Diet analysis of *Neotoma* middens from the last glacial period to present in the Snake Range, NV

By

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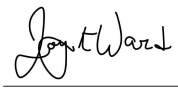
B.Sc., University of Central Florida, 2018

Submitted to the graduate degree program in Ecology and Evolutionary Biology and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Arts.



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Date Defended: 13 May 2020

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Diet analysis of *Neotoma* middens from the last glacial period to present in the Snake Range, NV

Chair: Dr. Joy K. Ward

Date Approved: 15 May 2020

Abstract

Atmospheric [CO_2] is increasing at an unprecedented rate from fossil fuel emissions, resulting in rapid climate shifts and subsequent changes in plant community composition. Ancient climactic excursions and [CO_2] change in the geological record may have also driven community shifts in vegetation from non-human effects, posing challenges for mammalian herbivores. During the Last Glacial Maximum (LGM), Younger Dryas, and mid-Holocene, temperatures were ~8 °C colder than modern values, ~9°C colder than modern values, and ~2°C warmer than modern values, respectively. Ancient DNA (aDNA) analysis of ancient samples allows for the characterization of ancient packrat (*Neotoma sp.)* diet from these time periods that was previously only possible through visual observations of macrofossils and pollen. My research examines how *Neotoma* diet shifted over time between 27,000 and 3300 yrs BP (from ^{14}C dating) with respect to shifting vegetation due to changes in climate and atmospheric [CO_2] over this time at the Snake Range in Nevada. I do this through the analysis of packrat middens, ancient *Neotoma* deposits that contain fossilized vegetation and fecal matter that represent past local vegetation. *N. cinerea* is a midden building species inhabiting the Snake Range and is a dietary generalist, meaning its diet likely reflects local vegetation. Evidence shows local vegetation shifts with environmental conditions across time, thus the advantage of different photosynthetic pathways shifts. Warm conditions and growing season rain favor C_4 plants, which are also highly competitive under low CO_2 conditions. On the other hand, C_3 plants are generally favored under cooler conditions and show improved growth rates under elevated [CO_2] conditions. CAM plants are generally desert-adapted and can tolerate very dry conditions

and a wide range of temperatures in many cases. To interpret isotopic signatures of fecal pellets and to distinguish whether they reflected a mixed C_3-C_4 diet or a full CAM diet of *Neotoma*, I utilized a DNA metabarcoding approach to identify plant species directly from the DNA of ancient fecal pellets. This combination of stable isotope diet analysis and species identification through aDNA metabarcoding allows for a novel two-tier approach that details the presence / absence of plant species as well as gives insight into diet shifts with vegetation changes. I identified no CAM taxa within the samples but found strong evidence of a C_3 diet throughout time as supported by identified taxa and $\delta^{13}C$ isotope analyses. In addition, I observed shifts in present vegetation of the pack rats' diet over time, notably that *Juniperus* sp. became more utilized in the diet in the mid-Holocene, suggesting these packrats may have evolved adaptations to subsist on this toxic juniper over time. My research identifies past dietary shifts due to changing [CO_2] and climate which informs future work in understanding how modern and future plant /animal biodiversity will respond to new shifts in climate and rising [CO_2] levels.

Acknowledgements

First and foremost, I would like to thank Dr. Ward for taking a chance on a student with zero background in her field and giving me the opportunity to pursue such a fascinating master's project. Under her mentorship, I have gained immeasurable skills in research, professional development, scientific writing, leadership, and so much more. Dr. Ward always advocated for me and I felt assured that she always had my best interests in mind. I am very fortunate to have such an admirable woman mentor in both science and life.

I would like to thank my thesis committee members: Dr. Brian Atkinson, Dr. Bryan Foster, and Dr. Maggie Wagner. They all have been the most supportive committee and it has honestly made all the difference. Dr. Atkinson's paleo and plant biology expertise have strengthened this project. I thank him for his valuable feedback and for always giving me a thumbs up and encouragement when I clearly needed it. Dr. Foster's plant ecology expertise has been invaluable towards this project, especially for someone like me who has zero plant background. I thank him for his valuable feedback and perspectives. Dr. Wagner will never fully understand how critical her expertise and teaching was to my completion of this project. If it were not for her and her class, I would still be staring at the raw MiSeq output twiddling my thumbs today. I thank her for her excellent teaching, guidance, and constant encouragement.

I would like to thank Dr. Kinmonth-Schultz, Aleah Henderson-Carter, James Fischer, Timber Burnette, and Thomas Johnson of the Ward lab for making me feel at home from day one. I was always supported by all of them and I have lost track of the many times they all dropped everything to let me vent about my problems. In addition, they were all extremely

receptive and helpful in providing feedback / edits on all aspects of my project progression, applications, and even some personal decisions. I look forward to seeing what amazing things they all go on to do. I would also like to thank Dr. Katie Becklin for her previous contributions that made this work possible and her assistance throughout this process.

I would like to thank Dr. Dennis O'Rourke for allowing the use of the Department of Bioanthropology Ancient DNA facilities for this project. I would also like to especially thank Caroline Kisielinski for guiding me on experimental design, providing relevant literature, training me in proper ancient DNA protocols, dedicating so much of her time and energy to lab work for this project, and always helping me make informed decisions to best progress my project. Her immense contribution to this project made it possible.

I would like to thank Jennifer Hackett for conducting library preparation and sequencing work for this project. I would like to thank Bruce Barnett from the KECK-NSF Paleoenvironmental and Environmental Laboratory for conducting stable isotope analyses for this project. I would also like to thank the KU Genome Sequencing Core for providing grant funding that made library preparation and DNA sequencing possible for this project.

None of this would have been possible without the constant love and support from my friends and family. I thank my friends Paige, Taryn, Meagan, and Pablo for all the encouragement, feedback, and empathy. I thank my partner Austin for always supporting me, instilling confidence in me, and constantly reminding me what is important in life. I thank my parents for their endless love and support in everything I do.

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Introduction

Ancient Climate

Climate is currently changing rapidly and is expected to alter the composition of plant communities and animal biogeography. Modern climate change is showing consistent warming in temperatures, melting of ice sheets, shifting precipitation patterns, and rising atmospheric CO_2 levels due to anthropogenic causes. Species are experiencing changes in their habitats leading to range shifts, disruptions to biological and physiological events such as reproduction or photosynthesis, and trophic disruptions (Scholes et al., 2014). It is becoming increasingly important to understand how climate and vegetation shifted in the past to model how they may respond in the future. Ancient climactic excursions in the geological record can provide insights into how these community shifts may unfold.

There were a few notable climactic excursions in the past 30,000 years. The Last Glacial Maximum (LGM) occurred approximately 26,500 to 19,000 calendar years ago. During this time, much of the world, including large portions of North America, were covered in thick layers of ice (Clark et al., 2009). In addition, sea levels were 400 to 450 feet lower than modern times (Lambeck, Rouby, Purcell, Sun, & Sambridge, 2014), and atmospheric CO_2 was around 180 ppm (IPCC, 2007), which was likely limiting to plant growth in general and C_3 plants in particular (J. R. Ehleringer, Cerling, & Helliker, 1997; Ward et al., 2005). The climate was colder and drier than modern climate (Clark et al., 2009), with global temperatures approximately 3 – 8 °C cooler than modern (Rehfeld, Munch, Ho, & Laepple, 2018). After the last ice age ended, temperatures consistently warmed during the Transition period from 18,000 to 11,000 calendar years ago

(Elsig et al., 2009), until an abrupt period referred to as The Younger Dryas that occurred approximately 12,800 to 11,680 calendar years ago (Schenk et al., 2018). In this period, temperatures were approximately 7.5 – 8.7 °C cooler than modern day (Cole & Arundel, 2005) and CO_2 levels were around 265 ppm (Monnin et al., 2001). This period was considered an abrupt instance of climate change. After the Younger Dryas period ended, the Holocene began, a period of general warming where temperatures mirrored those of modern times, beginning about 11,700 years ago (Rehfeld et al., 2018). The Holocene Climatic Optimum was a period within the Holocene about 9000 to 5000 years BP where temperatures were 0 to 1 °C warmer than today in the Northern Hemisphere (Marcott, Shakun, Clark, & Mix, 2013). Atmospheric CO_2 levels in pre-industrial Holocene times were around 280 ppm (Elsig et al., 2009; Monnin et al., 2001).

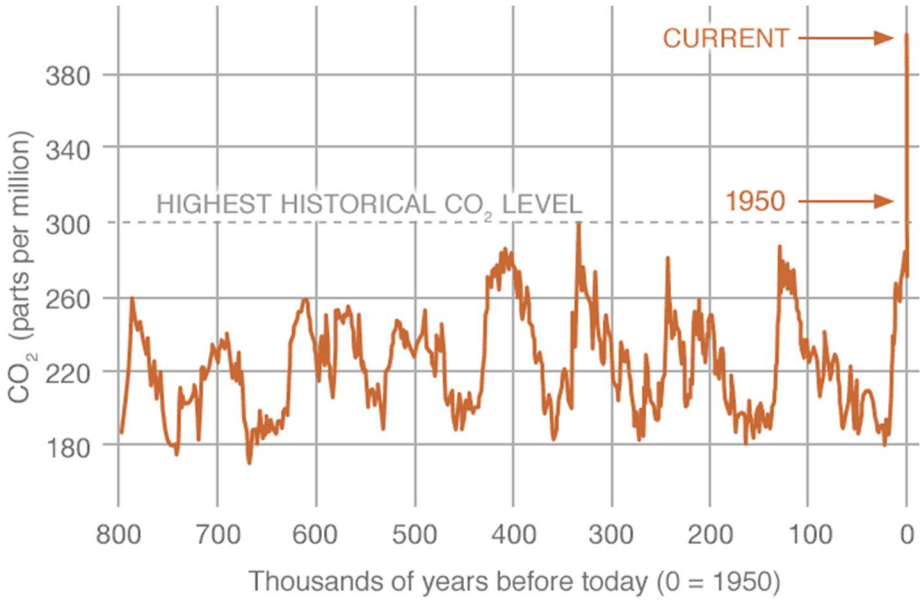

Figure 1. Atmospheric CO_2 levels over the past 800,000 years before 1950 (NOAA, NASA).

Photosynthetic Pathways

Climatic conditions have a significant impact on the performance and viability of different plant photosynthetic pathways. Previous research has focused on identifying environmental factors that affect plant geographic distributions and abundance (J. R. Ehleringer et al., 1997). Three metabolic photosynthetic pathways identified for plants are C_3, C_4, and CAM. C_3 photosynthesis utilizes the enzyme RuBisCO (RuBP) to fix CO_2 from the air and produce two 3-carbon molecules of 3-phosphoglyceric acid (3-PGA) within the chloroplast in the first step of CO_2 assimilation (Taiz & Zeiger, 2007). While the majority of plant species utilize the C_3 metabolic pathway, these plants are disadvantaged in hot climates and/or low [CO_2] conditions, leading to an increase in photorespiration, which reduces the efficiency of photosynthesis (R. F. Sage, Sage, & Kocacinar, 2012). Photorespiration is a process in plant metabolism where RuBisCo takes up oxygen, wasting some of the energy produced by photosynthesis. On the other hand, C_4 plants that eliminate photorespiration due to a CO_2 concentrating mechanism are more competitive in hot conditions and low CO_2 atmospheres due to the absence of photorespiration. In C_4 photosynthesis, atmospheric CO_2 is fixed to form oxaloacetate using the enzyme PEP-carboxylase in mesophyll cells. Oxaloacetate is then converted to malate and malate is translocated to the bundle sheath cells. Once in the bundle sheath cells CO_2 is carboxylated off of malate, and this ongoing progress produces a very high concentration of CO_2 at the site of rubisco in the Calvin cycle. Due to high CO_2 and lack of O_2, photorespiration is essentially eliminated in C_4 plants and therefore they are not subjected to photorespiration, however it is more energetically costly to undergo C_4 photosynthesis (R. F. Sage, 2004).

C_3 plants make up approximately 85% of all plant species and these include tree species (with the exception of two worldwide), bryophytes, ferns, cool season grasses, and most dicotyledons (McNab, 2008). C_3 plants are found to occur in cool and moist environments and can utilize higher atmospheric CO_2 concentrations (J. R. Ehleringer et al., 1997). C_4 plant species are far less common than C_3 species (J. R. Ehleringer et al., 1997). Globally, C_4 plant species account for approximately 18% of productivity (i.e., amount of biomass produced in an ecosystem) (Melillo et al., 1993) despite accounting for only 5% of plant species worldwide (McNab, 2008). A majority of C_4 species are monocots, mainly warm season grasses (Teeri & Stowe, 1976) and sedges (Teeri, Stowe, & Livingstone, 1980). C_4 plants typically occur in warm climates with seasonal rain corresponding with the growing season and are adapted to low atmospheric CO_2 concentrations (J. R. Ehleringer et al., 1997). CAM photosynthesis is found in approximately 10 percent of plant species (McNab, 2008). Characteristic of CAM photosynthesis, the stomata are closed during the day and allows these plants to perform well under drought (McNab, 2008). Therefore, many CAM species are desert plants, such as cactuses and agaves.

Carbon 13 ($\delta^{13}C$) isotope signatures can delineate C_3, C_4, and CAM plants from each other in organic tissue ranging from the plant tissue itself through components of animal systems and their waste products. All of these pathways differ in how they fractionate carbon isotopes and therefore exhibit unique $\delta^{13}C$ isotope signatures (Farquhar & Ehleringer, 1989). This allows for stable isotope analyses to provide an idea of community structure using ancient sub-fossils where organic tissue is still fully intact. C_3 plants are identified by approximately -22‰ to -30 ‰ $\delta^{13}C$, C_4 plants are identified by approximately -10 to -14 ‰ $\delta^{13}C$ (Cerling et al., 1997), and CAM plants are identified within the approximate range of -10 to -20 ‰ $\delta^{13}C$ (O'Leary, 1988). Many types of plants have been measured and there is a noticeable distinction between C_3 and C_4 plants with little overlap (Figure 2) (Cerling et al., 1997; Taiz & Zeiger, 2007). Challenges arise when isotope signatures fall in the middle of these two values in animal tissue (when proper offsets for tissue are also applied), it can be due to a mixture of C_3 or C_4 plants in the diet or a primarily CAM diet and it is challenging to distinguish these without further information about where all three types are able to co-occur. $\delta^{13}C$ isotope analyses are typically done on samples such as plant material, feces or coprolites, or bone. In particular, $\delta^{13}C$ isotopes of feces can be analyzed to determine animal diets and thus reconstruct plant communities (Cole & Arundel, 2005; Commendador & Finney, 2017; Jouy-Avantin, Debenath,

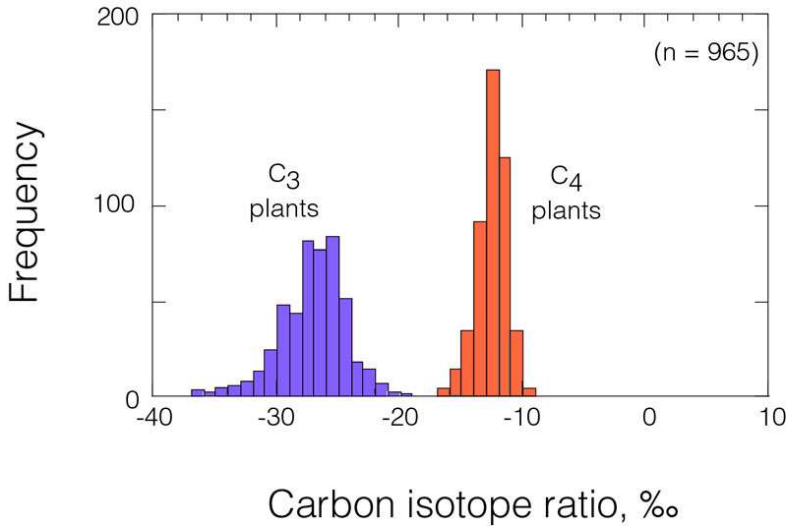

***Figure 2**. Frequency of carbon isotope ratios in C3 and C4 plant taxa (Cerling et al., 1997; Taiz & Zeiger, 2007)*

Moigne, & Mone, 2003), especially when generalists are studied that can rapidly switch diets based on plant availability.

A model was developed by Ehleringer (1997) that demonstrates how the quantum yield (ratio of photosynthetic gains in carbon to photons absorbed at the leaf-level)(J.R. Ehleringer & Bjorkman, 1977) for CO_2 uptake in C_3 plants and C_4 plants changes based on atmospheric CO_2 (ranging from 100 – 700 ppmV) and daytime growing-season temperature (ranging from 0 – 40 ° Celsius) (Figure 3). Ehleringer's model showed that there is no change to the quantum yield of CO_2 uptake for C_4 plants due to temperature and CO_2. Crossover temperatures were calculated that determined where the quantum yields of CO_2 were equal for the two pathways and once compiled were able to show that C_3 / C_4 transitions are based on CO_2 and temperature (J. R. Ehleringer et al., 1997). Increased levels of CO_2 cause lower amounts of stomatal conductance in both C_4 and C_3 plants (Polley, Mayeux, Johnson, & Tischler, 1997; R. F. Sage, Wedin, & Li, 1999). Due to C_4 plants being active during warm temperatures, the impact of lower amounts of stomatal conductance likely has a heavier impact on these species (R. F. Sage et al., 1999).

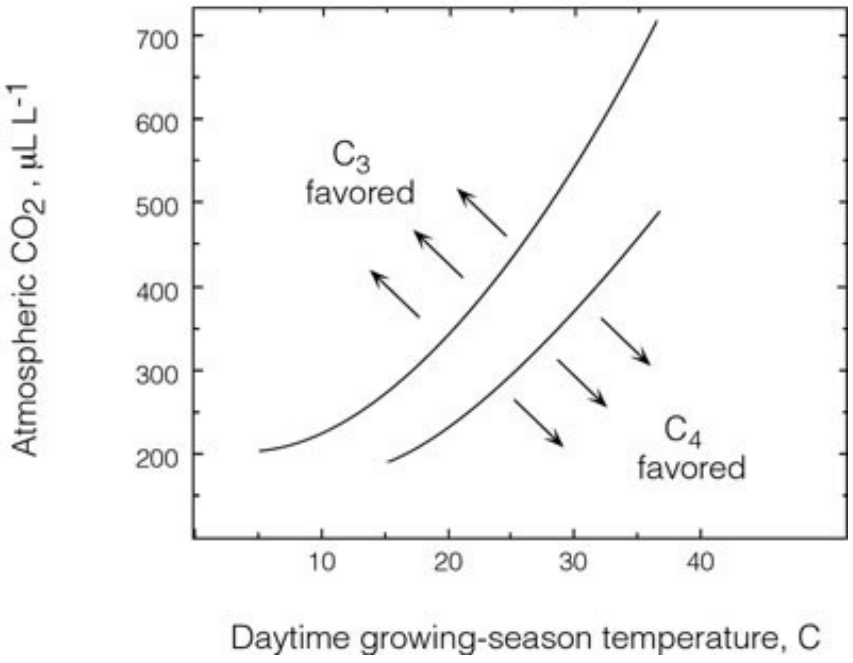

***Figure 3**. Ehleringer's model that predicts atmospheric CO2 levels and daytime growing season temperatures that will favor C3 versus C4 plants. (J. R. Ehleringer, Cerling, & Helliker, 1997)*

Based on this information and the model, it can be predicted that C_4 plants were more dominant in periods such as the glacial periods where there was much lower atmospheric CO_2 and daytime growing season temperatures higher than 17 ° Celsius (J. R. Ehleringer et al., 1997).

Paleoecological Methods

Previously, ancient climate and plant communities have been studied through the use of macrofossils, lake sediments, and pollen analyses. Plant macrofossils can consist of ancient seeds, leaves, twigs and other plant fragments and pollen has been analyzed from various sources, such as lake sediments, springs, and caves (Grayson, 2011). Palynologists, those who study pollen, prefer to analyze deposits from lake sediments that have remained consistently wet as they are usually better preserved and less susceptible to wind erosion that could lead to gaps in data (Maher, 1964). It is difficult to find viable ancient samples in a lot of these different media, because of requirements for well-preserved ancient DNA or sample material and lack of potential for contamination. An alternative exists in preserved plant material and animal feces found in packrat middens. The utility of using Packrat middens to understand past vegetation changes was first discovered by Philip Wells and Clive Jorgensen in 1960 in Nevada (J. L. Betancourt, Van Devender, & Martin, 1990). Pack rat middens have been studied thoroughly as they are found in cave deposits and can provide a variety of plant macrofossils, animal remains, and rodent feces over large temporal and spatial periods (Vaughan, 1990). Due to their discovery and useful application in paleoecology, the study of ancient pack rat middens has been coined as paleonidology (Spaulding, 1985). Due to being found in rock crevices, middens provide excellent

preservation of plant material from thousands of years of occupancy. This plant material provides a snapshot of surrounding vegetation for ancient times that otherwise would not be known.

These middens are created by *Neotoma* spp, a genus that can be found across North America, with varying range sizes and extents. For example, *N. cinerea* is a species of packrat that has a range extending from Canada all the way down to southern Arizona (Smith, 1997). With this variety of geographic location also comes a variety of climates and habitats for these species, some living in snowy woodlands with frigid winter temperatures and others living in deserts with extreme heat and dryness. According to Vaughan (1990), packrats have been found to inhabit alpine tundra, forests, chaparral, grasslands, desert scrub, and tropical thorn scrub. Notably, all *Neotoma* species have in common are their adaptability to dehydration, the act of building dens / nests for shelter, and the compulsory nature of collecting assorted things from around their nests (Vaughan, 1990), although there are species-specific differences in even these attributes and behaviors.

Packrat Ecology

Packrats are small, long-tailed rodents that have adapted feet for grasping rocks when climbing as well as flat-crowned molars and an enlarged caecum that allow for their diet of fibrous plant material (Smith, 1997; Vaughan, 1990). Packrats are adapted to survive in desert areas lacking drinking water because they maintain their water balance from eating plants with approximately fifty percent water weight (Schmidt-Nielsen & Schmidt-Nielsen, 1952). Many *Neotoma* species are dietary generalists and consume a wide variety of plants, however, there are a few species that are dietary specialists and have adapted to very specific diets of toxic

plants such as juniper (Skopec & Dearing, 2011). Packrats often consume CAM species such as prickly pear cactus (*Opuntia* spp.) and agave (*Agave* spp) and have also been found to consume a wide variety of mixtures of C_3 and C_4 plants such as C_3 juniper (*Juniperus* spp.) and C_4 saltbushes (*Atriplex)* (Cole & Arundel, 2005; Thompson, 1990). These dietary generalists are known to consume approximately 15 – 35 percent of juniper in their diet (Boyle & Dearing, 2003) and they do eat the toxic foliage portion of the juniper. There are particular adaptations to be able to subsist on this toxic vegetation, to include behaviors such as moderating the amount consumed and lengthening the time between meals (Torregrossa, Azzara, & Dearing, 2011), and also potential liver enzymes and gut microbes that allow for the increased consumption of the toxic vegetation (Kohl, Weiss, Cox, Dale, & Dearing, 2014). The middens of dietary generalists will likely be better indicators of the surrounding vegetation than those of a dietary specialist.

Members of the *Neotoma* genus universally exhibit collecting behavior to build their dens from surrounding plant material, twigs, rocks, and whatever is available (Finley, 1958). These middens serve as shelter, food storage, and trash receptacles, causing there to be a collection of fecal pellets, leaves, flowers, twigs, seeds, animal bones, etc. (Vaughan, 1990). Typically, a single packrat will occupy the den at any given time, except for times of breeding, but abandoned dens will often be taken over by newcomers (Dial &

Figure 4.*Chunk extracted from a Neotoma packrat midden in the Philip Wells Packrat Midden Collection at the University of Kansas.*

Cazaplewsk, 1990). These dens protect against predators, primarily birds and various mammals, and from temperature extremes (Vaughan, 1990). Each species of *Neotoma* will have different requirements for their dens based on their habitats and will therefore differ in how they construct their dens. Some species will construct their dens in rock crevices, some will construct small dens from plant material in the open, and some will build tall, spacious dens (Vaughan, 1990). A midden (Figure 4) is a part of the packrat's den and is usually where the rodent urinates and defecates as well as where they store any excess plant fragments (Dial & Cazaplewsk, 1990). Due to constant dehydration, the urine from the packrat saturates the midden, and when dried, solidifies all the material in a crystalline mass referred to as amberat and is the key factor in fossilization of midden contents (J. L. Betancourt et al., 1990). This is the section of the packrat den that fossilizes due to the crystallized urine, and researchers use the middens to identify plant macrofossils, animal remains, and fecal pellets for analyses and radiocarbon dating. The layers of a midden can accumulate over time and provide a stratified collection of ancient time that can be identified through radiocarbon dating, meaning middens can be aged from around 40,000 years old to modern (Grayson, 2011).

While there are differences in how and where some species construct their dens, all pack rats will gather plants, animal remains, and more from within an 100 m radius to either use as building materials or store for food, meaning the plant material found within these middens was likely found within that range (Dial & Cazaplewsk, 1990). Packrats are not known to hibernate, but they will collect excess plant material for food storage in case their foraging is disrupted (Vaughan, 1990). A study was done to compare diet to midden plant contents, finding that there was a significant correlation between diet and corresponding midden contents (Dial,

1988). This showed that the rodents were likely eating a majority of the variety of plants they were collecting and therefore their diet can be a good representation of the surrounding vegetation at that time.

Packrat midden Analyses

Packrat middens have been thoroughly studied by paleoecologists as they can provide insight into ancient climate and plant communities. Macrofossils from packrat middens have been analyzed from various regions around the world, to include much of western and southern North America, Africa, Australia and parts of South America (J. L. Betancourt et al., 1990; Chase et al., 2012; Diaz et al., 2019; Diaz, Latorre, Maldonado, Quade, & Betancourt, 2012; Pearson, Lawson, Head, McCarthy, & Dodson, 1999; Van Devender & Spaulding, 1979; Wells, 1983). Over 2000 *Neotoma* middens from western North America have been archived and analyzed since 1960 (Strickland, Thompson, & Anderson, 2001). Many analyses have been done from the careful process of extracting macrofossils of plant and animal materials from various midden layers. Once extracted, the macrofossils must be identified by a trained botanist. The material must also be radiocarbon dated to determine the age of the midden layer. From the macrofossils, inferences can be made about the plant vegetation that was surrounding the midden and how plants might have migrated in elevation and location. Many analyses have been done on presence / absence of plant species in a specific region or site and have greatly furthered the knowledge of ancient vegetation (J. L. Betancourt & Van Devender, 1981; Coats, Cole, & Mead, 2008; Emslie, Coats, & Oleksy, 2014; Holmgren et al., 2014; Lyford, Jackson, Gray, & Eddy, 2004; Thompson, 1984; Van Devender & Spaulding, 1979; Wells, 1983)

When first discovered, macrofossils from packrat middens were considered a check for previous pollen analyses. Due to the propensity for pollen to be widely dispersed through wind, it was thought that "pollen rain" may represent regional plants that were not readily found within the vicinity of the midden (Grayson, 2011). Pollen in lake deposits could also provide evidence of plants found within the lake's tributaries, possibly identifying plants at higher elevations as well as those nearby the water source (Maher, 1964). As it turns out, macrofossils and pollen from packrat middens provide very different information when it comes to vegetation and can be very powerful when combined in analysis. Due to pollen deposits often representing broader areas, being strongly continuous in time, and being found in large amounts, pollen can resolve issues of a packrat's selectivity of vegetation (Grayson, 2011). However, one drawback of pollen analysis is that pollen can usually only be identified to the family level whereas macrofossils can often be identified to the species level. Therefore, pollen can tell whether abundance in an area fluctuated over time, but macrofossils can tell you exactly what species were a part of that area's abundance. Many pollen analyses have been done from packrat middens to supplement the information obtained from macrofossils (Thompson, 1985; Thompson & Kautz, 1983; Wigand & Mehringer, 1985).

A less utilized approach used from packrat middens is the analysis of plant cuticles from pack rat fecal pellets. Plant cuticles, or epidermis fragments, can be identified under a microscope after the pellet material is ground up and placed on slides, allowing for an analysis of packrat diet and insight into how pack rats may present bias into midden analyses (Borrelli & Holmgren, 2016). Plant cuticles have been shown to identify taxa that were not detected as macrofossils in the middens sampled (Latorre, Betancourt, Rylander, & Quade, 2002). This method, while

requiring expertise in identifying the plant fragments, can provide a strong supplement to the existing macrofossil data found in a region. Another less utilized approach from packrat middens involves analyzing $\delta^{13}C$ isotopes from packrat fecal pellets to understand how vegetation and climate shifted over time (see description above about non-overlapping ratios between C_3 and C_4 species). A study done by Cole and Arundel 2005 looked at fecal pellets from packrat middens from Arizona to analyze the distribution of *Agave utahensis* during the Younger Dryas period (Cole & Arundel, 2005). They found extreme fluctuations in winter minimum temperatures occurred during the Younger Dryas in Arizona from isotopic results and corresponding elevational shifts of *Agave utahensis*. This was a key finding as previously these temperatures had not been generally accepted for this region and laid groundwork for considering carbon isotopes from *Neotoma* fecal pellets as a means to understand shifts in climate and vegetation.

A new and upcoming method has emerged in utilizing genomics to analyze ancient DNA (aDNA) extracted from packrat middens. Ancient DNA extracted from middens allowed for analysis of diet and biogeography of rare and extinct animals (Hofreiter et al., 2000; Kuch et al., 2002). Ancient DNA from middens has also been used to identify how papillomavirus infection spread and codiverged with packrats over time (Larsen, Cole, & Worobey, 2018) and how plant-pathogen communities responded to shifting climates over time (J. R. Wood et al., 2018). Ancient DNA metabarcoding is a more recent methodology that can be used to identify taxa by targeting the DNA of a specific gene using primers (Taberlet, Bonin, Zinger, & Coissac, 2018). This method can allow for the identification of taxa that may not have been detected using previous methods, such as macrofossils or pollen. It also allows for additional support of previous findings using these methods. The use of ancient DNA metabarcoding has been used previously on packrat

middens to identify rodent species (Hornsby, 2016; Kuch et al., 2002), but has only been done to assess plant communities over time in a few studies (Diaz et al., 2019; Moore, Tessler, Cunningham, Betancourt, & Harbert, 2020).

Great Basin

Most of what is known about the paleovegetation from the Great Basin comes from plant macrofossil analysis from packrat middens (Spaulding, 1985; Thompson, 1990; Wells, 1983). Typically, paleovegetation is determined from preserved remains in lakes and bogs, but in arid areas such as the Great Basin it is far less likely that extensive preservation will be seen (Spaulding, 1985). During the LGM, macrofossils from Great Basin pack rat middens show an abundance of subalpine vegetation such as bristlecone pine, limber pine, Engelmann spruce, white fir, juniper, pinyon pine, and accompanying steppe plants (Thompson, 1984; Wells, 1983). Some of these species are xerophytic, meaning they are adapted to utilize less water and suggests that this time period was more arid. During the Younger Dryas, macrofossils are greatly represented in the Great Basin and show a continued abundance of subalpine vegetation but an increase in abundance of juniper (Thompson, 1984; Wells, 1983). During this period, more mesophytic (requiring little water) species began to dominate as opposed to the xerophytic species of the LGM, suggesting that this period was wetter than the LGM. During the early Holocene, subalpine vegetation became very rare and the Great Basin consisted more of montane and pinyon-juniper vegetation such as quaking aspen, Rocky Mountain maple and Rocky Mountain juniper (Thompson, 1990). Interestingly during this time, there is evidence of lake levels rising in the Great Basin and grasses and sagebush becoming relatively more abundant

because of the wetter conditions (Grayson, 2011) . During the mid-Holocene, the Great Basin saw an introduction of joint fir, increases in pine, and increased abundance of sagebrush and western juniper (Thompson, 1990). The expansion of western juniper and fir pollen maximum of this time period suggests conditions with more effective moisture than modern (Thompson, 1990). When looking at how vegetation can respond to environmental change from the Pleistocene to modern times in the Great Basin, packrat middens have proven rich resources (Becklin, Medeiros, Sale, & Ward, 2014; Spaulding, 1985; Thompson, 1990; Wells, 1983).

Approach

The broad question I pose is in cases where plant communities are shifting in response to changing CO_2 and temperatures, how are animal diets responding? The objective of this study was to conduct a diet analysis of *Neotoma* over the time span of approximately 20,000 years at the Snake Range, Nevada, to ascertain potential shifts in diet due shifts in plant vegetation that were known to be occurring over time in response to climatic and CO_2 changes across that time period. We do this through a novel combination of methodologies using ancient DNA metabarcoding and stable isotope analyses on fecal pellets extracted from *Neotoma* middens in the Snake Range, Nevada. The middens that occur in the Snake Range and utilized in this study were likely constructed by *N. cinerea*, which is well documented as a dietary generalist. From $\delta^{13}C$ isotope signatures alone, we can get a sense of whether the packrat was eating mainly a C_3 or C_4 diet, but not determine whether it was eating a mixed C_3 or C_4 diet or a mainly CAM diet (Figure 2). In conjunction with ancient DNA metabarcoding, we can get a sense of plant community structure and identify the plants contributing to these

communities all in the perspective of the rodent's diet. The goals of this study are to: 1) Identify plant taxonomy from ancient DNA extracted from *Neotoma* fecal pellets over 27,000 to 3000 years BP; and 2) Determine the extent to which, if any, *Neotoma* diets changed over time due to changing vegetation that is predicted to have been responsive to CO_2 and climatic shifts. Because species of *Neotoma* are dietary generalists, we hypothesize that these species will show shifting diets that are related to changes in local vegetation in response to changing CO_2 levels and climate.

Methods

Study site

The middens in this study were extracted from the Snake Range, Nevada, which is found in the United States' Great Basin (Figure 5). In this area, mean annual temperatures in the LGM were approximately 2.5 °C (Braconnot et al., 2007), and in modern times are 8.6 °C (NOAA, 2011). Atmospheric CO_2 levels rose from approximately 180 ppm to 400 ppm from the LGM to modern times (IPCC, 2007). In addition, mean annual precipitation in the LGM was approximately 511 mm year^{-1} (Braconnot et al., 2007) and is now approximately 353 mm year^{-1} (NOAA, 2011).

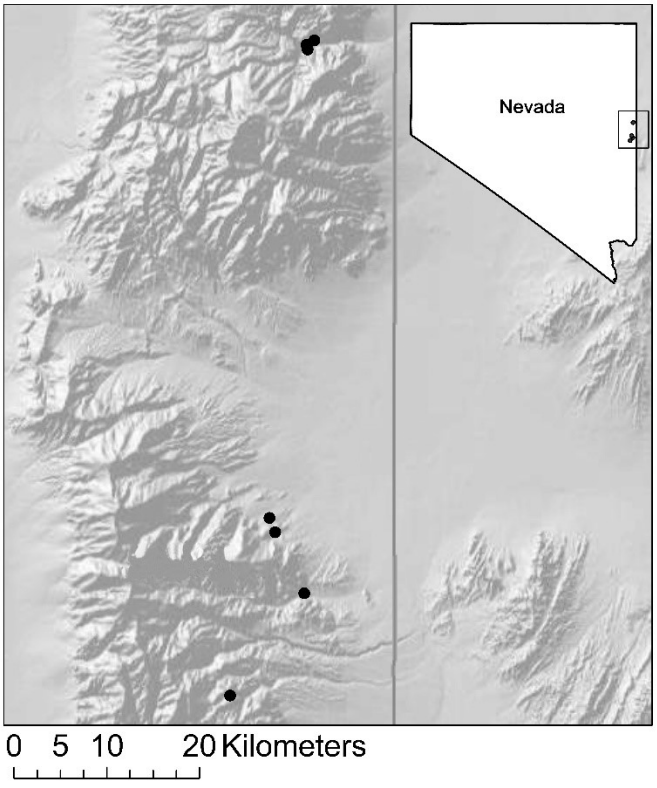

Figure 5. *Middens used in this study are represented by the black dots and were collected at the Snake Range, NV, USA.*

Midden Collection and Radiocarbon Dating

Ten midden layers from the Snake Range were previously identified for study within the Phillip Wells Pack Rat Midden Collection at the University of Kansas (curated by Dr. Joy K. Ward). All midden samples were previously extracted from a layer of midden following established protocols (Wells, 1983). Samples were thoroughly cleaned to remove any sources of outer contamination such as pollen, dust, and packrat urine (amberat) (Becklin et al., 2014). Samples were sent to the University of California Irvine KECK-CCAMS facility and the Woods Hole NOSAMS facility for radiocarbon (C^{14}) dating. There was not enough total fecal pellet sample material to complete radiocarbon(C^{14}) dating after completing other analyses, so we relied on dates from other fecal pellets and leaf tissue located in the same midden layers. Stratigraphic layering occurs in packrat middens which allows for different dates at different stratigraphic layers. Due to dates being consistent and low in variation among samples within a layer (Appendix Table 3), we will assume these dates for samples analyzed in this study.

Subsampling Pellets

Contamination from modern DNA is a major concern for ancient DNA analyses as very small amounts of endogenous DNA will remain in the samples and PCR amplification will preferentially amplify modern DNA over damaged ancient DNA molecules (Fulton, 2012). Due to the likely extent of damage to the ancient DNA and in order to avoid modern DNA contamination, subsampling, aDNA extractions, and PCR amplification were conducted in the University of Kansas Laboratory of Biological Anthropology ancient DNA clean room where no PCR amplification has taken place and specific protocols are in place to reduce contamination

(Knapp, Clarke, Horsburgh, & Matisoo-Smith, 2012). Our samples consisted of two pellet replicates per midden layer for 10 midden layers, resulting in 20 pellet samples (Table 1). Before entering the clean room, we adorned booties, hairnets, Tyvek full body suits, face masks, and gloves. Once dressed, we sprayed ourselves with bleach from head to toe and wiped the bleach to cover all surfaces. Then, we also adorned sleeves on top of the suits that were then bleached as well. We placed all samples into their own respective plastic bags and bleached the plastic bags before entering the clean room. In addition, we placed all protocols into laminated sleeves, taped, and bleached. Upon entering the clean room, we began to prepare all the tubes and reagents we would need for subsampling. We bleached all surfaces, tubes, instruments, reagent containers, and samples before placing them into the UV container hood. We separated out ten different plastic weigh boats and placed them under UVC light for 5 minutes. After the 5 minutes, we extracted the first fecal pellet from the tube and cut the pellet into sections using a razor blade until we had approximately 100 milligrams of sample for extraction. The remainder of the sample was returned to the initial tube to save for later analyses. We did not reuse any instruments in between samples and anything that was touched was bleached. Once the proper amount was attained, the sample was placed under UVC light for 5 minutes (J. R. Wood & Wilmshurst, 2016). We repeated this process for every pellet.

Table 1*. List of fecal pellet samples included in this study, including the midden layer they were extracted from, the Radiocarbon age, and the associated time period.*

Midden	Layer	Sample ID	Age (RC)	Time Period
264	A	264A-2	3295	Mid-Holocene
264	A	264A-3	3295	Mid-Holocene
253	A	253A-3	5615	Mid-Holocene
253	A	253A-2	5615	Mid-Holocene
253	F	253F-2	8795	Early Holocene
253	F	253F-4	8795	Early Holocene
253	K	253K-2	9200	Early Holocene
253	K	253K-3	9200	Early Holocene
262	A	262A-3	10190	Younger Dryas
262	A	262A-1	10190	Younger Dryas
270	I	270I-2	10950	Younger Dryas
270	I	270I-1	10950	Younger Dryas
187	I	187I-1	11650	Transition
187	I	187I-3	11650	Transition
185	C	185C-2	21600	LGM
185	C	185C-1	21600	LGM
271	F	271F-2	22300	LGM
271	F	271F-1	22300	LGM
271	D	271D-1	26080	LGM
271	D	271D-4	26080	LGM

Isotope Analysis

We conducted stable isotope analyses on the 20 fecal pellet samples in the Keck-NSF Paleoenvironmental and Environmental Laboratory at the University of Kansas using a ThermoFinnigan Mat 253 Isotope Ratio Mass Spectrometer. All samples were run to also determine Carbon (C), Nitrogen (N), C:N, and % C. We loaded approximately 3 -5 mg of the innermost digested plant material of the sample into 4 x 6 mm aluminum tins using a tared microbalance. We cleaned the workbench and all tools between samples with count-off and

ethanol to prevent contamination. We stored any remaining sample in vials with proper and specific labeling for potential use in future analyses. $\delta^{13}C$ were calculated using the following formula:

$$\delta = R_{sample} / R_{standard} - 1 \quad \text{(equation 1)}$$

where R is the ratio of $^{13}C/^{12}C$, using belemnite carbonate from the Pee Dee Formation, Hemingway, SC (PDB) as the standard. Data were converted to "per mil" (‰) notation by multiplying δ values by 1000.

Due to anthropogenic effects (mainly caused by burning of fossil fuels) on modern carbon isotope compositions (Seuss effect) (Plint, Longstaffe, & Zazula, 2019), a correction can be applied to account for changing $\delta^{13}C$ over time. For this study, I calibrated radiocarbon years to calendar years in order to compare EPICA Dome C measurements of $\delta^{13}C$ since the LGM to modern $\delta^{13}C$ (~-8.4‰) (EPICA, 2004). I calculated the difference in ancient ‰ for each radiocarbon age and modern $\delta^{13}C$ and applied the difference to my $\delta^{13}C$ isotope data.

aDNA Extractions and Amplification

All DNA extractions and PCR amplification prep were performed in the University of Kansas Ancient DNA clean lab. We made a buffer consisting of .5M EDTA, Proteinase K, and DTT and aliquoted 1 mL of buffer into each sample tube, including one extraction blank. After mixing, the samples were incubated overnight with medium-high spinning for 19 – 22 hours in a rotisserie incubator at 37 °C. We spun the sample tubes in centrifuge for 2 minutes to break up the pellet particulates. We then added 350 µL of solution C2 from Qiagen DNeasy PowerMax soil kit to each sample, mixed, and incubated at 4 °C for 10 minutes. We transferred the

supernatant to a new collection tube, added 250 µL of solution C3 from Qiagen DNeasy PowerMax soil kit to each sample, mixed, and incubated at 4 ° C for 10 min. After these steps, we transferred the supernatant to Amicon Ultra-4 30 kDA filter tubes and spun the samples at 2,500x using a swinging bucket rocket of Sorval T8 centrifuge for approximately 20 – 30 minutes, until only about 100 ul of concentrated extract remained. We extracted the 100 µL concentrations and moved them into previously prepared DeHyb A tubes and vortexed. We added the total amount to Zymo DCC-5 silica columns and completed 2 alcohol washes for each tube. The columns were then placed in 2.0mL low-bind tubes and the DSB+T Buffer was added to each tube. We incubated the columns for 5 minutes at room temperature, spun the tubes, and then stored the DNA extracts at -20 °C.

For PCR Amplification, we used the Platinum Taq HiFi kit. We used modified primers C (5'-CGAAATCGGTAGACGCTACG-3') and H (5'-CCATTGAGTCTCTGCACCTATC-3') to target the *trnL* gene (Diaz et al., 2019; Taberlet et al., 2007). To minimize sequencing error, we included a frameshift of 0 – 3 nucleotide base pairs for the forward and reverse primers. Each 25 µL reaction contained the following : 2.5 µL of 10x buffer, 1 µL MgSO4, 1.25 µL of BSA, 2 µL of dNTPs, 1 µL of both forward and reverse primer, 0.25 µL of Platinum Taq HiFi polymerase, 14 µL of water, and 2 µL of extracted DNA. Thermocycling conditions were as follows: 94 °C for 3:00, then 54 cycles at 94°C for 0:30, 62.9°C for 0:30, 68°C for 0:45, then 68°C for 10:00. We completed two replicates of PCR amplification to minimize error. We then ran gel electrophoresis on all replicates to check for DNA amplification.

Library Preparation and Sequencing

For library preparation, samples were sent to the Genome Sequencing Core at the University of Kansas where they were prepared following a modified version of the "16S Metagenomic Sequencing Library Preparation" protocol (Illumina, 2013). All amplicons were cleaned with AMPure XP Beads to remove any reaction mixture and leave only target fragments. After the clean-up, the amplicons were eluted into a volume of 15 µL of supernatant. Illumina index adapters were added to the 15 µL of amplicons and the full volume was included in the index PCR reaction Thermocycling conditions for this reaction were as follows: 95°C for 3 min, 8 cycles of 95°C for 0:30, 55°C for 0:30, and 72°C for 0:30, 72°C for 05:00, hold at 4°C. The completed amplicon library preps were then cleaned again using AMPure XP Beads to remove any reaction mixture leaving only the indexed amplicons. After the clean-up, all library preps were eluted into a volume of 15 µL, and quality checked with qPCR quantification. After normalizing and pooling the libraries, all samples were 2x300 paired-end sequenced using Illumina MiSeq technology.

Bioinformatic Analysis

I transferred the demultiplexed sequencing files received into my personal folder on the University of Kansas Community Cluster. I conducted initial bioinformatics on the raw sequencing data using the QIIME2 program (Bolyen et al., 2019) on the cluster. I imported the sequence data into QIIME2 using the input format of CasavaOneEightSingleLanePerSampleDirFmt. After importing the data, I trimmed primers and everything upstream using the Cutadapt tool (Martin, 2011) To quality-filter and denoise the data, I used the dada2 package (Callahan et al., 2016). I

truncated the reads to 110 base pairs for both forward and reverse reads. Then, I compiled the results into an amplicon sequence variant (ASV) table that could be imported to R. I assigned taxonomy to the rep sequences manually by using NCBI Blast of *trn*L gene entries (Appendix Table 4) and compiled a taxonomy table. I then combined the taxonomy and ASV tables together into a phyloseq object using the phyloseq package in R (McMurdie & Holmes, 2013). I performed basic quality control on the remaining ASVs, where I thresholded the ASVs to only include those that occurred in at least one sample and with at least 50 reads. Then, I calculated relative abundance (x / sum (x)) for the reads for each sample in R. I removed the four Blank samples from the analysis after determining contaminant taxa in them and removing those contaminant taxa that are not found in the study region (Araliaceae, Digitaria, Trisetum, Triticeae, Eritichium, Solidago, Setaria). I made all figures using ggplot2 and rioja packages in R (Juggins, 2017; Wickham, 2016).

Results

Relative abundance of taxa per sample

The sequencing protocol yielded an approximate total of 11 million raw reads. After quality filtering and removal of contaminants, the final data set consisted of 25 uniquely identified taxa among all samples (Figure 6). I identified 14 of these taxa to the Genus level but 11 were only identified to Family or subfamily level. No ASVs were identified to the species level. The most commonly occurring taxa within the samples was the genus *Juniperus,* comprising over 20% of the reads in 11 samples (Figure 6). Otherwise, the most abundant families and genera in order were *Penstemon* sp., Poaceae(Pooideae), Hydrangeaceae, *Castilleja* sp., Brassicaceae, Asteraceae(Asteroideae), and *Pinus* sp. (Figure 6).

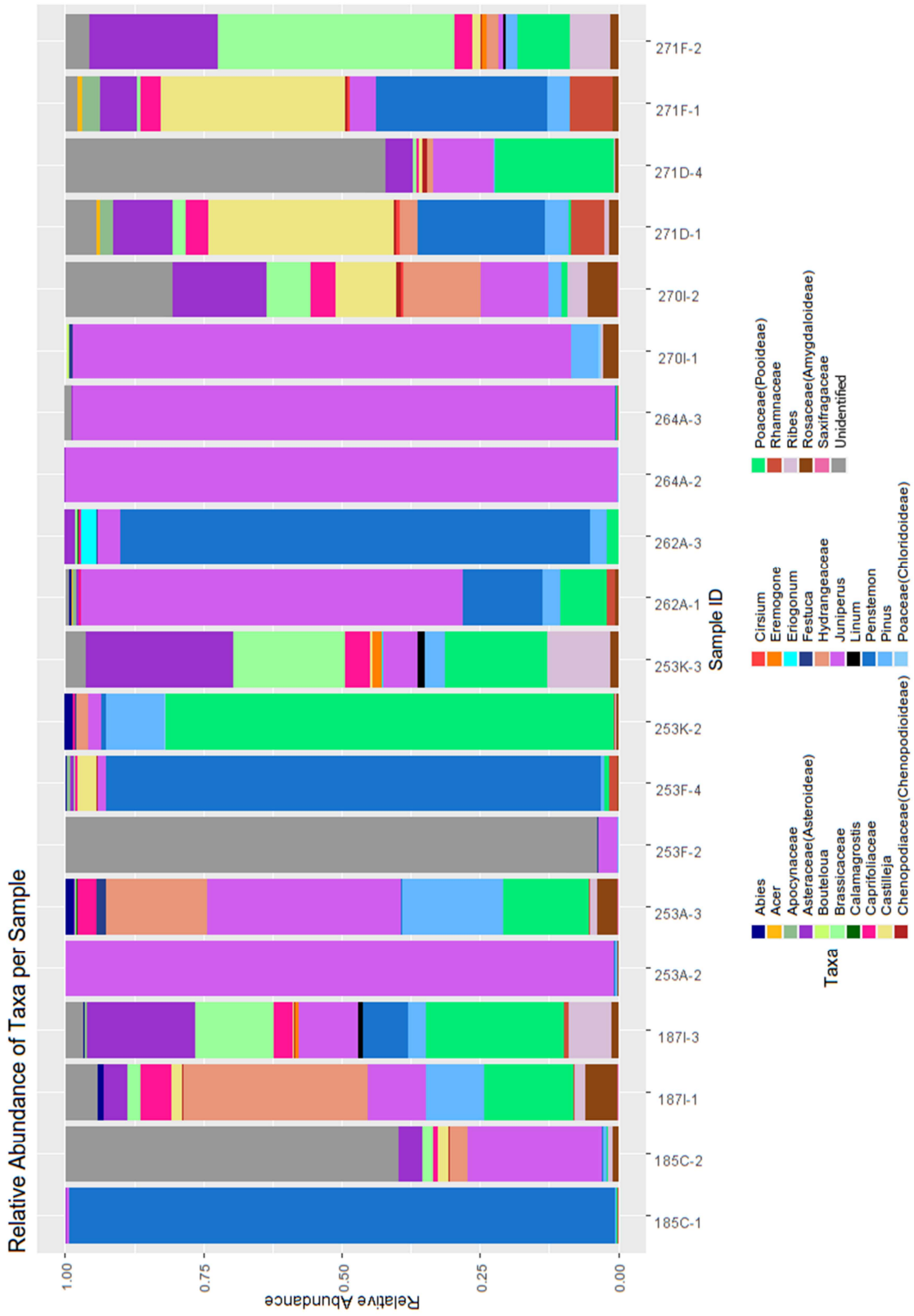

Figure 6. Calculated relative reads abundance (0-1) identified to taxonomic level per sample. Relative abundance was calculated using the formula x / sum(x).

Relative abundance of taxa over time

Relative read abundance varied by time period and genus and it is represented from 0 – 10 in Figure 7. I found 19 taxa within the Last Glacial Maximum (26800 to 21600 radiocarbon years) samples. Asteraceae(Asteroideae), Brassicaceae, and *Castilleja* sp. were identified at the highest relative read abundance in this time period. Rhamnaceae was identified at a higher relative read abundance in this time period than in any of the subsequent time periods (Figure 7). Notably, *Penstemon* sp. were identified at a relative read abundance of 9.155087 at 21,6000 radiocarbon years, the highest abundance this taxon occurred at in all samples (Figure 7). *Juniperus* sp. were identified during this time period, but at the lowest relative read abundance of all the time periods. In the Transition period (11650 radiocarbon years), Hydrangeace is identified at its highest relative read abundance and Poaceae(Pooideae) is identified at its second highest relative read abundance. *Pinus* sp. are also identified in this time period at 0.756 relative read abundance. In the Younger Dryas (10950 and 10190 radiocarbon years) period, *Juniperus* sp. appears at the second highest relative read abundance of the different time periods (Figure 7). This period also retains identification of *Penstemon* sp., Asteraceae(Asteroideae), *Castilleja sp.,* and Hydrangeaceae. In the Early Holocene (9200 radiocarbon years) period, Poaceae(Pooideae) was identified at a high (7.517) relative read abundance. The *Pinus* sp. taxon was also identified at the highest (0.996) relative read abundance of all the time periods (Figure 7). Overall, the least taxa were successfully identified from 8795 radiocarbon years ago. In the mid-Holocene samples, *Juniperus* made up most of the relative read abundance and was the highest relative read abundance of this taxon (Figure 7). *Pinus* sp., Hydrangeaceae, and Poaceae(Pooideae) were also identified during this time period.

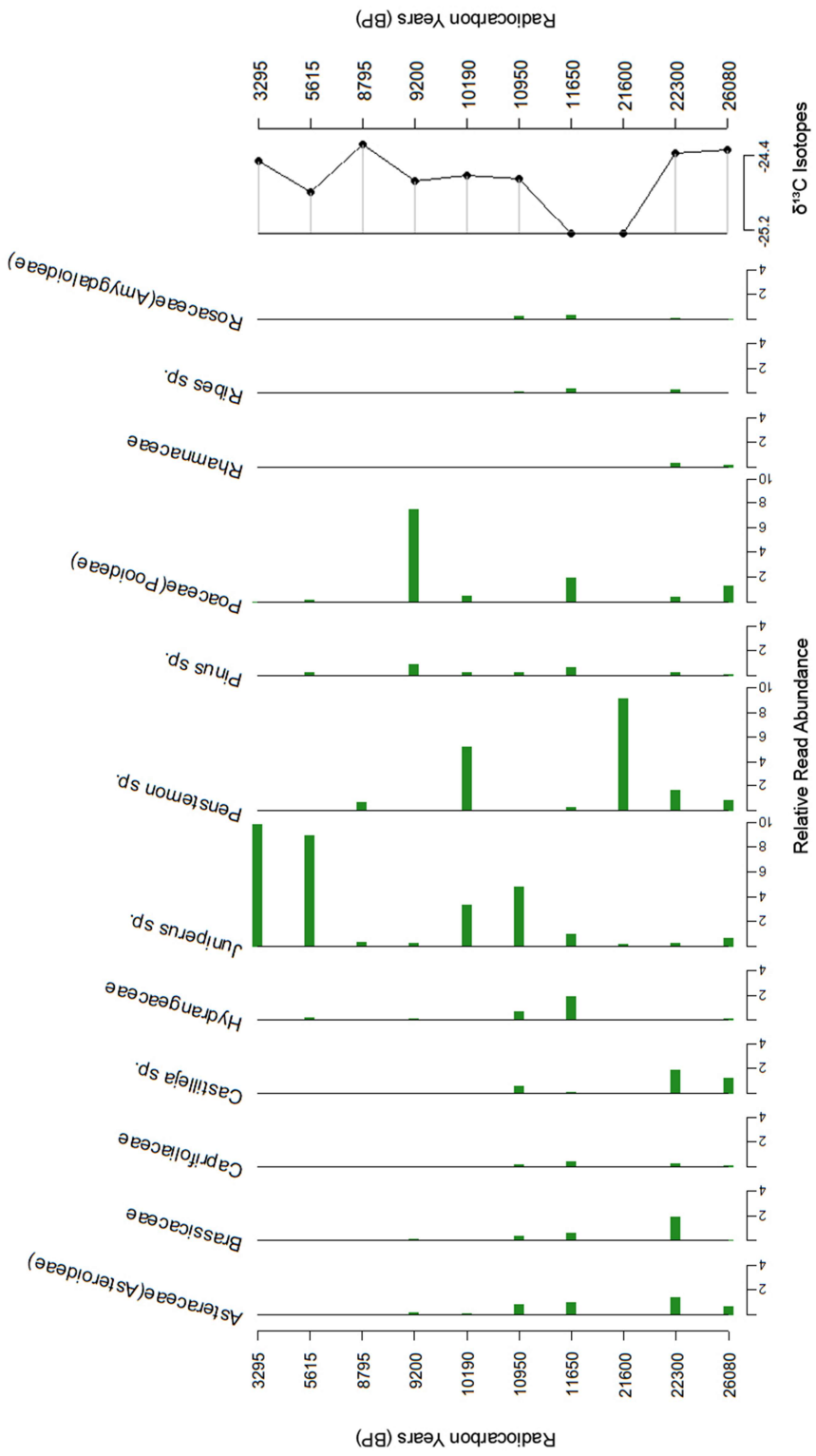

Figure 7. *Relative Read Abundance (0 - 10) for each taxon over Radiocarbon Years (BP) identified from the separate midden layers. Seuss corrected averaged isotopes values are presented over Radiocarbon years (BP).*

Pinus sp. and *Juniperus* sp. were identified in all samples. In the LGM (26080, 22300, and 21600 yrs BP), *Pinus* sp. and *Juniperus* sp. were identified at low relative read abundances (0.182, 0.314, and 0.028) and (0.697, 0.287, and 0.208), respectively (Figure 7). In the Transition period (11650 yrs BP), *Pinus* sp. was identified at a higher relative read abundance of 0.756, the second highest relative read abundance of this genera among the samples, while *Juniperus* sp. was identified at a relative read abundance of 1.065. In the Younger Dryas period (10950 and 10190 yrs BP), *Pinus* sp. appeared in the samples in consistent abundance (0.354 and 0.304) while *Juniperus* sp. appeared in much higher abundance in the samples (Figure 7). In the Early Holocene period, *Juniperus* sp. appeared in low relative read abundance while *Pinus* sp. appeared at its highest relative read abundance of all the samples (0.996). In the Middle Holocene time period, *Pinus* sp. appeared in lower abundance in the samples while *Juniperus* sp. appeared in both samples at high relative read abundance (Figure 7).

Isotopes

Due to changes in atmospheric $\delta^{13}C$ over the analyzed time periods, a correction was included for the $\delta^{13}C$ isotope values obtained from fecal pellets in this study. In Figure 8, $\delta^{13}C$ isotope values presented were corrected for changes in atmospheric $\delta^{13}C$ over the separate time periods. Corrected $\delta^{13}C$ isotope values for all samples ranged from -25.48‰ to -23.96 ‰, which is a relatively narrow range across such a wide time period, indicating a strong presence of a C_3 diet and potentially C_4 and/or CAM contributions. However, no CAM families or genera were taxonomically identified within these samples (Appendix Table 4). 21 of the identified families and genera contain almost exclusively C_3 species. Four of the identified families and genera are

known to contain some C_4 species: *Bouteloua sp.,* Asteraceae(Asteroideae), Chenopodiaceae, and Poaceae. The most negative isotope values (-25,47, -25.29, and -25.10) are from the 21600, 11650, and 10950 radiocarbon years, respectively (Figure 8). During these times, relatively abundant taxa identified consist of C_3 taxa such as *Penstemon* sp., Hydrangeace, *Pinus* sp. and *Juniperus* sp.. The least negative isotope values (-24.007, 24.029, -23.98, and -23.96) are from 26080, 22300, 8795, and 3295 radiocarbon years, respectively. Due to no CAM families or genera being identified, different C_4 families and genera may account for these more enriched values. However, even with the appearance of some possible C_4 families and genera, these isotope signatures are still more indicative of C_3 taxa. Asteraceae(Asteroideae) was present across my study periods and may have enriched isotope values. Chenopodiaceae was also present and likely contributed to enriched values for Early and Middle Holocene (3295, 8795) and LGM (22300, 26080) periods. Otherwise, *Bouteloua* sp. were present during the Early Holocene and Younger Dryas.

Table 2. *Seuss Corrected δ13C Isotope Values and Averaged δ13C Isotopes by Middens.*

Midden	Layer	Radiocarbon Age BP	Seuss Corrected $\delta^{13}C$ Isotopes	Averaged $\delta^{13}C$ Isotopes
264	A	3295	-23.9620	-24.4518
264	A	3295	-24.9417	
253	A	5615	-24.4728	-24.7863
253	A	5615	-25.0998	
253	F	8795	-23.9826	-24.2808
253	F	8795	-24.5790	
253	K	9200	-24.8060	-24.6667
253	K	9200	-24.5273	
262	A	10190	-24.8422	-24.6129
262	A	10190	-24.3836	
270	I	10950	-24.1929	-24.6453
270	I	10950	-25.0976	
187	I	11650	-25.2937	-25.2333
187	I	11650	-25.1729	

185	C	21600	-24.9866	-25.2324
185	C	21600	-25.4781	
271	F	22300	-24.0290	-24.3771
271	F	22300	-24.7252	
271	D	26080	-24.0070	-24.3370
271	D	26080	-24.6671	

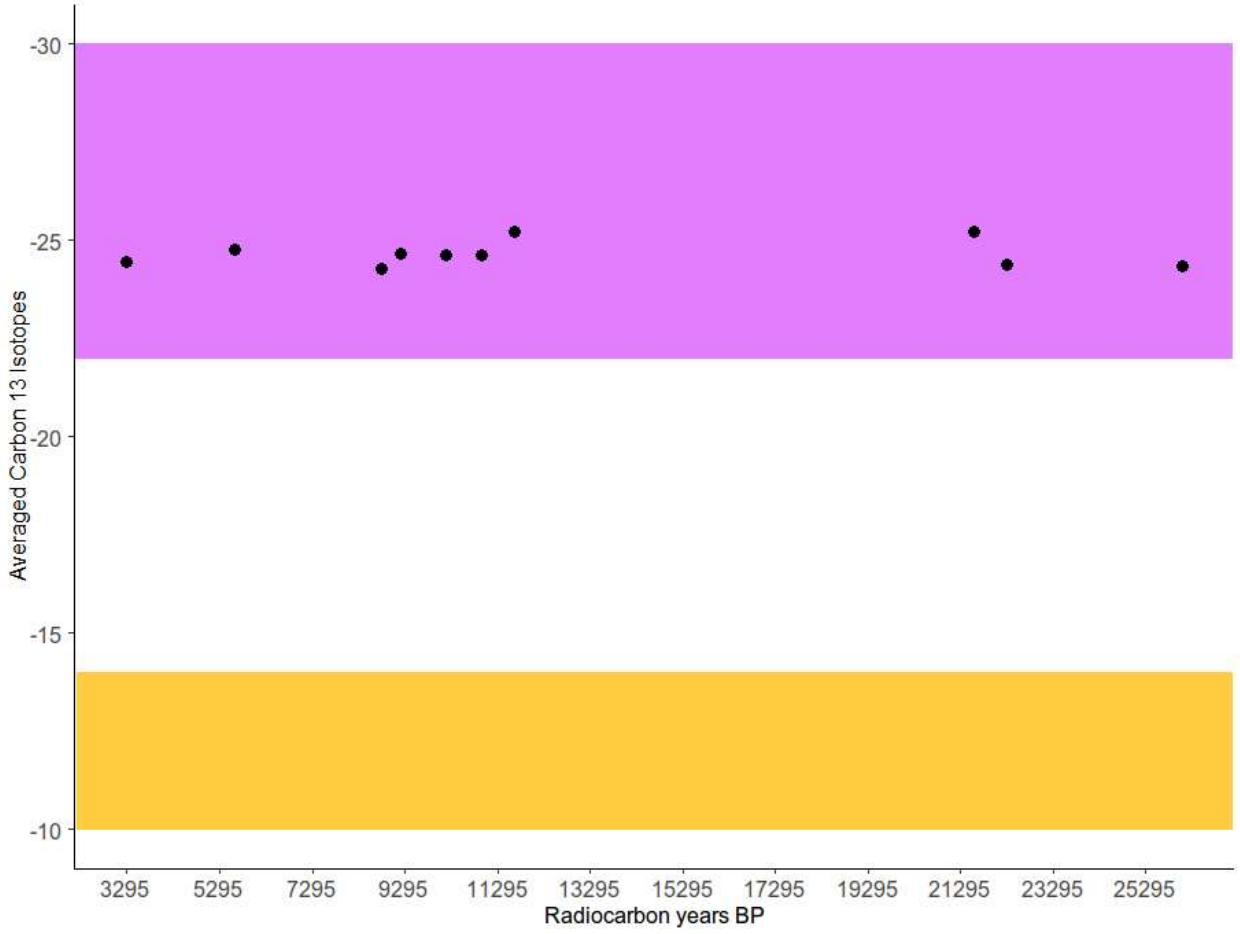

***Figure 8**. Seuss effect corrected $\delta^{13}C$ averaged isotope values over radiocarbon years BP. The isotope values of the two pellet replicates per midden layer are averaged and presented here. The orange box represents the range of C_4 carbon isotope values and the purple box represents C_3 carbon isotope values.*

Discussion

The primary goal of this project was to combine standard methods in paleoecology and plant biology to make novel inferences into plant community structures of the past in relation to climatic excursions. I used ancient DNA metabarcoding and stable isotope analyses on midden fecal pellets together to this avail. Overall, I was able to identify 25 taxa local to the Snake Range. While species level taxonomic identifications were not possible from these samples, likely due to the degradation of DNA as well as the lack of a site-specific reference database created from local vegetation, I have shown that it is possible to successfully extract and sequence ancient DNA from packrat midden fecal pellets and assign taxonomy to the Family and Genus level, a process that also allows us to make predictions about what specific species occurred at these time points and in these sites. In addition, identification to these taxonomic levels allows for the speculation of C_3 vs C_4 vs CAM plants. My findings also suggest some major genera and families to investigate in future work.

All taxa included in these analyses are known to occur at the study site. No taxa were identified that had not been previously identified from macrofossils extracted from these middens (Becklin et al., 2014; Wells, 1983). Unsurprisingly, many C_3 taxa were identified within these samples over time, especially *Juniperus* sp. which is well evidenced as occurring within the Snake Range (Becklin et al., 2014; Wells, 1983). No CAM taxa were identified from these samples even though the samples were referenced against various CAM reference *trn*L sequences (Appendix Table 4). This suggests that CAM was not responsible for any elevating of the observed isotopic signatures of the pellets and was not a main component of the packrats diet. Four taxa

identified could possibly represent C_4 species: *Bouteloua sp.,* Asteraceae(Asteroideae), Chenopodiaceae, and Poaceae. However, due to the consistent C_3 signatures of the $\delta^{13}C$ isotopes, the diet of the packrat primarily consisted of C_3 vegetation and any C_4 species present would have made up a very small portion of the diet.

Shifts in vegetation presence in the packrat's diet can be observed from each time period, which can be explained from changes in temperature, precipitation, and [CO_2]. In the LGM, when temperatures were around ~8 °C cooler than modern and atmospheric CO_2 was ~180 ppm, many of the identified taxa consisted of flowering plants. In the Younger Dryas period, when temperatures were around ~9 °C cooler than modern and atmospheric CO_2 was around ~265 ppm, the identified vegetation was a mixture of pines, conifers, flowering plants, and herbaceous plants. In the Early Holocene, where temperatures were close to modern and atmospheric CO_2 was around ~280 ppm, the vegetation identified at the highest relative read abundances consisted of flowering plants, pines, and grasses. In the Mid-Holocene, where temperatures were ~0-2°C warmer than modern times and atmospheric CO_2 remained around ~280 ppm, the main type of vegetation identified was the conifer *Juniperus*. My results provide additional support for previous work that has shown shifts in vegetation composition at the Snake Range over these time periods. Understanding shifts in past vegetation in response to changing temperatures and atmospheric CO_2 can inform how future vegetation may change in a constantly changing world.

There is a general trend of many of the analyzed time periods having a variety of taxa identified. This supports my assumption that the *Neotoma* species inhabiting these middens were likely dietary generalists such as *N. cinerea.* Notably, during the mid-Holocene time period

around 3295 and 5615 yrs BP, an overwhelming abundance of *Juniperus* genus was identified in both pellet samples. As this sample is the youngest in age and likely had the least amount of degraded DNA, it is interesting that one genus was identified in such abundance. This suggests that *Juniperus* could have been extremely prominent during this time. More work on pellets from this time frame is necessary to determine what drives this prominence of *Juniperus.*

Pinus sp. and *Juniperus* sp. have long been of interest to ecologists in the west. Unsurprisingly, these two genera were identified to some extent in all the samples in this study. Previous work at the Snake Range has identified presence / absence of a some particular species of these genera from the LGM through the Holocene from analyzing macrofossils from the same middens utilized in this study (Wells, 1983). Wells identified bristlecone pine (*Pinus longaeva)* at the site for all of these time periods, which would explain the consistent appearance of *Pinus* sp. in the packrat diet. He found limber pine (*Pinus flexilis)* began to appear in the Snake Range middens around 11800 yrs BP during the Transition period and persisted through until around 4640 yrs BP (Wells, 1983), matching up with the high relative abundance of *Pinus* sp. found in the Younger Dryas and late Holocene samples in this study. While we know juniper occurred at this site throughout time, Wells did not discover *Juniperus* sp. in abundance until around 11620 to 10600 yrs BP (*Juniperus communis)*. It disappeared from the middens again and did not return until around 4640 yrs BP in immense abundance (57 – 99 % composition by mass) as the first appearance of Utah juniper (*Juniperus osteosperma)* in the Snake Range (Wells, 1983). This huge increase in abundance of Utah juniper macrofossils in the middens around this time could explain the overwhelming abundance of *Juniperus* sp. found in the Mid-Holocene samples in this study. As Utah juniper became more abundant in the vegetation, the packrats likely developed

adaptations to be able to survive off the toxic juniper. The packrat does not really utilize these high levels of pine that were abundant on the landscape, which we know from middens and pollen. However, as juniper becomes more abundant at the site, the packrats do utilize juniper and this is fascinating because back in the LGM they did not utilize juniper but now they do and this suggests they may have adapted the behaviors that allow them to eat this plant.

My results support previous findings in this region and suggest potential successes using this method in the future. Ancient DNA can identify taxa that are not identified as macrofossils possibly from pollen, while macrofossils can identify taxa that might not be present as DNA. This could be due to the taxa not existing in the sample, the DNA of the taxa becoming so degraded that it is no longer identifiable from the sample, or limitations of the reference database leading to the inability to identify taxa. If more time were available to process more samples, new patterns and insights would likely emerge. In addition, with the inclusion of a specific reference database created from collected local vegetation, I am confident that new taxa could be identified and that taxa identified could be to a species level. Previous studies (Diaz et al., 2019; Moore et al., 2020) had varying success in identifying taxa from this *trn*L chloroplast gene. The utilization of different primer regions, such as *rbc*L, could lead to additional taxa identifications. However, these primer regions are also limited by what reference sequences are available and may be subject to varying levels of conservation within samples. In addition, different sequencing methods such as shotgun and metagenomics could potentially identify additional plant taxa while also providing insight on present animal, bacterial, and fungi taxa within the samples.

Paleoecological studies such as mine are frequently limited by sample sizes and persistence of DNA. Ancient samples must be processed in small batches to prevent contamination and therefore time becomes a major constraint on how many samples can be analyzed. In this study, I only extracted from twenty fecal pellets. Further, ancient DNA degrades significantly over time, so older pellets are likely limited in DNA. This means some of my samples may only represent a subset of taxa present at the site at a given time. While this study only utilized 20 fecal pellets in total, other paleodiet studies have ranged from 12 coprolite samples (James & Burney, 1997) to 43 coprolite samples (J. R. Wood et al., 2008). These samples may not be representative of full diet during these time periods, but they are a good starting point for allowing the speculation of shifts in diet over time.

There are limitations associated with amplicon sequencing of plant DNA. One of the major drawbacks of amplicon sequencing plant DNA is that you will be limited to the available reference sequences for that specific region. Ancient DNA analyses are limited by the barcodes obtained from modern vegetation. While the *trn*L intron is documented to be a well conserved chloroplast gene in most plant species, especially in ancient samples, there is still a relatively low resolution for the intron (Taberlet et al., 2007). This problem can be improved when a local reference database is generated from local vegetation, though for this study this was not possible. This study would likely have more taxa identified and to more specific taxonomic level if a site-specific reference database of local vegetation had been created. It would also be beneficial to supplement these analyses using a shotgun sequencing or metagenomics approach. With one of these alternative approaches, the samples would not be limited to the inconsistent plant genome reference databases. With a shotgun sequencing or metagenomics

approach, additional eukaryotic and prokaryotic species can be identified, such as additional plants, animals, bacteria, and fungi.

There are many ways this work can be expanded and improved upon in the future. Firstly, this work could be immediately improved by an increase in total samples. Fecal pellets are in no shortage in midden layers and if time and funding allowed, I would sample more pellets to identify deeper evidence of patterns in vegetation over time. In addition, it would be very beneficial to conduct DNA metabarcoding on material from the midden layer that the fecal pellets are extracted from so that comparisons could be made between the collecting and eating behaviors of these packrats. From past macrofossil analyses (Dial & Cazaplewsk, 1990), packrats are known to collect and store plant material for food so it is likely that some additional taxa could be identified from the midden layer itself. Additionally, when working with midden layer material it would be possible to extract DNA from a larger amount of sample material, suggesting that more taxa would be able to be identified. Previous work has been done to identify macrofossils from these midden layers (Becklin et al., 2014; Wells, 1983) and ancient DNA metabarcoding of the midden itself would provide additional support for past findings as well as the potential to identify new taxa that had not been previously observed. Lastly and perhaps most importantly, constructing a local reference database of vegetation found in the site is essential to improving the resolution of taxonomic identification of these samples.

More insight could be gained about the make-up of these fecal pellet samples if the rodent species were identified from ancient DNA. I would be interested to attempt identifying packrat species from ancient host DNA from these fecal pellets in the future as it would provide

biological insights that cannot currently be determined, such as if the *Neotoma* species can explain high abundance of one taxa in a given layer. In addition, I would sequence the packrat's genome to see if there were genes involved in these liver enzymes that allow for increased consumption of toxic vegetation and also sequence the microbiome to see if new microbes appeared in the time periods where toxic juniper was utilized more in the diet.

Conclusion

In this study, I identified indigenous taxa from ancient DNA extracted from Snake Range *Neotoma* midden fecal pellets from time periods ranging from 27,000 to 3500 radiocarbon years ago. No CAM species was identified within the samples, but many C_3 taxa and a few C_4 taxa were identified. There is strong evidence of a C_3 diet throughout the time periods in this study as supported by identified taxa and $\delta^{13}C$ isotope analyses. In addition, shifts in present vegetation of the pack rats' diet over time were observed and provide insight into how the rats adjusted their diet in response. I found that *Juniperus* sp. became increasingly present in the packrat's diet in the Younger Dryas period and in the mid-Holocene period, suggesting that they likely adapted to eating the ordinarily toxic plant material. In addition, the evidence of juniper and pine being dominant at the site across the 30,000 year range but only juniper being utilized heavily in the diet provides support to this theory that the packrats adapted to eat more of the juniper in their diet. I have shown that in combination, ancient DNA metabarcoding and stable isotope analyses on packrat fecal pellets can identify plant taxonomy to the Family or Genus level and provide insight into shifts in diet over time. These identified taxa provide additional support for previous findings at the Snake Range, NV, and can be utilized in future studies. Further work must be done to improve the resolution of these methods, to include improving reference plant databases and ancient sample preservation.

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Appendices

Appendix A.

***Table 3**. Radiocarbon ages of previously dated material from these middens.*

Facility	Midden	Species	Tissue Type	Age	Age Err
UCIAMS	264 A	Neotoma	Feces	3295	15
UCIAMS	253 B	ABCO	Leaf	4230	20
UCIAMS	253 B	JUOS	Leaf	4340	130
UCIAMS	253 A	Neotoma	Feces	5615	20
UCIAMS	253 K	PIMO	Leaf	7825	20
UCIAMS	253 F	Neotoma	Feces	8795	25
UCIAMS	253 K	OPPO	Spine	9220	25
UCIAMS	253 D	Neotoma	Feces	9320	25
UCIAMS	253 D	Neotoma	Feces	9395	25
UCIAMS	262 B	PIAR	Leaf	10115	20
UCIAMS	262 A	Neotoma	Feces	10190	25
UCIAMS	270 G	PIFL	Leaf	10940	35
Woods Hole	270 I	PILO	Leaf	10950	85
UCIAMS	270 G	SYMPH	Stem	10955	30
UCIAMS	270 G	PILO	Leaf	10955	30
UCIAMS	187 G	PIFL	Leaf	11135	30
UCIAMS	187 G	PILO	Leaf	11595	30
Woods Hole	187 I	PILO	Leaf	11650	90
UCIAMS	187 G	SYMPH	Stem	11715	35
UCIAMS	185 C	Neotoma	Feces	21600	90
Woods Hole	271 F	PILO	Leaf	22000	110
Woods Hole	271 F	PILO	Leaf	22600	420
UCIAMS	271 D	Neotoma	Feces	26080	140

***Table 4.** Plant taxonomy from the Great Basin with trnL gene reference entries.*

Family	***Genus***	***Species***
Pinaceae	*Abies*	*concolor*
Pinaceae	*Abies*	*sp.*
Pinaceae	*Abies*	*lasiocarpa*
Pinaceae	*Abies*	*magnifica*
Fabaceae	*Acacia*	*sp.*
Fabaceae	*Acacia*	*greggii*
Fabaceae	*Acacia*	*roemeriana*

Fabaceae	*Acacia*	*constricta*
Asteraceae	*Acamptopappus*	*sphaerocephalus*
Sapindaceae	*Acer*	*sp.*
Sapindaceae	*Acer*	*glabrum*
Sapindaceae	*Acer*	*grandidentatum*
Asteraceae	*Achillea*	*millefolium*
Poaceae	*Achnatherum*	*hymenoides*
Rosaceae	*Adenostoma*	*sp.*
Rosaceae	*Adenostoma*	*fasciculatum* var *obtusifolium*
Asparagaceae	*Agave*	*sp.*
Asparagaceae	*Agave*	*lecheguilla*
Asparagaceae	*Agave*	*americana*
Asteraceae	*Agoseris*	*glauca*
Poaceae	*Agropyron*	*sp.*
Poaceae	*Agropyron*	*cristatum*
Poaceae	*Agrostis*	*sp.*
Poaceae	*Agrostis*	*exarata*
Polemoniaceae	*Aliciella*	*latifolia*
Amaryllidaceae (formerly Alliaceae, Liliaceae)	*Allium*	*fimbriatum*
Amaryllidaceae (formerly Alliaceae, Liliaceae)	*Allium*	*nevadense*
Amaryllidaceae (formerly Alliaceae, Liliaceae)	*Allium*	*acuminatum*
Betulaceae	*Alnus*	*sp.*
Amaranthaceae	*Amaranthus*	*fimbriatus*
Asteraceae	*Ambrosia*	*sp.*
Asteraceae	*Ambrosia*	*dumosa*
Rosaceae	*Amelanchier*	*sp.*
Rosaceae	*Amelanchier*	*alnifolia*
Rosaceae	*Amelanchier*	*utahensis*
Boraginaceae	*Amsinckia*	*sp.*
Boraginaceae	*Amsinckia*	*tessellata*
Poaceae	*Andropogon*	*sp.*
Asteraceae	*Antennaria*	*sp.*
Asteraceae	*Antennaria*	*microphylla*
Apocynaceae	*Apocynum*	*cannabinum*
Ranunculaceae	*Aquilegia*	*formosa*
Brassicaceae	*Arabis*	*pulchra*
Ericaceae	*Arbutus*	*sp.*
Santalaceae	*Arceuthobium*	*sp.*
Ericaceae	*Arctostaphylos*	*uva-ursa*
Papaveraceae	*Argemone*	*sp.*
Rosaceae	*Argentina*	*anserina*

Poaceae	*Aristida*	*longiseta*
Asteraceae	*Artemisia*	*sp.*
Asteraceae	*Artemisia*	*arbuscula*
Asteraceae	*Artemisia*	*ludoviciana*
Asteraceae	*Artemisia*	*tridentata*
Asteraceae	*Artemisia*	*tripartita*
Apocynaceae (Asclepiadaceae)	*Asclepias*	*fascicularis*
Fabaceae	*Astragalus*	*layneae*
Fabaceae	*Astragalus*	*spp.*
Fabaceae	*Astragalus*	*eremiticus*
Fabaceae	*Astragalus*	*filipes (stenophyllus)*
Fabaceae	*Astragalus*	*cicer*
Fabaceae	*Astragalus*	*tenellus*
Amaranthaceae (Chenopodioideae)	*Atriplex*	*sp.*
Acanthaceae	*Beleperone (Justicia)*	*californica*
Berberidaceae	*Berberis*	*sp.*
Berberidaceae	*Berberis*	*trifoliolata*
Poaceae	*Bouteloua*	*sp.*
Poaceae	*Bouteloua*	*dactyloides*
Poaceae	*Bouteloua*	*ramosa*
Cucurbitaceae	*Brandegea*	*bigelovii*
Brassicaceae	*Brassica*	*tournefortii*
Poaceae	*Bromus*	*tectorum*
Poaceae	*Bromus*	*carinatus*
Poaceae	*Bromus*	*marginatus*
Poaceae	*Bromus*	*rubens*
Scrophulariaceae	*Buddleja*	*sp.*
Scrophulariaceae	*Buddleja*	*utahensis*
Burseraceae	*Bursera*	*microphylla*
Poaceae	*Calamagrostis*	*canadensis*
Poaceae	*Calamagrostis*	*rubescens*
Liliaceae	*Calochortus*	*kennedyi*
Liliaceae	*Calochortus*	*plummerae*
Liliaceae	*Calochortus*	*flexuosus*
Liliaceae	*Calochortus*	*macrocarpus*
Liliaceae	*Calochortus*	*nuttallii*
Liliaceae	*Calochortus*	*gunnisonii*
Liliaceae	*Calochortus*	*elegans*
Liliaceae	*Calochortus*	*nitidus*
Asparagaceae	*Camassia*	*quamash*
Asparagaceae	*Camassia*	*leichtlinii*

Onagraceae	*Camissonia*	*boothii*
Onagraceae	*Camissonia*	*claviformis*
Onagraceae	*Camissonia*	*campestris*
Celastraceae	*Canotia*	*holacantha*
Cyperaceae	*Carex*	*rostrata*
Cactaceae	*Carnegiea*	*gigantea*
Orobanchaceae	*Castilleja*	*chromosa*
Orobanchaceae	*Castilleja*	*angustifolia*
Orobanchaceae	*Castilleja*	*applegatei*
Orobanchaceae	*Castilleja*	*plagiotoma*
Orobanchaceae	*Castilleja*	*exserta*
Brassicaceae	*Caulanthus*	*pilosus*
Brassicaceae	*Caulanthus*	*coulteri*
Brassicaceae	*Caulanthus*	*inflatus*
Brassicaceae	*Caulanthus (Strepanthus)*	*cooperi*
Rhamnaceae	*Ceanothus*	*sp.*
Rhamnaceae	*Ceanothus*	*greggii*
Rhamnaceae	*Ceanothus*	*greggii* var *perplexans*
Cannabaceae	*Celtis*	*sp.*
Cannabaceae	*Celtis*	*reticulata*
Rosaceae	*Cercocarpus*	*sp.*
Rosaceae	*Cercocarpus*	*betuloides*
Asteraceae	*Chaenactis*	*sp*
Asteraceae	*Chaenactis*	*douglasii*
Asteraceae	*Chaetopappa*	*ericoides*
Rosaceae	*Chamaebatiaria*	*sp.*
Rosaceae	*Chamaebatiaria*	*millefolia*
Pteridaceae	*Cheilanthes*	*feei*
Bignoniaceae	*Chilopsis*	*linearis*
Rutaceae	*Choisya*	*sp.*
Polygonaceae	*Chorizanthe*	*sp.*
Polygonaceae	*Chorizanthe*	*brevicornu*
Polygonaceae	*Chorizanthe*	*rigida*
Asteraceae	*Chrysothamnus*	*viscidiflorus*
Asteraceae (Carduoideae)	*Cirsium*	*sp.*
Ranunculaceae	*Clematis*	*sp.*
Scrophulariaceae	*Collinsia*	*parviflora*
Polemoniaceae	*Collomia*	*linearis*
Rhamnaceae	*Condalia*	*sp.*
Cornaceae	*Cornus*	*sp.*
Cactaceae (Cactoideae)	*Coryphantha*	*vivipara*

Boraginaceae	*Cryptantha*	*sp.*
Boraginaceae	*Cryptantha*	*flavoculata*
Cucurbitaceae	*Cucurbita*	*palmata*
Convolvulaceae	*Cuscuta*	*sp.*
Cactaceae	*Cylindropuntia*	*sp.*
Apiaceae	*Cymopterus*	*deserticola*
Fabaceae	*Dalea*	*bicolor* var *argyraea*
Fabaceae	*Dalea*	*spinosa*
Fabaceae	*Dalea*	*molissima*
Fabaceae	*Dalea*	*ornata*
Poaceae (Danthonioideae)	*Danthonia*	*californica*
Poaceae (Danthonioideae)	*Danthonia*	*unispicata*
Poaceae (Danthonioideae)	*Danthonia*	*intermedia*
Poaceae (Danthonioideae)	*Danthonia*	*parryi*
Rosaceae	*Dasiphora*	*fruticosa*
Asparagaceae (Nolinoideae)	*Dasylirion*	*sp.*
Poaceae (Chloridoideae)	*Dasyochloa*	*pulchella*
Solanaceae	*Datura*	*wrightii*
Solanaceae	*Datura*	*stramonium*
Solanaceae	*Datura*	*discolor*
Solanaceae	*Datura*	*inoxia*
Ranunculaceae	*Delphinium*	*parishii*
Papaveraceae	*Dendromecon*	*rigida*
Poaceae (Pooideae)	*Deschampsia*	*caespitosa*
Brassicaceae	*Descurainia*	*sp.*
Asparagaceae (Brodiaeoideae)	*Dichelostemma*	*pulchellum*
Poaceae (Chloridoideae)	*Distichlis*	*spicata*
Crassulaceae	*Dudleya*	*abramsii*
Cactaceae	*Echinocactus*	*polycephalus*
Cactaceae (Cactoideae)	*Echinocereus*	*sp.*
Cactaceae (Cactoideae)	*Echinocereus*	*triglochidiatus*
Elaeagnus	*Elaeagnus*	*sp.*
Cyperaceae	*Eleocharis*	*quinqueflora*
Poaceae (Pooideae)	*Elymus*	*sp.*
Poaceae (Pooideae)	*Elymus*	*elymoides*
Poaceae (Pooideae)	*Elymus*	*macrourus*
Poaceae (Pooideae)	*Elymus*	*wawawaiensis*
Poaceae (Pooideae)	*Elymus*	*multisetus*
Poaceae (Pooideae)	*Elymus*	*trachycaulus*
Poaceae (Pooideae)	*Elymus*	*glaucus*
Asteraceae	*Encelia*	*sp.*

Ephedraceae	*Ephedra*	*sp.*
Ephedraceae	*Ephedra*	*viridis*
Ephedraceae	*Ephedra*	*aspera*
Ephedraceae	*Ephedra*	*californica*
Ephedraceae	*Ephedra*	*nevadensis*
Onagraceae	*Epilobium*	*canum*
Polemoniaceae	*Eriastrum*	*sapphirinum*
Asteraceae	*Ericameria*	*nauseosa*
Asteraceae	*Erigeron*	*sp.*
Boraginaceae	*Eriodictyon*	*sp.*
Polygonaceae (Eriogonoideae)	*Eriogonum*	*sp.*
Polygonaceae (Eriogonoideae)	*Eriogonum*	*caespitosum*
Polygonaceae (Eriogonoideae)	*Eriogonum*	*microthecum*
Polygonaceae (Eriogonoideae)	*Eriogonum*	*ovalifolium*
Polygonaceae (Eriogonoideae)	*Eriogonum*	*umbellatum*
Polygonaceae (Eriogonoideae)	*Eriogonum*	*vimineum*
Polygonaceae (Eriogonoideae)	*Eriogonum*	*compositum*
Geraniaceae	*Erodium*	*cicutarium*
Brassicaceae	*Erysimum*	*asperum*
Papaveraceae	*Eschscholzia*	*californica*
Loasaceae	*Eucnide*	*sp.*
Loasaceae	*Eucnide*	*urens*
Zygophyllaceae	*Fagonia*	*sp.*
Polygonaceae	*Fagopyrum*	*esculentum*
Rosaceae (Rosoideae)	*Fallugia*	*sp.*
Rosaceae (Rosoideae)	*Fallugia*	*paradoxa*
Hydrangeaceae (Hydrangeoideae)	*Fendlerella*	*sp.*
Hydrangeaceae (Hydrangeoideae)	*Fendlerella*	*utahensis*
Cactaceae (Cactoideae)	*Ferocactus*	*sp.*
Cactaceae (Cactoideae)	*Ferocactus*	*cylindraceus*
Cactaceae (Cactoideae)	*Ferocactus*	*haematacanthus*
Cactaceae (Cactoideae)	*Ferocactus*	*fordii*
Cactaceae (Cactoideae)	*Ferocactus*	*latispinus*
Poaceae (Pooideae)	*Festuca*	*idahoensis*
Poaceae (Pooideae)	*Festuca*	*occidentalis*
Oleaceae	*Forestiera*	*sp.*
Fouquieriaceae	*Fouquieria*	*sp.*
Fouquieriaceae	*Fouquieria*	*splendens*
Oleaceae	*Fraxinus*	*sp.*
Oleaceae	*Fraxinus*	*anomala*
Asteraceae (Asteroideae)	*Gaillardia*	*aristata*

Rubiaceae	*Galium*	*stellatum*
Rubiaceae	*Galium*	*sp.*
Garryaceae	*Garrya*	*sp.*
Garryaceae	*Garrya*	*ovata*
Geraniaceae	*Geranium*	*richardsonii*
Geraniaceae	*Geranium*	*viscosissimum*
Poaceae (Pooideae)	*Glyceria*	*sp.*
Asteraceae (Asteroideae)	*Gnaphalium*	*sp.*
Amaranthaceae (Chenopodioideae)	*Grayia*	*sp.*
Amaranthaceae (Chenopodioideae)	*Grayia*	*spinosa*
Asteraceae	*Gutierrezia*	*sp.*
Asteraceae	*Gymnosperma*	*sp.*
Asteraceae	*Gymnosperma*	*glutinosum*
Asteraceae	*Haplopappus*	*sp.*
Asteraceae (Hecastocleidoideae)	*Hecastocleis*	*sp.*
Asteraceae (Hecastocleidoideae)	*Hecastocleis*	*shockleyi*
Fabaceae	*Hedysarum*	*boreale spp. Utahensis*
Asteraceae (Asteroideae)	*Helianthus*	*sp.*
Asteraceae (Asteroideae)	*Helianthus*	*annuus*
Boraginaceae	*Heliotropium*	*curassavicum*
Asparagaceae (Agavoideae)	*Hesperocallis*	*undulata*
Poaceae (Pooideae)	*Hesperostipa*	*comata*
Asparagaceae (Agavoideae)	*Hesperoyucca*	*whipplei*
Poaceae (Panicoideae)	*Heteropogon*	*sp.*
Poaceae (Panicoideae)	*Heteropogon*	*contortus*
Asteraceae	*Heterotheca*	*villosa*
Poaceae (Chloridoideae)	*Hilaria*	*jamesii*
Rosaceae (Amygdaloideae)	*Holodiscus*	*sp.*
Rosaceae (Amygdaloideae)	*Holodiscus*	*microphyllus*
Lamiaceae (Nepetoideae)	*Hyptis*	*sp.*
Lamiaceae (Nepetoideae)	*Hyptis*	*emoryi*
Capparaceae	*Isomeris*	*arborea*
Hydrangeaceae (Jamesioideae)	*Jamesia*	*sp.*
Hydrangeaceae (Jamesioideae)	*Jamesia*	*americana*
Juglandaceae (Juglandoideae)	*Juglans*	*sp.*
Juglandaceae (Juglandoideae)	*Juglans*	*microcarpa*
Juncaceae	*Juncus*	*balticus*
Cupressaceae (Cupressoideae)	*Juniperus*	*osteosperma*
Cupressaceae (Cupressoideae)	*Juniperus*	*scopulorum*
Cupressaceae (Cupressoideae)	*Juniperus*	*communis*
Cupressaceae (Cupressoideae)	*Juniperus*	*sp.*

Cupressaceae (Cupressoideae)	*Juniperus*	*californica*
Cupressaceae (Cupressoideae)	*Juniperus*	*deppeana*
Cupressaceae (Cupressoideae)	*Juniperus*	*horizontalis*
Cupressaceae (Cupressoideae)	*Juniperus*	*monosperma*
Cupressaceae (Cupressoideae)	*Juniperus*	*pinchotii*
Cupressaceae (Cupressoideae)	*Juniperus*	*virginiana*
Cupressaceae (Cupressoideae)	*Juniperus*	*occidentalis*
Acanthaceae (Acanthoideae)	*Justicia*	*californica*
Poaceae (Pooideae)	*Koeleria*	*macrantha*
Amaranthaceae (Chenopodioideae)	*Krascheninnkovia*	*lanata*
Asteraceae	*Lactuca*	*spp.*
Polemoniaceae	*Langloisia*	*setosissima*
Boraginaceae	*Lappula*	*redowskii*
Zygophllaceae (Larreoideae)	*Larrea*	*sp.*
Zygophllaceae (Larreoideae)	*Larrea*	*divaricata*
Zygophllaceae (Larreoideae)	*Larrea*	*tridentata*
Fabaceae (Faboideae)	*Lathyrus*	*pauciflorus*
Brassicaceae	*Lepidium*	*sp.*
Brassicaceae	*Lepidium*	*fremontii*
Brassicaceae	*Lesquerella*	*sp.*
Fabaceae (Caesalpinioideae)	*Leucaena*	*sp.*
Fabaceae (Caesalpinioideae)	*Leucaena*	*retusa*
Poaceae	*Leymus*	*cinereus*
Poaceae	*Leymus*	*arenarius*
Poaceae	*Leymus*	*mollis*
Polemoniaceae	*Linanthus*	*sp.*
Linaceae	*Linum*	*lewisii*
Linaceae	*Linum*	*perenne*
Saxifragaceae	*Lithophragma*	*parviflora*
Boraginaceae (Boraginoideae)	*Lithospermum*	*sp.*
Boraginaceae (Boraginoideae)	*Lithospermum*	*incisum*
Boraginaceae (Boraginoideae)	*Lithospermum*	*ruderale*
Poaceae (Pooideae)	*Lolium*	*perenne ssp. Multiflorum*
Apiaceae	*Lomatium*	*dissectum*
Apiaceae	*Lomatium*	*grayi*
Apiaceae	*Lomatium*	*macrocarpum*
Apiaceae	*Lomatium*	*nuttallii*
Apiaceae	*Lomatium*	*triternatum*
Apiaceae	*Lomatium*	*cous*
Apiaceae	*Lomatium*	*ambiguum*
Caprifoliaceae (Caprifolioideae)	*Lonicera*	*sp.*

Cactaceae (Cactoideae)	*Lophocereus*	*schottii*
Fabaceae (Faboideae)	*Lotus*	*corniculatus*
Fabaceae (Faboideae)	*Lupinus*	*argenteus*
Fabaceae (Faboideae)	*Lupinus*	*sparsiflorus*
Fabaceae (Faboideae)	*Lupinus*	*polyphyllus*
Fabaceae (Faboideae)	*Lupinus*	*lepidus*
Fabaceae (Faboideae)	*Lupinus*	*leucophyllus*
Fabaceae (Faboideae)	*Lupinus*	*spp.*
Solanceae	*Lycium*	*andersonii*
Solanceae	*Lycium*	*pallidum*
Solanceae	*Lycium*	*shockleyi*
Asteraceae	*Lygodesmia*	*spp.*
Asteraceae	*Machaeranthera*	*sp.*
Loasaceae	*Mentzelia*	*sp.*
Loasaceae	*Mentzelia*	*reflexa*
Loasaceae	*Mentzelia*	*laevicaulis*
Loasaceae	*Mentzelia*	*involucrata*
Asteraceae	*Microseris*	*nutans*
Polemoniaceae	*Microsteris*	*gracilis*
Fabaceae (Caesalpinioideae)	*Mimosa*	*sp.*
Phrymaceae (formerly Scrophulariaceae)	*Mimulus*	*bigelovii*
Phrymaceae (formerly Scrophulariaceae)	*Mimulus*	*guttatus*
Phrymaceae (formerly Scrophulariaceae)	*Mimulus*	*rupicola*
Phrymaceae (formerly Scrophulariaceae)	*Mimulus*	*fremontii*
Phrymaceae (formerly Scrophulariaceae)	*Mimulus*	*mohavensis*
Phrymaceae (formerly Scrophulariaceae)	*Mimulus*	*parishii*
Phrymaceae (formerly Scrophulariaceae)	*Mimulus*	*suksdorfii*
Plantaginaceae (formerly Scrophulariaceae)	*Mohavea*	*confertiflora*
Plantaginaceae (formerly Scrophulariaceae)	*Mohavea*	*breviflora*
Celastraceae	*Mortonia*	*sp.*
Celastraceae	*Mortonia*	*scabrella*
Moraceae	*Morus*	*sp.*
Poaceae (Chloridoideae)	*Muhlenbergia*	*sp.*
Poaceae (Chloridoideae)	*Muhlenbergia*	*asperifolia*
Poaceae (Chloridoideae)	*Muhlenbergia*	*richardsonis*
Poaceae	*Muhlenbergia*	*porteri*
Liliaceae	*Muilla*	*maritima*
Liliaceae	*Muilla*	*coronata*
Hydrophyllaceae	*Nama*	*demissum*
Boraginaceae	*Nama*	*sp.*
Poaceae	*Nassella*	*viridula*

Asparagaceae (Nolinoideae)	*Nolina*	*sp.*
Asparagaceae (Nolinoideae)	*Nolina*	*microcarpa*
Onagraceae (Onagroideae)	*Oenothera*	*deltoides*
Onagraceae (Onagroideae)	*Oenothera*	*elata*
Onagraceae (Onagroideae)	*Oenothera*	*primiveris*
Onagraceae (Onagroideae)	*Oenothera*	*caespitosa*
Cactaceae (Opuntioideae)	*Opuntia*	*sp.*
Cactaceae (Opuntioideae)	*Opuntia*	*basilaris*
Cactaceae (Opuntioideae)	*Opuntia*	*engelmannii*
Cactaceae (Opuntioideae)	*Opuntia*	*erinacea*
Cactaceae (Opuntioideae)	*Opuntia*	*macrocentra*
Cactaceae (Opuntioideae)	*Opuntia*	*polyacantha*
Orobanchaceae	*Orobanche*	*fasciculata*
Orobanchaceae	*Orobanche*	*cooperi*
Poaceae (Pooideae)	*Oryzopsis*	*sp.*
Polygonaceae	*Oxytheca*	*perfoliata*
Asteraceae	*Packera*	*multilobatus*
Asteraceae	*Palafoxia*	*arida*
Fabaceae (Caesalpinioideae)	*Parkinsonia*	*aculeata*
Fabaceae (Caesalpinioideae)	*Parkinsonia*	*florida*
Poaceae (Pooideae)	*Pascopyrum*	*smithii*
Asteraceae (Asteroideae)	*Pectis*	*sp.*
Asteraceae (Asteroideae)	*Pectis*	*papposa*
Cactaceae (Cactoideae)	*Peniocereus*	*greggii*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*sp.*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*palmeri*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*pseudospectabilis*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*eatonii*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*incertus*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*centranthifolius*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*albomarginatus*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*acuminatus*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*deustus*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*pachyphyllus*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*rostriflorus*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*speciosus*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*barbatus*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*strictus*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*rydbergii*
Plantaginaceae (formerly Scrophulariaceae)	*Penstemon*	*whippleanus*
Rosaceae (Amygdaloideae)	*Peraphyllum*	*ramosissimum*

Asteraceae (Asteroideae)	*Perityle*	*emoryi*
Asteraceae	*Petradoria*	*pumila*
Rosaceae (Amygdaloideae)	*Petrophytum*	*sp.*
Rosaceae (Amygdaloideae)	*Petrophytum*	*caespitosum*
Boraginaceae	*Phacelia*	*distans*
Boraginaceae	*Phacelia*	*campanularia ssp. Vasiformis*
Boraginaceae	*Phacelia*	*tanacetifolia*
Boraginaceae	*Phacelia*	*crenulata*
Polemoniaceae	*Phlox*	*stansburyi*
Polemoniaceae	*Phlox*	*longifolia*
Polemoniaceae	*Phlox*	*speciosa*
Polemoniaceae	*Phlox*	*aculeata*
Boraginaceae (Lennooideae)	*Pholisma*	*arenarium*
Boraginaceae (Hydrophylloideae)	*Pholistoma*	*membranaceum*
Solanaceae (Solanoideae)	*Physalis*	*crassifolia*
Brassicaceae	*Physaria*	*sp.*
Pinaceae (Piceoideae)	*Picea*	*sp.*
Pinaceae (Piceoideae)	*Picea*	*engelmannii*
Pinaceae	*Picea*	*pungens*
Pinaceae (Piceoideae)	*Pinus*	*ponderosa*
Pinaceae (Piceoideae)	*Pinus*	*flexilis*
Pinaceae (Piceoideae)	*Pinus*	*sp.*
Pinaceae (Piceoideae)	*Pinus*	*contorta*
Pinaceae (Piceoideae)	*Pinus*	*muricata*
Pinaceae (Piceoideae)	*Pinus*	*albicaulis*
Boraginaceae (Boraginoideae)	*Plagiobothrys*	*sp.*
Boraginaceae	*Plagiobothrys*	*jonesii*
Plantaginaceae	*Plantago*	*patagonica*
Plantaginaceae	*Plantago*	*sp.*
Papaveraceae	*Platystemon*	*californicus*
Asteraceae (Asteroideae)	*Pluchea*	*sericea*
Poaceae (Pooideae)	*Poa*	*secunda*
Poaceae (Pooideae)	*Poa*	*annua*
Poaceae (Pooideae)	*Poa*	*fendleriana*
Poaceae (Pooideae)	*Poa*	*pratensis*
Poaceae (Pooideae)	*Poa*	*juncifolia*
Salicaceae (Salicoideae)	*Populus*	*sp.*
Salicaceae (Salicoideae)	*Populus*	*tremuloides*
Salicaceae (Salicoideae)	*Populus*	*fremontii*
Asteraceae	*Porophyllum*	*gracile*
Primulaceae (Primuloideae)	*Primula*	*specuicola*

Martyniaceae	*Proboscidea*	*altheaefolia / parviflora*
Fabaceae (Caesalpinioideae)	*Prosopis*	*sp.*
Rosaceae (Amygdaloideae)	*Prunus*	*sp.*
Rosaceae (Amygdaloideae)	*Prunus*	*andersonii*
Rosaceae (Amygdaloideae)	*Prunus*	*besseyi*
Rosaceae (Amygdaloideae)	*Prunus*	*fasciculata*
Rosaceae (Amygdaloideae)	*Prunus*	*fremontii*
Rosaceae (Amygdaloideae)	*Prunus*	*havardii*
Rosaceae (Amygdaloideae)	*Prunus*	*virginiana*
Poaceae	*Pseudoroegneria*	*spicata*
Pinaceae (Laricoideae)	*Pseudotsuga*	*menziesii*
Rutaceae	*Ptelea*	*sp.*
Rutaceae	*Ptelea*	*trifoliata*
Poaceae (Pooideae)	*Puccinellia*	*lemmonii*
Rosaceae (Dryadoideae)	*Purshia*	*sp.*
Rosaceae (Dryadoideae)	*Purshia*	*tridentata*
Fagaceae	*Quercus*	*sp.*
Fagaceae	*Quercus*	*arizonica x grisea*
Fagaceae	*Quercus*	*chrysolepis*
Anacardiaceae (Anacardioideae)	*Rhus*	*sp.*
Anacardiaceae (Anacardioideae)	*Rhus*	*trilobata*
Grossulariaceae	*Ribes*	*sp.*
Rosaceae (Rosoideae)	*Rosa*	*sp.*
Rosaceae (Rosoideae)	*Rosa*	*woodsii*
Salicaceae	*Salix*	*sp.*
Amaranthaceae (Salsoloideae)	*Salsola*	*kali*
Amaranthaceae (Salsoloideae)	*Salsola*	*tragus*
Lamiaceae (Nepetoideae)	*Salvia*	*sp.*
Lamiaceae (Nepetoideae)	*Salvia*	*dorrii*
Lamiaceae (Nepetoideae)	*Salvia*	*funerea*
Lamiaceae (Nepetoideae)	*Salvia*	*apiana*
Lamiaceae	*Salvia*	*mellifera*
Lamiaceae (Nepetoideae)	*Salvia*	columbariae
Brassicaceae	*Schoenocrambe*	*linifolia*
Cyperaceae	*Schoenoplectus*	*acutus*
Caryophyllaceae	*Scopulophila*	*rixfordii*
Selaginellaceae	*Selaginella*	*sp.*
Asteraceae (Asteroideae)	*Senecio*	*sp.*
Elaeagnaceae	*Shepherdia*	*argentea*
Malvaceae (Malvoideae)	*Sphaeralcea*	*sp.*
Malvaceae (Malvoideae)	*Sphaeralcea*	*coccinea*

Poaceae (Chloridoideae)	*Sporobolus*	*airoides*
Poaceae (Chloridoideae)	*Sporobolus*	*cryptandrus*
Brassicaceae	*Stanleya*	*pinnata*
Cactaceae (Cactoideae)	*Stenocereus*	*thurberi*
Asteraceae	*Stephanomeria*	*spp.*
Poaceae (Pooideae)	*Stipa*	*sp.*
Caprifoliaceae (Caprifolioideae)	*Symphoricarpos*	*sp.*
Caprifoliaceae (Caprifolioideae)	*Symphoricarpos*	*oreophilus*
Caprifoliaceae (Caprifolioideae)	*Symphoricarpos*	*occidentalis*
Caprifoliaceae (Caprifolioideae)	*Symphoricarpos*	*longiflorus*
Brassicaceae	*Sysimbrium*	*sp.*
Poaceae	*Taeniatherum*	*caput-medusae*
Tamaricaceae	*Tamarix*	sp.
Rutaceae	*Thamnosma*	*montana*
Malvaceae (Tilioideae)	*Tilia*	*sp.*
Anacardiaceae (Anacardioideae)	*Toxicodendron*	*rydbergii*
Asteraceae	*Tragopogon*	*dubius*
Poaceae (Chloridoideae)	*Tridens*	*sp.*
Poaceae (Chloridoideae)	*Tridens*	*muticus*
Fabaceae	*Trifolium*	*sp.*
Fabaceae	*Trifolium*	*macrocephalum*
Fabaceae	*Trifolium*	*variegatum*
Fabaceae	*Trifolium*	*gymnocarpon*
Fabaceae	*Trifolium*	*microcephalum*
Fabaceae	*Trifolium*	*eriocephalum*
Fabaceae	*Trifolium*	*thompsonii*
Pinaceae (Abietoideae)	*Tsuga*	*mertensiana*
Typhaceae	*Typha*	*latifolia*
Sapindaceae (Sapinoideae)	*Ungnadia*	*sp.*
Sapindaceae (Sapinoideae)	*Ungnadia*	*speciosa*
Urticaceae	*Urtica*	*dioica*
Urticaceae	*Urtica*	*gracilenta*
Urticaceae	*Urtica*	*urens*
Poaceae	*Ventenata*	*dubia*
Asteraceae	*Verbesina*	*sp.*
Fabaceae (Faboideae)	*Vicia*	*americana*
Vitaceae (Vitoideae)	*Vitis*	*sp.*
Vitaceae (Vitoideae)	*Vitis*	*arizonica*
Poaceae (Pooideae)	*Vulpia*	*octaflora*
Arecaceae (Coryphoideae)	*Washingtonia*	*filifera*
Asteraceae	*Xanthium*	*strumarium*

Asteraceae	*Xylorhiza*	*tortifolia*
Asparagaceae (Agavoideae)	*Yucca*	*sp.*
Asparagaceae (Agavoideae)	*Yucca*	*baccata*
Asparagaceae (Agavoideae)	*Yucca*	*brevifolia*
Asparagaceae (Agavoideae)	*Yucca*	*glauca*
Asparagaceae (Agavoideae)	*Yucca*	*harrimaniae*
Asparagaceae (Agavoideae)	*Yucca*	*rostrata*
Asparagaceae (Agavoideae)	*Yucca*	*schidigera*
Asparagaceae (Agavoideae)	*Yucca*	*elata*
Rutaceae (Rutoideae)	*Zanthoxylum*	*sp.*
Rhamnaceae	*Ziziphus*	*sp.*
Poaceae (Pooideae)	*Stipa*	*arida*
Poaceae (Pooideae)	*Oryzopsis*	*hymenoides*
Poaceae (Pooideae)	*Stipa*	*lemmonii*
Rosaceae	*Geum*	*triflorum*
Cleomaceae	*Peritoma*	*arborea*
Asteraceae	*Gutierrezia*	*sarothrae*
Fabaceae (Caesalpinioideae)	*Prosopis*	*glandulosa*
Poaceae	*Hilaria*	*rigida*
Poaceae (Pooideae)	*Festuca*	*octoflora*
Asteraceae	*Hymenoclea*	*salsola*
Berberidaceae	*Mahonia*	*sp.*
Asteraceae	*Lepidospartum*	*sp.*
Cactaceae	*Cereus*	*giganteous*
Pteridaceae	*Myriopteris*	*gracilis*
Scrophulariaceae	*Comandra*	*umbellata*
Cactaceae (Cactoideae)	*Escobaria*	*vivipara*
Cactaceae (Opuntioideae)	*Opuntia*	*leptocaulis*
Fabaceae	*Dalea*	*sp.*
Solanaceae	*Datura*	*metaloides*
Asteraceae	*Macheranthera*	*canescens*
Poaceae (Pooideae)	*Elymus*	*lanceolatus ssp. Psammophilus*
Euphorbiaceae	*Chamaesyce*	*albomarginata*
Verbenaceae	*Verbena*	*gooddingii*
Boraginaceae	*Cryptantha*	*circumscissa*
Poaceae (Pooideae)	*Stipa*	*comata*
Asparagaceae (Agavoideae)	*Yucca*	*whipplei*
Polemoniaceae	*Gilia*	*aggregata*
Amaranthaceae	*Eurotia*	*sp.*
Chenopodiaceae	*Ceratoides*	*lanata*
Polemoniaceae	*Phlox*	*gracilis*

Polygonaceae (Eriogonoideae)	*Eriogonum*	*simpsonii*
Moraceae	*Morus*	*microphylla*
Poaceae (Chloridoideae)	*Erioneuron*	*pulchellum*
Asteraceae	*Encelia*	*farinosa*
Boraginaceae	*Eriodictyon*	*angustifolium*
Apiaceae	*Perideridea*	*bolanderi*
Capparaceae	*Cleome*	*lutea*
Rosaceae (Amygdaloideae)	*Prunus*	*lyonii*
Fabaceae (Faboideae)	*Psoralea*	*lanceolata*
Asteraceae	*Tetradymia*	*sp.*
Amaranthaceae	*Tidestomia*	*oblongifolia*
Amaranthaceae (Salsoloideae)	*Salsola*	*iberica*
Gentianaceae	*Centaurium (Zeltnera venusta)*	*venustum*