

Review

New uses for ancient middens: bridging ecological and evolutionary perspectives

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Rodent middens provide a fine-scale spatiotemporal record of plant and animal communities over the late Quaternary. In the Americas, middens have offered insight into biotic responses to past environmental changes and historical factors influencing the distribution and diversity of species. However, few studies have used middens to investigate genetic or ecosystem level responses. Integrating midden studies with neoecology and experimental evolution can help address these gaps and test mechanisms underlying eco-evolutionary patterns across biological and spatiotemporal scales. Fully realizing the potential of middens to answer cross-cutting ecological and evolutionary questions and inform conservation goals in the Anthropocene will require a collaborative research community to exploit existing midden archives and mount new campaigns to leverage midden records globally.

A renewed vision for midden research

Anthropogenic shifts in climate, land use, and other factors threaten biodiversity and the overall stability and functioning of Earth's ecosystems. Consequently, considerable effort has gone into assessing the vulnerability of species and ecosystems to rapid environmental change. Although environmental change is nothing new and climate shifts during the late **Quaternary** (see [Glossary](#)) dwarf changes predicted over the next century [1], the magnitude and pace of anthropogenic climate change are accelerating [2]. Studying the past can help contextualize our understanding of the natural world today and improve predictions of biological responses to ongoing and future environmental change [3,4].

Predicting biological responses to environmental change across spatial, temporal, and biological scales requires tighter integration of **paleoecology**, **neoecology**, and evolutionary biology ([Figure 1](#)) [5–9]. Integrating these perspectives has been challenging because most fossil records lack the resolution to compare ancient and modern communities. The patchy distribution of some fossil records can also make it difficult to assess biological responses across spatial scales that are relevant to population, community, and ecosystem processes.

Middens, debris piles constructed by mammals, provide a source of spatially and temporally resolved data that can help overcome these challenges. In arid regions, middens can archive biological materials for tens of millennia ([Figure 2](#)), capturing environmental changes relevant to the problems we face today [4]. Moreover, the distribution of middens on different continents enables comparative studies across arid ecosystems. Although widespread, the preponderance of midden studies conducted to date, especially those focused on ecological and evolutionary processes, are from North and South America ([Figure S1](#) in the supplemental information online). As such, we focus this discussion on middens from the Americas.

Highlights

Environmental perturbations over the late Quaternary were similar in magnitude and scope to those predicted under scenarios of anthropogenic climate shifts.

Paleoecological records from this time frame, such as the midden record created by rodents in the Americas, have contextualized our understanding of biological responses to environmental change.

Recent technological advances have expanded the scope of midden research to investigate eco-evolutionary hypotheses and the adaptive potential of ecological systems.

However, ongoing human disturbances and climate change could jeopardize the preservation of middens and their surroundings for neoecological studies.

Integrating paleoecology with modern experimental studies is essential for understanding the impacts of environmental change. Achieving this goal will require a community of midden researchers focused on collaborative, cross-cutting questions.

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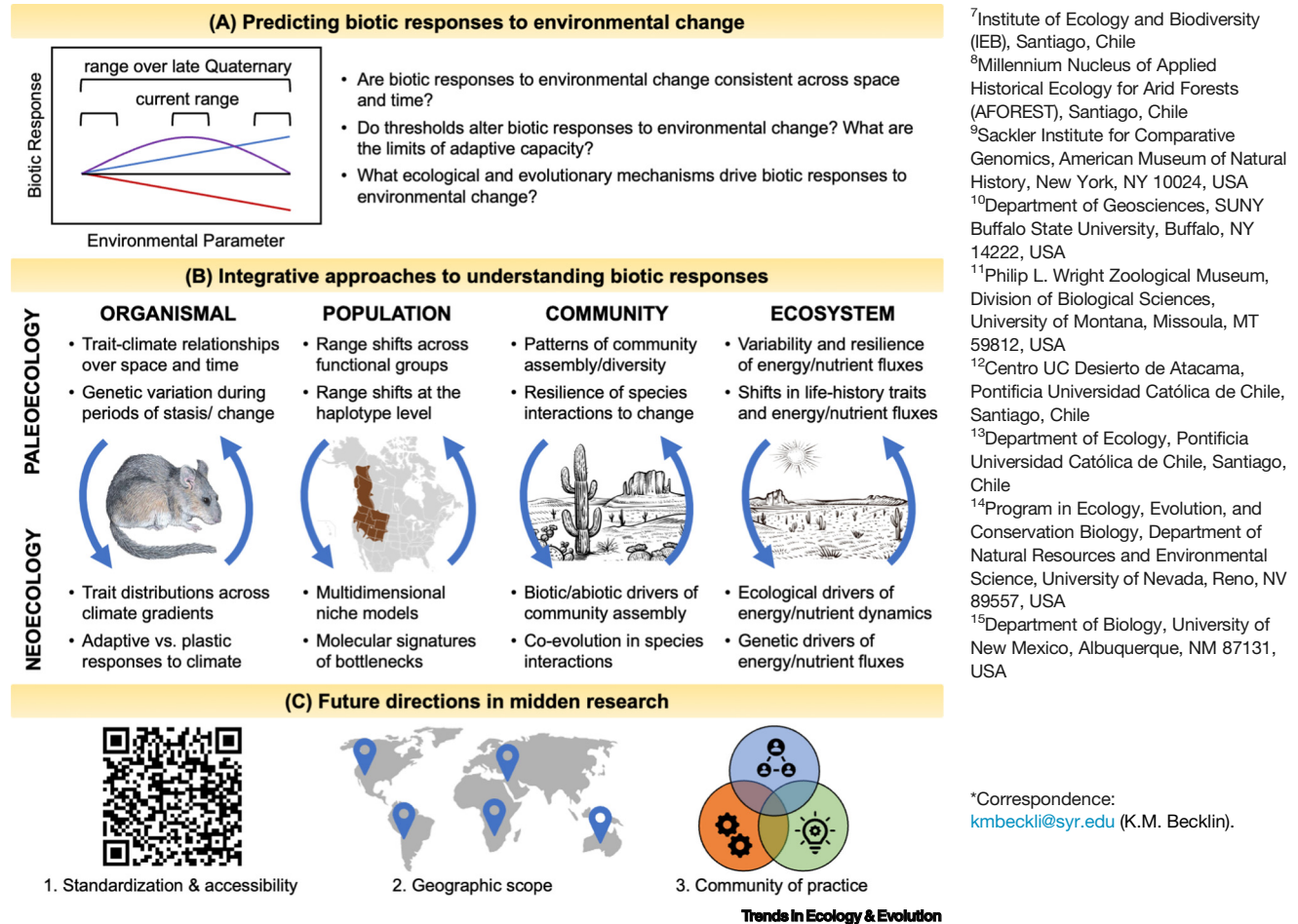


Figure 1. Integrative approaches to understanding the impacts of environmental change. (A) Paleoeological records that span spatiotemporal gradients in environmental conditions and neoeological and evolutionary experiments that manipulate biotic and abiotic factors are complementary approaches to detect and predict biological responses to environmental change. Responses to environmental conditions (e.g., change in body size, abundance, migration rate, diversity, energy flow) may range from positive to negative for different organisms and across biological scales. Paleoeological records that span a broader range of environmental conditions compared with modern systems provide important baseline information about the historical range of variability and stability of patterns through space and time. Neoeological studies can inform interpretation of midden data and provide insight into ecological and evolutionary mechanisms that drive biotic responses. (B) Here we outline examples of how data from midden and neoeological studies can be applied to advance our understanding of adaptive and plastic responses of desert organisms to their environment, mechanisms driving range expansions or contractions within and across species, dynamics of community assembly and species interactions, and the resilience of ecosystem processes to environmental change. (C) Building the capacity for integrative midden and neoeological research requires standardizing methodologies, as outlined in the companion protocols document [86]; increased accessibility of midden materials and data through regional depositories; expanded collection and study of middens and modern ecological communities globally; and a ‘community of practice’ focused on collaborative, interdisciplinary research that spans paleoeology, neoeology, and evolutionary domains.

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This review examines how midden records can advance our understanding of biological responses to environmental change by addressing questions at the frontier of ecology and evolutionary biology. In ‘What are middens’ we summarize the natural history and distribution of middens in the Americas and review seminal insights these records have yielded. In ‘Prospective

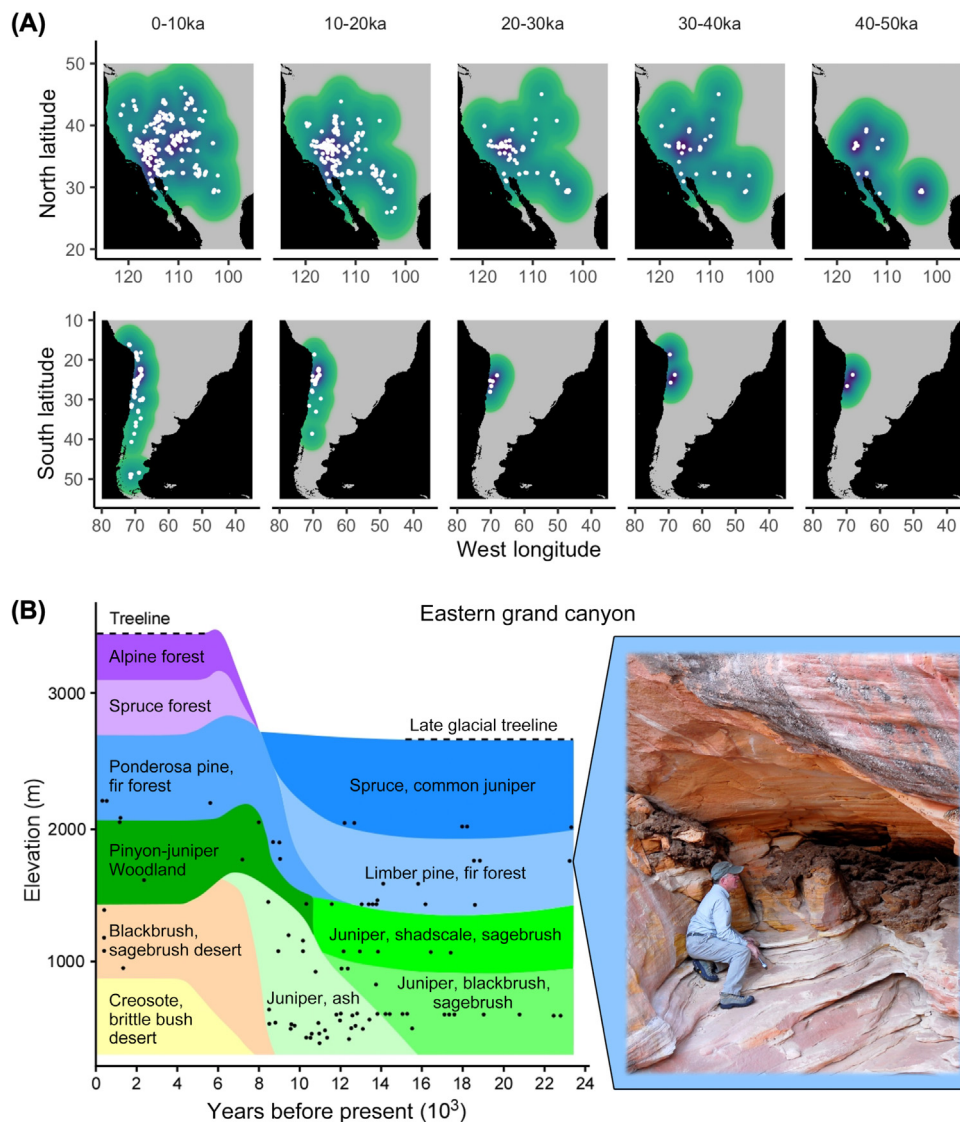


Figure 2. Spatiotemporal distribution of ^{14}C dated small mammal middens in the Americas. (A) In North America (top row), middens spanning the past 50 000 years have been found in all four major deserts and piedmonts of the Rocky Mountain, Sierra Nevada, Pacific Coast, and Sierra Madre Occidental ranges. In South America (bottom row), middens have been sampled from the central and southern Andes, with the hyperarid Atacama Desert yielding abundant Pleistocene deposits. Midden preservation is a function of climate and substrate, which together influence the density of middens across space and time. Midden locations and ages were compiled from the US Geological Survey North American Packrat Midden Database (<https://geochange.er.usgs.gov/midden/>) and catalogs of researchers working in South America. Ages were calibrated following Reimer *et al.* [98] for North American middens and Hogg *et al.* [99] for South American middens using methods available in the 'Bchron' R library, version 4.2.6 (<https://cran.r-project.org/package=Bchron>). (B) Middens can span large spatial and temporal scales even at a single location. In the Eastern Grand Canyon, middens (black circles) collected from different elevations were used to characterize transitions in the dominant vegetation (displayed as different colors) over time (adapted from [100]). Midden photo courtesy of Sandra Swift and Jim Mead.

questions in midden research' we discuss opportunities to integrate modern theories and tools with midden data. Finally, in 'The future of midden research' we discuss challenges associated with this work and strategies for expanding midden research globally.

Glossary

Ancient DNA (aDNA): DNA isolated from subfossils and characterized by short and damaged genetic material.

Anthropocene: unofficial period of time during which human activities have significantly affected the global environment.

Ecological baseline: reference state of an ecological system (e.g., species' distributions, abundances, biodiversity) before a disturbance or directional change.

Environmental DNA (eDNA): DNA isolated from soils, water, and other sources of genetic material; key reference for comparison to aDNA from plants, animals, and microbes.

Extinction: global death of all individuals of a species.

Extirpation: local disappearance of a species (i.e., death, migration).

Historical range of variability (HRV): the full range of ecological conditions occurring across multiple scales of space and time and the processes that drive these dynamics.

Macrofossil: preserved biological remains large enough to be visible with the naked eye or low-powered magnification.

Microfossil: preserved biological remains just at or below the size that are visible with the naked eye and that require microscopy to view.

Midden: pile of debris, mainly organic (e.g., plant material, bones, insects), accumulated in a cave or a rock shelter by a midden builder.

Midden builder: organisms responsible for constructing a particular midden.

Midden series: collection of middens from a geographic area that often span millennia of time.

Neoeecology: field of study that uses extensive sampling and controlled experiments to mechanistically understand ecological drivers, interactions, functioning, and resilience at relatively fine resolutions that are difficult to extrapolate across broad spatiotemporal scales.

Paleoecology: field of study that uses historical proxies to reconstruct species distributions, interactions, and dynamics of biotic communities and/or ecosystems in response to climate or land use changes over broad spatiotemporal scales.

Quaternary: the last 2.58 million years of geologic time; epochs within the Quaternary period are the Pleistocene

What are middens?

Middens are constructed by multiple rodent species in rocky arid and semi-arid habitats in the Americas and by other herbivores worldwide (Figure S2 in the supplemental information online). Although typically associated with a den, middens vary in size and form and can serve multiple purposes (e.g., nesting area, food cache, protection) [10,11]. Generations of small mammals inhabiting the same geographic area can produce a **midden series** that potentially captures >50 000 years of biotic change (Figure 2) [12]. Within a series, separate middens and **strata** in the same midden represent distinct depositional episodes or ‘snapshots’ in time. The duration of the depositional episode hinges on the researcher’s skill in the field and effort to avoid contamination from older or younger materials. Consistency in the timing and sequence of species’ occurrences across different midden series (e.g., Box 1) indicates that, while temporal mixing and contamination can occur, most material in a carefully sampled midden accumulated over a few months to a few decades [13]. Because middens must remain dry for long-term preservation, their distribution is restricted to rocky hillslopes with protective caves and crevices. Preservation of organic materials within middens is typically high, enabling detailed morphological, biogeochemical, and genetic analysis of **subfossils** tens of millennia after they were deposited.

The abundance and diversity of preserved materials are strengths of the midden record. Plant **macrofossils**, fecal pellets, bones, teeth, and other organic remains deposited by the **midden builder** are common, as are pollen **microfossils** and the remains of commensals, including arthropods, other small mammals, and lizards. While pollen can be blown in from many tens of kilometers [14–16], plant macrofossils such as leaves and seeds tend to originate within the foraging range (≥ 50 m) of the animal [17,18]. Although considered dietary generalists, rodent foraging preferences may influence the composition of preserved materials, causing some taxa to be over- or under-represented relative to the surrounding community [13,19,20]. However, multiple studies comparing midden composition against modern biota indicate bias is minimal [15,18,21–23].

Middens from North and South America have generated novel insights into ecological and evolutionary dynamics across diverse taxa and through major periods of climate change over the past 50 000 years (Boxes 1–3). These insights were aided by technological advances that enable more precise and detailed analysis of preserved materials (Appendix A in the supplemental information online).

Prospective questions in midden research

Organismal responses to climate change

In a changing environment, organisms can move, adapt, or go extinct. How they react is mediated by their genetic capacity, life history, ecology, and physical environment. Although middens have played a prominent role in characterizing climate tolerances and range dynamics for various organisms (Boxes 1–3), they cannot directly assess mechanisms underlying organismal responses to climate. The challenge of drawing causal inferences could be addressed by integrating paleoecological and neoecological studies (e.g., common garden experiments, experimental evolution, and phenotypic mapping) to produce a more complete picture of organismal responses to climate change.

Latitudinal and altitudinal range shifts, some of the most universal responses to climate change [24], are apparent in the North and South American midden records (Boxes 1 and 2). In these regions, middens provide direct evidence of range expansions, persistence of refugial populations, lineage replacements, and population expansions or contractions. If combined with molecular, morphological, or co-occurrence analyses, these records may reveal genetic (i.e., bottlenecks,

(2.58 million to 11 700 years ago) and Holocene (11 700 years ago until today).

Resilience: the rate at which a system variable returns to its reference condition following a perturbation or disturbance.

Stasis: a period of relative stability without activity or change in an ecological, evolutionary, or climatic system.

Strata: layers within a midden that can represent different depositional episodes.

Subfossil: ancient remains that are not mineralized due to insufficient time or poor conditions for mineralization; contains organic material for analyses such as radiocarbon dating or aDNA sequencing.

Transition state: gradual or abrupt changes that occur after a formerly stable ecological system is disturbed, or when a developing system gradually achieves new functions.

Box 1. Midden contributions to late Quaternary biogeography and vegetation dynamics in western North America

Middens from southern Canada to central Mexico indicate markedly different climate, hydrology, and vegetation during the late Pleistocene. Rocky environments now clad in desert scrub were dominated by pinyon–juniper woodlands. Desert species were displaced southward, or if present, co-occurred with different species than today [101,102]. By contrast, desert C_4 grasslands covered the USA–Mexico borderlands with the same composition and at similar elevations as today [40]. At mid-elevations, mixed-conifer woodlands replaced pinyon–juniper woodlands and boreal forests blanketed many mountaintops and plateaus where they are now missing [12].

Middens enable detailed mapping of species distributions and postglacial migrations, providing a frame of reference for modern population and community ecology. Migration of many desert shrubs was gradual and lagged, reaching northernmost limits only in the past 4000 years [40]. Different dispersal syndromes produced different colonization rates [103], with the most heat-adapted species, according to modern temperature tolerances, exhibiting the slowest rates of spread [35]. Holocene migration aligned diploid, tetraploid, and hexaploid populations of the now dominant creosote bush (*Larrea tridentata*) along a gradient of increasingly drier and hotter summers [41] with tetraploids only recently spreading from the Sonoran to western Chihuahuan Desert [104].

Populations of three pinyon pine varieties (*Pinus monophylla*, *Pinus edulis*, and *Pinus edulis* var. *fallax*) expanded 300–500 km northward at rates of 20–60 m y^{-1} , reaching northernmost outposts only within the past millennium [42]. In the northeastern Great Basin, increased wildfires and fire-related sedimentation accompanied *P. monophylla* infilling [105]. In the northern Colorado Plateau, local *P. edulis* spread was modulated by decadal precipitation variability [43]. Additionally, long-distance colonization by *P. edulis* along the Front Range of the Rocky Mountains yielded little loss in genetic diversity [44].

Focused midden surveys tracked in-tandem migrational histories of Utah juniper (*Juniperus osteosperma*) and Rocky Mountain ponderosa pine (*Pinus ponderosa* var. *scopulorum*) in the central Rocky Mountains (Figure 1). Utah juniper migration proceeded via long-distance dispersal and subsequent infilling by a single haplotype, paced by climate variability and structured by the distribution and connectivity of suitable habitat [45]. Ponderosa advanced as a ‘front’ with long delays in establishment followed by rapid expansion, indicating migration was limited by the timing of suitable climate rather than dispersal. Ponderosa migration in the central Rockies conflated the movements of two different haplotypes and established a novel zone of introgression between the main varieties (var. *scopulorum* and var. *ponderosa*) (Figure 1 [46]). Detailed migrational histories such as these offer myriad opportunities to investigate how the arrival and expansion of dominant and widespread species affect community assembly and disassembly and eventually modify the physical environment [106].

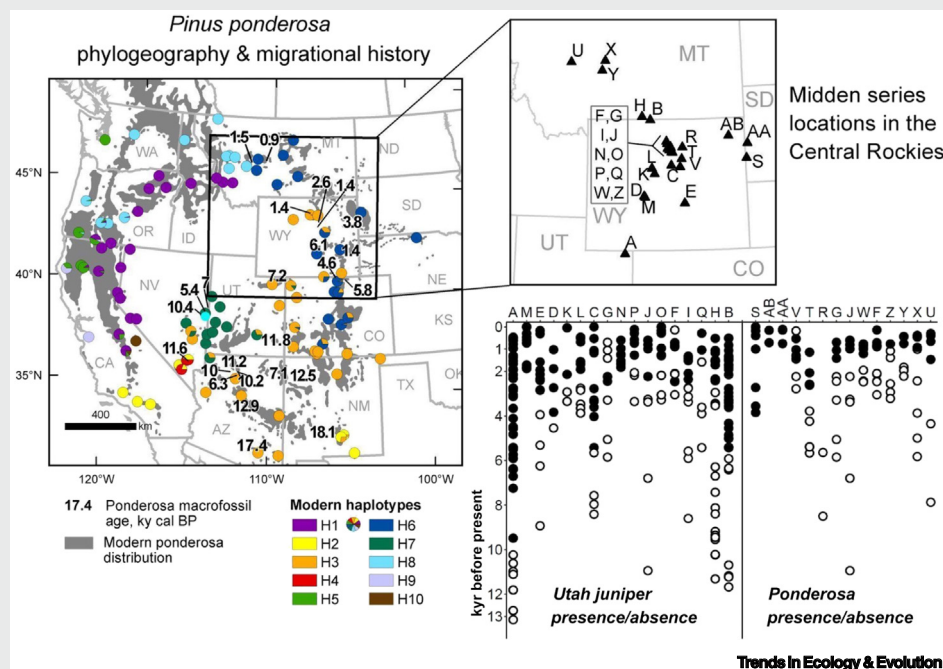


Figure 1. Migrational histories for ponderosa pine and Utah juniper. Upper left: distribution (gray shading) of Sierra Nevada ponderosa pine (*Pinus ponderosa* var. *ponderosa*; haplotypes H1, H2, and H8) and Rocky Mountain ponderosa pine (*Pinus ponderosa* var. *scopulorum*; H3–7, H9–10) with ages [kiloyear before present (BP)] of the earliest macrofossil occurrence of var. *scopulorum* in midden series. Location of ten modern haplotypes [107] indicated by colored circles. Upper right: inset map of the central Rocky Mountains showing midden sites/series (letters) sampled to reconstruct regional migrational histories of low-elevation Utah juniper (*Juniperus osteosperma*) [45] and *P. ponderosa* var. *scopulorum* [46]. Lower right: chronology of presence/absence of Utah juniper and ponderosa pine macrofossils at midden sites indicated by shaded/open circles. These examples indicate that species migration can involve either simultaneous or staggered spread of different haplotypes, with verification awaiting ancient DNA analysis. Early establishment of Utah juniper happened through long-distance dispersal to northernmost outputs followed by pulsed infilling in intervening areas. Ponderosa pine migration was mostly frontal with colonization of northwesternmost populations occurring ~1500 years ago, establishing a hybrid zone between var. *scopulorum* (H6) and var. *ponderosa* (H8).

Box 2. Linking past to current ecosystem dynamics in the Atacama Desert

The extreme hyperaridity of low elevation inland basins in the Atacama, the world's driest desert with virtually no rain or vascular plants, contrasts with cold temperatures in the high Andes. This generates opposing gradients of temperature and seasonal precipitation that vary consistently with latitude and elevation. Soil pH, solar radiation, and nutrient availability also vary systematically across these gradients, with distinct physiognomic belts that include fog-supported 'lomas' vegetation in the coastal ranges, absolute desert in the low-elevation longitudinal basin, sparse xeric shrubs and cacti communities on the Pacific slope, and extensive steppe grasslands that give way to cushion plants at the cold limits of vegetation [71,108].

The preservation of organic matter in middens facilitated reconstructions of past ecological change in the Atacama. During the Pleistocene, grasses and shrubs from the high Andean steppe descended >1000 m in elevation into what is now absolute desert [109–111]. C_4 grasses and summer annuals indicate that these 'pluvials' resulted from more frequent moist air mass incursions originating in the Gran Chaco or Amazon Basin that crossed the Bolivian and Chilean Altiplano and rained out farther down the Pacific slope of the Andes [112,113].

Paleoecological and neoecological approaches have been used in tandem to assess the impacts of late Quaternary and recent climate changes on desert communities in the Atacama. Arthropods sampled from modern and ancient middens helped reconstruct arthropod community dynamics over the Holocene, which led to key insights into the high ecological fidelity of fossil assemblages [23]. Ancient DNA (aDNA), first sequenced from an Atacama midden more than 20 years ago [114], identified the midden builder as an ancestral leaf-eared mouse (*Phyllotis limatus*) that today occurs 100 km to the north, indicating a modest range shift derived from a peripheral isolate. High-throughput sequencing of Atacama middens complemented morphological identification of pollen and macrofossil remains and revealed major vegetation changes across millennia (Figure 1) [115]. Comparing these paleorecords with modern soil **environmental DNA (eDNA)** [116] and vegetation surveys along environmental gradients [115] showed that the distribution of high-elevation shrubs and grasses was particularly sensitive to millennial-scale climatic variability (Figure 1) but insensitive to interannual and decadal-scale variability. In contrast, annual plants were more sensitive to interannual climate variation. These and other paleogenomic studies of plant pathogen [49] and rodent parasite assemblages [50] from the Atacama exemplify new approaches to examine community and ecosystem responses to climate change across timescales.

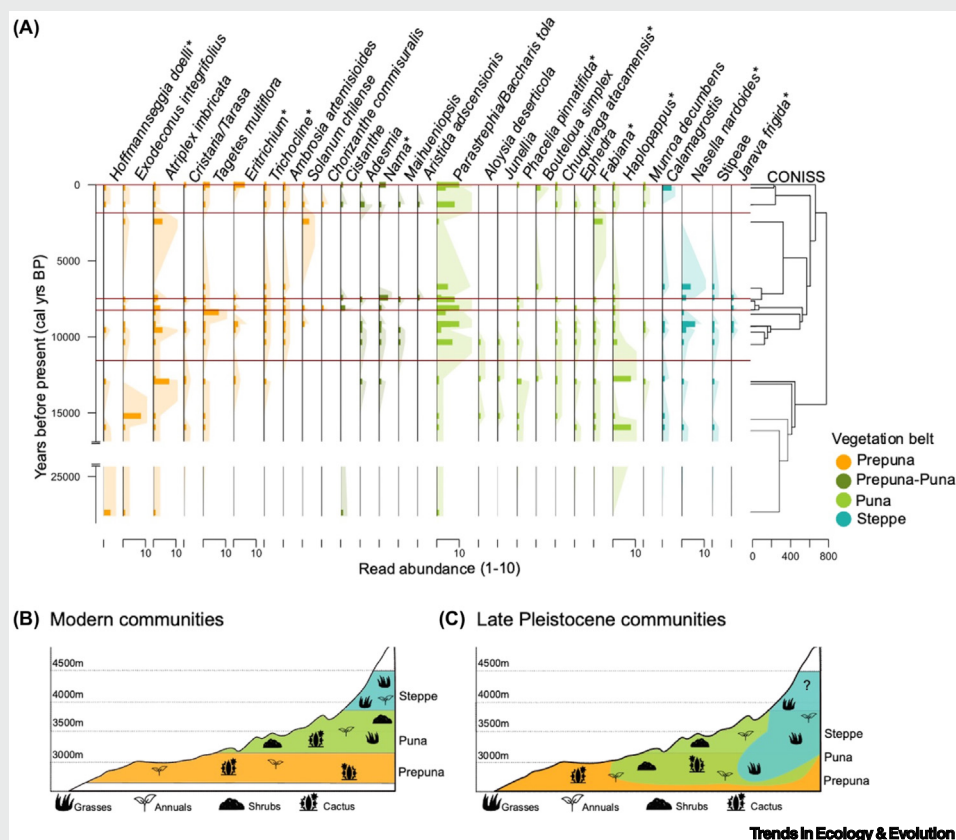


Figure 1. Time series of vegetation change in the central Atacama Desert. (A) Midden ancient DNA (aDNA) from high-throughput sequencing, the first such analysis of its kind for middens (published in [115]). *Indicates taxa not previously identified by macrofossil or pollen analyses. A stratigraphically constrained incremental sum of squares analysis (CONISS) identifies distinct temporal zones of similar vegetation affinity based on aDNA reads and species presence/absence. Comparing (B) modern and (C) late Pleistocene plant communities based on presence/absence data illustrates the dynamic nature of vegetation 'belts' over time on the Pacific slope of the Andes.

Box 3. Environmental drivers of body size in mammals: a tale of two continents

Mammal body size is highly heritable, easily measured, and strongly associated with multiple life history and ecological traits [117,118]. The influence of environmental factors such as temperature, precipitation, and primary productivity may vary across mammalian taxa and habitats [119], contributing to ongoing debate about environmental drivers of body size variation. For woodrats (*Neotoma*), body size closely tracks ambient temperature (Figure I) [120,121], indicating that changing temperature regimes can drive shifts in body size. Indeed, woodrats were the original empirical example of Bergmann's rule, an ecogeographic principle relating animal body size to environmental temperature [122,123].

Fecal pellets produced by different rodent species and preserved in middens provide an opportunity to examine body size–climate relationships over space and time. Pellet width is strongly correlated with body size in modern *Neotoma* populations (Figure I) [120,124]. Thus, the hundreds to thousands of fecal pellets contained within a typical midden series can provide a record of body size changes in ancient *Neotoma* populations. The relationship between pellet and body size is assumed for other midden-building species, although confirmation requires additional work in modern rodent populations.

North American midden studies have quantified morphological changes in multiple *Neotoma* species over the past 30 000 years, a time of substantial climate perturbation [32,34,120,124]. Patterns are remarkably congruent among sites across the geographic range: warming consistently led to smaller-bodied populations, whereas cooling led to larger-bodied populations (Figure I). Moreover, woodrats responded equally well to the challenges of both warming and cooling climates [32,124]. Only in rare instances, at the edge of their distribution, did climate fluctuations lead to extirpation, indicating adaptation coupled with elevational shifts allowed animals to cope with significant climate change during the late Quaternary [32].

Body size–climate relationships have yet to be as extensively explored elsewhere. However, recent work showing a positive correlation between precipitation and fecal pellet size from modern ashly chinchilla rat (*Abrocoma cinerea*) indicates that precipitation and primary productivity are more important drivers of rodent body size than temperature in the hyperarid Atacama Desert (Figure I) [33]. Based on this relationship, fecal pellet measurements from ancient *Abrocoma* middens were used to reconstruct precipitation anomalies that corresponded with known 'pluvial events' over the past 16 000 years (Figure I). Exploring body size–climate relationships in other regions would likely yield further insights into the climate tolerances and adaptive potential of different midden-building species.

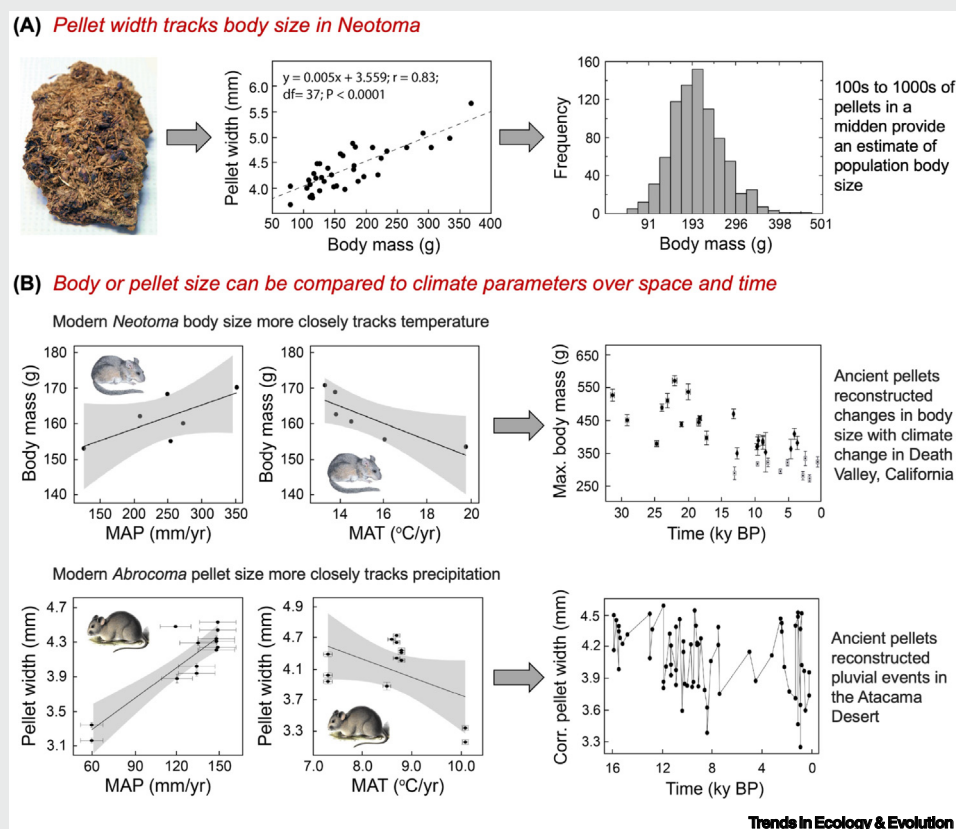


Figure I. Ancient middens provide a record of body size changes in rodent populations with climate change. (A) Fecal pellet width correlates strongly with rodent body size in modern woodrat (*Neotoma*) populations. Therefore, pellets preserved in middens provide a record of body size variation over space and time. (B) Modern rodent body size or pellet size can be compared with local climate parameters to identify important environmental drivers and calibrate ancient body size or paleoclimate reconstructions. Factors driving rodent body size variation can differ between rodent species and habitats, as shown in work using *Neotoma* (top row, western North America) and *Abrocoma* (bottom row, Atacama Desert, South America) middens (adapted from [33,120,121]). Ancient *Neotoma* pellets were used to reconstruct changes in body size with temperature changes in Death Valley, California. Ancient *Abrocoma* pellets were used to reconstruct pluvial events in the Atacama Desert. Abbreviations: ky BP, kiloyear before present; MAP, mean annual precipitation; MAT, mean annual temperature.

inbreeding, hybridization, introgression), phenotypic (i.e., body size), and ecological changes (i.e., species interactions) that accompanied past range shifts [25,26]. This historical perspective can inform interpretations of similar patterns in modern populations and identify processes constraining natural range shifts or assisted migration in the future.

Adaptive responses to climate changes over the late Quaternary have been inferred from morphological analysis of midden specimens (e.g., Box 3). **Ancient DNA (aDNA)** analysis could enable characterization of allele frequencies, signatures of selection, and genotype–environment associations that may constrain or drive adaptive responses [27–29]. The ability to link genotypes to morphological or physiological traits measured from midden specimens could provide additional insight into evolutionary processes when aligned with modern studies that assess climate responses across genetic and phenotypic variants [28]. Comparing trait distributions across environmental gradients in paleo and modern populations could also test the persistence of genotype–environment and genotype–phenotype associations through time. While most midden studies have focused on vascular plants [30,31] or the midden builders [32,33], other taxa frequently found in middens such as arthropods and microbes could provide additional insight into the adaptive capacity of desert organisms.

When neither migration nor adaptation are possible, organisms face the possibility of **extirpation** or **extinction**. Characterizing spatiotemporal patterns in the presence or absence of middens from a region can indicate potential extirpations of midden builders [32,34]. Likewise, analysis of aDNA could help detect cryptic plant, arthropod, and small vertebrate losses. However, few studies have explored these questions to date. Continued work in this area holds potential for advancing our understanding of leading and trailing edge dynamics, including whether extinctions are commonly preceded by range shifts or loss of phenotypic or genetic variation as predicted for modern systems.

Community assembly and biogeography

Middens have been used extensively to assess ecological and environmental drivers of plant community dynamics. Comparisons across periods of climate instability versus relative **stasis** have generated insight into the **resilience** and historical range of variation within C₄ grasslands, desert scrub, and mixed-conifer woodlands in western North America (Box 1). Data sets encompassing multiple midden series have further connected temporal inertia from colonization and local extinction lags to community–climate disequilibrium and reassembly [35]. The augmented ability to identify species using aDNA and to characterize the range of genotypic or phenotypic traits for multiple species in a midden could inform theories about how local diversity accumulates [36–38] and the degree to which species traits influence community assembly [39]. Migrational histories constructed for different plant species [40–47] could also be used to investigate how the arrival and persistence of dominant species affected community composition, diversity, and stability over time. Such information is relevant to neocological theories concerning the invasibility of communities, including how standing biodiversity alters invasibility [48] and the effects of long-distance colonization events on community trajectories. Although most midden studies of community dynamics have focused on plants, arthropod and microbial assemblages also have been examined in the Atacama [23,49,50], offering new opportunities to integrate midden data with modern ecological studies.

Eco-evolutionary dynamics driving species interactions

Midden series often encompass time spans necessary for assessing eco-evolutionary responses driven by shifts in species interactions due to climate change. However, modern experiments that study the strength of species interactions across environmental conditions and identify traits underlying potential fitness consequences [51,52] are needed to contextualize midden investigations.

Relationships between midden builders and local flora have been the most common form of species interaction investigated. Studies have helped characterize rodent dietary choices in different plant communities and indicate that small mammal populations are as sensitive to changes in local biotic interactions as they are to large-scale climate change [19,53]. Sequencing plant DNA retained in rodent fecal pellets may further resolve species-specific feeding behavior. This work could be paired with experiments in modern systems to study the eco-evolutionary consequences of variation in the dietary niche of midden builders and assess how climate conditions and resource availability shape plant–herbivore interactions [54].

Host–microbe interactions are increasingly recognized as an important factor underlying population dynamics and eco-evolutionary responses to climate change in modern systems [55,56]. Rodent fecal pellets provide information about gut microbiomes [57], which can influence host fitness and drive adaptation and co-diversification [52,58]. In modern systems, gut microbial diversity often shifts with changes in host diet and habitat [59,60], patterns that could be assessed over space and time using middens. Paleogenomic studies can also provide insight into other host–microbe interactions and their resilience to climate change. For example, plant pathogen [49] and animal parasite assemblages [50] in the Atacama differed in their resilience to large-scale climate fluctuations during the Pleistocene–Holocene, which may reflect variation in adaptive potential. Combining experimental manipulations of microbial evolution with long-term records of ancient microbial communities is a powerful approach for mapping eco-evolutionary dynamics in host–microbe interactions.

Nutrient cycling and energy transfer within ecosystems

Assessing the resilience of ecosystem processes to environmental change inherently requires integrating data across biological scales and over periods of stasis and change. By characterizing the **historical range of variability (HRV)**, midden studies could establish boundaries for modeling ecosystem function. However, few ecosystem-level studies have used middens to test modern theories linking biogeochemical cycles and energy or nutrient pathways to ecosystem stability. Challenges arise in both paleo and modern studies because some ecosystem components are often overlooked (microorganisms) or coarsely described and aggregated (species-rich macroinvertebrates). The potential for abiotic conditions to affect the chemical integrity of organic tissues over time presents an additional challenge [61]. Yet, modern theoretical and empirical approaches can be used to reconstruct biogeochemical cycles and trophic dynamics in paleoecological systems. Quantifying these processes in modern communities occurring near middens will be crucial to interpreting midden data.

Stable isotope analysis is often used to describe energy flow and trophic niches within modern food webs [62,63]. In combination with theories such as the metabolic theory of ecology, similar approaches could inform midden studies assessing drivers of millennial-scale fluctuations in energy and nutrient flow. For example, integrating metabolic principles and analysis of bone assemblages in owl roost deposits showed that compensatory dynamics driven by rodent species turnover stabilized energy flow in the Great Basin during the Pleistocene–Holocene transition despite rapid climate-driven changes in vegetation [64]. Although this work was not conducted using middens, it is an example of how neoecological theories can be applied to paleoecological data to infer energy and nutrient dynamics.

Chemical analysis of midden remains also offers insights into long-term dynamics of biogeochemical and hydrological cycles, which are strongly coupled in space and time in arid environments [65,66]. Nitrogen isotopes ($\delta^{15}\text{N}$), in particular, have been used to track changes in the global nitrogen cycle since the last glacial period [67,68]. In many terrestrial ecosystems, $\delta^{15}\text{N}$

is negatively correlated with precipitation [69,70]. Although the mechanistic link is somewhat unclear, this relationship is thought to reflect the influence of water availability and nutrient demand on the ‘openness’ of the nitrogen cycle. However, this relationship appears to break down in hyperarid environments [71,72]. Comparing $\delta^{15}\text{N}$ of midden specimens from different arid regions over glacial–interglacial periods may help resolve uncertainties about environmental drivers of the nitrogen cycle. Moving beyond the nitrogen cycle requires analytical approaches that more broadly map the spatiotemporal distribution of all essential elements for life and their stoichiometric niche [73]. For example, heavy metal concentrations in rodent pellets from middens have been used to study the effects of mining activity in the Atacama [74]. Chemical and isotopic analysis of leaf cuticle waxes in plant macrofossils also serve as a robust hydroclimate proxy and have been used to detect hydrological changes that affect resource pools and ecosystem dynamics [75]. Reconstructing the chemical composition of paleo-organisms at various time-scales can enhance our understanding of ecological and geological processes acting on past ecosystems and establish baseline data for predicting future changes in key ecosystem processes.

Applications of midden research for restoration

Knowledge of **ecological baselines** is more vital than ever as natural resource managers face challenges wrought by intensified land-use change, pollution, invasive species, wildfires, and climate change [76]. Middens provide a record of how communities responded to past disturbances, which can contextualize restoration goals by revealing the historical range of variability [77] and the pace and extent of major **transition states** [78]. Further, traits or species identified from the midden record that conferred community resilience in the past can inform restoration practices. For example, when reseeded after wildfires, community assembly dynamics gleaned from middens can indicate the optimal order in which species would be added to facilitate recovery and increase resistance to further disturbances. Migrational histories at the species or haplotype level could also address questions about extirpated populations and inform strategies for translocating species into emerging bioclimatic spaces with global change. For example, the intentional introduction of bighorn sheep (*Ovis canadensis*) to Tiburón Island in the Gulf of California was long regarded as a case of transplanting an alien species into a previously unoccupied ecosystem. However, aDNA analysis of ~1550-year-old bighorn dung matched a haplotype from southwest Arizona and southern California, indicating the extant population on Tiburón Island is an example of unintentional rewilding [79]. Similarly, middens from the northern Atacama identified ‘pluvial relict’ populations of large candelabra cacti (*Browningia candelaris*), which are now dwindling in response to climate change [80]. Identifying factors driving local extinctions and recovery from disturbance are major questions spanning basic and applied sciences, and even the most fundamental information extracted from middens (patterns of species arrivals) can take on new life in guiding natural resource management.

The future of midden research

To fully leverage the potential of middens, new studies are needed that explicitly align paleorecords with neoecological and evolutionary perspectives. Such efforts should include a comprehensive gap analysis of modern ecological data and existing midden data to identify opportunities for collaboration across disciplinary and geographic boundaries. Collaborative studies mining existing midden data or proposing new field campaigns to expand collections could target:

- (i) Neoecological and evolutionary theories requiring spatially or temporally resolved data or involving comparisons across diverse taxonomic groups (e.g., meta-community dynamics [81], diversity–stability relationships [82], molecular phylogeography [83,84]).

- (ii) Geographic areas where a historical perspective could provide context for understanding modern states. For example, coordinated midden studies and neoecological experiments across transition zones or in different arid regions are needed to characterize disruptive versus stabilizing forces important to management in the **Anthropocene**. Spatial models that predict the availability of different aged middens based on climate and landscape features [85] can help design coordinated field studies based on the likely availability of relevant middens.
- (iii) Major components of biodiversity that have been well-studied in modern systems but understudied in middens. Comparing species' traits, especially at the genetic level, across timescales can provide evidence of eco-evolutionary processes determining species' resilience and adaptive potential to ongoing environmental change. Although existing midden data could be used for such studies, new collections will likely be needed to target taxa of interest and to explicitly test hypotheses generated from molecular and experimental studies in modern systems.

As new field campaigns take shape, there is a pressing need to ensure standardized research methodologies. Past midden collections and processing were done primarily by individual researchers for specific projects focused on a subset of midden materials. Standardizing field and laboratory protocols is needed to preserve knowledge generated over decades of midden research and improve the ability to design studies that compare across midden series, regions, and taxa. To facilitate this process, we developed a midden processing guide that can be refined and updated with new developments in the field [86]. Along with a basic suite of field data, midden macrofossil measurements, and processing recommendations, preservation of aDNA from middens and expansion of reference DNA libraries are of particular interest as these data can help refine and streamline species identifications and facilitate genomic studies. A more comprehensive calibration of plant and animal assemblages preserved in middens, including detailed studies comparing modern midden assemblages with the diversity of living organisms (e.g., [23]), as well as data standards and networks for analyzing diverse midden materials are also needed to integrate studies across midden series and data types. Ultimately, standardizing protocols can ensure that researchers are able to exploit the midden records' full potential.

Establishing regional depositories for midden materials will be crucial to the long-term curation and accessibility of these irreplaceable resources. Most previously collected middens are housed at individual institutions (Appendix B in the supplemental information online) where they are at risk of being lost or discarded as researchers retire. Middens could be housed in existing natural history museums or contemporary biodiversity collections. However, specialized depositories are preferable given the unique nature of these collections, which span contemporary and paleoecological domains, the need for climate- and pest-controlled environments, and the risk of contamination with modern DNA if housed with contemporary specimens. We recognize that establishing midden depositories requires coordination across regions and stable funding sources and that different countries and institutions will likely have different data management, preservation, access, and use policies.

A similar coordinated approach is needed to increase the accessibility of midden data and ensure its compatibility and interoperability with other paleo and neoecological data. In North America, the United States Geological Survey (USGS) North American Packrat Midden Database (<https://geochange.er.usgs.gov/midden/>) will soon be phased out, with retirement of its principal architects. Records from this database could be incorporated into the global, multiproxy NEOTOMA Paleocology Database (<https://www.neotomadb.org/> [87]).

Expanding midden research globally is a key goal moving forward. Herbivore middens have been collected and studied in Africa since the 1950s [88], in the Middle East since the 1990s [89], and in Australia since the 1980s [90]. The unique environmental histories and biological and cultural settings in these regions could yield new insights into biological responses to environmental change. For example, Australia boasts the world's highest extinction rate for terrestrial mammals in the modern era [91]. Prime examples are stick-nest rats (*Leporillus*; Figure S2), for which former ranges can only be described from historical records, bone deposits, and middens. Approximately 125 middens have been dated and analyzed [90], with only one detailed series spanning the Pleistocene–Holocene transition [92]. Expanded collection and analysis of Australian middens could elucidate the impacts of past, recent, and future faunal extinctions on vegetation and arthropod dynamics. Midden builders in Africa and the Middle East include hyraxes (*Procavia capensis*) and dassie rats (*Petromus typicus*; Figure S2), the latter endemic to the Namib Desert. The abundant plant and arthropod macrofossils in dassie rat middens [93] remain unexploited by researchers, offering untapped potential for understanding late Quaternary ecological dynamics in the Namib. Although plant macrofossils are rare, pollen assemblages in hyrax middens [94,95] document early human impacts and local landscape degradation [89,96]. Additionally, feces from these middens could expand comparative studies of climate impacts on body size, diet, and microbiomes of midden builders. Collectively, the global distribution of midden records presents a range of late Quaternary climatic and vegetation histories to test ecological and evolutionary theory, from the dynamic arid and semi-arid ecosystems in western North America to the stable Australian drylands. The vast arid lands in Central Asia, which harbor middens made by mountain mole (*Alticola* [97]) and perhaps other animals, remain an unexplored midden frontier.

Concluding remarks

Middens are responsible for much of what is known about biotic responses to late Quaternary environmental changes in arid ecosystems in the Americas (Boxes 1–3) and this work has provided important historical context for neoecological and evolutionary studies. Yet, global change is already compromising comprehensive studies that rely on middens to understand the ecological history of arid ecosystems. Both middens and their contemporary surroundings are important end points for studying past and ongoing ecological change. However, these finite resources are susceptible to accelerating losses from land-use conversion, mineral resource extraction, biological invasions, increased wildfire frequency, and climate change. Identifying and protecting vulnerable middens and surrounding ecological sites is paramount.

Despite the importance of middens to research in global change biology, the publication rate for midden-based studies has waned over time (Figure S1) as researchers have retired or left the field. In the Americas, past midden research was loosely coordinated and carried out by a few researchers working independently in different regions to reconstruct paleoclimate and local vegetation histories. This emphasis reflects previous funding priorities and more focused training of midden researchers in geosciences and physical geography compared with ecology and evolutionary biology. However, many of the driving questions in global change biology are inherently interdisciplinary (see Outstanding questions). Multi-investigator teams that explicitly integrate paleoecology with modern experimental studies are ideal for addressing these questions. In addition to revitalizing midden research, integrating diverse perspectives enhances understanding of mechanisms underlying organismal to ecosystem-level responses to past, present, and future environmental changes (Figure 1). An emerging ‘community of practice’ of paleoecologists, neoecologists, and evolutionary biologists focused on collaboration, integrated science, and macroscale approaches can tackle the fundamental questions articulated in this review and identify other research priorities for the future.

Outstanding questions

How do eco-evolutionary processes shape genomic evolution and convergence or conservatism in species' responses to their environment across space and time? While the midden record is too recent to capture major speciation events, molecular evolutionary responses spurred by climate change may be tracked in selected taxa and lineages. Integrating modern and historical (from aDNA) phylogenies with evidence of functional diversity and niche evolution in the midden record may further identify long-term processes related to niche convergence, divergence, and conservatism.

In what contexts do genetic or phenotypic changes facilitate ecological stability in species distributions, community composition, and ecosystem function? To address this question, we recommend expanded field campaigns that collect middens from different arid regions, coupled with more in-depth organismal studies that leverage advanced technology to quantify genetic and phenotypic changes, and modeling approaches informed by neoecological theory and experiments.

What are the timescales, drivers, and consequences of synchronous and asynchronous responses to environmental change? How do these patterns vary across taxonomic and functional groups and what traits underlie these differences? Understanding mechanisms driving spatial synchrony of species responses could inform models of community assembly and ecosystem stability, with further implications for the conservation of contemporary biodiversity and management of invasive species.

What are the stabilizing forces in arid ecosystems and how might they operate in anthropogenic landscapes that are experiencing rapid climate change coupled with altered biogeochemical cycles and increased land use? Identifying characteristics that facilitate ecosystem stability despite gradual or abrupt environmental change could directly inform conservation and restoration of human-impacted systems in the future.

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Author contributions

All authors contributed to the conceptual design and initial drafts of this manuscript. K.M.B., J.L.B., F.P.D., R.H., A.D.H., C.A.H., C.L., and F.A.S. collaborated on manuscript figures. K.M.B. led revisions with input from all authors.

Declaration of interests

The authors declare no competing interests.

Supplemental information

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