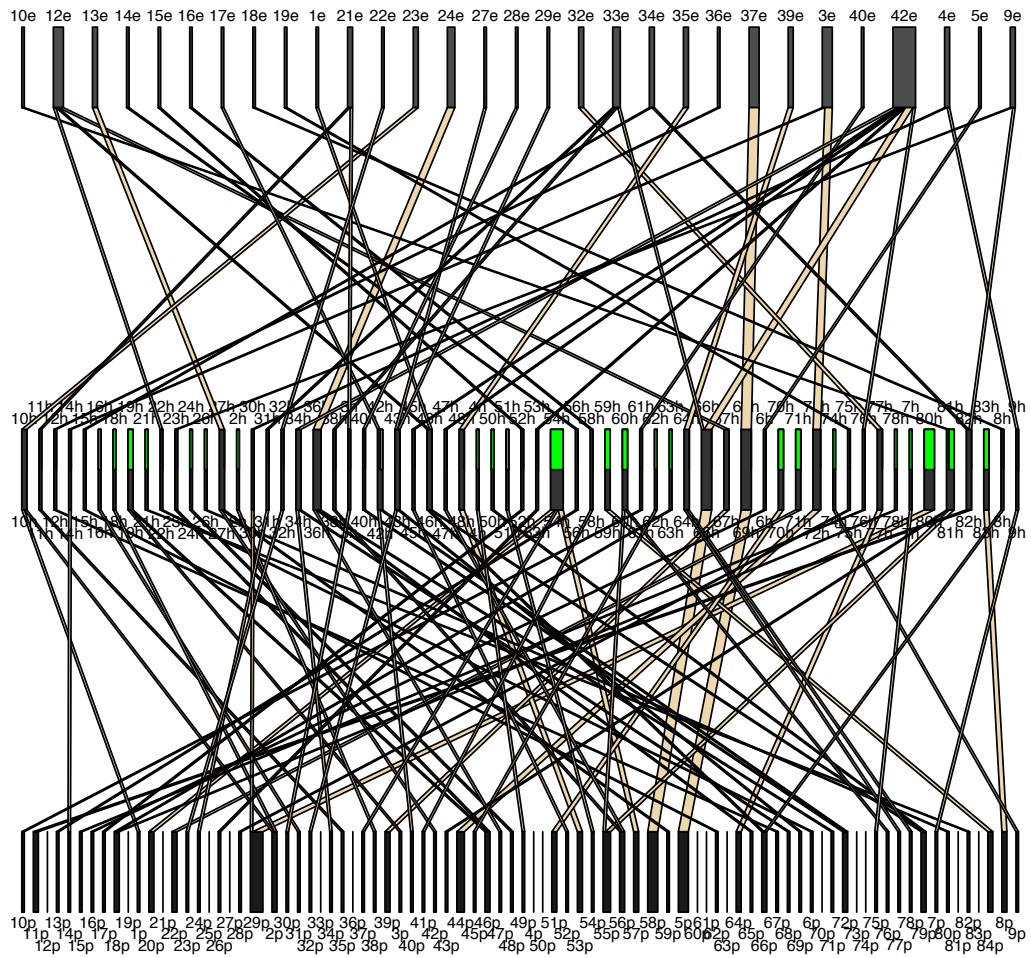


Figure 2

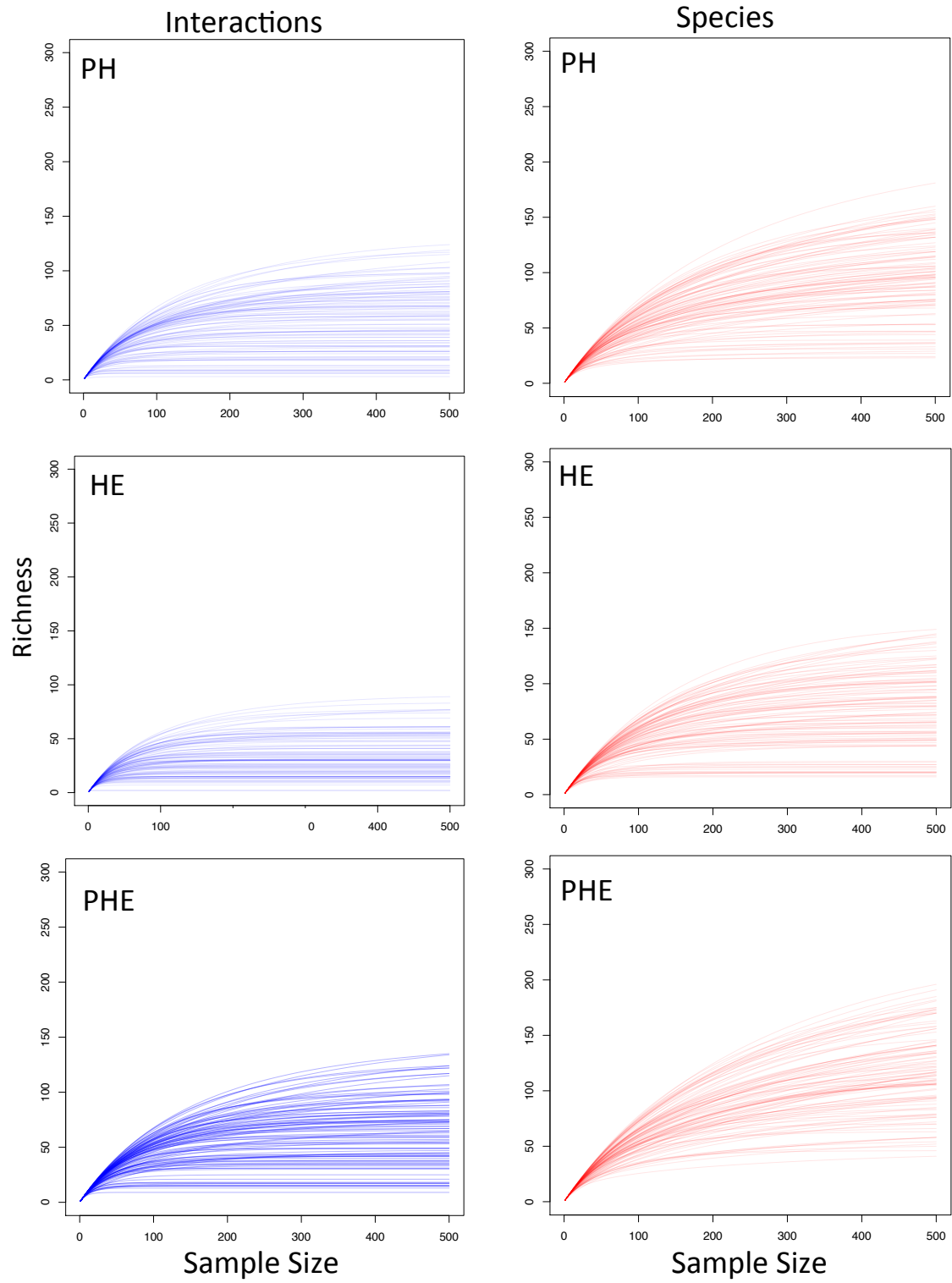


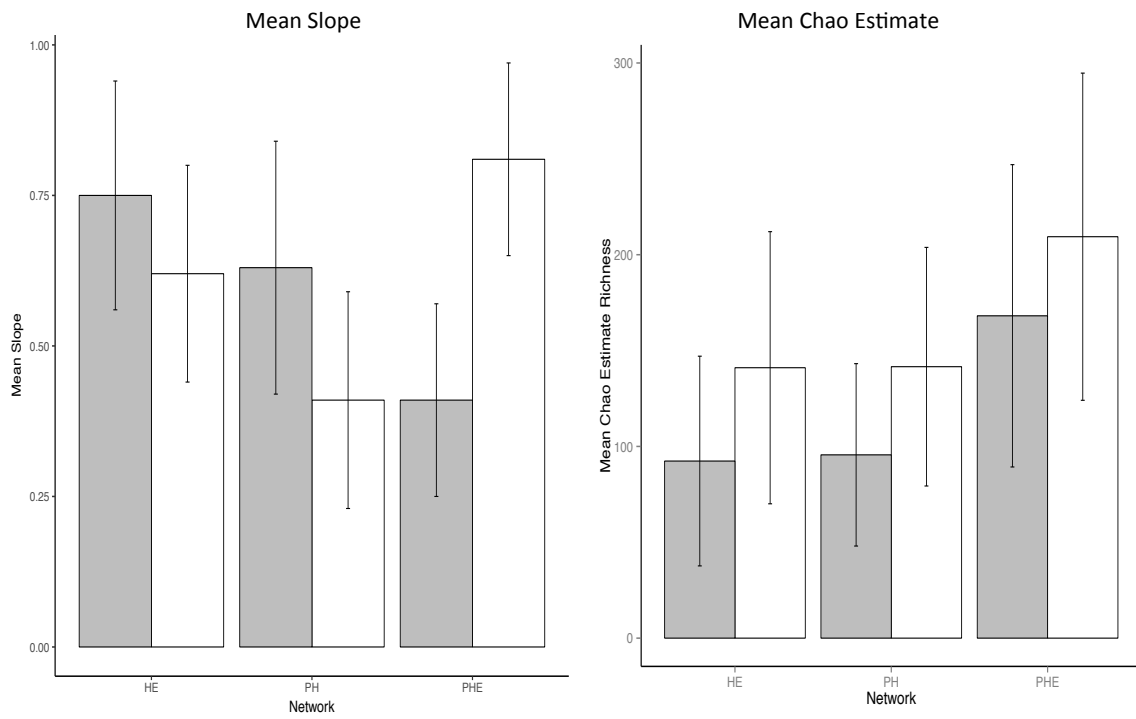
Figure 3:

Figure 4

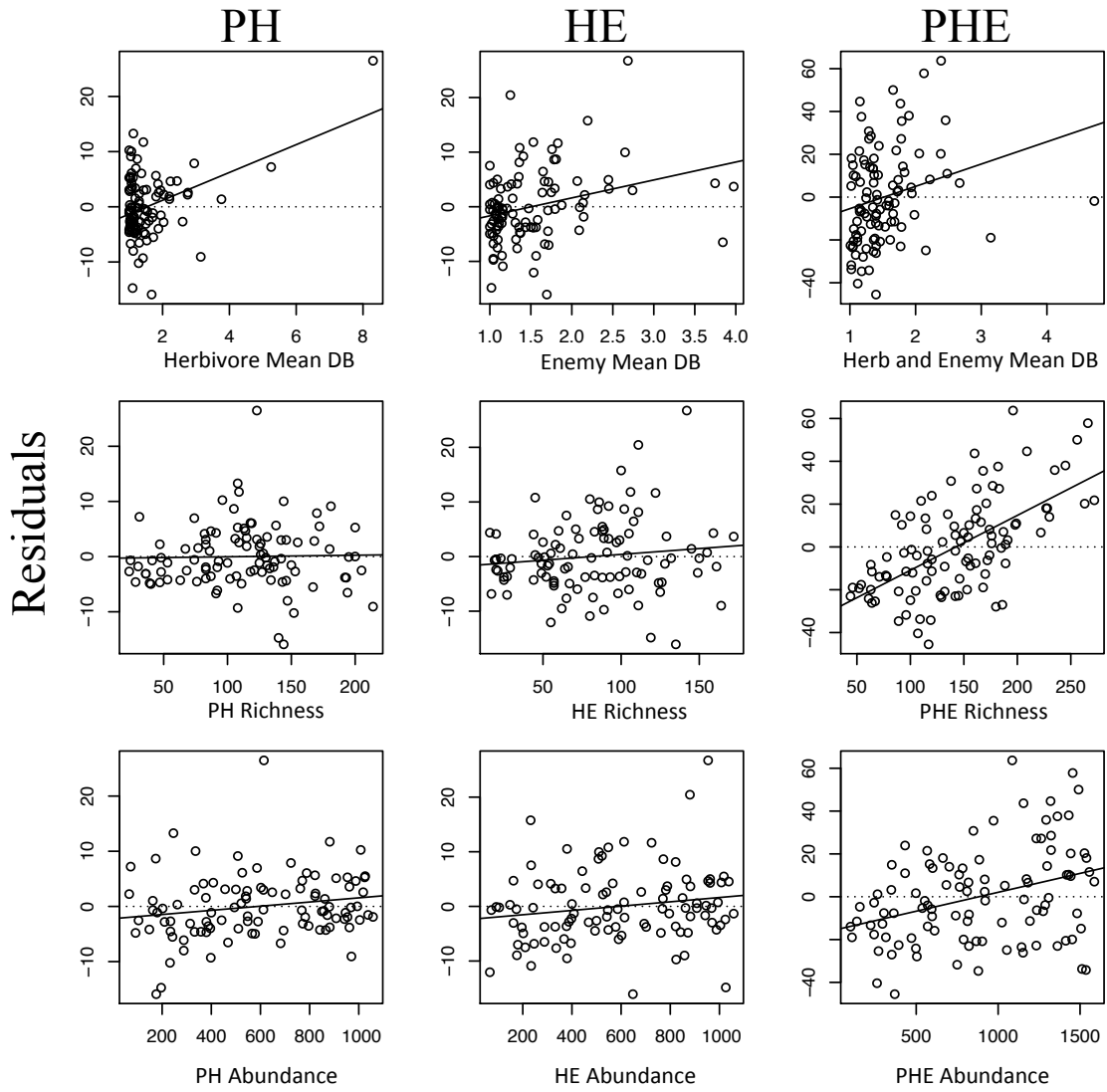


Figure 5

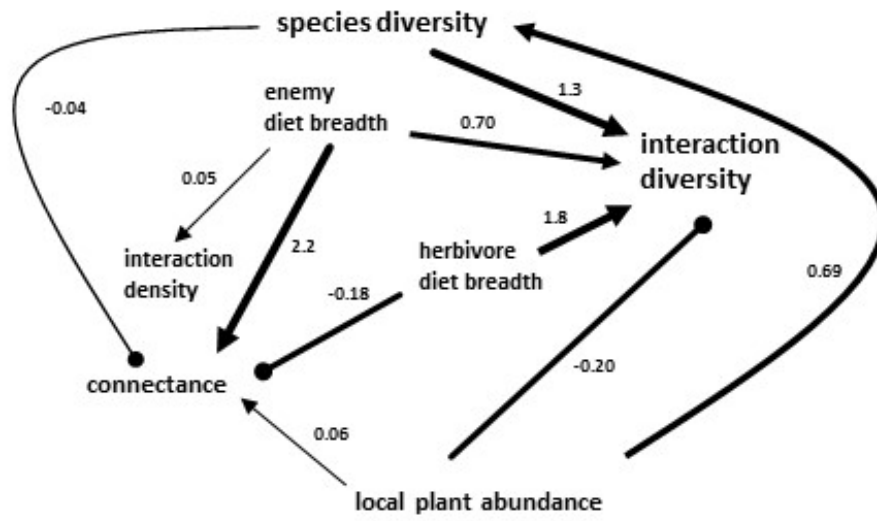
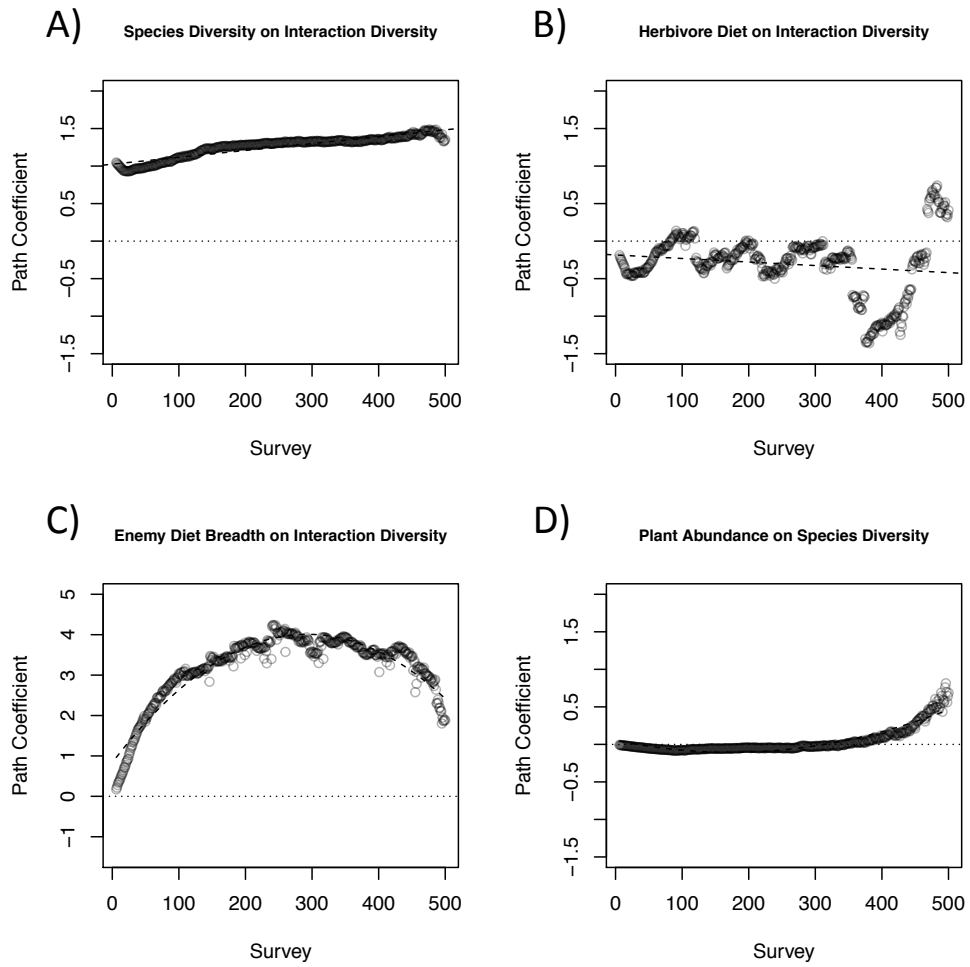


Figure 6



Supplementary Material

Chapter 4

Trophic interaction diversity: simulated networks generate relevant hypotheses

Nicholas A. Pardikes¹, Will Lumpkin¹, Paul J. Hurtado^{1,2}, and Lee A. Dyer¹

¹*Department of Biology, Program in Ecology, Evolution, and Conservation Biology,
Department of Biology, University of Nevada, Reno*

²*Department of Mathematics and Statistics, University of Nevada, Reno*

Tables**Table S1:** Direct relationship between species and interaction diversity. Correlation and the linear regression between log transformed species and interaction diversity

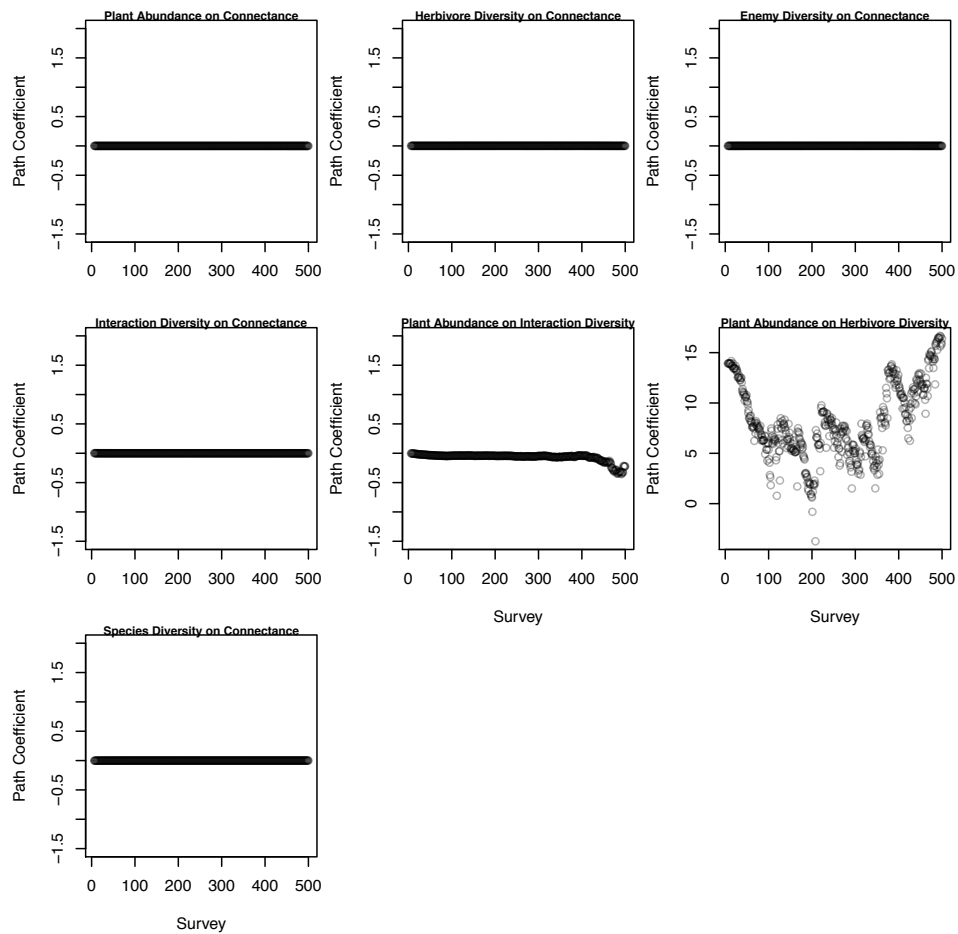
Network	Pearson's Correlation	Slope	R ²
PH	0.97	0.70	0.94
HE	0.94	0.70	0.90
PHE	0.40	0.25	0.16

Table S2: Slope and R² for between residuals from linear regression between species and interaction diversity and the variable of interest (diet, richness, abundance)

Network	Estimate Diet	R ² Diet	Estimate Richness	R ² Richness	Estimate Abund.	R ² Abund.
PH	2.5	0.16	0.003	0.009	0.0038	0.03
HE	3.3	0.07	0.02	0.005	0.004	0.02
PHE	10.4	0.05	0.26	0.37	0.017	0.11

Figures

Figure S1: Displays the remaining path coefficients from the path analysis not shown in Figure 6.



CONCLUSION

Using observational, experimental, and modeling approaches, my dissertation attempted to highlight how regional and local aspects of GEC can alter both abundances of species and species interactions. With the help of a long-term butterfly monitoring dataset, we identified associations between butterfly abundance and a large-scale climate pattern, El Niño Southern Oscillation (ENSO), across an elevational gradient in California. Global warming is predicted to increase the frequency of extreme ENSO conditions, yet little is known about how diverse butterfly assemblages, who exist across a heterogeneous landscape, are influenced by ENSO patterns. We established that butterfly populations, especially migratory species, are most sensitive to ENSO, and are more abundant in years with higher sea-surface temperatures. This work demonstrated the utility of large-scale climate indices for identifying biotic-abiotic relationships of migratory species, whose population dynamics are influenced by weather at spatial scales broader than those at local monitoring sites.

Several GEC factors have contributed to the global encroachment of woody plants into grass-dominated biomes. While the consequences of woody plant encroachment on primary productivity and plant community composition are well studied, few studies, if any, have investigated consequences of shrub encroachment on species interactions. Species interactions (e.g., pollination, herbivory, predation) provide the scaffolding of ecological communities and play an important role in the maintenance of biodiversity and stability of ecosystem processes (Dobson et al. 2006, Ives and Carpenter 2007), therefore it is critical to understand how species interactions will respond to shrub encroachment and determine the consequences of those responses.

In the Intermountain West, the areas occupied by juniper and piñon-juniper woodlands have increased dramatically and their expansion is displacing grassland and shrub steppe plant communities. Juniper is the sole host to a specialized community of caterpillars (larval Lepidoptera), yet no study had investigated how this expansion influenced trophic interactions associated with juniper. We determined that the influx of young, immature juniper trees on the landscape, due to recent expansion, provide a high quality resource for specialized juniper caterpillars. Old-growth juniper trees, across both *J. osteosperma* and *J. occidentalis*, contain a greater diversity of secondary metabolites and appear to be better chemically defended against specialized insect herbivores. However, the benefits that foliage from young, immature juniper trees provided were not consistent across both juniper species. Performance assays in the lab revealed that differences between young and old-growth trees was more pronounced in *J. osteosperma* than in *J. occidentalis*. We suggested that young trees are more essential to insect herbivores in *J. osteosperma* woodlands than *J. occidentalis*, since the decline in performance on foliage from old-growth trees is stronger on *J. osteosperma*.

The displacement of shrubs and grasses due to juniper expansion is likely to have significant impacts on the networks of interactions among species in these changing woodlands. We tested the *enemies hypothesis* in encroaching juniper woodlands, which predicts that predators and parasitoids are less efficient at controlling their hosts in less diverse systems. Specifically, we wanted to investigate whether declines in understory plant diversity, as a consequence of juniper encroachment (canopy cover), reduced parasitism rates and the species richness of parasitoids. We concluded that parasitism rates and species richness of parasitoids are negatively associated with understory plant

diversity, which is contradictory to what the ‘enemies hypothesis’ predicts. Parasitism rates and parasitoid species richness of juniper-feeding caterpillars were highest when the density and abundance of their host was greatest. We suggested that predictions from the ‘enemies hypothesis’ might not be applicable for highly specialized host-parasitoid interactions, such as those associated with juniper. However, our results support previous analysis, which show that species diversity within a trophic level can affect the associated multi-trophic structure of an ecosystem.

Finally, we developed a tri-trophic food web simulation model to investigate how taxonomic richness, abundance, and consumer diet breadth interact to determine the structure of ecological networks. We tested specific hypotheses about relationships between species and interaction diversity and disentangled the relative influences of fundamental processes, such as consumer specialization, as drivers of ecological network structure across various spatial scales. As species diversity declines globally, it is imperative that we are able to forecast the consequences of these losses on complex networks of biotic interactions. Our model identified consumer-diet breadth and species diversity as the strongest factors that determine interaction diversity. Interestingly, relationships between species and interaction diversity were not altered by changes in diet-breadth as much as we predicted.

In summary, this work revealed that GEC could influence biotic communities through local and regional processes and disentangling the effects of GEC on complex networks of species interactions will require detailed natural history information about each organism. The fact that not all species responded to ENSO similarly and that the same species responded to ENSO differently across its range, suggests that basic natural

history may help make sense of these patterns. Certain traits (natural history or life-history), such as being migratory or a specialized parasitoid, made a species more susceptible to GEC than others. Furthermore, if species interactions help mitigate consequences of GEC and can help maintain species diversity within an ecosystem, it is critical we start quantifying species interactions in nature. We know very little of who interacts with whom, and it is likely that responses to GEC are mediated through complex networks of biotic interactions. The fact that we know so little about basic interaction relationships among species makes predictions difficult and efforts to mitigate the effects of GEC on biotic communities virtually impossible.

REFERENCES

- Adams, R. P. 2014. *Junipers of the world: the genus Juniperus*. Trafford Publishing.
- Ali, J. G., and A. A. Agrawal. 2012. Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science* 17:293–302.
- Andow, D. A. 1991. Vegetational diversity and arthropod population response. *Annual Review of Entomology* 36:561–586.
- Andow, D. A., and D. R. Prokrym. 1990. Plant structural complexity and host-finding by a parasitoid. *Oecologia* 82:162–165.
- Andow, D. A., and S. J. Risch. 1985. Predation in diversified agroecosystems: relations between a coccinellid predator *Coleomegilla maculata* and its food. *Journal of Applied Ecology*:357–372.
- Aquilino, K. M., B. J. Cardinale, and A. R. Ives. 2005. Reciprocal effects of host plant and natural enemy diversity on herbivore suppression: an empirical study of a model tritrophic system. *Oikos* 108:275–282.
- ArcMap, E. 2010. 10.1. Computer software. Redlands, CA, ESRI.
- Auken, O. W., and F. Smeins. 2008. Western North American *Juniperus* communities: patterns and causes of distribution and abundance. *Western North American Juniperus Communities*:3–18.
- Awmack, C. S., and S. R. Leather. 2002. Host Plant Quality and Fecundity in Herbivorous Insects. *Annual Review of Entomology* 47:817–844.
- Barbour, M. A., M. A. Fortuna, J. Bascompte, J. R. Nicholson, R. Julkunen-Tiitto, E. S. Jules, and G. M. Crutsinger. 2016. Genetic specificity of a plant–insect food web:

- Implications for linking genetic variation to network complexity. *Proceedings of the National Academy of Sciences* 113:2128–2133.
- Barton, K. 2013. MuMIn: Multi-model inference. R package version 1.9. 5. R Project for Statistical Computing, Vienna, Austria.
- Barton, K. E., and K. Boege. 2017. Future directions in the ontogeny of plant defence: understanding the evolutionary causes and consequences. *Ecology Letters* 20:403–411.
- Barton, K. E., and J. Koricheva. 2010. The Ontogeny of Plant Defense and Herbivory: Characterizing General Patterns Using Meta-Analysis. *The American Naturalist* 175:481–493.
- Bascompte, J., and P. Jordano. 2006. The structure of plant-animal mutualistic networks. *Ecological networks: linking structure to dynamics in food webs*. Oxford University Press, Oxford, UK, 143-159.
- Bascompte, J., and D. B. Stouffer. 2009. The assembly and disassembly of ecological networks. *Philosophical Transactions of the Royal Society of London B Biological Sciences* 364:1781–1787.
- Bates, D., M. Maechler, B. Bolker, and Walker, Steven. 2014. lme4: Linear mixed-effects models using Eigen and S4 R package version 1.0-6.
- Bates, J. D., R. F. Miller, and T. Svejcar. 1998. Understory patterns in cut western juniper (*Juniperus occidentalis* spp. *occidentalis* Hook.) woodlands. *Great Basin Naturalist* 58:363–374.
- Beaver, R. A. 1985. Geographical variation in food web structure in *Nepenthes* pitcher plants. *Ecological Entomology* 10:241–248.

- Beckerman, A. P., O. L. Petchey, and P. H. Warren. 2006. Foraging biology predicts food web complexity. *Proceedings of the National Academy of Sciences* 103:13745–13749.
- Belsky, A. J. O. Y. 1996. Viewpoint : Western juniper expansion : threat to arid northwestern ecosystems? *Journal of Range Management* 49:53–59.
- Bernays, E. A. 2001. Neural Limitations in Phytophagous Insects: Implications for Diet Breadth and Evolution of Host Affiliation. *Annual Review of Entomology* 46:703–727.
- Björkman, M., P. A. Hambäck, R. J. Hopkins, and B. Rämert. 2010. Evaluating the enemies hypothesis in a clover-cabbage intercrop: effects of generalist and specialist natural enemies on the turnip root fly (*Delia floralis*). *Agricultural and Forest Entomology* 12:123–132.
- Blaauw, B. R., and R. Isaacs. 2012. Larger wildflower plantings increase natural enemy density, diversity, and biological control of sentinel prey, without increasing herbivore density. *Ecological Entomology* 37:386–394.
- Blois, J. L., P. L. Zarnetske, M. C. Fitzpatrick, and S. Finnegan. 2013. Climate change and the past, present, and future of biotic interactions. *Science* 341:499–504.
- Blüthgen, N. 2010. Why network analysis is often disconnected from community ecology: a critique and an ecologist's guide. *Basic and Applied Ecology* 11:185–195.
- Boege, K. 2005. Influence of plant ontogeny on compensation to leaf damage. *American Journal of Botany* 92:1632–1640.

- Boege, K., K. E. Barton, and R. Dirzo. 2011. Influence of Tree Ontogeny on Plant-Herbivore Interactions. Pages 193–214 *in* B. Lachenbruch and T. E. Dawson, editors. *Size- and Age-Related Changes in Tree Structure and Function*. Springer Netherlands, Dordrecht.
- Boege, K., and R. J. Marquis. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends in Ecology & Evolution* 20:441–448.
- Boege, K., and R. J. Marquis. 2006. Plant quality and predation risk mediated by plant ontogeny: consequences for herbivores and plants. *Oikos* 115:559–572.
- Boggs, C. L., and D. W. Inouye. 2012. A single climate driver has direct and indirect effects on insect population dynamics. *Ecology Letters* 15:502–508.
- Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution* 24:127–135.
- Bolnick, D. I., P. Amarasekare, M. S. Araújo, R. Bürger, J. M. Levine, M. Novak, V. H. W. Rudolf, S. J. Schreiber, M. C. Urban, and D. A. Vasseur. 2011. Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution* 26:183–192.
- Bommarco, R., and J. E. Banks. 2003. Scale as modifier in vegetation diversity experiments: effects on herbivores and predators. *Oikos*:440–448.
- Bowers, M. D., and N. E. Stamp. 1993. Effects of plant age, genotype and herbivory on *Plantago* performance and chemistry. *Ecology*:1778–1791.

- Bradley, B. A., and E. Fleishman. 2008. Relationships between expanding pinyon–juniper cover and topography in the central Great Basin, Nevada. *Journal of Biogeography* 35:951–964.
- Briggs, J. M., A. K. Knapp, J. M. Blair, J. L. Heisler, G. A. Hoch, M. S. Lett, and J. K. McCarron. 2005. An ecosystem in transition: causes and consequences of the conversion of mesic grassland to shrubland. *BioScience* 55:243–254.
- Bryant, J. P., P. Kuropat, P. Reichardt, and T. Clausen. 1991. Controls over the allocation of resources by woody plants to chemical antiherbivore defense. *Plant defenses against mammalian herbivory* 83.
- Bukovinszky, T., R. Gols, M. A. Posthumus, L. E. M. Vet, and J. C. Van Lenteren. 2005. Variation in plant volatiles and attraction of the parasitoid *Diadegma semiclausum* (Hellen). *Journal of Chemical Ecology* 31:461–480.
- Bukovinszky, T., E. H. Poelman, A. Kamp, L. Hemerik, G. Prekatsakis, and M. Dicke. 2012. Plants under multiple herbivory: consequences for parasitoid search behaviour and foraging efficiency. *Animal Behaviour* 83:501–509.
- Bunting, S. C., J. L. Kingery, and E. Stand. 1999. Effects of succession on species richness of the western juniper woodland/sagebrush steppe mosaic. Pages 76–81 SB Monsen, S. Richards, RJ Tausch, RF Miller, and C. Goodrich (compilers). *Proceedings Ecology and Management of piñon-juniper communities within the Interior West*. USDA Forest Service, RMRS-P-9.
- Burkle, L. A., and T. M. Knight. 2012. Shifts in pollinator composition and behavior cause slow interaction accumulation with area in plant–pollinator networks. *Ecology* 93:2329–2335.

- Burnham, K. P., and D. R. Anderson. 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer.
- Bybee, J., B. A. Roundy, K. R. Young, A. Hulet, D. B. Roundy, L. Crook, Z. Aanderud, D. L. Eggett, and N. L. Cline. 2016. Vegetation Response to Piñon and Juniper Tree Shredding. *Rangeland Ecology & Management* 69:224–234.
- Cabral, A. C., J. M. De Miguel, A. J. Rescia, M. F. Schmitz, F. D. Pineda, and S. Díaz. 2003. Shrub encroachment in Argentinean savannas. *Journal of Vegetation Science* 14:145–152.
- Cai, W., S. Borlace, M. Lengaigne, P. van Rensch, M. Collins, G. Vecchi, A. Timmermann, A. Santoso, M. J. McPhaden, L. Wu, M. H. England, G. Wang, E. Guilyardi, and F.-F. Jin. 2014. Increasing frequency of extreme El Nino events due to greenhouse warming. *Nature Climate Change* 4:111–116.
- Chacoff, N. P., D. P. Vazquez, S. B. Lomascolo, E. L. Stevani, J. Dorado, and B. Padron. 2012. Evaluating sampling completeness in a desert plant–pollinator network. *Journal of Animal Ecology* 81:190–200.
- Chao, A. 1984. Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics*:265–270.
- Chapin III, F. S., O. E. Sala, I. C. Burke, J. P. Grime, D. U. Hooper, W. K. Lauenroth, A. Lombard, H. A. Mooney, A. R. Mosier, S. Naeem, S. W. Pacala, J. Roy, W.L. Steffen, and D. Tilman. 1998. Ecosystem consequences of changing biodiversity. *Bioscience* 48:45–52.

- Chapin III, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. *Nature* 405:234–242.
- Cleary, D., and M. Genner. 2004. Changes in rain forest butterfly diversity following major ENSO-induced fires in Borneo. *Global Ecology and Biogeography* 13:129–140.
- Cohen, J. E., and Fredeiri. Briand. 1984. Trophic links of community food webs. *Proceedings of the National Academy of Sciences* 81:4105–4109.
- Cole, R. A. 1980. Volatile components produced during ontogeny of some cultivated crucifers. *Journal of the Science of Food and Agriculture* 31:549–557.
- Cornell, H. V., and J. H. Lawton. 1992. Species interactions, local and regional processes, and limits to the richness of ecological communities: a theoretical perspective. *Journal of Animal Ecology*:1–12.
- Coultrap, D. E., K. O. Fulgham, D. L. Lancaster, J. Gustafson, D. F. Lile, and M. R. George. 2008. Relationships Between Western Juniper (*Juniperus Occidentalis*) and Understory Vegetation. *Invasive Plant Science and Management* 1:3–11.
- Crutsinger, G. M., M. D. Collins, J. A. Fordyce, Z. Gompert, C. C. Nice, and N. J. Sanders. 2006. Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science* 313:966–968.
- D'Alessandro, M., M. Held, Y. Triponez, and T. C. Turlings. 2006. The role of indole and other shikimic acid derived maize volatiles in the attraction of two parasitic wasps. *Journal of Chemical Ecology* 32:2733–2748.

- Darwin, C. 1859. On the origin of species by means of natural selection, or, the preservation of favoured races in the struggle for life. 15th edition. J. Murray, London.
- Dáttilo, W., and L. Dyer. 2014. Canopy openness enhances diversity of ant–plant interactions in the Brazilian Amazon rain forest. *Biotropica* 46:712–719.
- Davidson, D. W. 1977. Species diversity and community organization in desert seed-eating ants. *Ecology* 58:712–724.
- Del-Claro, K., and H. M. Torezan-Silingardi. 2009. Insect-plant interactions: new pathways to a better comprehension of ecological communities in Neotropical savannas. *Neotropical Entomology* 38:159–164.
- Diaz, H. F., J. K. Eischeid, C. Duncan, and R. S. Bradley. 2003. Variability of freezing levels, melting season indicators, and snow cover for selected high-elevation and continental regions in the last 50 years. Pages 33–52 *Climate Variability and Change in High Elevation Regions: Past, Present & Future*. Springer.
- Didion, M., M.-J. Fortin, and A. Fall. 2007. Forest age structure as indicator of boreal forest sustainability under alternative management and fire regimes: a landscape level sensitivity analysis. *Ecological Modelling* 200:45–58.
- Dobson, A., D. Lodge, J. Alder, G. S. Cumming, J. Keymer, J. McGlade, H. Mooney, J. A. Rusak, O. Sala, V. Wolters, D. Wall, R. Winfree, and M. A. Xenopoulos. 2006. Habitat loss, trophic collapse, and the decline of ecosystem services. *Ecology* 87:1915–1924.
- D’odorico, P., G. S. Okin, and B. T. Bestelmeyer. 2012. A synthetic review of feedbacks and drivers of shrub encroachment in arid grasslands. *Ecohydrology* 5:520–530.

- Domínguez, C. A., R. Dirzo, and S. H. Bullock. 1989. On the function of floral nectar in *Croton suberosus* (Euphorbiaceae). *Oikos*:109–114.
- Donaldson, J. R., and R. L. Lindroth. 2004. Cottonwood leaf beetle (Coleoptera: Chrysomelidae) performance in relation to variable phytochemistry in juvenile aspen (*Populus tremuloides* Michx.). *Environmental Entomology* 33:1505–1511.
- Donaldson, J. R., M. T. Stevens, H. R. Barnhill, and R. L. Lindroth. 2006. Age-related shifts in leaf chemistry of clonal aspen (*Populus tremuloides*). *Journal of chemical ecology* 32:1415–29.
- Dormann, C. F., B. Gruber, and J. Fründ. 2008. Introducing the bipartite package: analysing ecological networks. *interaction* 1:0–2413793.
- Dormann, C., J. Fründ, N. Blüthgen, and B. Gruber. 2009. Indices, graphs and null models: analyzing bipartite ecological networks. *The Open Ecology Journal* 2:7–24.
- Downey, M. H., and C. C. Nice. 2011. Experimental evidence of host race formation in *Mitoura* butterflies (Lepidoptera: Lycaenidae). *Oikos* 120:1165–1174.
- Duffy, J. E., B. J. Cardinale, K. E. France, P. B. McIntyre, E. Thebault, and M. Loreau. 2007. The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecology Letters* 10:522–538.
- Dyer, L. A., C. D. Dodson, J. O. Stireman III, M. A. Tobler, A. M. Smilanich, R. M. Fincher, and D. K. Letourneau. 2003. Synergistic effects of three Piper amides on generalist and specialist herbivores. *Journal of Chemical Ecology* 29:2499–2514.
- Dyer, L. A., and D. K. Letourneau. 1999. Trophic cascades in a complex terrestrial community. *Proceedings of the National Academy of Sciences* 96:5072–5076.

- Dyer, L. A., M. S. Singer, J. T. Lill, J. O. Stireman, G. L. Gentry, R. J. Marquis, R. E. Ricklefs, H. F. Greeney, D. L. Wagner, H. C. Morais, I. R. Diniz, T. A. Kursar, and P. D. Coley. 2007. Host specificity of Lepidoptera in tropical and temperate forests. *Nature* 448:696.
- Dyer, L. A., T. R. Walla, H. F. Greeney, J. O. Stireman III, and R. F. Hazen. 2010. Diversity of Interactions: A Metric for Studies of Biodiversity. *Biotropica* 42:281–289.
- Dyer, L., and D. Letourneau. 2013. Can Climate Change Trigger Massive Diversity Cascades in Terrestrial Ecosystems? *Diversity* 5:479–504.
- Dyer, L., D. Wagner, H. Greeney, A. M. Smilanich, T. Massad, M. Robinson, M. Fox, R. F. Hazen, A. Glassmire, N. Pardikes, K. Fredrickson, C. Pearson, G. Gentry, and J. O. Stireman III. 2012. Novel Insights into Tritrophic Interaction Diversity and Chemical Ecology Using 16 Years of Volunteer-Supported Research. *American Entomologist* 58:15–19.
- Eldridge, D. J., M. A. Bowker, F. T. Maestre, E. Roger, J. F. Reynolds, and W. G. Whitford. 2011. Impacts of shrub encroachment on ecosystem structure and functioning: towards a global synthesis. *Ecology Letters* 14:709–722.
- Elger, A., D. G. Lemoine, M. Fenner, and M. E. Hanley. 2009. Plant ontogeny and chemical defence: older seedlings are better defended. *Oikos* 118:767–773.
- Elton, C. S. 2000. *The ecology of invasions by animals and plants*. University of Chicago Press.
- Evans, H. F. 1976. The searching behaviour of *Anthocoris confusus* (Reuter) in relation to prey density and plant surface topography. *Ecological Entomology* 1:163–169.

- Fang, Q., and S.Q. Huang. 2016. Plant-pollinator interactions in a biodiverse meadow are rather stable and tight for three consecutive years. *Integrative zoology* 11(3):199-206.
- Finke, D. L., and R. F. Denno. 2002. Intraguild predation diminished in complex-structured vegetation: implications for prey suppression. *Ecology* 83:643–652.
- Fletcher, T. D. 2012. QuantPsyc: quantitative psychology tools. R package version 1.
- Fordyce, J. A., Z. Gompert, M. L. Forister, and C. C. Nice. 2011. A Hierarchical Bayesian Approach to Ecological Count Data: A Flexible Tool for Ecologists. *PLoS ONE* 6:e26785.
- Forister, M. L. 2004. Oviposition preference and larval performance within a diverging lineage of lycaenid butterflies. *Ecological Entomology* 29:264–272.
- Forister, M. L., J. P. Jahner, K. L. Casner, J. S. Wilson, and A. M. Shapiro. 2011. The race is not to the swift: Long-term data reveal pervasive declines in California's low-elevation butterfly fauna. *Ecology* 92:2222–2235.
- Forister, M. L., A. C. McCall, N. J. Sanders, J. A. Fordyce, J. H. Thorne, J. O'Brien, D. P. Waetjen, and A. M. Shapiro. 2010. Compounded effects of climate change and habitat alteration shift patterns of butterfly diversity. *Proceedings of the National Academy of Sciences* 107:2088–2092.
- Forister, M. L., V. Novotny, A. K. Panorska, L. Baje, Y. Basset, P. T. Butterill, L. Cizek, P. D. Coley, F. Dem, I. R. Diniz, P. Drozd, M. Fox, A. E. Glassmire, R. Hazen, J. Hrcek, J. P. Jahner, O. Kaman, T. J. Kozubowski, T. A. Kursar, O. T. Lewis, J. Lill, R. J. Marquis, S. E. Miller, H. C. Morais, M. Murakami, H. Nickel, N. A. Pardikes, R. E. Ricklefs, M. S. Singer, A. M. Smilanich, J. O. Stireman, S.

- Villamarín-Cortez, S. Vodka, M. Volf, D. L. Wagner, T. Walla, G. D. Weiblen, and L. A. Dyer. 2015. The global distribution of diet breadth in insect herbivores. *Proceedings of the National Academy of Sciences* 112:442–447.
- Fox, J., D. Bates, D. Firth, M. Friendly, G. Gorjanc, S. Graves, R. Heiberger, G. Monette, H. Nilsson, and D. Ogle. 2009. CAR: Companion to applied regression, R Package version 1.2-16.
- Fox, J., S. Weisberg, D. Adler, D. Bates, G. Baud-Bovy, S. Ellison, D. Firth, M. Friendly, G. Gorjanc, S. Graves, R. Heiberger, R. Laboissiere, G. Monette, D. Murdoch, H. Nilsson, D. Ogle, B. Ripley, W. Venables, A. Zeileis, and R-Core. 2015. car: Companion to Applied Regression.
- Fritz, R. S., C. G. Hochwender, D. A. Lewkiewicz, S. Bothwell, and C. M. Orians. 2001. Seedling herbivory by slugs in a willow hybrid system: developmental changes in damage, chemical defense, and plant performance. *Oecologia* 129:87–97.
- Fründ, J., K. S. McCann, and N. M. Williams. 2016. Sampling bias is a challenge for quantifying specialization and network structure: lessons from a quantitative niche model. *Oikos* 125:502–513.
- Garcia, R. A., M. Cabeza, C. Rahbek, and M. B. Araújo. 2014. Multiple Dimensions of Climate Change and Their Implications for Biodiversity. *Science* 344:1247579.
- Giavalisco, P., Y. Li, A. Matthes, A. Eckhardt, H.-M. Hubberten, H. Hesse, S. Segu, J. Hummel, K. Köhl, and L. Willmitzer. 2011. Elemental formula annotation of polar and lipophilic metabolites using ^{13}C , ^{15}N and ^{34}S isotope labelling, in combination with high-resolution mass spectrometry: *Isotope labelling for unbiased plant metabolomics*. *The Plant Journal* 68:364–376.

- Gibson, R. H., B. Knott, T. Eberlein, and J. Memmott. 2011. Sampling method influences the structure of plant–pollinator networks. *Oikos* 120:822–831.
- Gingras, D., and G. Boivin. 2002. Effect of plant structure, host density and foraging duration on host finding by *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae). *Environmental Entomology* 31:1153–1157.
- Gingras, D., P. Dutilleul, and G. Boivin. 2002. Modeling the impact of plant structure on host-finding behavior of parasitoids. *Oecologia* 130:396–402.
- Girling, R. D., A. Stewart-Jones, J. Dherbecourt, J. T. Staley, D. J. Wright, and G. M. Poppy. 2011. Parasitoids select plants more heavily infested with their caterpillar hosts: a new approach to aid interpretation of plant headspace volatiles. *Proceedings of the Royal Society of London B: Biological Sciences*:rsob20102725.
- Godfray, H. C. J. 2004. Parasitoids. *Current Biology* 14:R456.
- Gols, R., R. Wagenaar, T. Bukovinszky, N. M. van Dam, M. Dicke, J. M. Bullock, and J. A. Harvey. 2008. Genetic variation in defense chemistry in wild cabbages affects herbivores and their endoparasitoids. *Ecology* 89:1616–1626.
- Gompert, Z., and J. A. Fordyce. 2012. BayesPref: heirarchical Bayesian analysis of ecological count data. R package version 1.
- Goodman, D. 1975. The Theory of Diversity-Stability Relationships in Ecology. *The Quarterly Review of Biology* 50:237–266.
- Gotelli, N. J., and R. K. Colwell. 2001. Quantifying biodiversity: Procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters* 4:379–391.

- Gripenberg, S., P. J. Mayhew, M. Parnell, and T. Roslin. 2010. A meta-analysis of preference–performance relationships in phytophagous insects. *Ecology Letters* 13:383–393.
- Gross, T., L. Rudolf, S. A. Levin, and U. Dieckmann. 2009. Generalized models reveal stabilizing factors in food webs. *Science* 325:747–750.
- Haddad, N. M., G. M. Crutsinger, K. Gross, J. Haarstad, J. M. H. Knops, and D. Tilman. 2009. Plant species loss decreases arthropod diversity and shifts trophic structure. *Ecology Letters* 12:1029–1039.
- Hagen, M., W. D. Kissling, C. Rasmussen, M. A. M. De Aguiar, L. E. Brown, D. W. Carstensen, I. Alves-Dos-Santos, Y. L. Dupont, F. K. Edwards, J. Genini, P. R. Guimaraes, G. B. Jenkins, P. Jordano, C. N. Kaiser-Bunbury, M. E. Ledger, K. P. Maia, F. M. D. Marquitti, O. McLaughlin, L. P. C. Morellato, E. J. O’Gorman, K. Trojelsgaard, J. M. Tylianakis, M. M. Vidal, G. Woodward, and J. M. Olesen. 2012. Biodiversity, Species Interactions and Ecological Networks in a Fragmented World. Pages 89–210 in U. Jacob and G. Woodward, editors. *Advances in Ecological Research*, Vol 46, Pt 1.
- Halkka, A., L. Halkka, O. Halkka, K. Roukka, and J. Pokki. 2006. Lagged effects of North Atlantic Oscillation on spittlebug *Philaenus spumarius* (Homoptera) abundance and survival. *Global Change Biology* 12:2250–2262.
- Hallett, T. B., T. Coulson, J. G. Pilkington, T. H. Clutton-Brock, J. M. Pemberton, and B. T. Grenfell. 2004. Why large-scale climate indices seem to predict ecological processes better than local weather. *Nature* 430:71–75.

- Hambäck, P. A., and G. Englund. 2005. Patch area, population density and the scaling of migration rates: the resource concentration hypothesis revisited. *Ecology letters* 8:1057–1065.
- Harmon, J. P., A. R. Ives, J. E. Losey, A. C. Olson, and K. S. Rauwald. 2000. *Coleomegilla maculata* (Coleoptera: Coccinellidae) predation on pea aphids promoted by proximity to dandelions. *Oecologia* 125:543–548.
- Hawkins, B. A. 2005. Pattern and process in host-parasitoid interactions. Cambridge University Press.
- Hebblewhite, M., and E. Merrill. 2008. Modelling wildlife–human relationships for social species with mixed-effects resource selection models. *Journal of Applied Ecology* 45:834–844.
- Herrick, J. E., V. C. Lessard, K. E. Spaeth, P. L. Shaver, R. S. Dayton, D. A. Pyke, L. Jolley, and J. J. Goebel. 2010. National ecosystem assessments supported by scientific and local knowledge. *Frontiers in Ecology and the Environment* 8:403–408.
- Hodde, M. S. 2003. The effect of prey species and environmental complexity on the functional response of *Franklinothrips orizabensis*: a test of the fractal foraging model. *Ecological Entomology* 28:309–318.
- Holmgren, M., M. Scheffer, E. Ezcurra, J. R. Gutiérrez, and G. M. Mohren. 2001. El Niño effects on the dynamics of terrestrial ecosystems. *Trends in Ecology & Evolution* 16:89–94.
- Holopainen, J. K. 2004. Multiple functions of inducible plant volatiles. *Trends in Plant Science* 9:529–533.

- Holt, R. D., and M. Loreau. 2001. Biodiversity and ecosystem functioning: The Role of trophic interactions and the importance of system openness. Pages 246–262 *in* A. P. Kinzig, S. W. Pacala, and D. Tilman, editors. *Monographs in Population Biology*. The functional consequences of biodiversity: Empirical progress and theoretical extensions. Princeton University Press.
- Huffman, D. W., M. T. Stoddard, J. D. Springer, J. E. Crouse, and W. W. Chancellor. 2013. Understory plant community responses to hazardous fuels reduction treatments in pinyon-juniper woodlands of Arizona, USA. *Forest Ecology and Management* 289:478–488.
- Hughes, L. 2012. Climate change impacts on species interactions: assessing the threat of cascading extinctions. Pages 337–359 *Saving a Million Species*. Springer.
- Hunter, M. D. 2016. *The Phytochemical Landscape: Linking Trophic Interactions and Nutrient Dynamics*. Princeton University Press.
- Hunter, M. D., and P. W. Price. 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* 73:724–732.
- Hutchinson, G. E. 1959. Homage to Santa Rosalia or why are there so many kinds of animals? *The American Naturalist* 93:145–159.
- Ings, T. C., J. M. Montoya, J. Bascompte, N. Bluethgen, L. Brown, C. F. Dormann, F. Edwards, D. Figuerola, U. Jacob, J. I. Jones, R. B. Lauridsen, M. E. Ledger, H. M. Lewis, J. M. Olesen, F. J. F. van Veen, P. H. Warren, and G. Woodward. 2009. Ecological networks - beyond food webs. *Journal of Animal Ecology* 78:253–269.

- Ishii, Y., and M. Shimada. 2010. The effect of learning and search images on predator–prey interactions. *Population Ecology* 52:27.
- Ives, A. R., and S. R. Carpenter. 2007. Stability and diversity of ecosystems. *Science* 317:58–62.
- Jackman, S., with contributions from A. Tahk, A. Zeileis, and C. M. and J. Fearon. 2015. *pscl*: Political Science Computational Laboratory, Stanford University.
- Jaeger, D. M., J. B. Runyon, and B. A. Richardson. 2016. Signals of speciation: volatile organic compounds resolve closely related sagebrush taxa, suggesting their importance in evolution. *New Phytologist* 211:1393–1401.
- Jaksic, F. M. 2001. Ecological effects of El Niño in terrestrial ecosystems of western South America. *Ecography* 24:241–250.
- Janzen, D. H. 1974. The deflowering of Central America. *La deforestación de Centroamérica. Natural History* 83.
- Janzen, D. H., M. Hajibabaei, J. M. Burns, W. Hallwachs, E. Remigio, and P. D. N. Hebert. 2005. Wedding biodiversity inventory of a large and complex Lepidoptera fauna with DNA barcoding. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360:1835–1845.
- Jiang, L., H. Joshi, and S. N. Patel. 2009. Predation alters relationships between biodiversity and temporal stability. *The American Naturalist* 173:389–399.
- Jiménez-Alfaro, B., M. Chytrý, L. Mucina, J. B. Grace, and M. Rejmánek. 2016. Disentangling vegetation diversity from climate–energy and habitat heterogeneity for explaining animal geographic patterns. *Ecology and evolution*.

- Johnson, D. D., and R. F. Miller. 2008. Intermountain presettlement juniper: distribution, abundance, and influence on postsettlement expansion. *Rangeland Ecology & Management* 61:82–92.
- Jost, L. 2006. Entropy and diversity. *Oikos* 113:363–375.
- Kaiser, H. 1983. Small scale spatial heterogeneity influences predation success in an unexpected way: model experiments on the functional response of predatory mites (Acarina). *Oecologia* 56:249–256.
- Karban, R. 1990. Herbivore outbreaks on only young trees - testing hypotheses about aging and induced resistance. *Oikos* 59:27–32.
- Kareiva, P. 1987. Habitat fragmentation and the stability of predator–prey interactions. *Nature* 326:388–390.
- Karlik, J. F., A. H. McKay, J. M. Welch, and A. M. Winer. 2002. A survey of California plant species with a portable VOC analyzer for biogenic emission inventory development. *Atmospheric Environment* 36:5221–5233.
- Kearsley, M. J., and T. G. Whitham. 1989. Developmental changes in resistance to herbivory: implications for individuals and populations. *Ecology*:422–434.
- Keddy, P. A. 1992. Assembly and response rules: two goals for predictive community ecology. *Journal of Vegetation Science* 3:157–164.
- King, C., G. Ballantyne, and P. G. Willmer. 2013. Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution* 4:811–818.

- Knapp, A. K., J. M. Briggs, S. L. Collins, S. R. Archer, M. S. Bret-Harte, B. E. Ewers, D. P. Peters, D. R. Young, G. R. Shaver, E. Pendall, and others. 2008. Shrub encroachment in North American grasslands: shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. *Global Change Biology* 14:615–623.
- Knapp, P., and P. T. Soulé. 1998. Recent *Juniperus occidentalis* (western juniper) expansion on a protected site in central Oregon. *Global Change Biology* 4:347–357.
- Knowles, N., M. D. Dettinger, and D. R. Cayan. 2006. Trends in snowfall versus rainfall in the western United States. *Journal of Climate* 19:4545–4559.
- Koch, B., P. J. Edwards, W. U. Blanckenhorn, T. Walter, and G. Hofer. 2015. Shrub encroachment affects the diversity of plants, butterflies, and grasshoppers on two Swiss subalpine pastures. *Arctic, Antarctic, and Alpine Research* 47:345–357.
- Kramer, P., and T. Kozłowski. 2012. *Physiology of woody plants*. Elsevier.
- Latif, M., and N. S. Keenlyside. 2009. El Niño/Southern Oscillation response to global warming. *Proceedings of the National Academy of Sciences* 106:20578–20583.
- Lavandero, B., S. Wratten, P. Shishehbor, and S. Worner. 2005. Enhancing the effectiveness of the parasitoid *Diadegma semiclausum* (Helen): movement after use of nectar in the field. *Biological Control* 34:152–158.
- Lawrence, R., B. M. Potts, and T. G. Whitham. 2003. Relative importance of plant ontogeny, host genetic variation, and leaf age for a common herbivore. *Ecology* 84:1171–1178.

- Letourneau, D. K. 1987. The Enemies Hypothesis: Tritrophic Interactions and Vegetational Diversity in Tropical Agroecosystems. *Ecology* 68:1616–1622.
- Liebhold, A., W. D. Koenig, and O. N. Bjørnstad. 2004. Spatial Synchrony in Population Dynamics. *Annual Review of Ecology, Evolution, and Systematics* 35:467–490.
- Liebhold, A. M., D. M. Johnson, and O. N. Bjørnstad. 2006. Geographic variation in density-dependent dynamics impacts the synchronizing effect of dispersal and regional stochasticity. *Population Ecology* 48:131–138.
- López-Carretero, A., C. Díaz-Castelazo, K. Boege, and V. Rico-Gray. 2014. Evaluating the spatio-temporal factors that structure network parameters of plant-herbivore interactions. *PloS ONE* 9:e110430.
- Loreau, M., A. Downing, M. Emmerson, A. Gonzales, J. Hughes, P. Inchausti, J. Joshi, J. Norberg, and O. Sala. 2002. A new look at the relationship between diversity and stability.
- Lucas, A. M., C. F. Scholl, D. D. Murphy, C. R. Tracy, and M. L. Forister. 2014. Geographic distribution, habitat association, and host quality for one of the most geographically restricted butterflies in North America: Thorne’s hairstreak (*Mitoura thornei*). *Insect Conservation and Diversity* 7:343–354.
- MacArthur, R. 1955. Fluctuations of animal populations and a measure of community stability. *Ecology* 36:533–536.
- Magurran, A. E. 2013. *Measuring biological diversity*. John Wiley & Sons.
- Marion, Z., J. Fordyce, B. Fitzpatrick, and M. Z. Marion. 2015. Package “hierDiversity.”
- Markham, C. G., and D. R. McLain. 1977. Pacific sea-surface temperature related to rain in California. *Nature* 269:501–504.

- Mazerolle, M. J. 2011. AICcmodavg: model selection and multimodel inference based on (Q) AIC (c). R package version 1:17.
- McCann, K. 2007. Protecting biostructure. *Nature* 446:29–29.
- McCann, K. S. 2000. The diversity–stability debate. *Nature* 405:228–233.
- McIver, J., and E. Macke. 2014. Short-Term Butterfly Response to Sagebrush Steppe Restoration Treatments. *Rangeland Ecology & Management* 67:539–552.
- McPhaden, M. J., S. E. Zebiak, and M. H. Glantz. 2006. ENSO as an integrating concept in earth science. *Science* 314:1740–1745.
- MEA. 2005. Millennium ecosystem assessment. *Ecosystems and Human Well-Being: Biodiversity Synthesis*, Published by World Resources Institute, Washington, DC.
- Meredith, M., and J. Kruschke. 2015. Bayesian Estimation Supersedes the t-Test.
- Miller, R. F. 2005. Biology, ecology, and management of western juniper (*Juniperus occidentalis*). Corvallis, Or.: Agricultural Experiment Station, Oregon State University.
- Miller, R. F., J. D. Bates, T. J. Svejcar, F. B. Pierson, and L. E. Eddleman. 2007. Western juniper field guide: asking the right questions to select appropriate management actions. *US Geological Survey Circular* 1321:61.
- Miller, R. F., and J. A. Rose. 1995. Historic expansion of *Juniperus occidentalis* (western juniper) in southeastern Oregon. *Western North American Naturalist* 55:37–45.
- Miller, R. F., R. J. Tausch, E. D. McArthur, D. D. Johnson, and S. C. Sanderson. 2008. Age structure and expansion of pinon-juniper woodlands: a regional perspective in the Intermountain West.

- Miller, R., R. Tausch, and W. Waichler. 1999. Old-growth juniper and pinyon woodlands. Pages 375–384 USDA Forest Service Proceedings RMRS-P-9.
- Mokany, K., T. D. Harwood, K. J. Williams, and S. Ferrier. 2012. Dynamic macroecology and the future for biodiversity. *Global Change Biology* 18:3149–3159.
- Moreira, X., L. Abdala-Roberts, S. Rasmann, B. Castagneyrol, and K. A. Mooney. 2016. Plant diversity effects on insect herbivores and their natural enemies: current thinking, recent findings, and future directions. *Current Opinion in Insect Science* 14:1–7.
- Mote, P. W. 2006. Climate-driven variability and trends in mountain snowpack in western North America. *Journal of Climate* 19.
- Mougi, A., and M. Kondoh. 2012. Diversity of interaction types and ecological community stability. *Science* 337:349–351.
- Naeem, S., M. Loreau, and P. Inchausti. 2002. Biodiversity and ecosystem functioning: The emergence of a synthetic ecological framework.
- Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in Ecology and Evolution* 4:133–142.
- Natural Resources Conservation Service and Grazing Lands Technology Institute. 1997. Inventorying, classifying and correlating juniper and pinyon communities to soils in Western United States. Pages 1–39. U.S. Department of Agriculture.

- Nice, C. C., M. L. Forister, Z. Gompert, J. A. Fordyce, and A. M. Shapiro. 2014. A hierarchical perspective on the diversity of butterfly species' responses to weather in the Sierra Nevada Mountains. *Ecology* 95:2155–2168.
- Nielsen, A., and J. Bascompte. 2007. Ecological networks, nestedness and sampling effort. *Journal of Ecology* 95:1134–1141.
- Novotny, V., Y. Basset, S. E. Miller, G. D. Weiblen, B. Bremer, L. Cizek, and P. Drozd. 2002. Low host specificity of herbivorous insects in a tropical forest. *Nature* 416:841–844.
- Novotny, V., P. Drozd, S. E. Miller, M. Kulfan, M. Janda, Y. Basset, and G. D. Weiblen. 2006. Why are there so many species of herbivorous insects in tropical rainforests? *Science* 313:1115–1118.
- Novotny, V., S. E. Miller, L. Baje, S. Balagawi, Y. Basset, L. Cizek, K. J. Craft, F. Dem, R. A. I. Drew, J. Hulcr, J. Leps, O. T. Lewis, R. Pokon, A. J. A. Stewart, G. A. Samuelson, and G. D. Weiblen. 2010. Guild-specific patterns of species richness and host specialization in plant-herbivore food webs from a tropical forest. *Journal of Animal Ecology* 79:1193–1203.
- Novotny, V., S. E. Miller, J. Hulcr, R. A. I. Drew, Y. Basset, M. Janda, G. P. Setliff, K. Darrow, A. J. A. Stewart, J. Auga, B. Isua, K. Molem, M. Manumbor, E. Tamtiai, M. Mogia, and G. D. Weiblen. 2007. Low beta diversity of herbivorous insects in tropical forests. *Nature* 448:692.
- NRCS, U. 2008. Geospatial data gateway.
- Ode, P. J. 2006. Plant Chemistry and Natural Enemy Fitness: Effects on Herbivore and Natural Enemy Interactions. *Annual Review of Entomology* 51:163–185.

- Ohgushi, T., T. P. Craig, and P. W. Price. 2007. Ecological communities: plant mediation in indirect interaction webs. Cambridge University Press.
- Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, H. Wagner, and others. 2015. Package "vegan." <http://CRAN.R-project.org/package=vegan>.
- Oliver, T., D. B. Roy, J. K. Hill, T. Brereton, and C. D. Thomas. 2010. Heterogeneous landscapes promote population stability. *Ecology Letters* 13:473–484.
- Paine, R. T. 1966. Food web complexity and species diversity. *American Naturalist*:65–75.
- Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421:37–42.
- Petanidou, T., A. S. Kallimanis, J. Tzanopoulos, S. P. Sgardelis, and J. D. Pantis. 2008. Long-term observation of a pollination network: fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization. *Ecology Letters* 11:564–575.
- Pfister, C., and F. Stevens. 2003. Individual variation and environmental stochasticity: implications for matrix model predictions. *Ecology* 84:496–510.
- Poethig, R. S. 1990. Phase change and the regulation of shoot morphogenesis in plants. *Science* 250:923.
- Poethig, R. S. 2003. Phase change and the regulation of developmental timing in plants. *Science* 301:334–336.
- Poisot, T., E. Canard, D. Mouillot, N. Mouquet, D. Gravel, and F. Jordan. 2012. The dissimilarity of species interaction networks. *Ecology Letters* 15:1353–61.

- Post, E., and M. C. Forchhammer. 2002. Synchronization of animal population dynamics by large-scale climate. *Nature* 420:168–171.
- Preisser, E. L., and D. R. Strong. 2004. Climate Affects Predator Control of an Herbivore Outbreak. *The American Naturalist* 163:754–762.
- Price, P. W., C. E. Bouton, P. Gross, B. A. McPheron, J. N. Thompson, and A. E. Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics* 11:41–65.
- Quintero, C., and M. D. Bowers. 2012. Changes in plant chemical defenses and nutritional quality as a function of ontogeny in *Plantago lanceolata* (Plantaginaceae). *Oecologia* 168:471–481.
- R Core Team. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Raffa, K. F., B. H. Aukema, B. J. Bentz, A. L. Carroll, J. A. Hicke, M. G. Turner, and W. H. Romme. 2008. Cross-scale drivers of natural disturbances prone to anthropogenic amplification: the dynamics of bark beetle eruptions. *Bioscience* 58:501–517.
- Raffaelli, D., W. H. van der Putten, L. Persson, D. A. Wardle, O. L. Petchey, J. Koricheva, M. van der Heijden, J. Mikola, and T. Kennedy. 2002. Multi-trophic dynamics and ecosystem processes.
- Ratajczak, Z., J. B. Nippert, and S. L. Collins. 2012. Woody encroachment decreases diversity across North American grasslands and savannas. *Ecology* 93:697–703.

- Ratnasingham, S., and P. D. Hebert. 2007. BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular ecology notes* 7:355–364.
- Reinkensmeyer, D. P., R. F. Miller, R. G. Anthony, V. E. Marr, and C. M. Duncan. 2008. Winter and early spring bird communities in grasslands, shrubsteppe, and juniper woodlands in central Oregon. *Western North American Naturalist* 68:25–35.
- Richards, L. A., L. A. Dyer, M. L. Forister, A. M. Smilanich, C. D. Dodson, M. D. Leonard, and C. S. Jeffrey. 2015. Phytochemical diversity drives plant–insect community diversity. *Proceedings of the National Academy of Sciences* 112:10973–10978.
- Richards, L. A., L. A. Dyer, A. M. Smilanich, and C. D. Dodson. 2010. Synergistic effects of amides from two *Piper* species on generalist and specialist herbivores. *Journal of Chemical Ecology* 36:1105–1113.
- Ricklefs, R., and D. Schluter. 1993. Species diversity in ecological communities: historical and geographical perspectives.
- Riihimäki, J., P. Kaitaniemi, J. Koricheva, and H. Vehviläinen. 2005. Testing the enemies hypothesis in forest stands: the important role of tree species composition. *Oecologia* 142:90–97.
- Risch, S. J., D. Andow, and M. A. Altieri. 1983. Agroecosystem diversity and pest control: data, tentative conclusions, and new research directions. *Environmental Entomology* 12:625–629.
- Roland, J., and S. F. Matter. 2012. Variability in winter climate and winter extremes reduces population growth of an alpine butterfly. *Ecology* 94:190–199.

- Romme, W. H., C. D. Allen, J. D. Bailey, W. L. Baker, B. T. Bestelmeyer, P. M. Brown, K. S. Eisenhart, M. L. Floyd, D. W. Huffman, and B. F. Jacobs. 2009. Historical and modern disturbance regimes, stand structures, and landscape dynamics in pinon-juniper vegetation of the western United States. *Rangeland Ecology & Management* 62:203–222.
- Root, R. B. 1973. Organization of a Plant-Arthropod Association in Simple and Diverse Habitats: The Fauna of Collards (*Brassica Oleracea*). *Ecological Monographs* 43:95–124.
- Rosenstock, S. S., and I. I. I. Charles Van Riper. 2001. Breeding bird responses to juniper woodland expansion. *Journal of Range Management*:226–232.
- Rosenthal, G. A., and M. R. Berenbaum. 2012. *Herbivores: their interactions with secondary plant metabolites: ecological and evolutionary processes*. Academic Press.
- Rosenzweig, M. L. 1968. Net primary productivity of terrestrial communities: prediction from climatological data. *American Naturalist*:67–74.
- Russell, E. P. 1989. Enemies hypothesis: a review of the effect of vegetational diversity on predatory insects and parasitoids. *Environmental Entomology* 18:590–599.
- Rzanny, M., and W. Voigt. 2012. Complexity of multitrophic interactions in a grassland ecosystem depends on plant species diversity: Plant diversity and multitrophic interactions. *Journal of Animal Ecology* 81:614–627.
- Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker,

- and D. H. Wall. 2000. Global biodiversity scenarios for the year 2100. *Science* 287:1770–1774.
- SAS. 2010. Version 9.4.
- Scheffer, M., S. Carpenter, J. A. Foley, C. Folke, and B. Walker. 2001. Catastrophic shifts in ecosystems. *Nature* 413:591–596.
- Scherber, C., N. Eisenhauer, W. W. Weisser, B. Schmid, W. Voigt, M. Fischer, E.-D. Schulze, C. Roscher, A. Weigelt, E. Allan, and others. 2010. Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature* 468:553–556.
- Schuldt, A., S. Both, H. Bruelheide, W. Härdtle, B. Schmid, H. Zhou, and T. Assmann. 2011. Predator Diversity and Abundance Provide Little Support for the Enemies Hypothesis in Forests of High Tree Diversity. *PLoS ONE* 6:e22905.
- Schweiger, O., J. Settele, O. Kudrna, S. Klotz, and I. Kühn. 2008. Climate change can cause spatial mismatch of trophically interacting species. *Ecology* 89:3472–3479.
- Shang, H., J. Yan, and X. Zhang. 2011. El Niño–Southern Oscillation influence on winter maximum daily precipitation in California in a spatial model. *Water Resources Research* 47:W11507.
- Sheehan, W. 1986. Response by specialist and generalist natural enemies to agroecosystem diversification: a selective review. *Environmental Entomology* 15:456–461.
- Sheehan, W., and A. M. Shelton. 1989. Parasitoid response to concentration of herbivore food plants: finding and leaving plants. *Ecology* 70:993–998.

- Shiojiri, K., and R. Karban. 2006. Plant age, communication, and resistance to herbivores: young sagebrush plants are better emitters and receivers. *Oecologia* 149:214–220.
- Smilanich, A. M., L. A. Dyer, J. Q. Chambers, and M. D. Bowers. 2009a. Immunological cost of chemical defence and the evolution of herbivore diet breadth. *Ecology Letters* 12:612–621.
- Smilanich, A. M., L. A. Dyer, and G. L. Gentry. 2009b. The insect immune response and other putative defenses as effective predictors of parasitism. *Ecology* 90:1434–1440.
- Srygley, R. B., R. Dudley, E. G. Oliveira, R. Aizprua, N. Z. Pelaez, and A. J. Riveros. 2010. El Niño and dry season rainfall influence hostplant phenology and an annual butterfly migration from Neotropical wet to dry forests. *Global Change Biology* 16:936–945.
- Srygley, R. B., R. Dudley, E. G. Oliveira, and A. J. Riveros. 2014. El Niño, Host Plant Growth, and Migratory Butterfly Abundance in a Changing Climate. *Biotropica* 46:90–97.
- Stacklies, W., H. Redestig, M. Scholz, D. Walther, and J. Selbig. 2007. *pcaMethods*—a bioconductor package providing PCA methods for incomplete data. *Bioinformatics* 23:1164–1167.
- Stenseth, N. C., G. Ottersen, J. W. Hurrell, A. Mysterud, M. Lima, K.-S. Chan, N. G. Yoccoz, and B. Adlandsvik. 2003. Studying climate effects on ecology through the use of climate indices: the North Atlantic Oscillation, El Niño Southern

- Oscillation and beyond. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270:2087–2096.
- Stewart, W. C., T. R. Whitney, E. J. Scholljegerdes, H. D. Naumann, N. M. Cherry, J. P. Muir, B. D. Lambert, J. W. Walker, R. P. Adams, K. D. Welch, D. R. Gardner, and R. E. Estell. 2015. Effects of species and stage of maturity on nutritional, in vitro digestibility, and plant secondary compound characteristics. *Journal of Animal Science* 93:4034–4047.
- Stireman, J., L. A. Dyer, and R. Matlock. 2005a. Top-down forces in managed versus unmanaged habitats. *Ecology of Predator-Prey Interactions*:303–323.
- Stireman, J. O., III, L. A. Dyer, D. H. Janzen, M. S. Singer, J. T. Li, R. J. Marquis, R. E. Ricklefs, G. L. Gentry, W. Hallwachs, P. D. Coley, J. A. Barone, H. F. Greeney, H. Connahs, P. Barbosa, H. C. Morais, and I. R. Diniz. 2005b. Climatic unpredictability and parasitism of caterpillars: Implications of global warming. *Proceedings of the National Academy of Sciences* 102:17384–17387.
- Stireman, J. O., and M. S. Singer. 2003. Determinants of parasitoid–host associations: insights from a natural tachinid–lepidopteran community. *Ecology* 84:296–310.
- Stoelinga, M. T., M. D. Albright, and C. F. Mass. 2010. A new look at snowpack trends in the Cascade Mountains. *Journal of Climate* 23:2473–2491.
- Strand, M. R. 2008. The insect cellular immune response. *Insect Science* 15:1–14.
- Suttle, K. B., M. A. Thomsen, and M. E. Power. 2007. Species interactions reverse grassland responses to changing climate. *Science* 315:640–642.
- Svoma, B. M. 2011. Trends in snow level elevation in the mountains of central Arizona. *International Journal of Climatology* 31:87–94.

- Tausch, R. J., R. F. Miller, and J. C. Chambers. 2009. Pinon and juniper field guide: asking the right questions to select appropriate management actions. US Geological Survey Reston, VA, USA.
- Tewksbury, J. J., J. G. Anderson, J. D. Bakker, T. J. Billo, P. W. Dunwiddie, M. J. Groom, S. E. Hampton, S. G. Herman, D. J. Levey, N. J. Machnicki, C. Martinez del Rio, M. E. Power, K. Rowell, A. K. Salomon, L. Stacey, S. C. Trombulak, and T. A. Wheeler. 2014. Natural history's place in science and society. *BioScience* 64:300–310.
- Thébault, E., and C. Fontaine. 2010. Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science* 329:853–856.
- Thompson, J. N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia experimentalis et applicata* 47:3–14.
- Thompson, J. N. 1996. Evolutionary ecology and the conservation of biodiversity. *Trends in Ecology and Evolution* 11:300–303.
- Thompson, J. N. 1997. Conserving interaction biodiversity. Pages 285–293 *The Ecological Basis of Conservation*. Springer.
- Thompson, M. T., and M. Toone. 2012. Estimating root collar diameter growth for multi-stem western woodland tree species on remeasured forest inventory and analysis plots.
- Tipping, M. E., and C. M. Bishop. 1999. Probabilistic Principal Component Analysis. *Journal of the Royal Statistical Society. Series B (Statistical Methodology)* 61:611–622.

- Turlings, T. C. J., and F. Wackers. 2004. Recruitment of predators and parasitoids by herbivore-injured plants. *Advances in insect chemical ecology* 2:21-75
- Tylianakis, J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. *Ecology Letters* 11:1351–1363.
- Tylianakis, J. M., R. K. Didham, and S. D. Wratten. 2004. Improved fitness of aphid parasitoids receiving resource subsidies. *Ecology* 85:658–666.
- Tylianakis, J. M., E. Laliberte, A. Nielsen, and J. Bascompte. 2010. Conservation of species interaction networks. *Biological Conservation* 143:2270–2279.
- Tylianakis, J. M., T. Tschardtke, and O. T. Lewis. 2007. Habitat modification alters the structure of tropical host–parasitoid food webs. *Nature* 445:202–205.
- Val, E. D., and R. Dirzo. 2003. Does ontogeny cause changes in the defensive strategies of the myrmecophyte *Cecropia peltata*? *Plant Ecology* 169:35–41.
- Valiente-Banuet, A., M. A. Aizen, J. M. Alcántara, J. Arroyo, A. Cocucci, M. Galetti, M. B. García, D. García, J. M. Gómez, P. Jordano, R. Medel, L. Navarro, J. R. Obeso, R. Oviedo, N. Ramírez, P. J. Rey, A. Traveset, M. Verdú, and R. Zamora. 2015. Beyond species loss: the extinction of ecological interactions in a changing world. *Functional Ecology* 29:299–307.
- Van Auken, O. W. 2000. Shrub invasions of North American semiarid grasslands. *Annual Review of Ecology and Systematics*:197–215.
- Van Auken, O. W. 2009. Causes and consequences of woody plant encroachment into western North American grasslands. *Journal of Environmental Management* 90:2931–2942.

- Van Bael, S. A., J. D. Brawn, and S. K. Robinson. 2003. Birds defend trees from herbivores in a Neotropical forest canopy. *Proceedings of the National Academy of Sciences* 100:8304–8307.
- Van Dam, N. M., and G. M. Poppy. 2007. Why plant volatile analysis needs bioinformatics—detecting signal from noise in increasingly complex profiles. *Plant Biology* 9:e12–e19.
- Vandenbosch, R. 2003. Fluctuations of *Vanessa cardui* butterfly abundance with El Niño and Pacific Decadal Oscillation climatic variables. *Global Change Biology* 9:785–790.
- Vargas, R. I., J. D. Stark, G. K. Uchida, and M. Purcell. 1993. Opiine parasitoids (Hymenoptera: Braconidae) of oriental fruit fly (Diptera: Tephritidae) on Kauai island, Hawaii: islandwide relative abundance and parasitism rates in wild and orchard guava habitats. *Environmental Entomology* 22:246–253.
- Vázquez, D. P., and M. A. Aizen. 2004. Asymmetric specialization: a pervasive feature of plant-pollinator interactions. *Ecology* 85:1251–1257.
- Vazquez, D. P., N. P. Chacoff, and L. Cagnolo. 2009. Evaluating multiple determinants of the structure of plant–animal mutualistic networks. *Ecology* 90:2039–2046.
- Vehviläinen, H., J. Koricheva, and K. Ruohomäki. 2007. Tree species diversity influences herbivore abundance and damage: meta-analysis of long-term forest experiments. *Oecologia* 152:287–298.
- Venables, W. N., and B. D. Ripley. 2013. *Modern applied statistics with S-PLUS*. Springer Science & Business Media.

- Vinson, S. B. 1976. Host Selection by Insect Parasitoids. *Annual Review of Entomology* 21:109–133.
- Violle, C., B. J. Enquist, B. J. McGill, L. Jiang, C. H. Albert, C. Hulshof, V. Jung, and J. Messier. 2012. The return of the variance: intraspecific variability in community ecology. *Trends in Ecology & Evolution* 27:244–252.
- Vitousek, P. M. 1994. Beyond global warming: ecology and global change. *Ecology* 75:1861–1876.
- Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of Earth's ecosystems. *Science* 277:494–499.
- Vogler, U., A. S. Rott, C. Gessler, and S. Dorn. 2009. Terpene-mediated parasitoid host location behavior on transgenic and classically bred apple genotypes. *Journal of Agricultural and Food Chemistry* 57:6630–6635.
- Wallace, A. R. 1878. *Tropical nature, and other essays*. Macmillan and Company.
- Wang, X.-G., and M. A. Keller. 2002. A comparison of the host-searching efficiency of two larval parasitoids of *Plutella xylostella*. *Ecological Entomology* 27:105–114.
- Wehrens, R., G. Weingart, and F. Mattivi. 2014. metaMS: An open-source pipeline for GC–MS-based untargeted metabolomics. *Journal of Chromatography B* 966:109–116.
- Weiher, E., and P. Keddy. 2001. *Ecological assembly rules: perspectives, advances, retreats*. Cambridge University Press.
- Weisberg, P., E. Lingua, and R. Pillai. 2007. Spatial patterns of pinyon-juniper woodland expansion in central Nevada. *Rangeland Ecology & Management* 60:115–124.

- Weisser, W. W. 1995. Within-patch foraging behaviour of the aphid parasitoid *Aphidius funebris*: plant architecture, host behaviour, and individual variation. *Entomologia experimentalis et applicata* 76:133–141.
- Wenninger, E. J., and R. S. Inouye. 2008. Insect community response to plant diversity and productivity in a sagebrush-steppe ecosystem. *Journal of Arid Environments* 72:24–33.
- Wilson, J. K., A. Kessler, and H. A. Woods. 2015. Noisy communication via airborne infochemicals. *BioScience* 65:667–677.
- Wilson, J. K., and H. A. Woods. 2016. Innate and Learned Olfactory Responses in a Wild Population of the Egg Parasitoid *Trichogramma* (Hymenoptera: Trichogrammatidae). *Journal of Insect Science* 16:110.
- Winemiller, K. O. 1989. Must connectance decrease with species richness? *The American Naturalist* 134:960–968.