

Mutualistic Networks Over Time: The Effects of Changing Floral Abundances on Plant-Pollinator Interactions

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Abstract

Plant-pollinator networks have been shown to have a general structure that is constant across time and geographic range. However, the identities of the plants and pollinators within these networks and the ways in which they interact are highly variable. We investigated a possible mechanism for this variability: niche breadth changes driven by changing levels of intraspecific competition. If intraspecific competition can partially explain changing network composition, we can use it to predict future ecosystem changes and help maintain ecosystem and species stability in a changing climate. We examined the effects of changing floral abundance on niche breadth throughout one growing season. We predicted that increasing floral abundance, accompanied by greater intraspecific competition, would increase plant species niche breadth above that expected due to increased abundance of inflorescences alone; probabilistically species will interact with the most abundant species the most even if abundance were the only factor. We found that the pollination networks in the sites investigated are all highly nested and found evidence that a species' niche breadth varies across sites (as predicted by the literature). The data do not currently support the claim that niche breadth increases with increasing abundance due to increased intraspecific competition; finer pollinator species identification may reveal further trends in the data. Once pollinators are identified to species, the analysis will be redone and the hypothesis revisited.

Introduction

In the last two decades, ecologists have begun to examine the macrostructure of communities. Ecologists have studied food webs (e.g. Dunne *et al.* 2002, Krause *et al.* 2003) and, more recently, mutualistic networks (such as plant-pollinator and seed dispersal networks) as methods of understanding community structure and the interactions that maintain it (Bascompte and Jordano 2007, Bascompte 2009, Blüthgen 2010). A network is broadly defined as the species in a community and the interactions between them. A range of structural properties can be defined from these interactions, including the number of links each species has to other species (degree distributions), the percentage of total possible links that exist in the network (connectance), and the identities of the species that interact (Bascompte and Jordano 2007, Bascompte 2009). Analyses of these whole-network properties informs conservation efforts by revealing the robustness of systems and the effects of factors such as phenology, perturbations, and climate on network structure and function (Tylianakis *et al.* 2008, Kaiser-Bunbury *et al.* 2010, Burkle and Alarcón 2011, Burkle and Knight 2012, Burkle *et. al.* 2013, Simanonok *et al.* 2014).

For plant-pollinator networks, various studies have found a relatively constant network structural property of *binary nestedness* (Bascompte *et. al.* 2003) over time and across geographic regions (Alarcón *et al.* 2008, Olesen *et al.* 2008, Bascompte 2009, Burkle and Alarcón 2011). A binary-nested network is one in which specialist species interact with a subset of the species with which generalist species interact. However, despite this relatively constant network structure, the species identities in a system exhibit significant turnover between years or seasons (Olesen *et al.*

2008, Petanidou *et al* 2008, Dupont *et al.* 2009, Burkle and Alarcón 2011). Simanonok *et al.* (2014) showed that much of the network turnover within a season could be explained by plant flowering phenology and the resulting temporal changes in resources for pollinators. However, little has been done to investigate the mechanisms through which plant phenology affects interactions within networks.

Plant phenology involves the change in floral abundance from growth to bloom to fruiting. Changing plant and pollinator abundances have also been shown to have significant effects on network structure in a seasonal and yearly analysis (Olesen *et al.* 2008, Alarcón *et al.* 2008, Burkle and Alarcón 2011, Burkle *et al.* 2013). However, an investigation of the effects of intra-season abundance changes on network structure is lacking.

One such potential mechanism is the relationship between competition and niche breadth. Intraspecific competition has been rigorously studied as leading to increased niche breadth both experimentally and theoretically (Rosenzweig 1991). The ability to assess food density greatly affects the effect of competition on niche breadth and habitat selection; the effects are moderated for species that can accurately assess food density (Rosenzweig 1991). Optimal-foraging theory also predicts conditions for competition stabilization in that species tend to choose the least time intensive and safer option in terms of habitat and foraging when at low population densities (Sih 1986). Intraspecific competition leads to specialization of the individuals in the species both in terms of habitat and diet, and increased niche breadth for the species overall (Diamond 1978, Rosenzweig 1991).

Plant abundances, and subsequently the levels of plant intraspecific competition, vary from year to year in many systems (Tillman 1999, Alarcón *et al.* 2008). It has been shown that as abundance of a pollinator species increases, there is more intraspecific competition that leads to niche broadening (Fontaine *et al.* 2008, Tur *et al.* 2014, Brosi 2016). If this occurs for plants as well, when a plant is more abundant or has higher floral density in a given year, it would have greater intraspecific competition and so a wider niche breadth; it would bring in more pollinators. A plant would also form different interactions throughout its phenology, acting as a more specialized species at low flower abundances (interacting with fewer species of pollinators) and acting as a more generalized species at high abundances (interacting with more species of pollinators) (Olesen and Jordano 2002, Dupont *et al.* 2003, Vazquez and Aizen 2003, Tylianakis *et al.* 2008, Blüthgen 2010). As species abundances vary all across the network, the network structure should remain binarily nested while the species composition and interactions change. The literature indicates that a species' level of generalization is not a static property of a species or may in some cases be a sampling artifact (Blüthgen 2010, Brosi 2016). Thus, the degree of generalization by both plant species and pollinator species should vary across both time and across geographic region (Petanidou *et al* 2008).

Here we analyzed the changes in niche breadth (NB) and Shannon's diversity index (S-DIV) for 10 plants species in three separate plant-pollinator networks to test whether increasing abundances (by increasing levels of intraspecific competition and so niche breadth) can explain part of the dynamic nature of species interactions while maintaining binary nestedness. We

predicted that plant species' niche breadth would increase along with floral abundance and so be greatest at full bloom (with the greatest intraspecific competition) and lower both before and after (with lower rates of intraspecific competition).

Materials and Methods

Study Sites

We looked at three study plots at high elevation in the Colorado Rocky Mountains. The plots are at least 1 km apart and are as follows: the southern edge of Maxfield Meadow ("MM", 9428 ft in elevation), a site near the Avery Picnic area in the upper Gothic River valley ("AP", 9733 ft in elevation), and a site in Virginia Basin ("VB", 11268 ft in elevation); see Fig.1. Each site was comprised of two rows of four 20m x 1m transects (i.e., eight total) separated by 2m. Transects were assigned to either insect or plant sampling in an alternating manner. The four plant-focused transects were used to assess plant abundance and diversity as well as visitation data.

Plant Species Under Focus

Though we collected data on the entire network, we focused on a few plant species per site that have periods of both high and low abundance within the period of data collection, have more than 15 interactions at high abundance and also have at least one day of data collection with niche breadth above three. We chose plants with at least one day with higher niche breadth values and several interactions because significant deviations from the expected values are difficult to detect at low niche breadth values and we wanted a reasonable sample size of interactions from which to derive the null model. In MM, the plant species of focus are *Delphinium barbeyi*, *Potentilla gracilis*, and *Linum lewisii*. At AP, the plant species of focus are *Delphinium barbeyi*, *Erigeron elatior*, *Hymenoxys hoopesii*, and *Potentilla gracilis*. At VB, the plants of focus are *Castilleja sulphurea*, *Linum lewisii*, *Potentilla gracilis*, *Senecio crassulus*, *Helianthella quinquinervis*, *Achillea millefolium*, and *Ligusticum porteri*. We examined *Potentilla gracilis* across all sites to assess the constancy of patterns we detect. The additional species that vary between study sites allow for breadth of investigation.

Collection of Abundance and Interaction Data

We collected data once a week for each site. We determined plant abundances by counting the number of flowers of each species in bloom in the plant-focused transects. We examined the changing abundances of floral resources of each plant species using simple counts (i.e. we assumed that the pollen and nectar loads are constant for each flower of a given species).

We collected interaction data by recording the pollination events that occurred, capturing the pollinator for identification and determining the plant species in the field. Butterfly species were identified and then released. *Bombus* and *Osmia* species were captured, marked for identification in the case of recapture, and then released. We observed each 2m x 1m section of the plant-focused transect for 3 total minutes (1 minute 30 seconds from each orientation, north

and south), stopping our timers each time we attempted to catch an observed pollinator. The gross ID of the pollinator species was recorded regardless of whether capture was successful and used for this preliminary data analysis. The gross ID categories were chosen for ease of identification, not consistent diversity within each category. Specimens will later be identified to species to allow for more accurate network analysis.

Analysis of Network Data

Each week of collection was analyzed with a different null model to capture the finer changes in floral abundance across the blooming season. We created a null model that predicts the plant species' niche breadth (NB) and Shannon's Diversity Index (S-DIV) using only abundance estimates from interaction data (the number of interactions a category of pollinators or plants had). We used a null model because the number of plant-pollinator interactions will increase with increasing plant abundance even without an increase in niche breadth – more abundant plants will interact with many pollinator species because there are more individuals available for interaction (Vázquez *et al.* 2009, Blüthgen 2010). The null model accounts for this baseline increase due to abundance alone. If NB patterns are significantly increased at high abundances relative to the null model via permutation tests, this will indicate that niche broadening may be occurring as abundance increases. The null model was programmed in R (code attached as Appendix B). The null model we created is based on holding both the row sums and the column sums of the matrix constant, i.e. the number of interactions per plant species and per pollinator species remained constant in each permutation of the null model.

We used permutation tests to assess significance, comparing the observed data value to 9,999 permutations of a null model specific to that week of data collection and site.

Results

All three of the sites had highly nested network structures (see Fig.2). MM had the highest nestedness with an average temperature of 0.01275 ± 0.01287 (standard deviation). The temperature for AP was 0.03065 ± 0.00703 and for VB it was 0.11930 ± 0.05325 (mean $\pm$ standard deviation for both). The specialist species thus interacted with a subset of those with which the generalist species interacted.

The data indicate variation in plant NB and S-DIV both across time and between sites. *Potentilla gracilis* had significantly different NB and S-DIV in different locations (see Table 10). *P. gracilis*' NB was significantly greater at VB than AP (1-way ANOVA, p-value: 0.0274) and S-DIV was significantly greater at VB than MM (1-way ANOVA, p-value: 0.00763). This indicates that though the overall network structure was constant, any particular plant's placement within the network was not. In contrast to our predictions, at high abundance NB and S-DIV were significantly or marginally significantly *below* those predicted by the null model for *P. gracilis* in two of the three sites (VB, NB $P = 0.015$ and 0.0002 , S-DIV $P = 0.0002$; MM, NB $P = 0.0602$) and were not significant in the remaining (AP) site (see Tables 9 and 13).

The interaction data were inconclusive as to the effect of intraspecific competition on NB or S-DIV. We categorized floral abundances as either low (less than 50), medium (between 50 and 150) and high (150 or above). The greatest number of deviations from the expected were at high abundance, where 27.3% of the plants had at least one sample day's NB significantly greater than that predicted by the null model (as in Fig.4 B) and 9.1% had NB significantly lower than predicted (as in Fig.4 A). For S-DIV, 27.3% of plants had at least one day's above the predicted, and 27.3% had below the predicted. Though there was deviation from the expected, especially for S-DIV at high abundances, there was no consistent correlation between abundance and the direction of said deviation.

Discussion

There is a gap in our understanding of the mechanisms that drive network structure and maintain its constancy across ecosystems. Our study of niche breadth and intraspecific competition supported the nestedness of networks with significant variation in the NB and S-DIV. The data do not, however, support the hypothesis that intraspecific competition's effects on niche breadth can explain the variation in plant NB and S-DIV across a flowering season. One challenge is that, at low niche breadth values, it is difficult (if not impossible) to detect NB or S-DIV significantly below the expected because there is a bottom limit to both those values. As shown in Fig. 3, the distributions for both NB and S-DIV are constraining at low NB and tend to be dominated by the lowest possible value (1 and 0 respectively). Therefore these data cannot be used to test to see if niche breadth is significantly lower than expected at low abundances due to a lack of intraspecific competition unless the plant has a reasonably high niche breadth, even at low abundances. There is a data-driven limit to our understanding of the network at low abundances.

At medium and high abundances, the data are inconclusive. At medium floral abundances, there are more plants that have NB values below the expected than above and the same is true of S-DIV. However, the majority of plants are still well within the expected ranges. The same is true at high floral abundances. The data preliminarily indicate that both NB and S-DIV are somehow limited such that they are below that expected by abundance alone. One possible explanation is that there are forbidden links or interactions that are not possible due to morphological or phenological mismatches; however the literature indicates that this effect is not significant (Vázquez 2005, Petanidou *et al* 2008).

The pollinator data are also currently organized by gross ID, rather than by species or functional groups. These groups were defined for ease of identification in the field and do not contain equal species diversity. This skews the interaction data by making the niche breadth for those that interact with the larger gross ID categories (such as "Other Fly") lower than they are in reality. Significantly higher than expected niche breadths may currently be masked within the gross ID categories. Once the captured pollinators are identified to species, the analyses will be redone to test for significantly higher niche breadths than expected at high abundance.

It is also possible that the relationship between intraspecific competition and niche breadth does not explain the variation in network composition across time. Other factors such as changing climactic conditions may explain more of the variation in plant NB and S-DIV across geographic ranges and across time (Devoto *et al.* 2005, Alarcón *et al.* 2008, Tylianakis *et al.* 2008, Gonzales *et al.* 2009). Further studies should investigate other possible mechanisms for changing network composition within a constant structure and should also look at other, non-alpine environments.

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Appendix A

Tables and Figures:

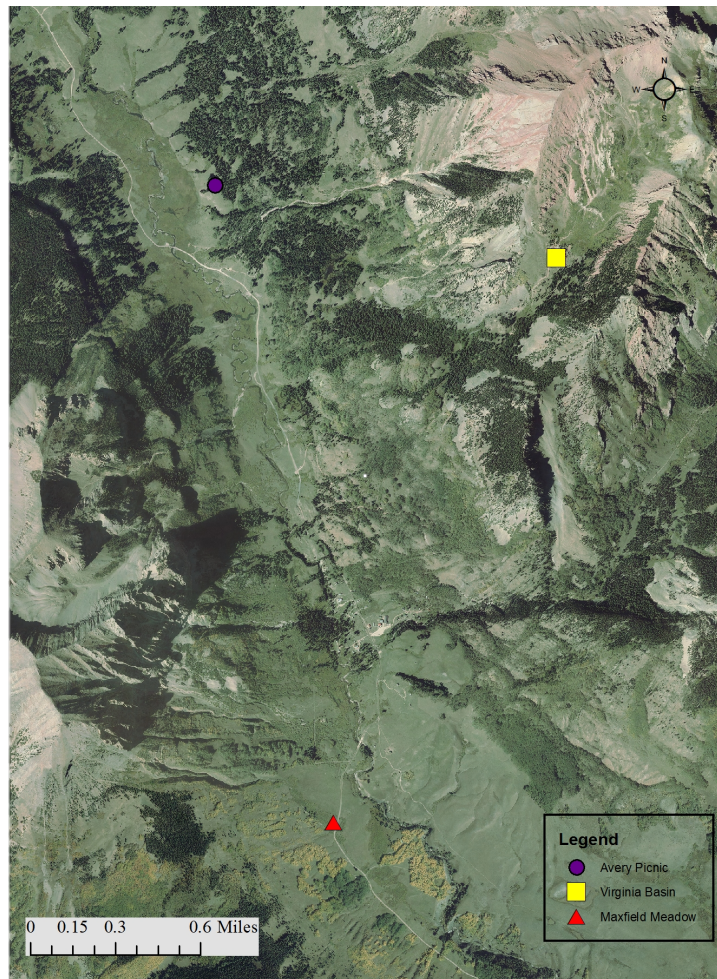


Fig.1: Map of Research Sites: Maxfield Meadow (9428ft), Avery Picnic (9733 ft) and Virginia Basin (11268ft). They are at least one kilometer apart to try and access separate pollinator population pools.

For Tables 1 – 9, the text colors correspond to different classifications of floral abundance. Low [0, 50) = **Blue**; Medium [50 , 150) = **Purple**; High [150, infinity) = **Red**.

* Indicates that the data are significantly above that expected by the null model (p-values below 0.025)

* Indicates that the data are significantly below that expected by the null model (p-values above 0.975)

⁺ Indicates that the value was the highest in the 10,000 replicates (9999 from the null model and one from the experimental data)

⁺⁺ Indicates that all 10,000 values were the same

Table 1: Floral Abundance and Number of Interactions for Focus Plants at Maxfield Meadow Site

Plants	7/6/16		7/12/16		7/20/16		7/25/16	
	Floral Count	Interactions	Floral Count	Interactions	Floral Count	Interactions	Floral Count	Interactions
<i>Delphinium barbeyi</i>	8	1	155	14	723	60	891	39
<i>Potentilla gracilis</i>	89	81	123	35	326	25	169	180
<i>Linum lewisii</i>	151	95	64	44	41	12	14	13

Table 2: Niche Breadth and Shannon's Diversity Index for Focus Plants at Maxfield Meadow Site

Plants	7/6/16		7/12/16		7/20/16		7/25/16	
	Niche Breadth	Shannon Diversity	Niche Breadth	Shannon Diversity	Niche Breadth	Shannon Diversity	Niche Breadth	Shannon Diversity
<i>Delphinium barbeyi</i>	1	0 ⁺⁺	4*	1.09130*	3	1.01339	6	1.12262
<i>Potentilla gracilis</i>	5	0.82408*	2	0.45814	4	0.86227	8	1.02664
<i>Linum lewisii</i>	3*	0.11670*	2	0.18491	2	0.28684*	2	0.27119*
Nestedness (Temperature)	0.03105		0.00235		0.00542		0.01216	

Table 3: P-Values for Niche Breadth and Shannon's Diversity Index for Focus Plants at Maxfield Meadow Site

Plants	7/6/16		7/12/16		7/20/16		7/25/16	
	Niche Breadth P-value	Shannon Diversity P-value	Niche Breadth P-value	Shannon Diversity P-value	Niche Breadth P-value	Shannon Diversity P-value	Niche Breadth P-value	Shannon Diversity P-value
<i>Delphinium barbeyi</i>	0 ⁺⁺	0 ⁺⁺	0.0006*	0.0005*	0.9439	0.9154	0.1844	0.3316
<i>Potentilla gracilis</i>	0.6578	0.0130*	0.5843	0.7012	0.2145	0.9446	0.811	0.9699
<i>Linum lewisii</i>	0.9944 *	0.9999*	0.6989	0.9989*	0.8959	0.9995*	0.8649	0.9974*

Table 4: Floral Abundance and Number of Interactions for Focus Plants at Avery Picnic Site

Plants	7/11/16		7/16/16		7/21/16		7/27/16	
	Floral Count	Interactions	Floral Count	Interactions	Floral Count	Interactions	Floral Count	Interactions
<i>Delphinium barbeyi</i>	408	29	578	22	208	24	56	11
<i>Erigeron elatior</i>	22	13	68	44	154	46	241	137
<i>Hymenoxys hoopesii</i>	193	94	213	88	190	79	139	88
<i>Potentilla gracilis</i>	30	24	90	17	46	2	33	3

Table 5: Niche Breadth and Shannon's Diversity Index for Focus Plants at Avery Picnic Site

Plants	7/11/16		7/16/16		7/21/16		7/27/16	
	Niche Breadth	Shannon Diversity	Niche Breadth	Shannon Diversity	Niche Breadth	Shannon Diversity	Niche Breadth	Shannon Diversity
<i>Delphinium barbeyi</i>	4	0.83735	3	0.57638	4	1.053878	2	0.58595
<i>Erigeron elatior</i>	2	0.66628	3	0.68709	3	0.78064	7	0.91639
<i>Hymenoxys hoopesii</i>	3	0.68866	4	0.74526	5	0.86966	7	0.95563
<i>Potentilla gracilis</i>	3	0.79238	3	0.75297	1	0.00000	2	0.63651
Nestedness (Temperature)	0.02042		0.03235		0.03593		0.03446	

Table 6: P-Values for Niche Breadth and Shannon's Diversity Index for Focus Plants at Avery Picnic Site

Plants	7/11/16		7/16/16		7/21/16		7/27/16	
	Niche Breadth P-value	Shannon Diversity P-value	Niche Breadth P-value	Shannon Diversity P-value	Niche Breadth P-value	Shannon Diversity P-value	Niche Breadth P-value	Shannon Diversity P-value
<i>Delphinium barbeyi</i>	0.0718	0.2220	0.2609	0.8393	0.1215	0.1193	0.5841	0.8575
<i>Erigeron elatior</i>	0.4000	0.4685	0.6096	0.7296	0.7556	0.8095	0.843	0.9283
<i>Hymenoxys hoopesii</i>	0.9013	0.8964	0.7797	0.7597	0.6397	0.7124	0.349	0.6511
<i>Potentilla gracilis</i>	0.2246	0.3068	0.1645	0.3634	0.5533	0.5533	0.1108	0.1108

Table 7: Floral Abundance and Number of Interactions for Focus Plants at Virginia Basin Site

Plants	7/7/16		7/15/16		7/23/16		7/28/16	
	Floral Count	Interactions	Floral Count	Interactions	Floral Count	Interactions	Floral Count	Interactions
<i>Castilleja sulphurea</i>	137	3	206	3	227	15	201	6
<i>Linum lewisii</i>	35	2	157	8	113	54	28	1
<i>Potentilla gracilis</i>	97	74	448	128	598	325	530	229
<i>Senecio crassulus</i>	24	4	335	52	663	219	270	20
<i>Helianthella quinquenervis</i>	1	0	31	8	126	48	119	13
<i>Achillea millefolium</i>	15	0	177	2	913	34	1223	35
<i>Ligusticum porteri</i>	2	9	45	37	73	156	91	87

Table 8: Niche Breadth and Shannon's Diversity Index for Focus Plants at Virginia Basin Site

Plants	7/7/16		7/15/16		7/23/16		7/28/16	
	Niche Breadth	Shannon Diversity	Niche Breadth	Shannon Diversity	Niche Breadth	Shannon Diversity	Niche Breadth	Shannon Diversity
<i>Castilleja sulphurea</i>	2	0.63651	2	0.63651	6*	1.41418*	4*	1.32966*
<i>Linum lewisii</i>	1	0	2	0.56234	2	0.09222*	1	0
<i>Potentilla gracilis</i>	3*	0.19546*	8	0.41748*	7*	0.27981*	5*	0.24653*
<i>Senecio crassulus</i>	1	0	6	0.60370	8	0.26930*	6*	1.34731*
<i>Helianthella quinquenervis</i>	0	0	6*	1.66746*	6	0.98468*	4	1.03177
<i>Achillea millefolium</i>	0	0	1	0	3	0.35460	4	0.38668
<i>Ligusticum porteri</i>	3	0.68374	4	0.80024	5	0.63673	7	0.93746
Nestedness (Temperature)	0.03946		0.14450		0.14792		0.14533	

Table 9: P-Values for Niche Breadth and Shannon's Diversity Index for Focus Plants at Virginia Basin Site

Plants	7/7/16		7/15/16		7/23/16		7/28/16	
	Niche Breadth P-value	Shannon Diversity P-value	Niche Breadth P-value	Shannon Diversity P-value	Niche Breadth P-value	Shannon Diversity P-value	Niche Breadth P-value	Shannon Diversity P-value
<i>Castilleja sulphurea</i>	0.0646	0.0646	0.0744	0.0744	0.0004*	0.0000*+	0.0025*	0.0025*
<i>Linum lewisii</i>	0.3352	0.3352	0.4188	0.4264	0.9623	0.9781*	0 ⁺⁺	0 ⁺⁺
<i>Potentilla gracilis</i>	0.9997*	0.9999*	0.9735	0.9999*	0.9925*	0.9999*	0.9999*	0.9999*
<i>Senecio crassulus</i>	0.5707	0.5707	0.6089	0.8434	0.7136	0.9986*	0.0121*	0.0029*
<i>Helianthella quinquenervis</i>	0 ⁺⁺	0 ⁺⁺	0.0000*+	0.0005*	0.0678	0.0011*	0.0621	0.0669
<i>Achillea millefolium</i>	0 ⁺⁺	0 ⁺⁺	0.3383	0.3383	0.5522	0.5979	0.6190	0.8278
<i>Ligusticum porteri</i>	0.1362	0.2254	0.7895	0.4256	0.9685	0.0739	0.6512	0.0404

Table 10: Niche Breadth and Shannon's Diversity Index for *Potentilla gracilis* Across Sites

Location	Niche Breadth Mean	Niche Breadth Standard Deviation	Shannon's Diversity Index	Shannon's Diversity Index Standard Deviation
Maxfield Meadow	4.75	2.50	0.79278*	0.23978
Avery Picnic	2.25*	0.96	0.54546	0.36962
Virginia Basin	5.75*	2.22	0.28482*	0.09500

*Indicates significant difference across sites

Potentilla gracilis was used as an example of the change in species NB and S-DIV across sites since it satisfied all plant-choice criteria in all three sites.

Table 11: Percentage of Total Plants and Observations with Niche Breadth and Shannon's Diversity Index Significantly Deviating from that Predicted by the Null Model

Abundance Level	Niche Breadth				Shannon's Diversity Index			
	Lower		Higher		Lower		Higher	
	Plants	Datasets	Plants	Datasets	Plants	Datasets	Plants	Datasets
Low	0%	0%	10%	6.3%	10%	12.5%	20%	12.5%
Medium	9.1%	7.6%	0%	0%	27.3%	23.1%	9.1%	7.7%
High	9.1%	11.1%	27.3%	14.8%	27.3%*	18.5%	27.3%*	14.8%

* Indicates that one plant is in both categories (has one day at high abundance with niche breadth below predicted and one with it above predicted, for example).

Percentages for plants are based upon the number of plants with at least one day at the given abundance level. Percentages for datasets are based upon the sum of the number of days for each plant at the given abundance level.

Table 12: Significant Deviations Above the Expected Values for Niche Breadth and Shannon's Diversity Index

Value-Type	Location	Plant Species	Date	Value	P-Value	Abundance Category
NB	Maxfield Meadow	<i>Delphinium barbeyi</i>	7/12/16	4	0.0012	High
NB	Virginia Basin	<i>Castilleja sulphurea</i>	7/23/16	6	0.0008	High
NB	Virginia Basin	<i>Castilleja sulphurea</i>	7/28/16	4	0.0050	High
NB	Virginia Basin	<i>Senecio crassulus</i>	7/28/16	6	0.0242	High
NB	Virginia Basin	<i>Helianthella quinquinervis</i>	7/15/16	6	< 0.0001*	Low
S-DIV	Maxfield Meadow	<i>Potentilla gracilis</i>	7/6/16	0.82408	0.0260	Low
S-DIV	Maxfield Meadow	<i>Delphinium barbeyi</i>	7/12/16	1.09130	0.0010	High
S-DIV	Virginia Basin	<i>Castilleja sulphurea</i>	7/23/16	1.41418	< 0.0001*	High
S-DIV	Virginia Basin	<i>Castilleja sulphurea</i>	7/28/16	1.32966	0.0050	High
S-DIV	Virginia Basin	<i>Senecio crassulus</i>	7/28/16	1.24731	0.0058	High
S-DIV	Virginia Basin	<i>Helianthella quinquinervis</i>	7/15/16	1.66746	0.0010	Low
S-DIV	Virginia Basin	<i>Helianthella quinquinervis</i>	7/23/16	0.98468	0.0022	Medium

*Was the lowest value of 10,000 analyzed

Table 13: Significant Deviations Below the Expected Values for Niche Breadth and Shannon's Diversity Index

Value-Type	Location	Plant Species	Date	Value	P-Value	Abundance Category
NB	Maxfield Meadow	<i>Linum lewisii</i>	7/6/16	3	0.1120	High
NB	Virginia Basin	<i>Potentilla gracilis</i>	7/7/16	3	0.0006	Medium
NB	Virginia Basin	<i>Potentilla gracilis</i>	7/23/16	7	0.0150	High
NB	Virginia Basin	<i>Potentilla gracilis</i>	7/28/16	5	0.0002	High
S-DIV	Maxfield Meadow	<i>Linum lewisii</i>	7/6/16	0.11670	0.0002	High
S-DIV	Maxfield Meadow	<i>Linum lewisii</i>	7/20/16	0.28684	0.0010	Low
S-DIV	Maxfield Meadow	<i>Linum lewisii</i>	7/25/16	0.27119	0.0052	Low
S-DIV	Virginia Basin	<i>Linum lewisii</i>	7/23/16	0.09222	0.0438	Medium
S-DIV	Virginia Basin	<i>Potentilla gracilis</i>	7/7/16	0.19546	0.0002	Medium
S-DIV	Virginia Basin	<i>Potentilla gracilis</i>	7/15/16	0.41748	0.0002	High
S-DIV	Virginia Basin	<i>Potentilla gracilis</i>	7/23/16	0.27981	0.0002	High
S-DIV	Virginia Basin	<i>Potentilla gracilis</i>	7/28/16	0.24853	0.0002	High

P-values have been converted such that any below 0.05 are considered statistically significant

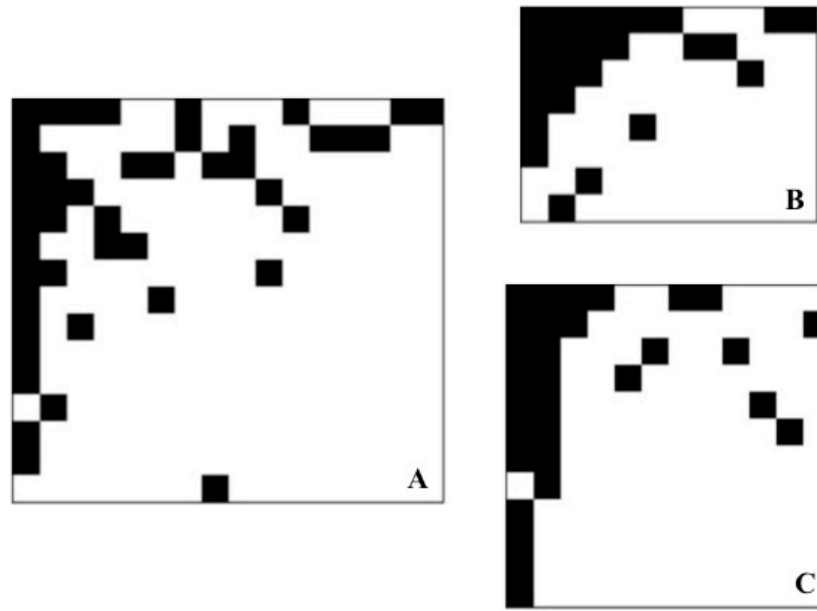


Fig.2: Representations of the nestedness of the networks at the three sites: Virginia Basin (A), Maxfield Meadow (B) and Avery Picnic (C). Each square represents one interaction pair with each row representing a plant species and each column representing a pollinator species. The darkened boxes represent pairs with observed interactions and white boxes represent pairs without observed interactions. The images are representations of the interaction matrix derived from experimental data for the days with the nestedness values closest to the mean for each site (A: 7/15/16, B: 7/25/16, C:7/16/16) not including the plants and pollinators with no observed interactions. For highly nested networks, the specialist species interact with a subset of species with which the generalists interact. As such, the top left corner is dark (generalist plants and generalist pollinators) with that continuing along the top (generalist plant) and left sides (generalist pollinator) of the matrix. The bottom right corner is white in highly nested networks since it represents the interactions between more specialized species.

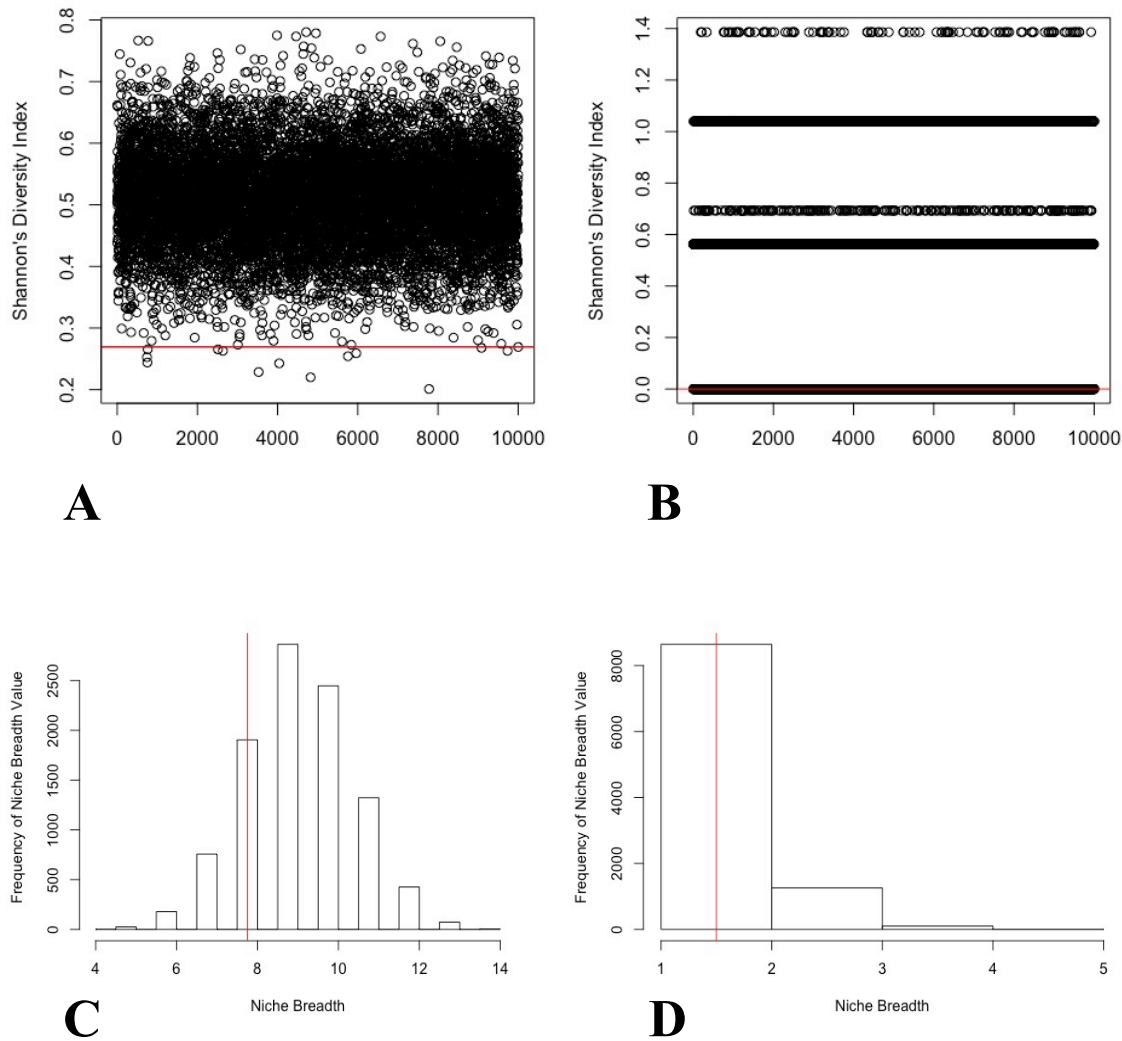


Fig.3: Shannon's Diversity Index and Niche Breadth Distribution at Low and High Niche Breadth for *Senecio crassulus* (A and C: 7/23/16 and B and D: 7/7/16). The vertical and horizontal red lines represent the position of the experimental value within the distribution of those generated by the null model. At a low niche breadth of 1, most of the null model simulated niche breadths were also 1 and so a significantly lower than expected niche breadth would be impossible to detect (D). The same is visible in Shannon's Diversity Index at low niche breadth where the values are clustered, the most popular being a value of 0, the same as the experimental value (B). Once again, it is not possible for the experimental value to be significantly lower than expected. At higher niche breadth values, there is a distribution is more spread out and so allows for the detection of significantly lower than expected scores for both niche breadth (C) and Shannon's Diversity Index (A).

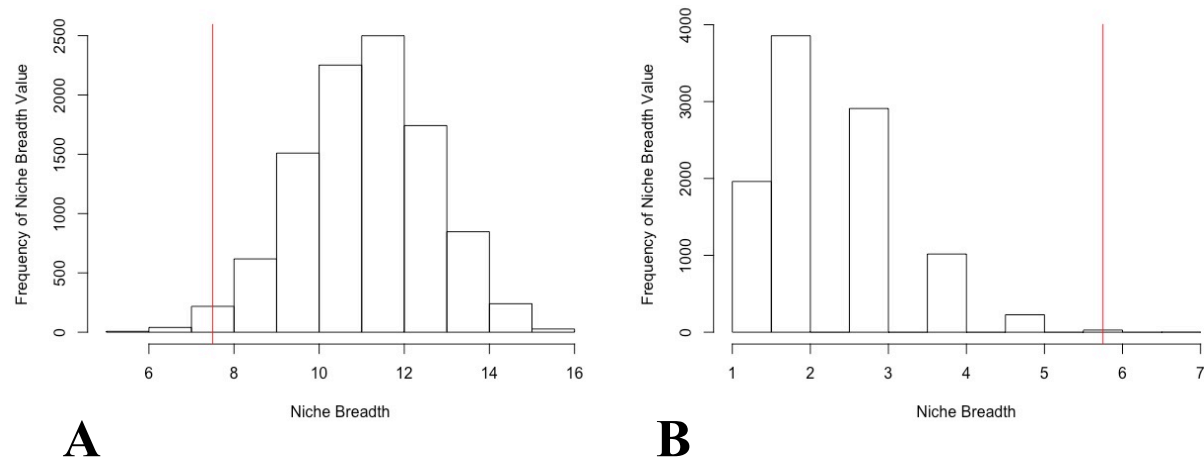


Fig.4: Sample niche breadth distributions for A: lower than expected niche breadth (*Potentilla gracilis* on 7/15/16 at VB) and B: higher than expected niche breadth (*Castilleja sulphurea* on 7/23/16 at VB). The vertical red line represents the placement of the experimental data within those generated by the null model.

Appendix B

Null Model Function

```
swap_matrix = function(matrix_data, pl_of_interest, num_loops=9999){  
  #Inputs:  
  #pl_of_interest is a list of the names of the focus plants to be examined  
  #matrix_data is the interaction matrix, including a total for total number of interactions  
  #and niche breadth for each plant and pollinator.  
  #num_loops is the number of iterations or simulations the model should run. Defaults to 9999  
  
  #Outputs  
  #results_list is a list that contains two vectors, niche_breadth and s_div. The former contains all  
  #the niche breadth values for the simulated matrices and for the experimental interaction matrix  
  #and the later contains all the Shannon's Diversity Index Values.  
  
  #Take the values from the matrix  
  cols = length(matrix_data[1,])  
  rows = length(matrix_data[,1])  
  plants = levels(matrix_data[,1])  
  total_ind = match("Total", plants)  
  niche_breadth_ind = match("Num_Spp_Int", plants)  
  plants = plants[-total_ind][-niche_breadth_ind]  
  pollin = names(matrix_data)  
  pollin = pollin[2:(cols)] #Get rid of the empty first column  
  total_obs = matrix_data[rows-1, cols-1]  
  
  #Create the lists that will store niche breadth and the diversity index  
  shan_div = matrix(0L, nrow =(length(pl_of_interest)), ncol= num_loops + 1)  
  niche_breadth = matrix(0L, nrow =(length(pl_of_interest)), ncol= num_loops + 1)  
  
  #Run the null model  
  for (rep in 1:num_loops){  
    #Create interaction matrix  
    plant_tots = data.matrix(matrix_data[1:(rows - 2), cols - 1])  
    pollin_tots = data.matrix(matrix_data[rows - 1, 2:(cols - 2)])  
    int_matrix = matrix(0L, nrow =(length(plants) + 2), ncol= (length(pollin)))  
    plant_data = rep("", total_obs)  
    pollin_data = rep("", total_obs)  
  
    tot_col = cols - 2  
    tot_row = rows - 1
```

#Create Plant List

```
pl_lp_ind = 0
tot_loop_pl = 0
for (pl in plant_tots) {
  pl_lp_ind = pl_lp_ind + 1
  if (pl == 0){
    next
  }
  for (loop in 1:pl){
    tot_loop_pl = tot_loop_pl + 1
    plant_data[tot_loop_pl] = plants[pl_lp_ind]
    #append(plant_data, plants[pl_lp_ind])
  }
}
```

#Create Pollinator List

```
poll_lp_ind = 0
tot_loop_pol = 0
for (poll in pollin_tots){
  poll_lp_ind = poll_lp_ind + 1
  if (poll == 0){
    next
  }
  for (loop_2 in 1:poll){
    tot_loop_pol = tot_loop_pol + 1
    pollin_data[tot_loop_pol] = pollin[poll_lp_ind]
  }
}
```

#Assignment List

```
decision_list = sample(1:total_obs, total_obs, replace=F)
```

```

#Randomize Matrix
choice_ind = 0
for (p in plant_data){
  choice_ind = choice_ind + 1
  if (choice_ind > total_obs){
    break
  }
  pl_ind = match(p, plants)
  choice = decision_list[choice_ind]
  i = pollin_data[choice]
  ins_ind = match(i, pollin)

  if (int_matrix[pl_ind, ins_ind] == 0) {
    int_matrix[tot_row + 1, ins_ind] = int_matrix[tot_row + 1, ins_ind] + 1
    int_matrix[pl_ind, tot_col + 1] = int_matrix[pl_ind, tot_col + 1] + 1
  }
  int_matrix[pl_ind, ins_ind] = int_matrix[pl_ind, ins_ind] + 1
  int_matrix[tot_row, tot_col] = int_matrix[tot_row, tot_col] + 1

#For debugging purposes/to show errors
int_matrix[tot_row, ins_ind] = int_matrix[tot_row, ins_ind] + 1
int_matrix[pl_ind, tot_col] = int_matrix[pl_ind, tot_col] + 1
}

#Calculate Shannon's Diversity Index
s_div = diversity(int_matrix[1:length(plants), 1:(length(pollin) - 2)], index = "shannon",
MARGIN = 1, base = exp(1))

#Store Niche Breadth and Diversity Index
for (item in 1:length(s_div)) {
  if (plants[item] %in% pl_of_interest) {
    plt_ind = match(plants[item], pl_of_interest)
    shan_div[plt_ind, rep] = s_div[item]
  }
}

for (plt in pl_of_interest){
  plt_ind_int = match(plt, plants)
  plt_ind_niche = match(plt, pl_of_interest)
  niche_breadth[plt_ind_niche, rep] = int_matrix[plt_ind_int, tot_col + 1]
}
}

```

```

#Shannon's Diversity For the Experimental Data
s_div_exp = diversity(matrix_data[1:(rows-2),2:(cols-2)], index = "shannon", MARGIN = 1,
base = exp(1))
for (item in 1:length(s_div_exp)) {
  if (plants[item] %in% pl_of_interest) {
    plt_ind = match(plants[item], pl_of_interest)
    shan_div[plt_ind, num_loops + 1] = s_div_exp[[item]]
  }
}

#Niche Breadth for Experimental Data
plt_ind_niche = 0
for (plt in pl_of_interest){
  plt_ind_niche = plt_ind_niche + 1
  plt_ind_int = match(plt, plants)
  niche_breadth[plt_ind_niche, num_loops + 1] = matrix_data[(plt_ind_int), cols]
}

#Store Results in a list and return
results_list = list("Niche_Breadth" = niche_breadth, "Shannons_Div" = shan_div)
return(results_list)
}

```