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Consequences of the Megafauna Extinction: Changes in Food Web Networks on the Edwards Plateau Across the Pleistocene-Holocene Transition

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CONSEQUENCES OF THE MEGAFAUNA EXTINCTION: CHANGES IN FOOD WEB NETWORKS ON THE EDWARDS PLATEAU ACROSS THE PLEISTOCENE-HOLOCENE TRANSITION

by

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CHANGES IN FOOD WEB NETWORKS ON THE EDWARDS PLATEAU ACROSS THE PLEISTOCENE-HOLOCENE TRANSITION

Quentin A. Smith Jr., M.S.
University of Nebraska, 2024

Advisors: S. Kathleen Lyons and Daizaburo Shizuka

We are experiencing biodiversity loss due to climate change and human impacts, which is not only harmful to the environment but can also alter the composition of communities and interactions among species. The late Pleistocene experienced a loss of large-bodied mammals which resulted in significant changes in community structure due to changes in body size, diet, and species associations. The impact of these changes on species interactions and community structure across the Pleistocene-Holocene transition remains poorly understood. Using a robust data set of species composition, stable isotopes, body size, and climate variables, we constructed and compared ecological networks of mammal paleocommunities on the Edwards Plateau, Texas. One site, Hall's Cave, has a temporal resolution that allows an in-depth analysis by separating the last 22,000 years into 16 time intervals. We measured the structure of food webs using modularity, and species contributions to modularity. Additionally for Hall's Cave, we measured an index of node overlap and segregation for each time interval. We find consistent decreases in modularity across all sites with food webs shifting from having multiple clusters in the Pleistocene to fewer clusters in the Holocene. The less complex network post extinction is largely attributed to the loss of particular species interactions.

In Hall’s Cave, we find node overlap increases and modularity decreases over time. Spearman-Rank correlation analyses indicate that changes in modularity were not driven by changes in species richness or climate change. The degree of node overlap shifted across the Pleistocene-Holocene transition and was significantly different from null model expectations in the Holocene but not in the Pleistocene. These results suggest that the transition from a modular network to a network of less complexity with an overlap of interacting species may have been driven by other factors that changed the food web, such as species composition. Thus, the change in mammal food web structure of Hall’s Cave was mainly a consequence of the megafauna extinctions.

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Lastly, I would like to thank my family for always being supportive and encouraging on my journey! You have all constantly motivated me to learn more and to keep chasing after goals. Your excitement to learn about my academic and research career has and will continue to be guiding light. To my friends, thank you for always being there to share thoughts, support, and laughs.

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CHANGES IN MAMMALIAN NETWORKS ACROSS THE PLEISTOCENE-HOLOCENE TRANSITION AS A RESULT OF THE MEGAFAUNA EXTINCTION

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Current climate and human impacts have the potential to fundamentally alter the composition of mammal communities and the interactions of their members. The megafauna extinctions of the Late Pleistocene represent an analogous period of mammal community disruption which may provide insight into how modern communities will respond to global anthropogenic change. The loss of megafauna at the terminal Pleistocene (~13 kya) resulted in distinct changes in body size distributions, diet, and species associations; however, the impact of these changes on species interactions remains poorly understood. Ecological networks are a representation of the interactions between species within a community and can be used to measure properties such as how communities are partitioned into clusters of interacting species (modularity), and how densely species are connected by consumer-resource interactions (connectedness). Past work has linked network connectedness to community resilience and modularity to ecological complexity. Using a robust data set of community composition, stable isotope information, and body size, we constructed and compared ecological networks of mammal paleocommunities across 9 sites in the Edwards Plateau, Texas before and after the Pleistocene-Holocene transition. We find consistent increases in connectedness and decreases in modularity across all sites with food webs shifting from having multiple clusters in the Pleistocene to typically one cluster in the Holocene. Examination of

individual contributions to modularity shows that the loss of a few key species has large effects in most sites, *Canis lupus* and sometimes *Panthera onca* have the largest contribution to the change in empirical modularity. These results suggest that the shift from a meso-predator to an apex predator by these species significantly impacted mammalian community structure and species interactions. Counterintuitively, the observed decrease in modularity and increase in connectedness imply an increase in resilience of the network after the extinction but comes at the expense of ecological complexity.

Introduction:

Current climate change and human impacts are altering the composition of communities and may fundamentally alter the interactions of their members. During Earth's history, assemblages of mammals have changed dramatically from periods of diversification and high diversity to periods of mass extinction and low diversity (Smith *et al.* 2010). The megafaunal extinctions of the Late Pleistocene represent a period of mammal community disruption which may provide insight into how modern communities will respond to global anthropogenic change. Fifty thousand years ago, continents were populated with more than 150 genera (374 species) of megafauna (animals >44 kg) (Smith *et al.* 2003). By 12,000 years ago, at least 97 of those genera (175 species) were gone (Barnosky *et al.* 2004; Lyons *et al.* 2004; Smith *et al.* 2018). Hypotheses that have been proposed to explain this extinction include human hunting and other impacts (Martin 1973), climate change (Graham and Grimm 1990, Graham and Lundelius 1984), disease (McPhee and Marx 1997), and a bolide impact (Firestone *et al.* 2011), and much effort has been aimed at determining the relative contributions of each potential driver on the extinction event (Lyons *et al.* 2004; Koch and Barnosky 2006; Barnosky *et al.* 2009). Regardless of the causes of the megafaunal extinction, it is clear that mammalian communities underwent dramatic changes at the terminal Pleistocene such as altered species associations (Lyons *et al.* 2016; Toth *et al.* 2019), diversity, function, and interactions (Barnosky *et al.* 2004; Lyons *et al.* 2004; Koch and Barnosky 2006; Lyons *et al.* 2019; Smith *et al.* 2019; Smith *et al.* 2023) such that modern mammal community structure is likely distinct from extinct mammal communities.

During the Late Pleistocene 53 species of large mammals inhabited North America. However by 12,000 years ago, 76%-80% of these species had gone extinct (Martin 1967; Martin and Klein 1984; Barnosky *et al.* 2004; Lyons *et al.* 2004; Smith *et al.* 2016; Smith *et al.* 2018). The extinction of megafauna could have had major effects on the structure of mammalian communities, given their potentially important roles in their ecosystems. Modern megafauna are ecosystem engineers and are essential to their communities because they help maintain their habitats and facilitate interactions between species (Janzen and Martin 1982; Owen-Smith 1992; Smith *et al.* 2016). Large herbivores, omnivores, and carnivores each play a role in a community and provide different ecosystem services. Herbivores can be key determinants of grassland plant species composition and diversity through foraging and the movement of nutrients throughout the ecosystem (Dublin *et al.* 1990; Owen-Smith 1992; Bakker *et al.* 2006; Goheen *et al.* 2010; Goheen *et al.* 2018; Doughty *et al.* 2016). For example, African elephants help maintain open conditions in savannah by suppressing woody regeneration and create grassy openings in forests and thickets simply by roaming around (Owen-Smith 1992; Goheen *et al.* 2010; Goheen *et al.* 2018). Rhino grazing maintains short-grass lawns within mosaics of tussock grass and thickets, which impede the spread of fire thus protecting woody patches (Owen-Smith 1999). Grazing by bison maintains high species diversity in tallgrass prairie (Knapp *et al.* 1999). Carnivores in mammalian communities can also have impacts on the structure and diversity of communities they inhabit (Owen-Smith 1988, Estes *et al.* 2011). They are often categorized as either apex predators or meso-predators. Apex predators typically benefit ecosystems through top–down control of meso-predators (often omnivorous) and herbivores, which ultimately influences the abundance and behavior of other apex predators and meso-predators (Estes *et al.* 2011, Brook *et al.* 2012).

Extinct megafauna would likely have performed similar roles in communities during the late Pleistocene. Losing key species, in this case, megafauna, has been shown to cause a cascade of effects such as, shifts in predator diets (Smith *et al.* 2022), and changes in mammalian body size distributions over time (Lyons *et al.* 2004; Lyons and Smith 2013; Smith *et al.* 2019; Smith *et al.* 2023). Thus, their loss would have altered plant community composition (Gill *et al.* 2009), seed dispersal (Janzen and Martin 1982; Pires *et al.* 2018), rates and patterns of nutrient cycling (Doughty *et al.* 2016), affected soil compaction and thus water tables and the composition and the identity of burrowing animals (Smith *et al.* 2023) in their communities. Moreover, their loss led to significant changes in mammalian ecological networks that were less resilient to future extinctions (Fricke *et al.* 2022; Pires *et al.* 2015; Nenzen *et al.* 2014).

Ecological network analysis is a powerful approach to study how the mammalian community has changed due to changes in community composition. Ecological networks are a representation of the interactions between species within a community and can quantify potential interactions based on a combination of body size, abundance, diet and other traits of species in a community (Montoya *et al.* 2006; Fortuna *et al.* 2010; Bascompte 2010; Delmas et. al. 2018). Analyses of food webs as ecological networks have revealed important function and structure of trophic relationships (e.g. predator-prey interactions) (Dunne *et al.* 2002; Pascual and Dunne 2006; Montoya *et al.* 2006; Ings *et al.* 2009). One important structural property of food webs is compartmentalization, which measures the degree to which groups of species are more likely to interact with each other rather than interacting with other individuals or groups in the food web (May 1972, Girvan and Newman 2002, Krause *et al.* 2003). The degree of compartmentalization of a food web, often measured by a metric called modularity (Newman and Girvan 2004, Guimerà *et al.* 2010) has been shown to have important impacts on the stability and dynamics of food webs (May 1972; Pimm 1979; Pimm and Lawton 1980; Yodzis 1982; Girvan and Newman 2002; Krause *et al.* 2003; Allesina and Pascual 2009; Rezende *et al.* 2009). Thus, changes in the compartmentalization of the mammal communities could not only inform us on how modern mammal communities differ from their ancient relatives, but also provide insight into which extinct taxa were essential to maintaining a stable network and help predict how modern communities will respond to global anthropogenic change.

Here, we address how mammalian food web networks for 9 sites on the Edwards Plateau of Texas changed after the megafauna extinctions across the Pleistocene-Holocene transition. Specifically, we quantify the modularity of mammalian food web networks before and after extinction. We also calculate the contribution to modularity for each species in the network before and after extinction to determine species roles in the overall observed network modularity.

Methods:

Study Sites

The Edwards Plateau is a region in Texas that contains many fossiliferous cave sites that are the focus of our study (**Fig. 1.1**). The Texas Memorial Museum at the

University of Texas contains archived fossil data collected from many sites from the Edwards Plateau. To assemble our mammal communities at each site, we used fossil data from 9 well sampled sites in the Edwards Plateau. All sites included in the study record a full assemblage of mammals with multiple trophic groups and all have a record encompassing the extinction event.

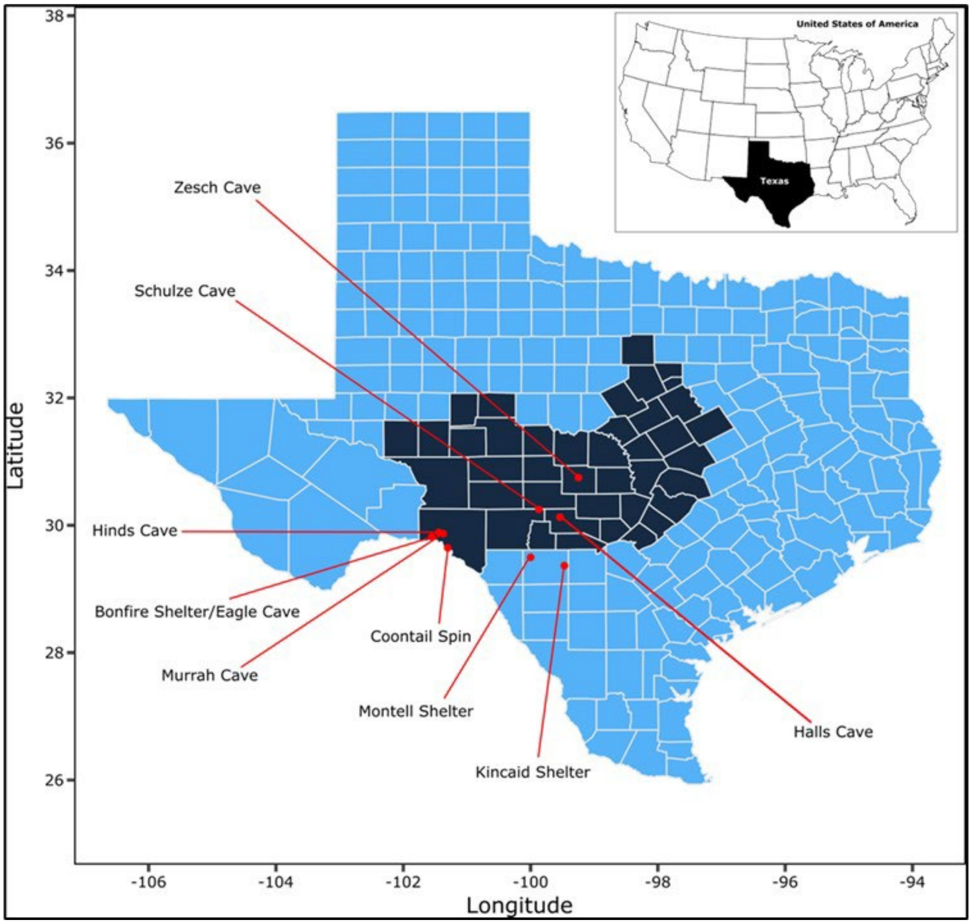

Figure 1.1: ***Map of Texas and 9 sites****. The Edwards Plateau in dark navy blue. All 9 sites are indicated in red.*

Preparing Food Web Networks

We used a robust data set of species composition, stable isotope information (Smith *et al.* 2022; Tomé *et al.* 2020; Tomé *et al.* 2022), and body size (Smith *et al.*

2022; Smith *et al.* 2003) to construct and compare ecological networks of mammal paleocommunities across 9 sites in the Edwards Plateau, Texas before and after the Pleistocene-Holocene transition. Networks are constructed from a matrix of diet and body size data that infers what each species is likely to eat. Inferences about trophic interactions were made directly by comparing the stable isotope values of primary and secondary consumers (Smith *et al.* 2022; Tomé *et al.* 2020, Tomé *et al.* 2022). For species for whom stable isotope information is not available, we inferred diet from species accounts and other primary literature. Dietary inferences for predators were further refined by using the expected scaling relationships between the body size of predators and their prey (Carbone *et al.* 1999). For example, if an apex- or meso-predator has $\delta^{13}C$ values similar to that of a putative prey species, and their body size scaling relationship is such that the predator could survive on that prey species, that interaction is assumed to be one that could occur in the network. From the matrix, we created a food web network of each Pleistocene and Holocene site. We use the R package “igraph” (Csardi G and Nepusz T 2006), to construct directed food web networks for each site in the Pleistocene and Holocene.

Quantifying Food Webs

To observe how the food web network structure changed across the Pleistocene-Holocene transition, we quantify the network modularity before and after extinction. Modularity measures how communities are partitioned into clusters of interacting species. While there are many algorithms to calculate modularity that take different approaches to the computational problem of finding partitions in the network, many of them are not suited for directed networks (i.e., networks in which relationships are asymmetric–as food webs are). Here, we use a simplified method that can handle directed networks: the *community detection algorithm based on edge betweenness*. This method is described in one of the earliest papers on modularity, by Newman & Girvan (2004). This method identifies clusters, or ‘compartments’ as partitions of the network that maximize the number of edges that link nodes within the same compartment and minimize the number of edges between compartments. We implemented this approach on directed networks including primary producers in the R package “igraph” (Csardi G and Nepusz T 2006).

We used simulated removals of each species to measure their relative contribution to the observed network structure. Such simulated removal methods have been used to resolve species roles in the stability of food webs (Dunne *et al.* 2002; Staniczenko *et al.* 2010). Here, we calculate an index of the ‘Effect of Removal’ = (ΔM_{-i}) to demonstrate individual species contributions to the empirical modularity of each network across the 9 sites. This is calculated this by subtracting the network's empirical modularity from the recalculated value of modularity of the network after a species node has been removed. Thus,

$$\Delta M_{-i} = M_{-i} - M$$

where M is the modularity of the empirical network and M_{-i} the modularity of the network after removing species i. Thus, a positive effect of removal indicates that removing that species would result in an increase in modularity. In other words, a species with positive effect of removal is a species that tends to connect compartments of the food web that would otherwise be weakly connected (**Fig. 1.2**). In contrast, species with a negative effect of removal decreases the network's modularity when removed (**Fig. 1.2**). This is likely to occur when a species is connected only within its own compartment, thus removing the species reduces the number of connections within compartments but does not affect the number of connections between compartments. The effect of removal on modularity for each species in all networks where they were present, and these values were compared between time periods across all sites, between trophic groups for Pleistocene and Holocene networks.

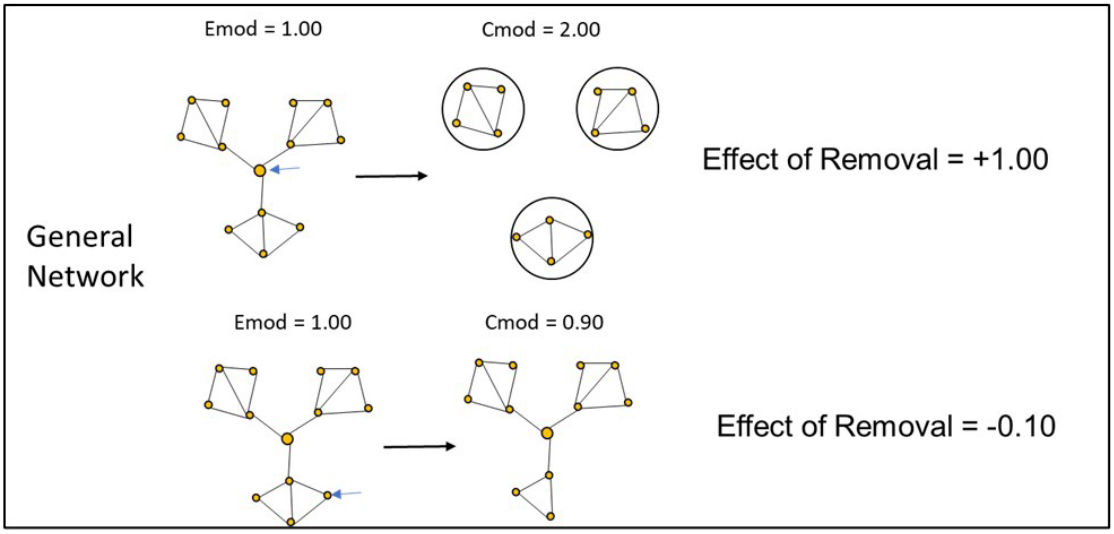

Figure 1.2: ***A conceptual model of modularity 'Effect of Removal'.*** *A positive effect of removal means an increase in modularity occurred after that species node was removed and vice-versa. The blue arrow indicates the species node that is being removed in a simulation.*

We used a Generalized Linear Mixed-Effects Model to model the effects of multiple variables on the extinctions that occurred at the terminal Pleistocene. Generalized Linear Mixed-Effects Models allow us to model the dependency of a variable given a fixed or random effect under varying assumptions (Field, Miles, and Field 2012). For our model, we use a logistic regression for binary data: (1) A Response Variable = species presence (extant = 1, extinct = 0), (2) Three Predictor Variables = Mass of each species, Degree (i.e, the number of species an individual is interacting with), and their ΔM_{-i} values, (3) A Random Variable = The Site.

Results:

Changes in food web structure from Pleistocene to Holocene

Food Web Structure - The food web networks illustrated differences in structure across the 9 sites (**Fig. 1.3**). Pleistocene food webs were more spread out showing distinct areas of compartmentalization of different species and between respective trophic groups. Values of modularity showed significant differences between the Pleistocene and Holocene networks with modularity significantly higher in Pleistocene networks (**Fig. 1.4**) (Wilcoxon Signed-Rank Test: p-value = 0.0039).

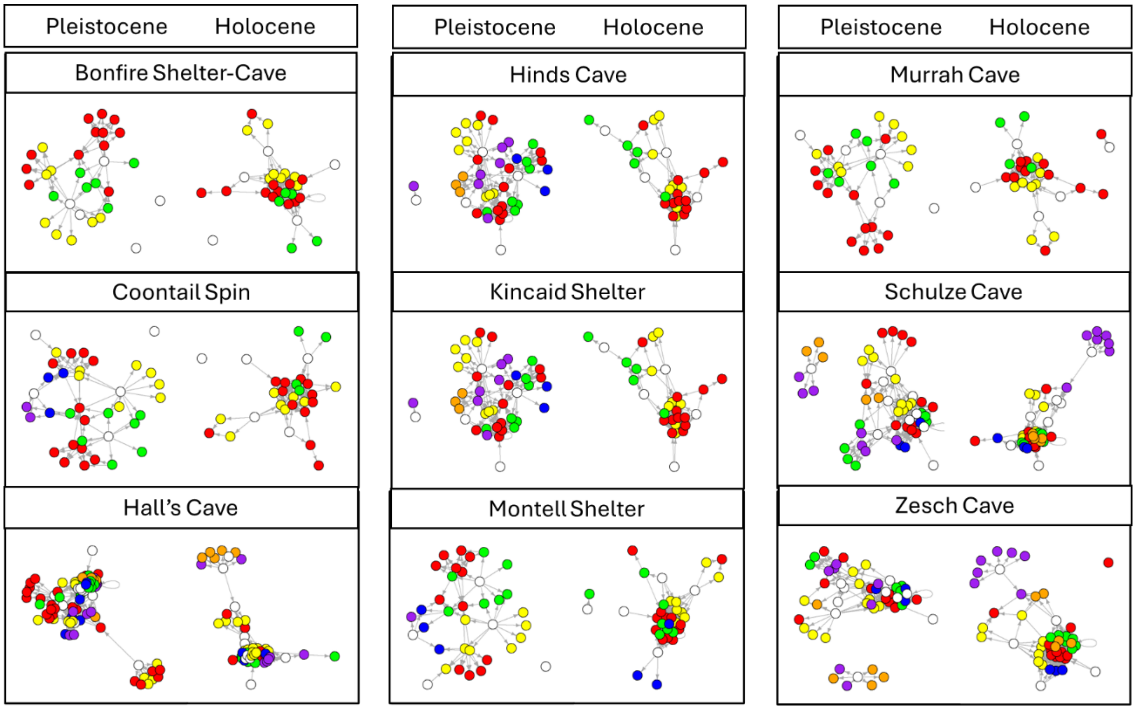

Figure 1.3: ***Pleistocene and Holocene food web networks.*** *Nodes are color coded by trophic groups: browsers (yellow), grazers (blue), carnivores (red), mixed feeders (brown), omnivores (orange), frugivores/granivores (green), insectivores (purple), primary resources (white).*

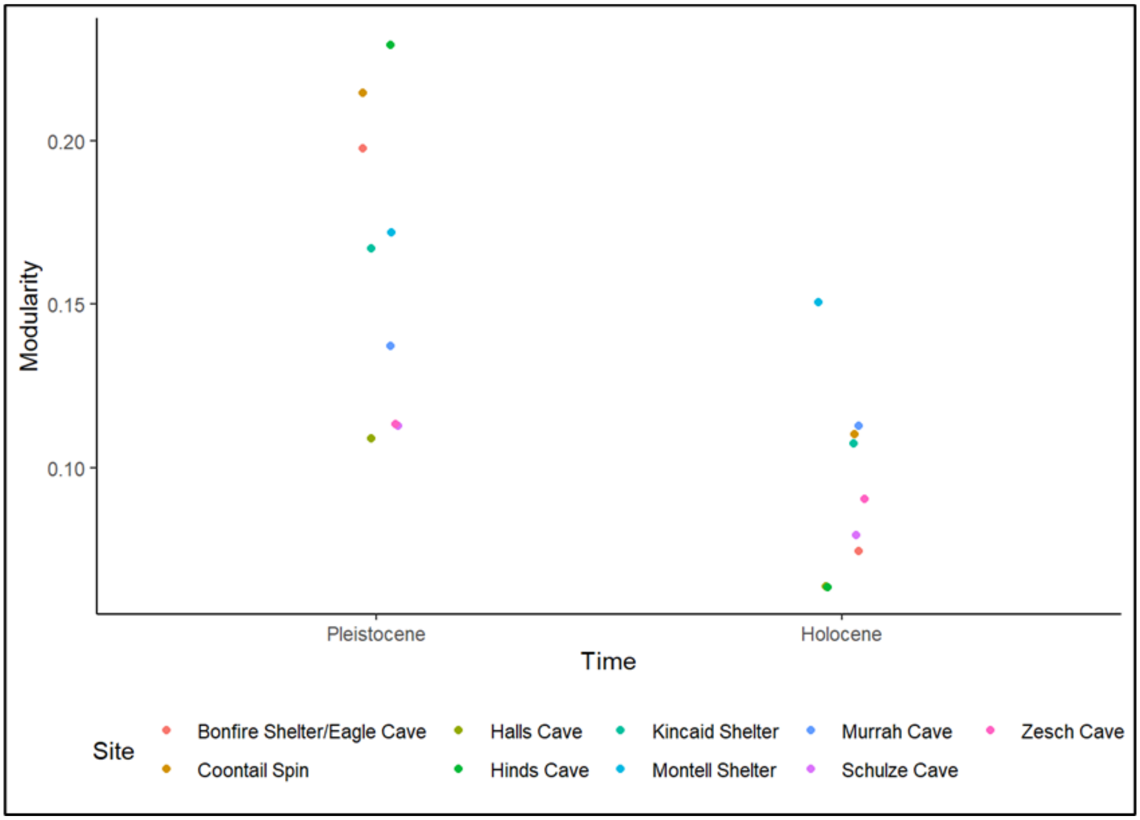

Figure 1.4: ***Empirical modularity across the 9 sites for the Holocene and Pleistocene.***

Species influence on food web structure

Effect of Removal - We represent the effect of removal on modularity (ΔM_{-i}) across all 9 sites for both the Pleistocene and Holocene (**Fig. 1.5,1.6**). The Pleistocene networks typically yielded a modular structure with one species (*Canis lupus*) with a highly positive effect of removal (**Fig. 1.5**). Most sites had multiple species with a weak negative effect of removal (**Fig. 1.5,1.6**). Interestingly, the structure of the Holocene networks typically had multiple species with positive and negative effects of removal suggesting that networks had little structure to disrupt by a single species loss (**Fig. 1.6**). Comparison of the distributions of the effects of removal shows the Holocene with a large concentration of species with a contribution to modularity near zero compared to the Pleistocene with fewer species contributions to modularity near zero and a few strong outliers (**Fig. 1.7**). The highly positive contribution to modularity values in the Pleistocene arises from *Canis lupus* (0.31-0.12) across 6 sites. On the low end, C3 plants make up the higher negative area of the Pleistocene (-0.09, -0.05).

The effect of removal for the different trophic groups was compared between the Pleistocene and Holocene (**Fig. 1.8**). The Pleistocene and Holocene were significantly different in their effects of removal for all trophic groups (Kolmogorov-Smirnov Test: $P < 0.03$ aside from carnivores, $P = 0.08$) and mixed feeders (**Fig. 1.8**). However, the direction of the difference was not consistent among trophic groups. In some cases, values were higher for the Pleistocene (e.g., grazers, frugivores, omnivores), whereas in others, values were lower for the Pleistocene (e.g., browsers, insectivores). Carnivores have a large distribution of the effect of removal on modularity due to many outliers, mainly consisting of *Canis lupus* in the Pleistocene and many different species in the Holocene. We see a complete extinction of mixed feeders across all 9 sites as there were none in the Holocene to calculate the effects of removal. Comparisons between trophic groups by their presence (extant/survivor) or absence (extinct/extirpated) show similar distributions of effect of removal (**Fig. 1.9**). For some trophic groups, surviving species had higher values than extinct and extirpated (e.g. carnivores, browsers, frugivores, granivores). All insectivores were survivors across all 9 sites, therefore there were no extinct species to compare effects of removal. Mixed feeders underwent complete extinction across the 9 sites, thus, there were no extant species to compare effects of removal. Only grazers had lower values of effect of removal for survivors compared to the extinct species.

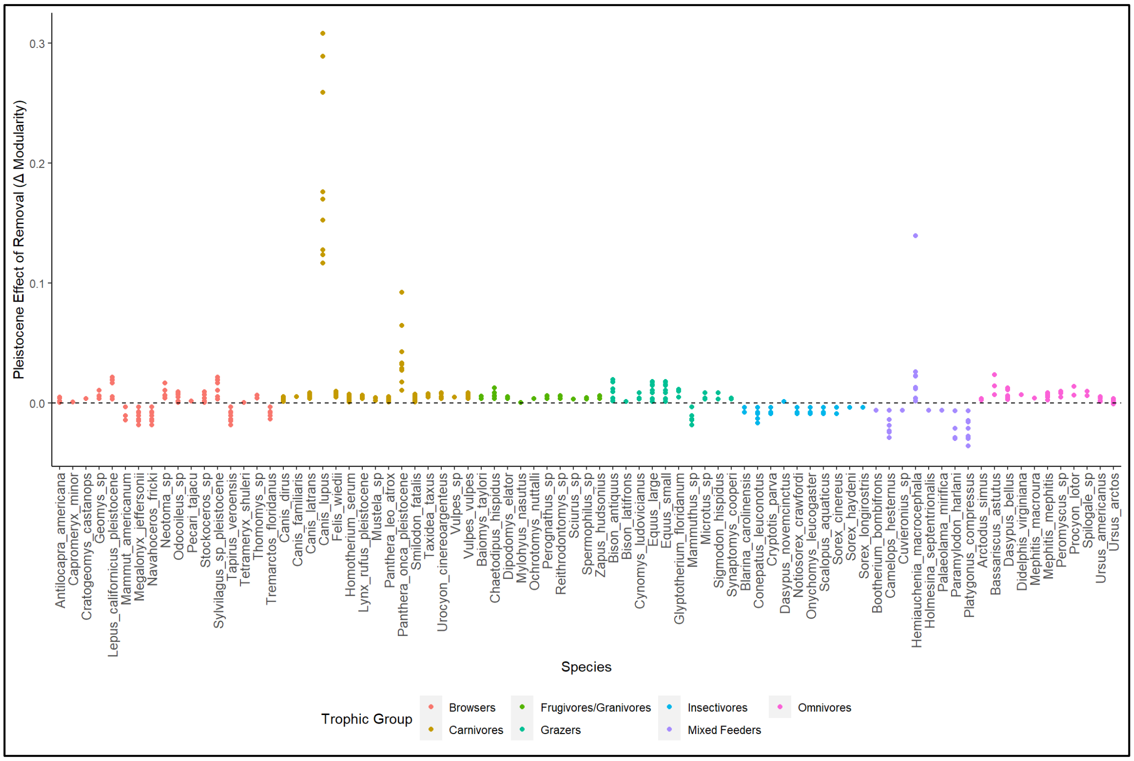

Figure 1.5: ***Pleistocene species modularity across the 9 sites.*** *Each color represents the species trophic group: browsers (red), carnivores (yellow), frugivores/granivores (lime green), grazers (turquoise), insectivores (blue), mixed feeders (purple), omnivores (pink).*

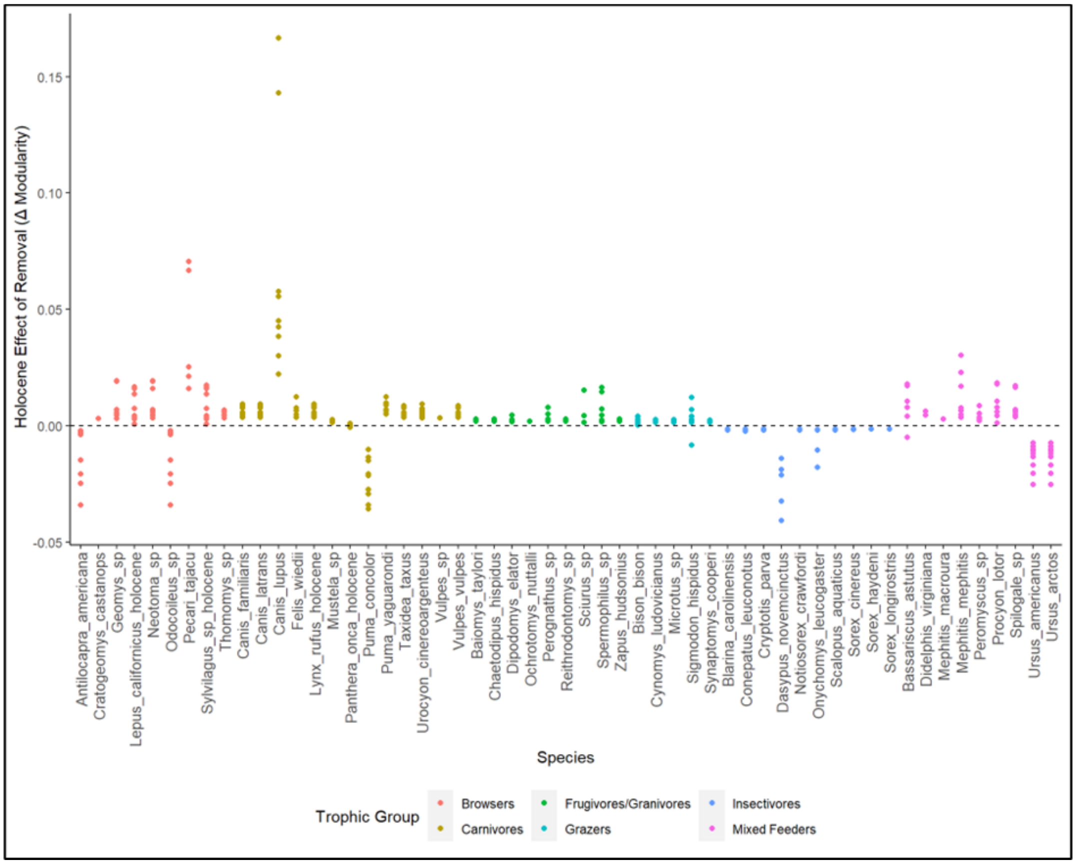

Figure 1.6: ***Holocene species modularity across the 9 sites.*** *Each color represents the species trophic group: browsers (red), carnivores (yellow), frugivores/granivores (lime green), grazers*

(turquoise), insectivores (blue), mixed feeders (purple), omnivores (pink).

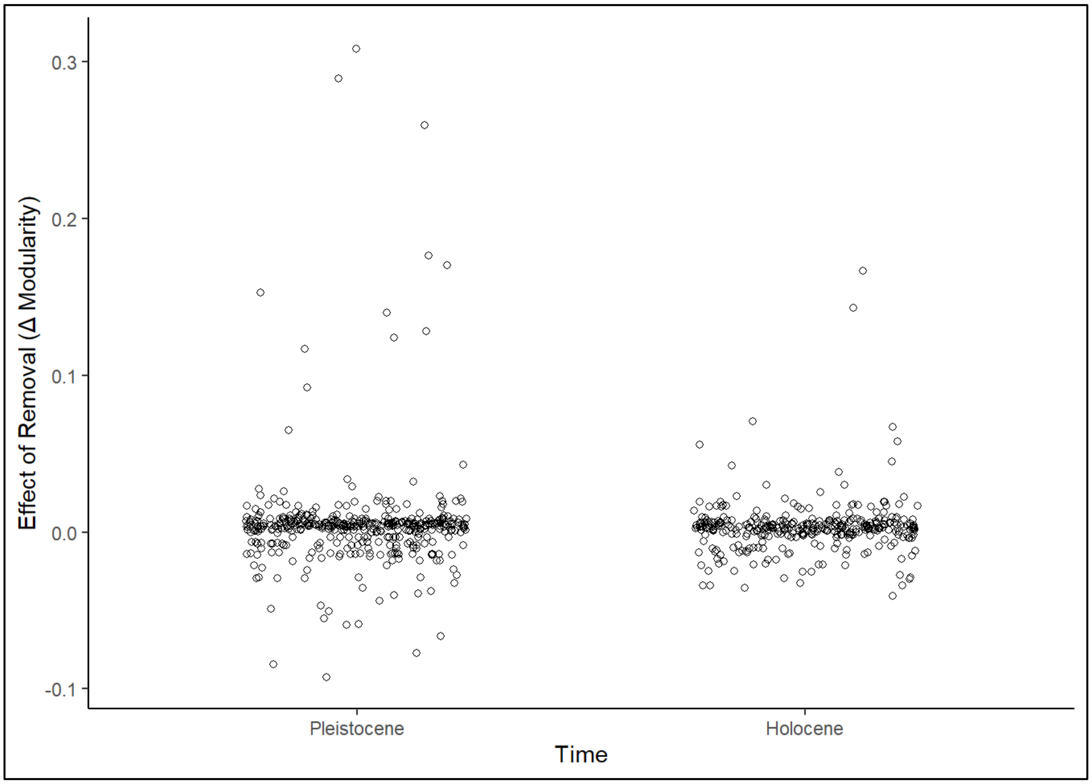

Figure 1.7: ***Change in modularity across all species and sites between the Holocene and Pleistocene.***

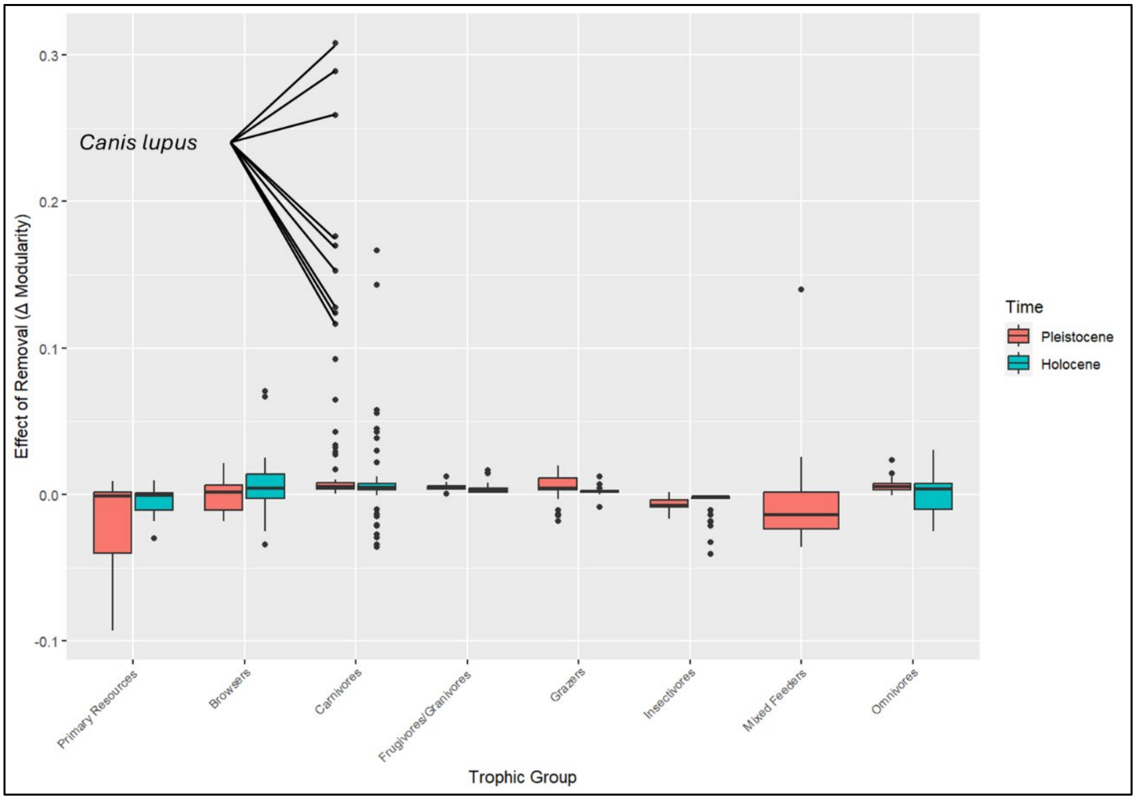

Figure 1.8: ***Comparison of the effect of removal for the Pleistocene (red box plots) and Holocene (blue box plots) by trophic level****. Nine out of the top ten values for the effect of removal on modularity are attributed to Canis lupus (across the 9 sites). There are no mixed feeders in any of the 9 sites in the Holocene.*

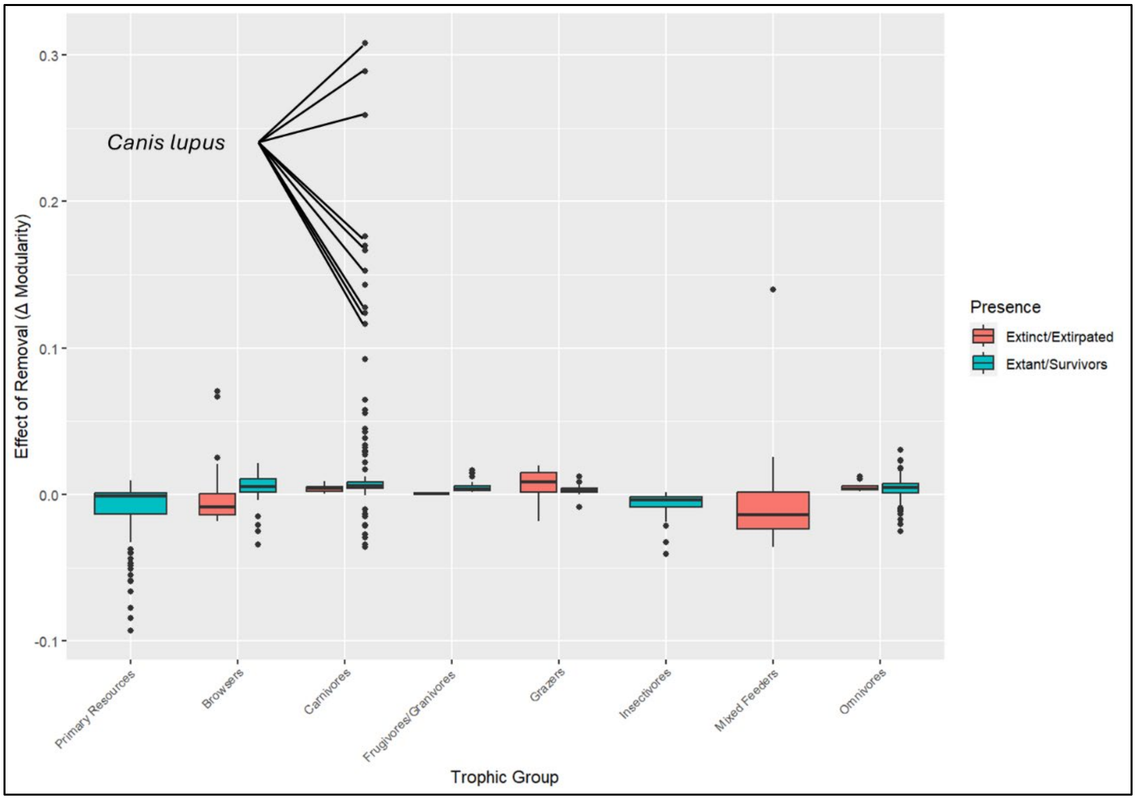

Figure 1.9: ***Comparison of the effect of removal of each trophic group by their presence****. Extant (blue box plots) and Extinct/Extirpated (red box plots). Nine out of the top ten values for the effect of removal on modularity are attributed to Canis lupus (across the 9 sites). There are no mixed feeders in any of the 9 sites in the Holocene.*

Global Mixed-Effects Model - When we test a global model combining data from all sites, we find a strong effect of body mass as a predictor of extinction, with larger mammals having a higher probability of extinction ($P < 0.001$). The effect of removal on modularity, ΔM_{-i} is a weaker but significant predictor of extinction, with species with higher values having a lower probability of extinction ($P = 0.045$; **Table 1.1**). Network degree is not a significant predictor of extinction ($P = 0.35$). When we analyze the same model for each site separately, body mass is the only significant predictor of extinction at all 9 sites ($0.001 < P < 0.03$ at all sites). This is unsurprising given that we know the megafauna extinction was size biased (Smith *et al.* 2003; Barnosky *et al.* 2004; Lyons *et* *al.* 2004; Smith *et al.* 2018). In contrast, the model shows that a species role in the food web network has a weaker effect, if any, on probability of extinction during the Pleistocene-Holocene transition.

Table 1.1: Results of mixed-effects model

Variable	**Estimate**	**Z-value**	**P-value**
Body mass	-37.06	-7.96	$1.80e^{-15}$
Effect of removal on modularity (ΔM_{-i})	0.622	2.01	0.045
Network degree	0.241	0.926	0.354

Discussion:

We find significant changes to the structure of mammalian food web networks on the Edwards Plateau due to the megafauna extinction (**Figs. 1.3,1.4**). Food web networks in the Holocene have collapsed relative to the Pleistocene and are less compartmentalized with a reduced modularity. Our findings for mammal communities on the Edwards Plateau are consistent with findings from other parts of the world, in which megafaunal extinctions have been associated with major shifts in the structure of food web networks (Fricke *et al.* 2022; Pires *et al.* 2015; Nenzen *et al.* 2014). Our study provides support for this pattern using a uniquely fine-scaled dataset in which the same change in network structure can be detected in paired samples of Pleistocene and Holocene food webs from multiple sites, providing robust evidence for these shifts in the community. Similarly, our results suggest that food webs after the extinction are more stable and resilient to future species loss; multiple species contribute to the modularity of the networks after the extinction (**Fig. 1.7**), whereas typically only one had a strong contribution to modularity prior to the extinction (**Fig. 1.6**).

We suggest that the patterns of species loss caused the observed changes in the food web networks and that there was little to no feedback between the collapse of the food web networks and the extinction. That is, we see no evidence that the importance of species in the compartmentalized structure of the Pleistocene food web affected their probability of extinction. Mixed-Effects models of the Pleistocene find body size as a main driver of the extinction event when considering other factors such as, the number of species an individual species is interacting with (degree) and the changes in modularity contributions of particular species. Other studies have argued that the unique size bias of this extinction event in mammalian evolutionary history (Smith *et al.* 2018) requires a direct mechanism such as human hunting of large-bodied species to explain the size bias (Lyons *et al.* 2004a, Lyons *et al.* 2004b, Smith and Lyons 2011, Smith *et al.* 2018). Our results are consistent with that interpretation. If climate change were a primary driver of the megafauna extinction, we would expect to see feedback between the ecological collapse of the food web networks and the extinction, yet we do not.

Trophic Groups

Individual species' effect of removal on each site's network provides us insight on the importance of the roles played by trophic guilds in terms of the composition of a mammal food web network. As results showed, we see significant differences in the distribution of each trophic group's effect of removal transitioning from the Pleistocene to the Holocene (**Fig. 1.8**). In particular, *Canis lupus* occupied a unique role in the Pleistocene food web by having an extremely high effect on modularity. This implies they positively influence the network's structure by connecting the food web's compartments through their interactions. This contrasts with the roles of apex carnivores that went extinct (e.g. *Smilodon fatalis*, *Homotherium serum*, *Panthera leo atrox*, *Canis dirus*), which had relatively low effects of removal (**Figs. 1.6,1.7**) suggesting that they were primarily interacting with species within their compartments and were more specialized. In an analysis of the stable isotopes of large mammals at Hall's Cave, Smith *et al.* (2022) found that the extinct felid apex predators were specialized on baby grazers (e.g., horses, mammoth and bison). Their extinction led to changes in the diets of the surviving large felids (i.e., *P. onca* and *Puma concolor*). The jaguar shifted its diet into the space previously occupied by the extinct cats and the mountain lion occupied the dietary niche vacated by the jaguar (Smith *et al.* 2022). Although they also documented a shift in the diet of the surviving grey wolf (*C. lupus*) toward the dietary niche previously occupied by the dire wolf (*C. dirus),* it was not significant (Smith *et al.* 2022). The differences in the effects of removal we find in the extinct and surviving large carnivores on the Edwards Plateau are consistent with the meso-predator release found at Hall's Cave. The surviving species were more generalist and interacting with more clusters in the Pleistocene, perhaps because of competition with extinct carnivores. Indeed*,* the surviving species have a lower effect of removal in the Holocene (**Fig. 1.5,1.8**) suggesting that they became more specialized with the reduction in potential prey species and the reduced competition with other surviving carnivores. Considering the role carnivores play in their community, species with positive effects of removal like *C. lupus* were likely interacting with multiple compartments of species and were more capable of adapting to the changes occurring in their habitat than the carnivores that went extinct.

Browsers that increased in their effect of removal in the Holocene suggest that extant species have more interactions with other compartments in the network than extinct species from the Pleistocene. Like the extinct carnivores, extinct browsers (e.g. *Mammut* americanum, *Megalonyx jeffersonii, Tapirus veroensis, Navahoceros fricki, Tremarctos floridanus*) had lower effects of removal indicating that they were interacting only within their compartments (**Fig. 1.6,1.7**). Isotopic analyses of browsers at Hall's Cave found that found that large-bodied surviving species (e.g. deer, antelope) maintained relatively similar diets transitioning into the Holocene (Smith *et al.* 2022). Moreover, the deer (*Odocoileus*) significantly grew in body size in the Holocene which is likely attributed due to less competition and lower predation after the shifts in diet of meso-predators that took over apex predator roles (Smith *et al.* 2022). Strikingly, large-bodied grazers that went extinct (e.g. *Bison latifrons, Bison antiquus, Glyptotherium floridanum, Equus*) were mostly significantly higher in effect of removal in comparison to the survivors in the Holocene except for the mammoth (*Mammuthus*) (**Figs. 1.6,1.7,1.9**). These interactions were due to predation by apex predators and would have been lost even if these grazers survived. The lone survivor, bison (*B. bison*), did not shift in its diet and was the smallest of the three species (Smith *et al.* 2022), and has as positive an influence on the network as its extinct relatives (**Fig. 1.6,1.7**). Moreover, the decreases in effect of removal of herbivore trophic groups coincides with the shifts in predator diets after the megafauna extinction. The consequences of losing the megaherbivores, such as the grazers, may have contributed to the extinction of the specialist predators and shifts in meso-predator diets through lower competition and less prey.

Conclusions

Our work documenting the shifts in a food web across the Edwards Plateau has important implications for potential changes to come for modern food webs. Following the megafauna extinction, mammal communities across the Edwards Plateau shifted from a highly modular network in the Pleistocene to a significantly less modular network in the Holocene. We found that the consequences of the loss of megafauna resulting in a less complex network are largely attributed to loss of particular species interactions. Although trophic groups varied in their effects of removal, we still see a collapse in network structure across the Edwards Plateau transitioning into the Holocene. We believe this may be due to the loss of specific biotic interactions between specialist carnivores and their preferred prey resulting in an overall decline in network modularity. These interactions likely were important for the ecological complexity and function of the mammal community on the Edwards Plateau.

LARGE STRUCTURAL CHANGES IN A FOOD WEB NETWORK OVER THE LAST 22,000 YEARS

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We are experiencing rapid biodiversity loss due to climate change and human impacts. Such biodiversity loss is not only harmful to the environment but can also alter the composition of communities and the interactions of their members. The Late Pleistocene experienced a drastic loss of large-bodied mammals which resulted in significant changes in community structure due to changes in body size, diet, and species associations. However, the effect of climate change on species interactions and community structure across the Pleistocene-Holocene transition remains poorly understood. Using a robust data set of species composition, stable isotopes, body size, and multiple climate variables, we constructed and compared ecological networks of mammal paleocommunities across the last 22,000 years at Hall's Cave, Texas. In each of 16 time intervals, we measured the structure of the food web using modularity and an index of node overlap and segregation. We find node overlap increases and modularity decreases over time. Spearman-Rank and Partial correlation analyses indicate that changes in modularity were not driven by changes in species richness across time, nor was it driven by climate change. The degree of node overlap shifted dramatically across the Pleistocene-Holocene transition and was significantly different from null model expectations in the Holocene but not in the Pleistocene. These results suggest that the transition from a diverse and modular network to a network of less complexity with an

overlap of interacting species may have been driven by other factors that changed the food web, such as species composition. This implies that the change in mammal food web structure of Hall's Cave was mainly a consequence of the megafauna extinctions and not coupled with climate change.

Introduction:

The late Pleistocene (22,000-11,700 years) was a unique period where approximately 47% of large-bodied (megafauna) species went extinct globally (Barnosky *et al.* 2009; Smith *et al.* 2018). This extinction event heavily impacted North America as approximately 76%-80% of megafauna species went extinct around 11,500-10,000 years ago (Martin 1967; Martin and Klein 1984; Barnosky *et al.* 2004; Lyons *et al.* 2004; Smith *et al.* 2016). Several hypotheses have been proposed to explain the extinction including climate change (Graham and Grimm 1990, Graham *et al.* 1996, Graham and Lundelius 1984), human hunting (Martin 1973; Martin 1990), and disease (McPhee and Marx 1997). Although the causes are still debated, ongoing research on the causes of the megafauna extinctions suggest human impacts as a driving factor (Lyons *et al.* 2004; Koch and Barnosky 2006; Barnosky *et al.* 2009; Smith *et al.* 2019).

Regardless of the causes, megafaunal extinction is likely to have had major consequences for the structure of ecological communities. Modern megafauna are ecosystem engineers and are important to their communities as they help maintain their habitats and facilitate interactions between species (Janzen and Martin 1982; Owen-Smith 1992; Smith *et al.* 2016). Their roles range from megaherbivores maintaining diversity of plant species compositions, suppression of woody regeneration and nutrient cycling throughout the ecosystem (Dublin *et al.* 1990; Owen-Smith 1992; Bakker *et al.* 2006; Goheen *et al.* 2010; Goheen *et al.* 2018; Doughty *et al.* 2016). Meanwhile, apex predators influence the abundance of herbivores, mesopredators and other apex predators through top-down control over their ecosystem (Johnson 2009). Extinct megafauna likely played similar roles: thus, the extinction event would have impacted ecosystems. Indeed, losing megafauna species have cascading effects in communities such as shifting diets, changes in community assembly and body size distributions (Lyons *et al.* 2004; Lyons and Smith 2013; Smith *et al.* 2016; Smith *et al.* 2019; Tomé *et al.* 2019; Smith *et al.* 2022; Smith *et al.* 2023). Their loss also led to significant changes in mammalian food webs and ecological networks that were less resilient to future extinctions (Fricke *et al.* 2022, Pires *et al.* 2015, Nenzen *et al.* 2014).

Ecological networks are a representation of the interactions between species within a community and can quantify potential interactions based on a combination of body size, abundance, diet and other traits of species in a community (Montoya *et al.* 2006; Fortuna *et al.* 2010; Bascompte 2010; Delmas et. Al. 2018). In particular, food web networks have been widely used in ecological studies to understand trophic relationships such as predator-prey interactions, the structure of communities, ecosystem function and more (Dunne *et al.* 2002; Pascuel and Dunne 2006; Montoya *et al.* 2006; Ings *et al.* 2009). One important structural property of food webs that has gained much attention is compartmentalization, which measures the degree to which groups of species are more likely to interact with each other rather than with other individuals or groups in the food web (May 1972, Girvan and Newman 2002, Krause *et al.* 2003). The degree of compartmentalization of a food web has been shown to have important impacts on the stability and dynamics of food webs (May 1972; Pimm 1979; Pimm and Lawton 1980; Yodzis 1982; Girvan and Newman 2002; Krause *et al.* 2003; Allesina and Pascual 2009; Rezende *et al.* 2009). Studies of compartmentalization in ecological networks have often analyzed modularity, which measures the degree to which connections between nodes are clustered into discrete subunits (Newman and Girvan 2004, Guimerà *et al.* 2010). A complementary approach is to measure node overlap, or the degree to which species share interacting partners (Strona & Veech 2015). Examining the changes in the modularity and node overlap of mammal food web networks could shed light on how modern mammal communities differ from their ancient relatives as well as what factors are related to the stability or instability of food web structure and how modern communities will respond to global climate and anthropogenic change.

Concurrently with the megafauna extinction, North America experienced climatic shifts from a cool climate to a warmer climate that was followed by events such as the last glacial maximum and the younger dryas cooling period (Alley 2000, Clark *et al.* 2012, IPCC 2014). Climate changes have the potential to alter the composition of communities and the species interactions within them by affecting the environment they inhabit (Lyons 2003; Lyons 2005; Blois *et al.* 2010; Smith *et al.* 2016; Tóth *et al.* 2019; Tomé *et al.* 2019). Alongside these climatic changes, recent studies have shown human presence in North America to be as early as 23,000-21,000 years ago, demonstrating a broader range of coexistence between humans and megafauna before extinctions occurred (Bennett *et al.* 2021). It is unclear whether these early dates represent isolated forays into North America, but evidence suggests that humans were not widespread until ~ 16,000 cal yr BP (Surovell *et al.* 2022, Potter *et al.* 2020). Considering the environmental and ecological changes occurring during the megafauna extinction, the consequences of the extinction event may provide a baseline understanding of what may happen to future mammal communities if we lose our remaining megafauna.

One approach to resolving the consequences of megafaunal extinctions and climate change on the structure of ecological communities is to use sites with fossil records with finely calibrated temporal resolution. In this study, we use such a dataset from Hall's Cave, Texas, to: (1) Understand how the structure of a North American mammalian food web has changed over the past 22,000 years, a time period spanning the megafauna extinction, (2) test the potential drivers of the changes in food webs, (3) determine whether and when the food web structure departs from expectations due to changes in the species assemblage, and (4) ask whether introduced herbivores that have naturalized populations in Texas have restored the food webs at Hall's Cave. Specifically, we use an ecological network approach to measure the changes in the modularity, or compartmentalization, of the food web.

Data & Methods:

Study Site

Hall's Cave is located on the Edwards Plateau in Kerrville County, Texas (**Fig. 2.1**). Modern vegetation of the region consists of woodland and savanna dominated by oak, junipers, and various tall and short grasses (Toomey 1993; Joines 2011; Cordova and Johnson 2019; Tomé *et al.* 2019). Prior to deglaciation, the area around Hall's Cave was cooler and wetter and was dominated by pine, oak and cool season grasses (Cordova and Johnson 2019). By the early Holocene, the site became warmer and more arid (Cordova and Johnson 2019). Hall's Cave has fantastic preservation of the mammal community with a fossil record spanning the last 22,000 years (Toomey 1993; Smith *et al.* 2016). In addition to mammals (Toomey 1993, Smith *et al.* 2016, Hedberg *et al.* 2022; Tomé *et al.* 2020; Tomé *et al.* 2022; Smith *et al.* 2022), studies have been done ranging from paleoclimate reconstructions to opal phytoliths (Toomey 1993; Toomey *et al.* 1993; Cooke *et al.* 2003; Joines 2011; Smith *et al.* 2016; Cordova and Johnson 2019). The record of flint and charcoal at the site indicates that humans were continuously present near Hall's Cave starting around ~15,500 years ago. The megafauna extinctions at the site are loosely coupled with a directional change in climate, however, they decouple in many instances (e.g. Smith *et al.* 2016). In addition, Hall's cave has a well resolved age model which allows the record to be finely divided into 16 time bins of approximately 1,300 years each (Tomé *et al.* 2020).

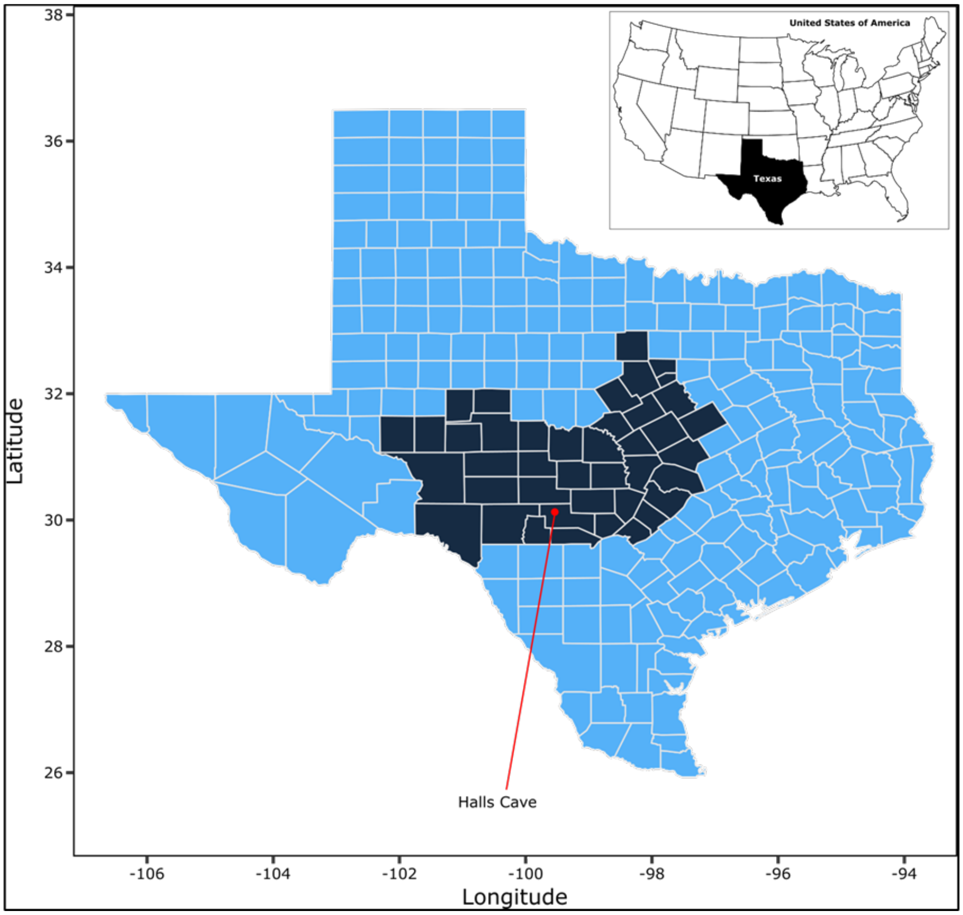

Figure 2.1: Map of Texas and Hall's Cave. *The Edwards Plateau in dark navy blue and Hall's Cave indicated in red.*

Climate Data

This study utilized downscaled and debiased paleoclimatic simulation data of the Community Climate System Model (CCSM3) developed by Liu *et al.* 2009. It is a unified dataset from climate simulations that spans the last 22,000 years in North America (Lorenz *et al.* 2016). Similar to previous studies, the climate data for the region surrounding Hall's Cave was extracted in 500 year intervals (Tomé *et al.* 2020; Tomé *et al.* 2022). Climate data were then averaged for each of our 16 time bins. The climate variables include mean daily maximum temperature, standard deviation of the mean daily maximum temperature, mean daily minimum temperature, standard deviation of the mean daily minimum temperature and total monthly precipitation.

Preparing Food Web Networks

Food webs were composed using a matrix of interacting species based on stable isotopes, diet and body size data. Species lists for each time period were taken from Smith *et al.* (2016) and Hedberg *et al.* (2022). The final time bin includes introduced species on the Edwards Plateau (Hedberg *et al.* 2022). Dietary inferences for each species were made using $\delta^{13}C$ and and $\delta^{15}N$ stable isotopes from specimens from Hall's Cave and the Edwards Plateau (Smith *et al.* 2022, Tomé *et al.* 2020, Tomé *et al.* 2022, Gearty *et al.* in prep.). For example, $\delta^{13}C$ was used to infer whether a primary consumer was eating primarily C3 or C4 vegetation or whether they were a mixed feeder. Similarly, $\delta^{13}C$ indicated whether a secondary consumer was primarily eating browsers, grazers or mixed feeders (Smith *et al.* 2022; Gearty *et al.* in prep). $\delta^{15}N$ indicated where in the trophic chain primary and secondary consumers fell and was used to differentiate apex and meso-predators. Inferred feeding relationships were also constrained using scaling relationships between the body size of predators and prey (Carbone *et al.* 1999). If the body size of a potential prey was too large or too small for a particular predator, it was excluded as a food source for that species. Using the stable isotope and inferred scaling relationships, we created a food web network for each of the 16 time bins at Hall's Cave. To form the food webs, we used the R package "igraph" (Csardi G and Nepusz T 2006).

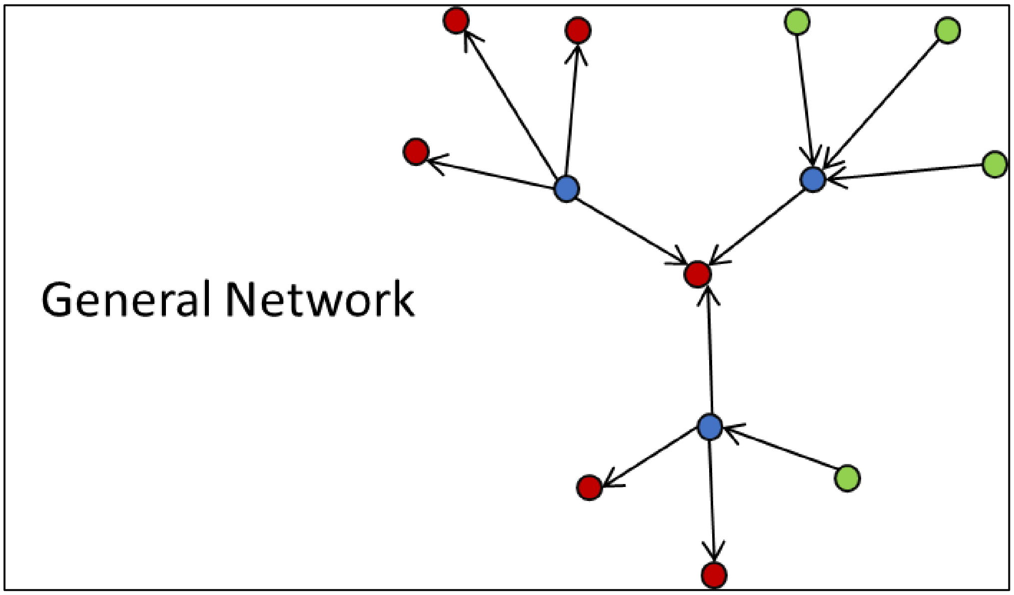

Figure 2.2: ***Conceptual model of a general food web network.*** *Nodes (circles) represent a species, edges (arrows) represent the connection between nodes where the arrow points to the species that is eating the species the arrow extends from. Carnivores (red), Herbivores (blue), Plants (green).*

Quantifying the structure of Food Webs - We quantified the changes in the structure of the food web by examining modularity, which quantifies how communities are partitioned into clusters of interacting species. We used two methods to measure the modularity of the food web across time: (1) community detection using the whole food web, and (2) node overlap using a bipartite network of consumers and resources. As we show, the two approaches are complementary, with different strengths and weaknesses.

First, we measured the modularity of the whole food web using *the edge-betweenness community detection algorithm* (Newman & Girvan, 2004). Briefly, this method identifies clusters, or 'node communities' as partitions of the network that maximize the number of edges that link nodes within the same community and minimize the number of edges between communities. This approach can be implemented on directed networks including primary producers. However, the drawback is that permutations of consumer-resource relationships on a directed network are complicated, making null hypothesis testing difficult.

To overcome some of the obstacles of null hypothesis testing of the whole food web, we took a second approach, by converting the food web into bipartite networks of consumer-resource relationships. A bipartite network consists of two types of nodes (e.g., 'consumer' vs. 'resource'), with edges only connecting nodes of different types. Here, we created a simple consumer-resource network of only a predator eating prey, while excluding primary resources. We assigned omnivores as 'consumer' or 'resource' based on their predator-prey ratio: omnivores were considered 'resource' if it had more predator species that consumed it than prey species that it consumed (e.g., *Peromyscus* mice), while they were considered 'consumer' if it had fewer predator species than its prey species (e.g., bears). We then measured the Node Overlap and Segregation (NOS) index, which measures the tendency of species to share or not share interacting partners and measures how much overlap there is between the pairs of interacting partners, and then compares that to the random expectation (Strona & Veech 2015). We show here that node overlap (smaller NOS) is strongly negatively correlated with modularity measured by edge-betweenness community detection described above, but NOS allows us to use permutation-based statistical tests, as the bipartite network approach simplifies null model simulations.

Statistical Analyses

Null Model Testing of NOS - Here, we are interested in determining how the observed structure of the food web may change due to changes in species composition across time. Therefore, we tested whether the observed NOS index of the bipartite network differed from what could have been expected if species composition were randomized. To do this, we first assembled a list of all species found in Hall's Cave during the entire sampling period (i.e., over the past 20,000+ years) and assigned each species to 'consumer' or 'resource' using the same criteria as above (namely, 'consumers' consisted of carnivores and omnivores who had more prey species than predators). Then, for each time interval, we randomly selected consumers and resources from the global pool of species, matching the number of consumers and resources observed in that time interval. We then measured the NOS index of the resulting 'null' bipartite network. We repeated this procedure 1,000 times to generate a null distribution of the NOS index for each time interval. We generated P-values, through a one-tailed test, as the proportion of times the NOS index of the null model equaled or exceeded the NOS index of the observed network at that time interval.

Testing the impact of climate change on food web structure - Climate variables may be auto correlated with time. To account for any temporal auto correlations, we took the first difference between time bins to get the change that occurs in climate. This ultimately removed a time bin, resulting in 15 total. We used Spearman-Rank Correlations, a non-parametric statistical test, to understand how well correlated two variables are. In this case, we tested the relationship between the change in temperature, precipitation, and species richness to the change in modularity over time. Partial correlations and multiple regressions were also used to further test the relationship between climate and modularity.

Results:

Changes in Hall's Cave Food Web structure from Pleistocene to Holocene

We represent the food webs across all 16 time bins as directed networks (**Fig. 2.3**). The modularity of this network decreases as time moves from the Pleistocene to the Holocene, as species richness fluctuates (**Fig. 2.4A-D**). Modularity began to decrease ~18,000 years ago and was very low by the beginning of the Holocene (**Fig. 2.4A, B**). Modularity remains low throughout the Holocene and never recovers (**Fig. 2.4A**). Species richness is low at the beginning of our time series and increases across the Pleistocene to the extinction interval (**Fig. 4C-D**). Richness declines after the extinction and remains low throughout the Holocene and never recovers despite the inclusion of introduced species in the final time bin (**Fig. 2.4C**). The first time bin contains ~29 species and there is a peak of ~60 species in the seventh time bin, then it rapidly declines to ~34 species in the last time bin (**Fig. 2.4C**). However, modularity is not significantly correlated with species richness (Spearman correlation = -0.004, P = 0.99) (**Fig. 2.6**).

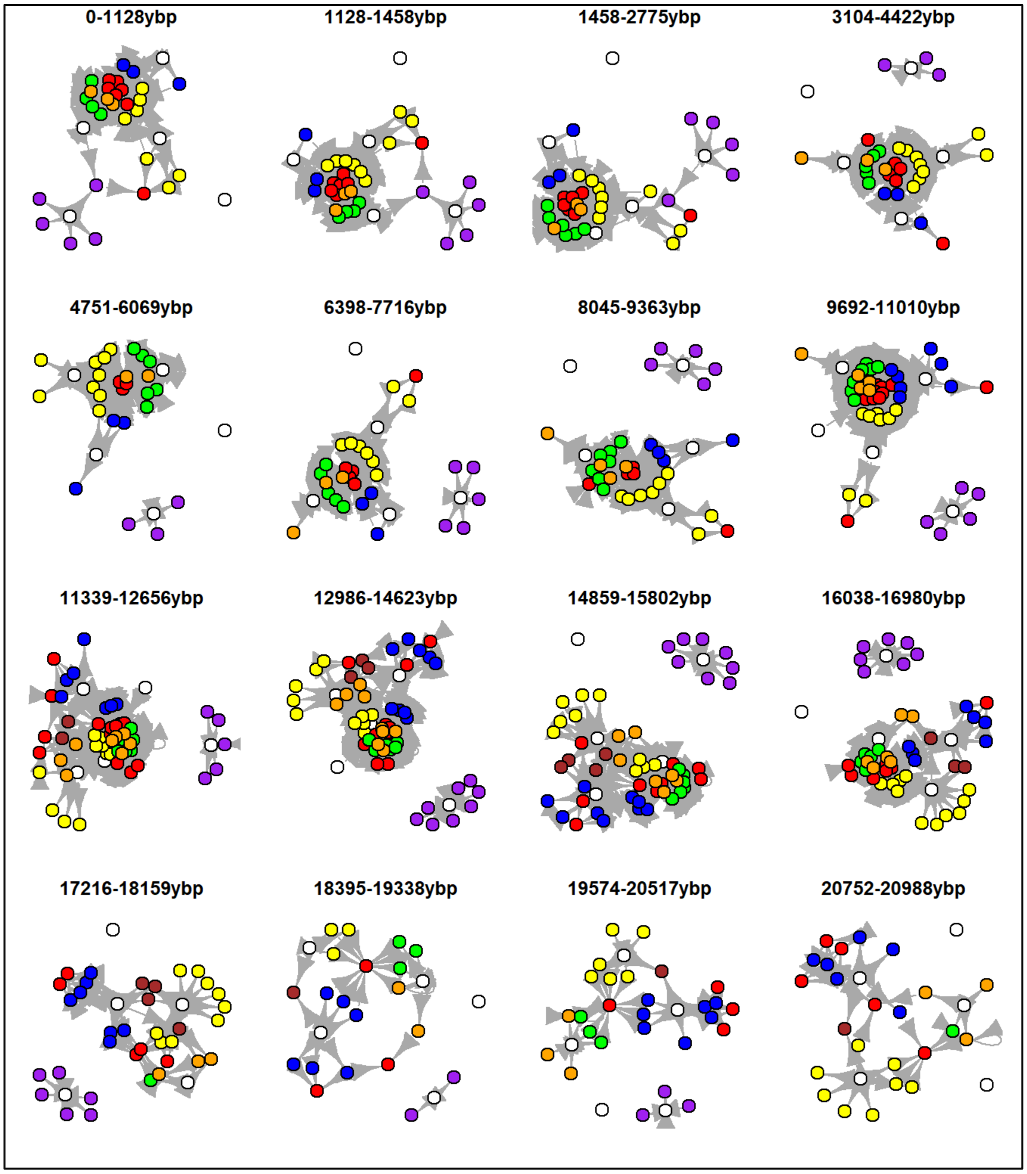

Figure 2.3: ***Hall's Cave Food Web Networks from the Pleistocene to Holocene.*** *Time bins 1-7 = Holocene transition (Present-11,339), 8-16 = Pleistocene (12,986-20,988 years). Nodes are color coded by trophic groups: browsers (yellow), grazers (blue), carnivores (red), mixed feeders (brown), omnivores (orange), frugivores/granivores (green), insectivores (purple), primary resources (white).*

Comparing modularity and node overlap

Modularity of the directed networks and node overlap in the bipartite networks were strongly inversely correlated (Spearman correlation = -0.68, P = 0.005): modularity decreases as NOS increases over time **(Fig. 2.4A)**. During the Pleistocene, NOS fluctuates, but does not change in a directed manner. NOS increases beginning in the extinction interval and remains high across the Holocene, however, modularity and NOS overlap in the most recent time bin (**Fig. 2.4A**).

Null Model Testing - Observed NOS indices were significantly higher than expected by chance for all the Holocene time intervals ($P < 0.025$) and the Pleistocene-Holocene transition (**Fig. 2.5: P = 0.038**). In contrast, the observed NOS indices were not significantly different from expected for any of the Pleistocene time intervals (**Fig. 2.5: P > 0.27 for all)**. This suggests that the changes in the species assembly at the Pleistocene-Holocene transition rapidly increased node overlap and reduced the level of compartmentalization of the food web.

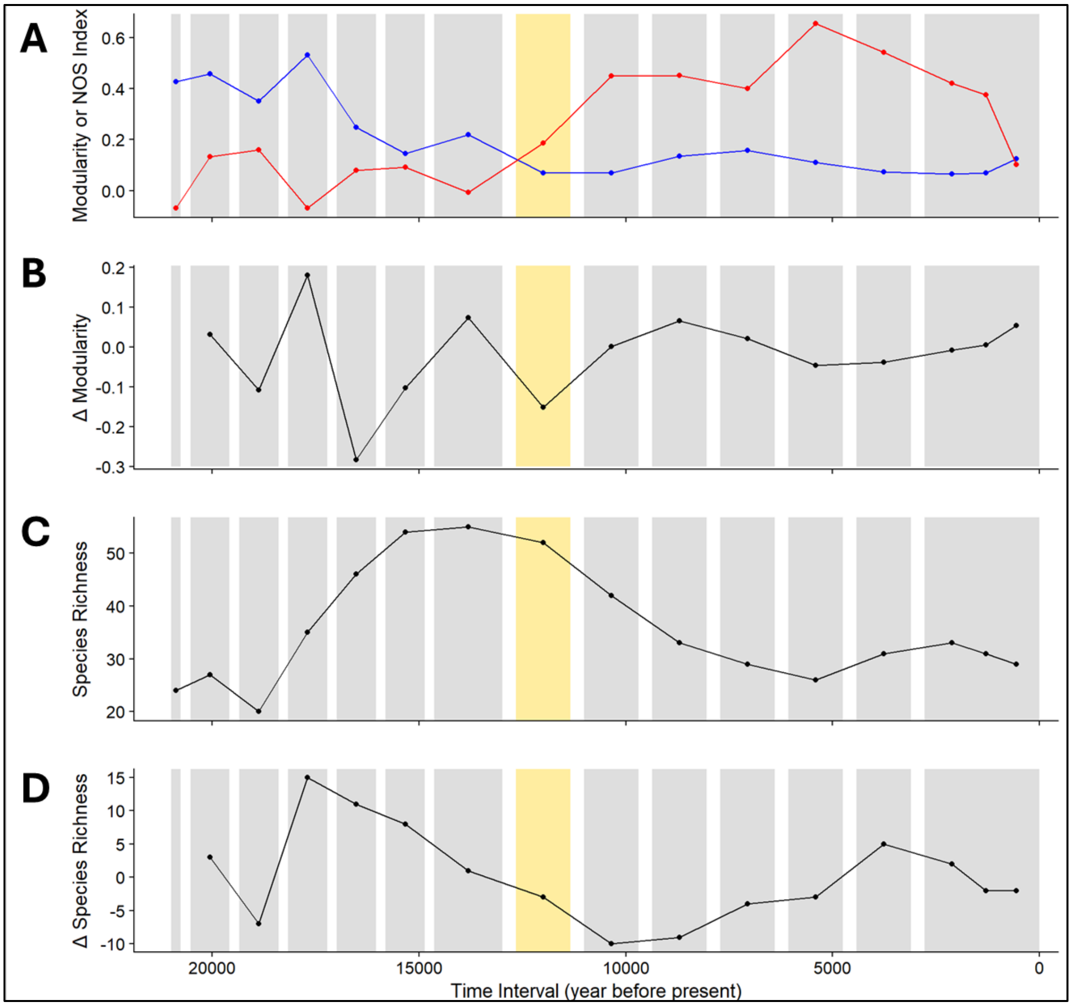

Figure 2.4 ***Correlation between modularity and species richness at Hall's Cave****. Width of the gray and yellow bars represent the length of a time bin. The yellow bar indicates the transition from the Pleistocene to the Holocene and is the extinction interval.* ***(A)*** *Modularity (Blue) and NOS (Red) over time. Modularity and NOS have an inverse relationship. When modularity declines, NOS increases and vice-versa.* ***(B)*** *First differences of modularity over time.* ***(C)*** *Species richness over time.* ***(D)*** *First differences of species richness over time.*

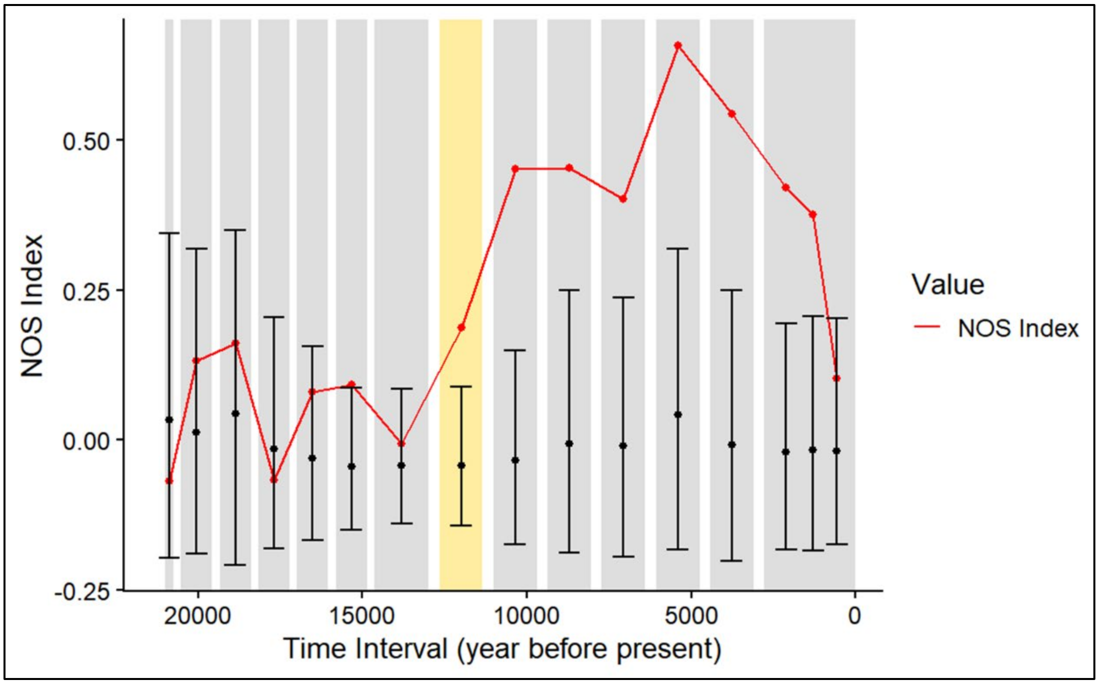

Figure 2.5: ***Observed Node Overlap and Segregation Index (NOS) plotted against expected NOS index over time.*** *Observed NOS deviates from expected by chance only after the transition into the Holocene except for the most recent time bin.*

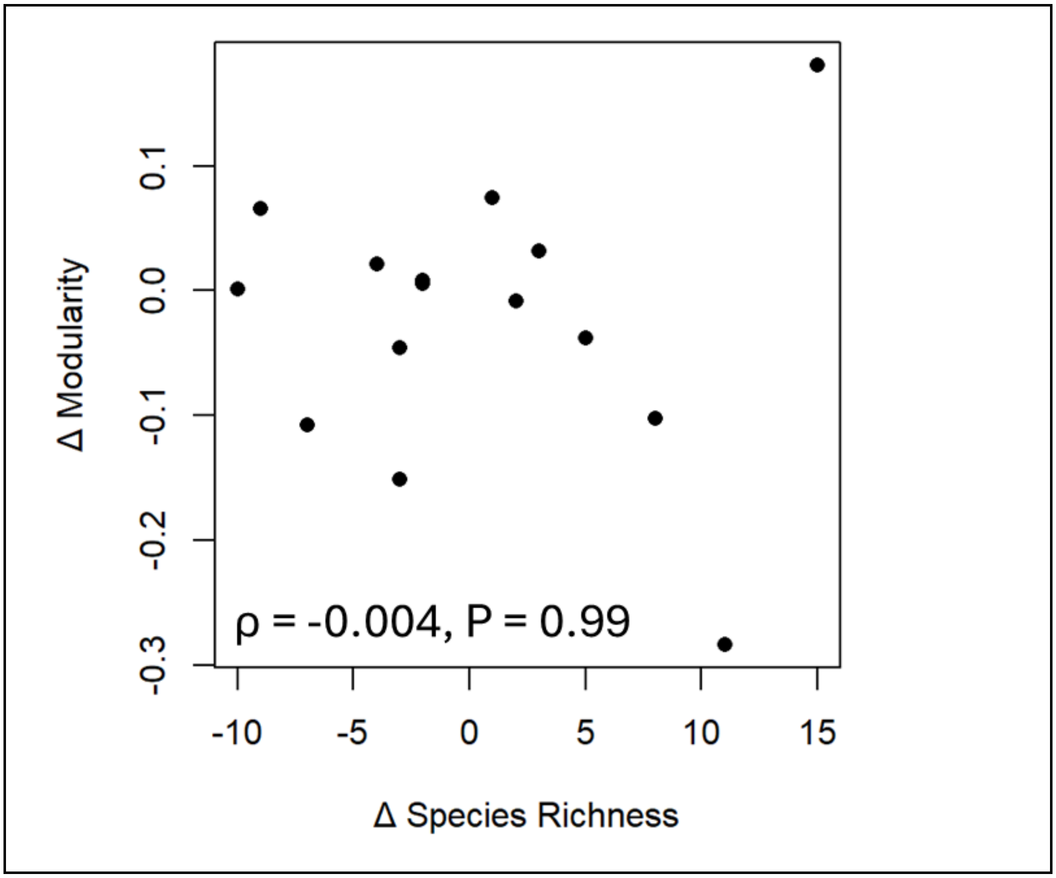

Figure 2.6: ***Spearman-Rank correlation between modularity and species richness****. Species richness is not significantly correlated with network modularity (ρ = -0.004, P = 0.99).*

Local Climate Associations with Food Web Modularity

Spearman-Rank correlations yielded no significant relationships between local climate and modularity for any climate variables **(Fig 2.7)**. Partial correlations and multiple regressions give similar results of no significant relationships between climate and modularity.

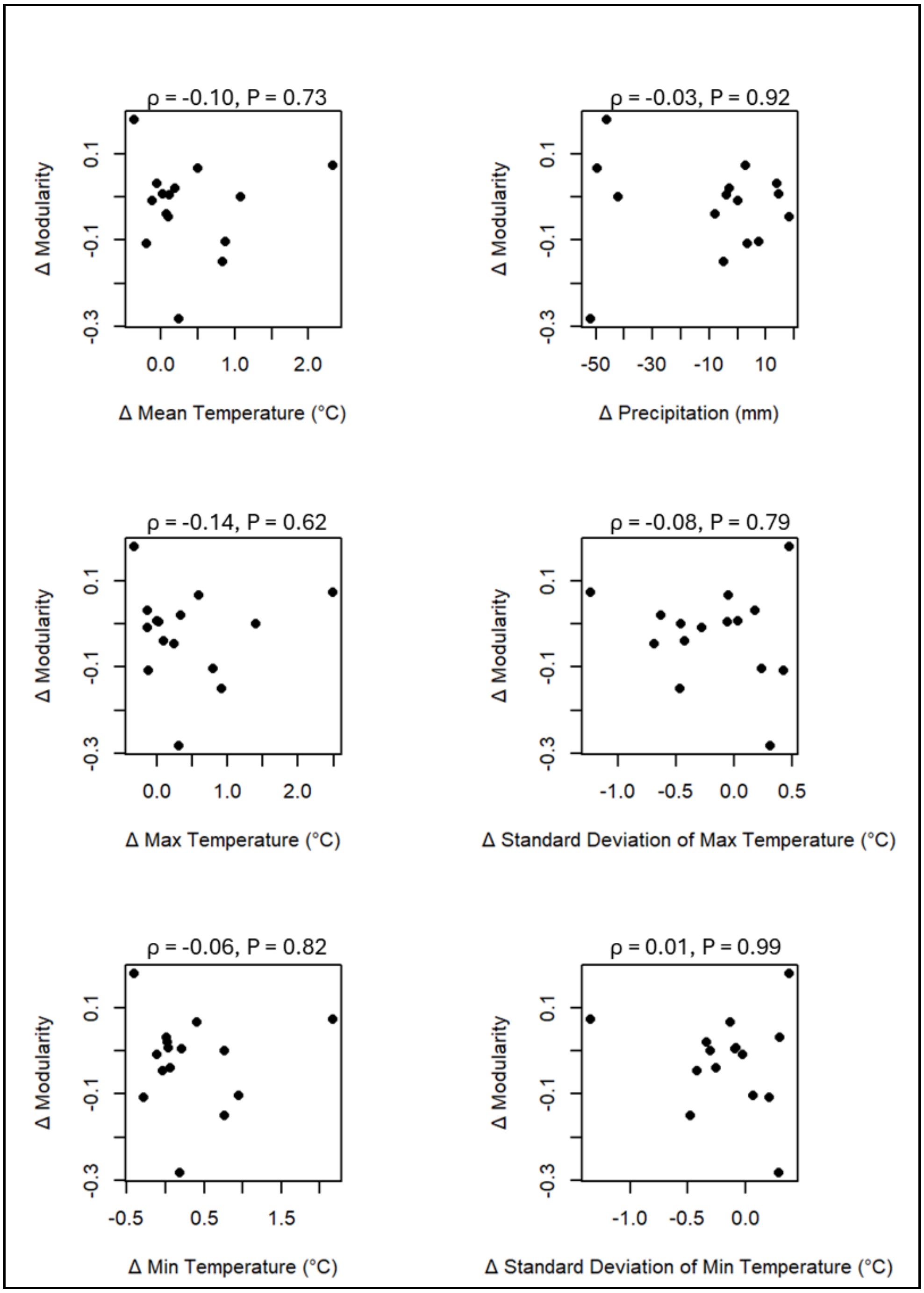

Figure 2.7: ***Spearman-Rank Correlations between Climate and Modularity****. No temperature variables were significantly correlated with network modularity (P > 0.05).*

<u>Discussion</u>:

Our results demonstrate significant changes in the Hall's Cave food web network structure during the Pleistocene-Holocene transition. We see a modular network with low levels of node overlap in the Pleistocene transform into a significantly less modular network with high levels of node overlap in the Holocene (**Fig. 2.4A-B**). In particular, the degree of node overlap shifted dramatically from low to high at the Pleistocene-Holocene transition, with the food webs in the Holocene, but not the Pleistocene, showing significant difference in node overlap from null expectations (**Fig. 2.4A**). Interestingly, modularity began to decline around 18,000 years ago coincident with deglaciation (Clark *et al.* 2012). Modularity reaches a low level by the extinction interval and remains low thereafter (**Fig. 2.4-A-B**). Although our data shows substantial changes in the structure of the food web network in Hall's Cave during the time of megafaunal extinction, we found that these changes were not correlated with changes in species richness (**Fig 2.6**), nor was it correlated with changes in climate (**Fig 2.7**).

Our results suggest that there are other factors involved in the changes in network structure we observe over the past 22,000 years. One potential hypothesis relates to anthropogenic change at Hall's Cave. The record of flint flakes indicates that the earliest evidence of humans at Hall's Cave occurred ~18,000 years ago and by ~15,500 years ago humans were continuously present at the site. Shortly after, by ~11,000-11,500 years, most megafauna at the site were extinct (Smith *et al.* 2016; Smith *et al.* 2022). Just after this transition, we see low species richness without much recovery as the richness in the Pleistocene (**Fig. 2.4C-D**). While we did not detect strong correlations between changes in species richness and changes in food web network modularity (**Fig. 2.6**), it is plausible that anthropogenic changes in species composition such as the loss of specific megafauna species could have been a major driver for changes in the food web structure. An examination of the functional diversity of Hall's Cave over time found that the extinct megafauna was functionally unique and a whole region of functional space was lost as a result of the extinction (Hedberg *et al.* 2022). If the unique ecological roles of the extinct megafauna were contributing significantly to the modularity of the network, the composition of the species that were lost was likely more important than overall species richness in maintaining the food web.

The relationship between species richness and modularity are similar to trends in our null model simulations. The simulations imply that patterns of node overlap became highly non-random in the Holocene. This highlights how the specific megafauna species lost during the extinction event were highly important for the structure of the Hall's Caves' food web network. Given the roles that each modern megafauna trophic group plays, the extinct species were likely interacting in a similar fashion. The extinct megaherbivores (e.g. *Mammut americanum, Mammuthus, Bison latifrons, Bison antiquus*) would have affected the abundance of C3 and C4 plant vegetation (Owen-Smith 1992; Bakker *et al.* 2006; Goheen *et al.* 2010; Goheen *et al.* 2018; Doughty *et al.* 2016). The apex predators (e.g. *Smilodon fatalis, Homotherium serum, Panthera leo atrox, Canis dirus*) were likely controlling the abundance of the prey and other predators and preventing overgrazing/over predation (Johnson 2009). Indeed, loss of the apex felid guild resulted in a classic case of meso-carnivore release in the extant felids (Smith *et al.* 2022). Furthermore, we previously found that each species in the network has a contribution to the observed network modularity by simulating random removals of each species (Chapter 1). Species that had a positive value of contribution were possibly connecting different clusters in the food web network by interacting with many species, which increased observed modularity after removal and species with a negative value of contribution likely only interacted with the cluster of species they were in, meaning modularity of the network decreased after removal. The extinct megafauna was more likely to be found interacting with the cluster of species they were in (**Fig. 2.3**). Thus, their removal contributed strongly to the decrease in modularity. Here, the consequences of megafauna extinctions were the loss of important species (e.g. ecosystem engineers) in the community that led to a less diverse and less complex food web network structure.

The Texas hill country where Hall's Cave is located has been rewilded with some African antelope species because they have been introduced to private lands for big game hunting. Several of these species have naturalized including Blackbuck antelope (*Antilope cervicapra*), axis deer (*Axis axis*), cattle (*Bos taurus*), horse (*Equus caballus*), sika deer (*Cervus nippon*), fallow deer (*Dama dama*), sheep (*Ovis aries*), the oryx (*Oryx dammah*), and the wild boar (*Sus scrofa*) (Hedberg *et al.* 2021). Although they have been shown to partially restore the lost functional diversity of the Hall's Cave community (Hedberg *et al.* 2021), their presence does provide partial restoration by the overlap in modularity and NOS in the most recent time bin but does not increase modularity or decrease the NOS index to the levels they attained prior to the extinction (**Fig. 2.4A**, last time bin). This is likely because their predators were not introduced and the extant predators in the area are not preying on them. Without the restoration of predator-prey interactions, the missing links and modularity of the Hall's Cave food web cannot be restored.

Our work documenting the shifts in a food web across the past 22,000 years has important implications for potential changes to come for modern food webs. We are currently experiencing rapid diversity loss which many scientists are calling the sixth mass extinction due to global climate change and anthropogenic impacts (Barnosky *et al.* 2011). Current food web networks have demonstrated similar responses in decreased modularity due to climate and anthropogenic change. Takemoto and Kajihara (2016) found that global warming and human impacts has caused decreased network modularity for not only food webs, but also pollination, and seed-dispersal networks. Marine food webs demonstrated a decline in network modularity as the density of humans increase in the Caribbeans (Gilarranz *et al.* 2016). There have been many efforts to restore structure to face biodiversity loss due to these global effects, such as rewilding of species back into communities. Rewilding efforts occurred around the world such as, rewilding extirpated species in Europe (Pereira and Navarro 2015), and rewilding of African mammals in Texas environments (Schmidly and Bradley 2016). In addition, the Hall's Caves' Holocene community includes introduced species from ~1,128 years ago, showing early rewilding of species brought in with early humans (e.g. *Canis familiaris*). Our data demonstrates that rewilding efforts may help the structure of food web networks, however, they cannot restore lost ecosystem function completely. This study highlights the importance of not only understanding the impact of mass extinctions on ecosystems, but also the effect megafauna has within those ecosystems. This understanding of the consequences of the megafauna extinctions changing the structure of food web networks may contribute to future studies on the effects of megafauna decline and biodiversity loss.

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