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Multiscale climate change impacts on plant diversity in the Atacama Desert

Francisca P. Díaz[1,2] | Claudio Latorre[3,4] | Gabriela Carrasco-Puga[1,2] | Jamie R. Wood[5] | Janet M. Wilmshurst[5,6] | Daniela C. Soto[1,2] | Theresa L. Cole[5,7] | Rodrigo A. Gutiérrez[1,2]

[1]Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Santiago, Chile

[2]FONDAP Center for Genome Regulation & Millennium Institute for Integrative Biology (iBio), Santiago, Chile

[3]Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile

[4]Institute of Ecology and Biodiversity (IEB), Ñuñoa, Santiago, Chile

[5]Manaaki Whenua – Landcare Research, Lincoln, New Zealand

[6]School of Environment, The University of Auckland, Auckland, New Zealand

[7]Department of Zoology, University of Otago, Dunedin, New Zealand

Correspondence
Rodrigo A. Gutiérrez, Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Santiago, Chile.
Email: rgutierrez@bio.puc.cl

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Abstract

Comprehending ecological dynamics requires not only knowledge of modern communities but also detailed reconstructions of ecosystem history. Ancient DNA (aDNA) metabarcoding allows biodiversity responses to major climatic change to be explored at different spatial and temporal scales. We extracted aDNA preserved in fossil rodent middens to reconstruct late Quaternary vegetation dynamics in the hyperarid Atacama Desert. By comparing our paleo-informed millennial record with contemporary observations of interannual variations in diversity, we show local plant communities behave differentially at different timescales. In the interannual (years to decades) time frame, only annual herbaceous expand and contract their distributional ranges (emerging from persistent seed banks) in response to precipitation, whereas perennials distribution appears to be extraordinarily resilient. In contrast, at longer timescales (thousands of years) many perennial species were displaced up to 1,000 m downslope during pluvial events. Given ongoing and future natural and anthropogenically induced climate change, our results not only provide baselines for vegetation in the Atacama Desert, but also help to inform how these and other high mountain plant communities may respond to fluctuations of climate in the future.

KEYWORDS
ancient DNA, Atacama Desert, biodiversity, biogeography, climate change, desert plants, elevational gradients, fossil middens, metabarcoding, paleoecology

1 | INTRODUCTION

Climate has a major role in the distribution of ecosystems and species range on Earth. The last few decades have revealed overwhelming evidence that climate is changing in the world (Pachauri et al., 2014; Root et al., 2003). How climate change is affecting species, biodiversity and ecosystem structure, function and services, both now and in the future, are questions of utmost

importance (Root et al., 2003). Moreover, how existing adaptations could potentially buffer plants and other species from ongoing and future climate change remains unresolved. Understanding the impact of climate change is particularly relevant in arid regions, where ecosystems are often vulnerable to natural or anthropogenic ecosystem changes in water availability (Diaz, Grosjean, & Graumlich, 2003). Moreover, plants in such regions display remarkable adaptations to such demanding environmental conditions, and local communities can be surprisingly diverse and unique providing rich genetic resources of global interest.

On the western slopes of the Andes, the ecosystems of the Atacama Desert (which range from hyperarid to high Andean) can be found across an elevational range spanning more than 6,000 m which encompasses pronounced precipitation and temperature gradients over relatively short distances (~300 km). Elevational gradients are important tools for understanding climatic and even evolutionary controls on biodiversity (Arroyo, Squeo, Armesto, & Villagran, 1988; Lenoir, Gégout, Marquet, De Ruffray, & Brisse, 2008; Whittaker, Willis, & Field, 2001). The Atacama is thus an excellent model with which to study biodiversity dynamics and their climatic controls. On interannual timescales and during the late Holocene, Atacama climate variability is driven mainly by El Niño Southern Oscillation (ENSO) (Vuille, Bradley, & Keimig, 2000). After large precipitation events, annual blooms are frequent, increasing plant richness and cover, and soil microbial activity (Schulze-Makuch et al., 2018). Ancient DNA (aDNA) of plant pathogens also increased in abundance and diversity in the Atacama during wetter events in the past (Wood et al., 2018). On much longer (millennial) timescales, the Atacama has experienced major hydroclimate variations. Air temperatures started to warm from glacial to interglacial conditions at ~19,000 cal year BP coupled with a c. 4°C increase in regional sea surface temperature (SST) (Kaiser, Schefuss, Lamy, Mohtadi, & Hebbeln, 2008) along with a 6°C increase in terrestrial tropical ecosystems (Thompson, 2000). Moisture or precipitation changes followed, with a period of increased precipitation (by around 500 mm), originally described as the Central Andean Pluvial Event (CAPE) in two distinct intervals (CAPE I: 15,900–13,800 and CAPE II: 12,700–9,700 cal year BP) (Quade et al., 2008). Here, we follow the recommendation of a recent revision of different paleoclimate records (De Porras, Maldonado, De Pol-Holz, Latorre, & Betancourt, 2017) from the central-southern Atacama Desert, which defines widespread wet conditions from ~17,500–13,800 (CAPE I) and 12,700–8,500 cal year BP (CAPE II).

As each plant species currently inhabits a preferred temperature and precipitation range (determined mainly by elevation in this environment), the exact identification of the plants that lived in the desert through different time periods can provide information about past climate change (Díaz, Latorre, Maldonado, Quade, & Betancourt, 2012; Latorre, Betancourt, Rylander, & Quade, 2002). DNA metabarcoding is a molecular technique for identifying species using molecular markers (barcodes) obtained from a sample containing DNA of more than one organism (Hebert, Cywinska, & Ball, 2003; Taberlet, Coissac, Pompanon, Brochmann, & Willerslev, 2012) and combined with aDNA can be used to reconstruct past diversity from ancient or fossil samples like lake sediments and other substrates (Alsos et al., 2016; Murray et al., 2012; Willerslev et al., 2003).

The recovery of aDNA or paleoenvironmental DNA from a variety of organisms is a technique that has been greatly improved during the last decade (and now often termed paleogenomics) (Rawlence et al., 2014; Willerslev & Cooper, 2005). The highest success rate for aDNA extraction has been attained with organisms from frozen sites (Gould, León, Buffen, & Thompson, 2010) and generally from dry or cold environments, rather than hot or humid sites (Grealy et al., 2016; Haouchar et al., 2014; Poinar et al., 1998; Rawlence et al., 2014). The Atacama Desert, due to its hyperaridity, therefore offers a potentially perfect environment to preserve aDNA (Kuch et al., 2002), but the high diurnal temperatures and strong radiation could also limit this.

Past variations in climate and vegetation can be reconstructed by analyzing the contents of fossil middens of herbivorous rock-dwelling rodents (Betancourt & Saavedra, 2002; Pearson & Betancourt, 2002), which are found in deserts around the world (Betancourt & Davis, 1984; Gil-Romera, Scott, Marais, & Brook, 2007; Murray et al., 2012). Middens are amalgamations of plant, animal, and other organic remains, embedded in a matrix of crystallized urine (Betancourt & Saavedra, 2002). They can be preserved for tens of millennia in the arid Atacama (with the oldest samples dating to >50,000 years BP), offering exceptional spatiotemporal and taxonomic resolution. Middens provide a unique source of ancient material preserved in ideal conditions, as they are usually found in rock crevices which offer some protection against the effects of high diurnal temperatures and direct radiation.

Sequence information from aDNA can be used to identify taxa that are often not resolved by morphological analyses (plant macrofossils, cuticles, or pollen) and has the potential of providing a complementary view of biodiversity over time as compared to more traditional methodologies (Rawlence et al., 2014; Wood et al., 2012). Although aDNA from one 11,700-year-old fossil rodent midden from the Atacama desert has previously been used to reconstruct past rodent distributions (Kuch et al., 2002), we present the first application of DNA metabarcoding and next-generation sequencing for entire plant communities, from a time series of middens spanning 27,590–200 years before present.

Our goal is to understand how different magnitudes of climate change affect plant communities in the Andean Atacama. To integrate spatial and temporal changes in plant biodiversity from annual to millennial timescales, we compare aDNA data from rodent middens with contemporary plant biodiversity information over the course of eight consecutive years across an altitudinal gradient (from 2,500 to 4,500 m a.s.l.) with strong precipitation differences (from 10 to 300 mm/year). Plant community composition should be affected in proportion to the magnitude of climate change, but the challenge behind this hypothesis is to define the sensitivity of the system. For instance, how has vegetation responded to the last decade of climate change in contrast to much longer timescales with much greater magnitudes of climate change (i.e., across the Last Glacial–interglacial transition) (Nolan et al., 2018).

Our results provide valuable insights into the determinant factors of survival and biodiversity of Atacama plants. Our integrated approach and data generated should also contribute to understanding the impacts of different magnitudes of climate change on mountain and desert plant

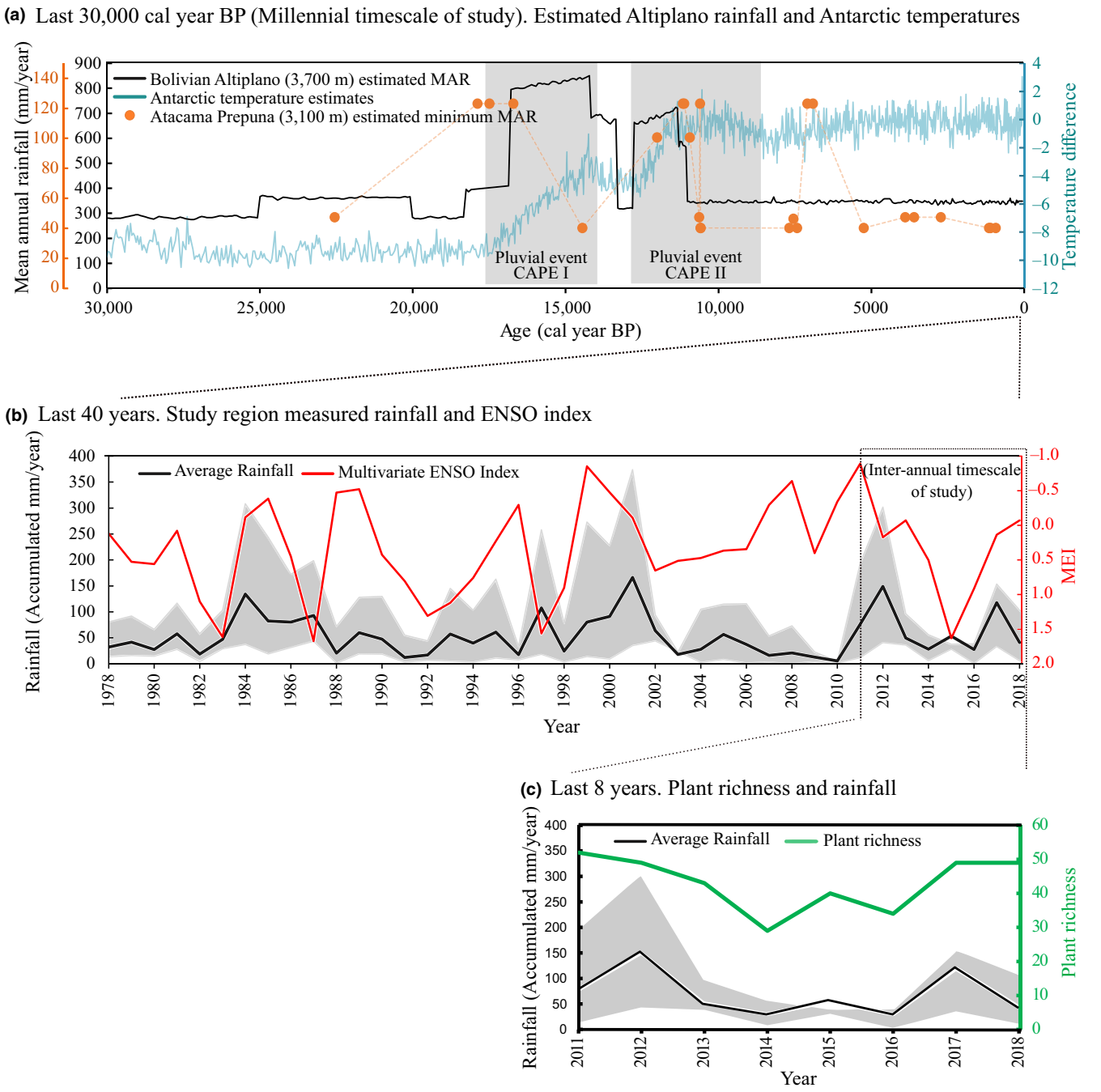

FIGURE 1 Climate change over the different timescales present in our study. (a) Last 30,000 cal year BP. Black line: Modeled precipitation rates for the Bolivian Altiplano at 3,700 m a.s.l. (m) (Placzek, Quade, & Patchett, 2013). Rainfall scale (0–900 mm/year) is shown in black on the left. Orange dots: Minimum Mean annual rainfall (MAR) estimated from fossil vegetation at 3,100 m (Latorre et al., 2006). Rainfall scale (0–140 mm/year) is shown in orange on the left. Blue line: Temperature estimated from the Antarctica Epica Dome C (temperature difference from the average of the last 1,000 years) (Jouzel & Masson-Delmotte, 2007). Gray boxes: Central Andean Pluvial Event (CAPE) Includes CAPE I (17,500–13,800 cal year BP) and CAPE II (12,700–8,500 cal year BP) (De Porras et al., 2017, modified from Quade et al., 2008). (b) Last 40 years. Black line: Average total annual rainfall (Data obtained from 20 DGA stations at 22–24° lat S and 2,300–4,400 m, Antofagasta region). Upper gray limit represents average precipitation at 4,400–4,000 m and lower gray limit represents average precipitation at 2,300–2,700 m. Red line: Annual Multivariate ENSO Index (Data obtained from www.esrl.noaa.gov) (Wolter & Timlin, 2011). Correlation between annual average rainfall and MEI is not significant ($r^2 = 0.04$, $p > 0.05$). (c) Last 8 years. Black line and gray limits: Same as in B. Green line: Total plant richness observed in the study area after the rainy season (Talabre-Lejía transect, 2,500–4,500 m). Correlation between average rainfall and plant richness is $r^2 = 0.21$, $p > 0.05$. Correlation between plant richness and MEI is $r^2 = 0.39$, $p > 0.05$

communities; vital information for designing conservation management plans and predictive models of global change (Jackson & Blois, 2015).

2 | MATERIALS AND METHODS

2.1 | Vegetation surveys

In northern Chile's region of Antofagasta lie the driest areas in the world as it corresponds to the maximum penetration of the Atacama Desert into the interior from the Pacific coast. Seasonal timing of precipitation determines a single season productivity pulse in the Atacama (after summer, during March to April). Mean annual rainfall, obtained from 20 Dirección General de Aguas, Chile (DGA) weather stations located at 22–24° lat S and 2,300–4,400 m is 54 mm/year (Figure 1b). The elevation gradient and their close interaction with increasing rainfall and decreasing temperatures (among other factors such as grazing or human impacts) give rise to distinct vegetation belts which can be consistently recognized across the region, based on overall plant physiognomy (Figure 2a). However, species assemblages of those belts could be ephemeral or even anomalous over long time periods. Nevertheless, the current distribution of flora constitutes a reference to which we can compare vegetation and thus estimate degree of similarity or changes that occurred over time from the paleo record. The lowermost vegetation belt is the Prepuna between 2,600 and 3,300 m and is vegetated mostly by cushion cacti, a few xerophytic shrubs and annuals, with a mean annual temperature of ~14°C, but strong daily fluctuations from 32 to −4°C. Mean annual rainfall is close to 0 mm/year at the upper margin of the absolute desert (defined by the general absence of vegetation) at 2,500 m and ~60 mm/year at 3,300 m. The Puna (or tolar) lies between 3,300 and 4,000 m and is dominated by shrubs, but also contains the highest richness and plant cover along the elevation gradient. Steppe dominates at our study sites (23°24′ lat. S) from 4,000 to 4,500 m and is characterized by perennial grasses, low mean annual temperatures close to 4°C and a mean annual precipitation of ~160 mm.

Present relationships between environmental variables and plant distributions were established with vegetation surveys performed during the first week of April over eight consecutive years

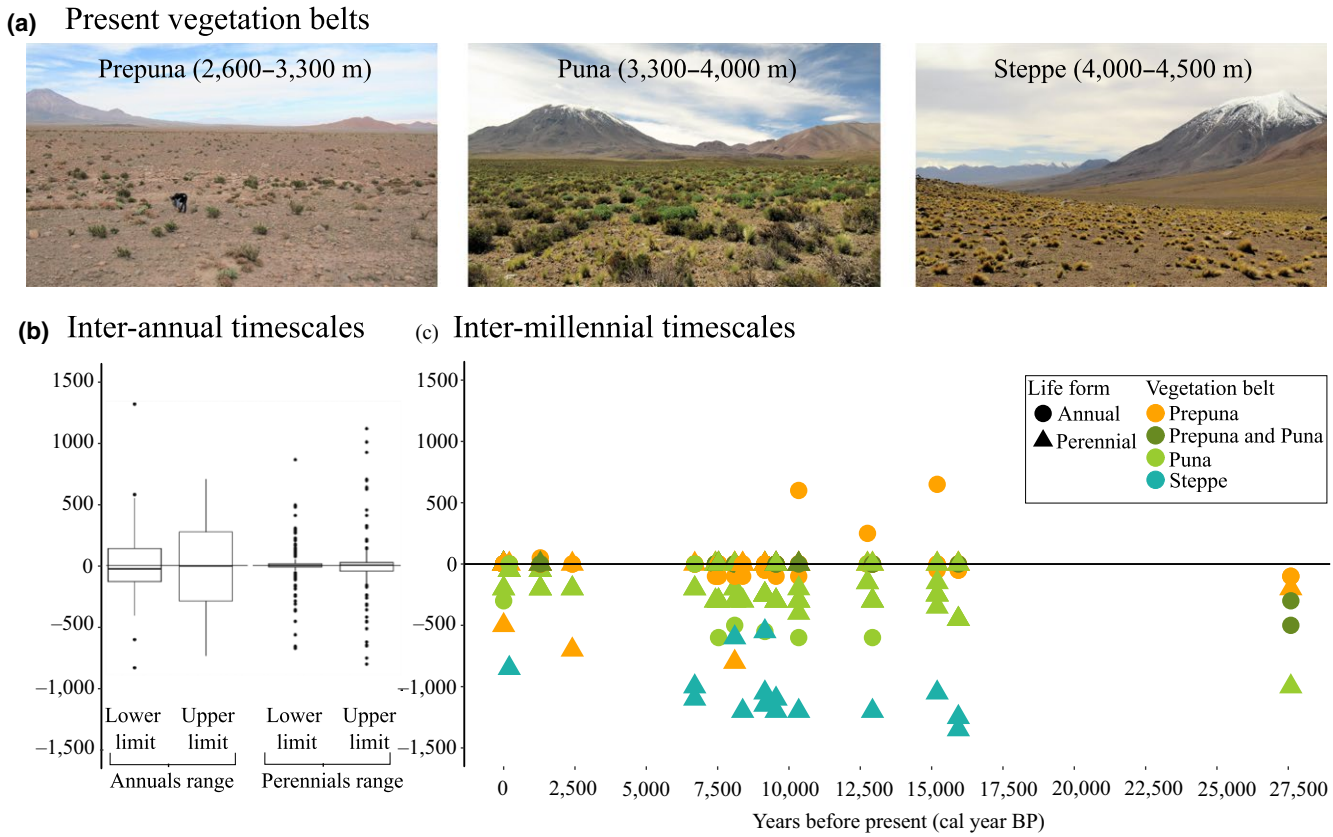

FIGURE 2 Vegetation belts in the Andean Atacama Desert and comparison across different timescales showing major elevational ranges of plant taxa in the study sites. (a) Photographs of vegetation belts in the Andean Atacama. (b) Boxplots show the interannual variability of range distributions of annual and perennial taxa observed during the 8 years of vegetation surveys. Elevation change is shown as annual variations along the upper and lower limits of distributions for each species, compared to the average present-day elevation (averaged over the 8 years of the study). Outliers represent species with low coverage (only found in some years). (c) Long-term range changes compared to present-day ranges (meters below or above their current distribution). Zero here is defined as the maximum distribution range estimated from A. For instance, if a species was found in a 10,000 cal year BP midden collected at 3,000 m and that species grows today between 4,000–4,200 m, it will appear as a point at −1,000 m (the actual elevational change). As most middens were collected along the lower limit of the vegetation, these limits tend to descend in the past, which gives a good estimate of their past lower limit of distribution

(2011–2018) and following every 100 m of elevation (22 sites) across an elevation gradient from approximately 2,500 to 4,500 m a.s.l. (Talabre-Lejía transect, TLT) (see Díaz, Frugone, Gutiérrez, & Latorre, 2016 for more details). A total of 61 plant species were collected and frozen to construct a local DNA reference database (*Atacama database*) across this gradient (Table S1). Plant species richness was measured using two 250 m^2 plots at each of the 22 sites over all the years of the study (Figure 1c). Species observed permanence (i.e., how many years the same species appeared at each site) was calculated for each site and along each species distribution range (min and max altitude) as well as for the average upper and lower limit of distribution of each plant species in the last 8 years' surveys. Additional data regarding perennial species distribution (using a similar collection method) were available from May 1998 along the same transect (Latorre, 2002). Simple linear models were used for assessing any possible correlations with Pearson's correlation coefficient, analyses were performed in the R Programming Language (R Core Team, 2018).

2.2 | Barcodes database

Using local DNA barcode databases can significantly increase the resolution or capacity to identify taxa from a complex sample (Taberlet et al., 2007). To improve resolution and subsequent analysis of aDNA reconstruction, we constructed a local barcode *Atacama database* using the species collected during the plant surveys and some additional species that we collected previously from lower altitude sites (Table S1). These plants were identified, and specimens were deposited at the Herbarium at Universidad de Concepción, Chile ("Herbario de la Universidad de Concepción CONC"; species list and codes in Table S1). We extracted DNA from these specimens using the PureLink Plant total DNA purification kit (Invitrogen) following the manufacturer's protocol. We amplified the chloroplast *trnL* region using primers c and d as described by Taberlet (Taberlet et al., 2007). Amplification was performed in 25 μl containing 0.4 μl of Phusion High-Fidelity DNA Polymerase (Thermo Scientific, EEUU); 8.0 μl of enzyme buffer; 0.2 μl of each 10 mM dNTP (Thermo Scientific, EEUU); 2.0 μl from each 10 mM primer and 4.0 μl of extracted DNA (between 10 and 50 ng/μl). Following an activation step of 30 s at 95°C for the enzyme, the PCR mixture underwent 25 cycles of 5 s at 95°C, 30 s at 55°C and 5 min at 72°C on a 2720 Thermal Cycler (Applied Biosystems, EEUU).

We obtained *trnL* sequences or barcodes from those 61 species to construct the *Atacama database* (GenBank accession numbers MH115328–MH115388). The resolution of the selected barcode (*trnL c-h*) using only the global *DDBJ database* was around 25% for species, almost 50% for genera and 80% for families. Using our local *Atacama database*, the resolution increased to around 80% for species and genera and 100% for families.

2.3 | Fossil rodent middens collections and radiocarbon dating

All middens (N:26) were previously collected in Cerros de Aiquina (Antofagasta region, northern Chile, from 2,414 to 3,380 m a.s.l.) and radiocarbon dated by C. Latorre and collaborators (Latorre, Betancourt, Rylander, Quade, & Matthei, 2003) (see Table S2 for details). Middens are deposited episodically and mostly discontinuously, nevertheless our ages distribution covers the different climatic events described in Figure 1a. After collection, middens were processed to identify macrofossils and pollen (results published in De Porras et al., 2017; Latorre et al., 2003, comparisons in Figure 3) and an unprocessed subsample of each midden was retained as a voucher specimen (used here to extract aDNA). Figure S1 shows photographs and a workflow summary for the aDNA recovery and analyses. Accelerator mass spectrometry radiocarbon dating was performed at different facilities, see details in Table S2. Radiocarbon dates for the middens were calibrated using ShCal13 (Hogg et al., 2013) in OxCal v.4.3.2 (Ramsey, 1995) and median ages were used for analyses (Table S1). Two of the middens were determined to be modern (after ^{14}C dating) and the others are from 49,600 to 200 ^{14}C year BP. However, in this study we consider only samples from 27,590 to 200 cal year BP, because we could not amplify plant DNA from the older sample (see Section 3.2 in Results).

2.4 | Midden subsampling, aDNA extraction and amplification

To recover the damaged aDNA and avoid modern DNA contamination, subsampling, extraction and amplification were performed at the specialized aDNA laboratory at Manaaki Whenua - Landcare Research Long-Term Ecology Laboratory, Lincoln, New Zealand (Figure S1). First, we isolated an inner piece of material from 24 fossil rodent middens, following the protocol for subsampling late Quaternary coprolites (detailed in Wood & Wilmshurst, 2016). The subsampling procedure was carried out in a sterilized subsampling hood and consisted of: irradiating the sample with UVC, removing the outer surface, irradiating again, bisecting the sample and taking a sample for aDNA analysis from the interior of one half. Tools and the sampling box were sterilized between samples using bleach, ethanol, and UVC.

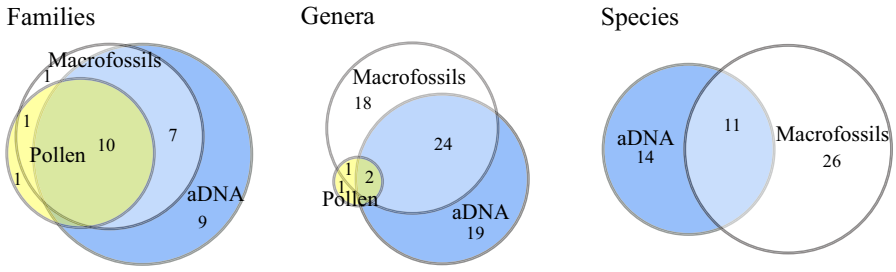

FIGURE 3 Number of taxa identified from the same fossil rodent middens (Cerros de Aiquina or CDA sites) using different techniques. Macrofossil data were obtained from (Latorre et al., 2003) and pollen data were obtained from (De Porras et al., 2017)

Subsampling was undertaken inside the physically isolated aDNA laboratory where portions weighing 5 g were rehydrated in H_2O for 24 hr. To check for possible contamination (Cooper & Poinar, 2000; Rawlence et al., 2014; Wood & Wilmshurst, 2016), we carried out extraction blanks alongside actual template samples. The aDNA extraction was performed using the PowerMax soil DNA isolation kit (MoBio). From two middens, we did extraction replicates to detect heterogeneity inside the midden and in sample size. Since suitable metabarcodes for aDNA studies should be no longer than 300 bp, but typically around ~130 bp for plants (Rawlence et al., 2014), we amplified a smaller section of trnL (163–228 bp), using primers c and h (including the P6 loop) described in (Taberlet et al., 2007). As some inconsistent results between PCRs of the same sample have been shown in amplifications from environmental or complex samples (Ficetola et al., 2015), we did two independent amplifications using the same primers for each sample. We modified the primers adding a linker sequence. To avoid incorrect detection of clusters of our low nucleotide diversity libraries during next-generation sequencing, we used a mixed set of four different length primers with the same target (trnL c-h), but with 0, 1, 2, or 3 nucleotide spacers between the trnL primers and linker sequence. The aDNA PCR protocol for one reaction (25 μl) was: BSA 2.5 μl, Buffer 10× 2.5 μl, $MgSO_4$ 1 μl, dNTP 0.2 μl, F primer 0.5 μl, R primer 0.5 μl, Taq Platinum HiFi 0.25, H_2O 15.3 μl and DNA 2 μl (PCR protocol: 94° 3:00 then 50–55 cycles [94° 0:30; 55° 0:30; 68° 0:45] and 68° 10:00). We also followed a Shrimp DNAase protocol (0.25 μl: DNAase diluted 10 times, 15 min at 37° and 15 min at 65°) to remove possible contaminant DNA from master mix before adding the aDNA. Extraction controls and PCR negative controls were included in all PCR reactions. We checked the extraction and amplification success by visualizing the amplicons in an agarose gel electrophoresis. DNA amplification and all downstream procedures (quantification, purification, and sequencing) were performed in laboratories physically separated from the aDNA laboratory. We sequenced both amplification replicates from each sample. To control for possible contamination, we also sequenced three extraction blanks, which were done in parallel with the extracted samples. As we used PCR to amplify, it was not possible to investigate the quality of DNA preservation because the ends of DNA molecules are not revealed. But we follow other criteria of authenticity for aDNA (Paabo et al., 2004) such as the mentioned extraction controls, PCR controls and repeated amplifications from all extracts.

2.5 | Libraries preparation and sequencing

To prepare the libraries, we followed the protocol "16S Metagenomic Sequencing Library Preparation" (Illumina, 2013), adapted to our marker trnL (c-h). We did a PCR clean-up using magnetic beads (Agencourt AMPure XP), cleaning both size extremes. We ran a second amplification using Illumina adaptors with the Illumina indices (Nextera XT) and we ran a second Clean-up using the same method. We quantified the libraries using the Illumina Library Quantification Kit, Universal qPCR Mix (KAPA Biosystems) and real-time PCR and validate them through capillary electrophoresis. Finally, we normalized and pool libraries for sequencing. We sequenced using Illumina's next-generation pair-end sequencing technology (MiSeq, Reagent Kit v3, 600 cycles).

2.6 | Bioinformatic analysis

To generate the databases and analyze the sequencing results, we used the OBITools package (Boyer et al., 2016). We processed the data, constructing a local *Atacama database* with our sequences obtained from the vegetation surveys and a global DDBJ database performing an in silico PCR to DDBJ (Mashima et al., 2016) (mirror of GenBank) with our primers using ecoPCR tool (Ficetola et al., 2010). The aDNA sequences were processed pairing forward and reverse reads, removing "joined" pairs. Then, we dereplicated the reads, and filtered them by count number (more than 5 reads) and by length (between 60 and 240 bp). Finally, we identified and removed possible PCR errors and did the taxonomic assignation (top score taxon, more than 95% of identity) to both databases using NCBI taxonomy database as reference (Federhen, 2011). The *Atacama database* was used to identify in the first place. The DDBJ database was used to identify missing taxa, higher taxonomic levels and contaminants (Table S4). Three low-quality samples (with more than 10% of contaminants) were not included in posterior analyses. We selected 29 (out of 73) of the most informative plant taxa to construct the results diagram in Figure 4 (mostly identified to species or genus level and representative of Atacama vegetation belts).

Considering that our midden series are not continuously deposited, and have a relatively low sample size, we were not able to do time-series statistical analyses. Results are presented as taxa abundance charts and taxa elevation displacement compared to the present-day ranges (meters below or above their current distribution). To compare PCR and extraction replicates, we calculated a Bray–Curtis dissimilarity index, and then compared groups using an ANOVA followed by a Tukey mean differences test (Figure S2). All analyses and charts were performed in the R Programming Language (R Core Team, 2018), using package rioja (Juggins, 2007).

2.7 | Data availability statement

Sequence data (local plant barcodes) that support the findings of this study have been deposited in GenBank with the accession codes MH115328–MH115388. Unprocessed reads from each midden sample (.fastq) of this study are available from the corresponding author upon reasonable request.

3 | RESULTS

3.1 | Perennial plant species in high Andean Atacama exhibit resilience to short-term climate changes

How vegetation adjusts to short-term and interannual environmental changes is relevant for the development of comprehensive

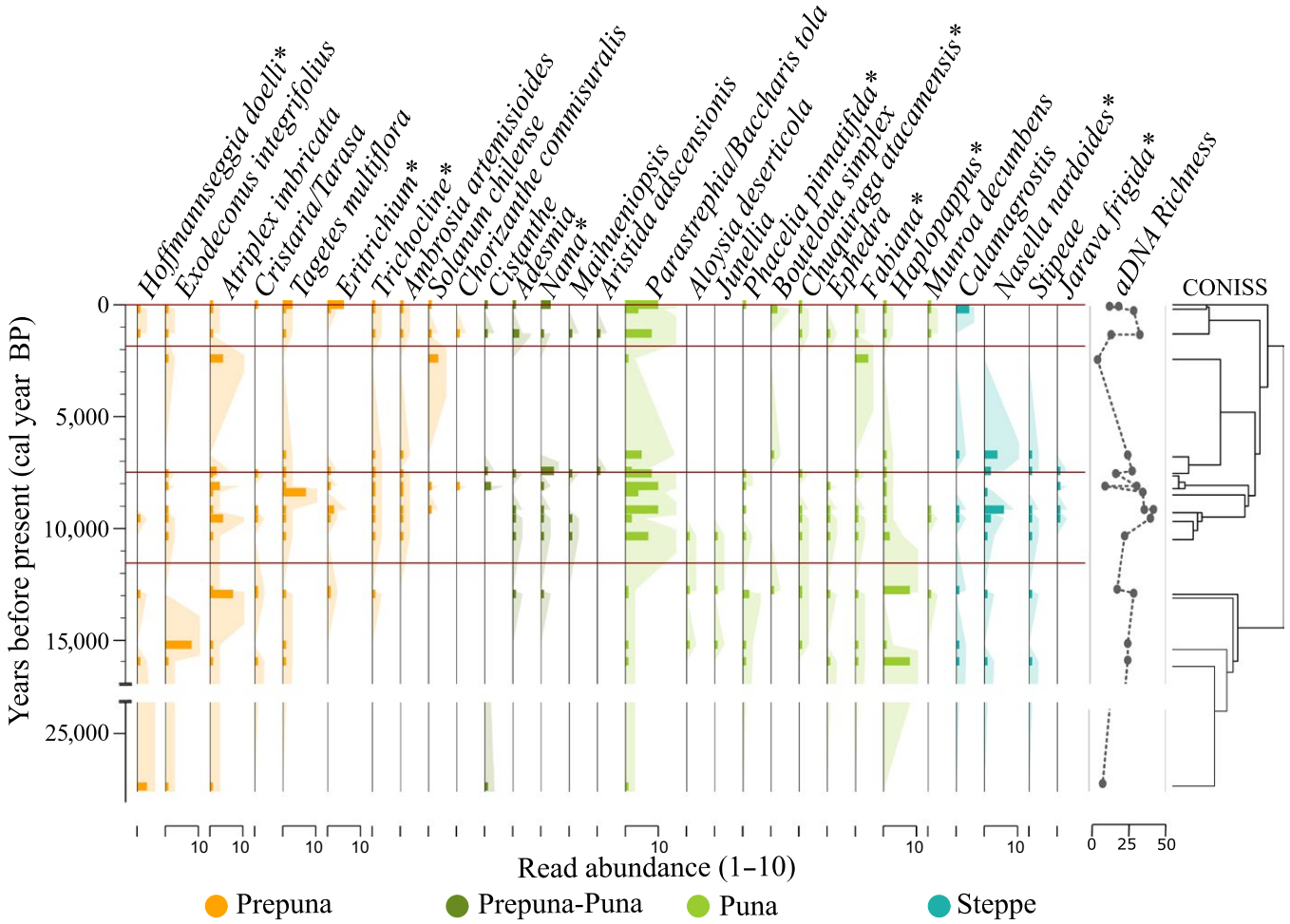

FIGURE 4 Selected plant taxa identified in 21 middens using aDNA. Bars represent read abundance (normalized from 1 to 10) and color shadows behind bars are exaggerated curves to show tendencies. (*) Highlight new taxa, not previously identified by macrofossil or pollen analyses. Constrained Incremental Sums of Squares cluster analysis (CONISS) indicates the similarity among middens within similar groups (red lines). All middens were collected around 3,100 m a.s.l. (from 3,000 to 3,400 m a.s.l.), except the oldest midden (27,590 cal year BP) which was collected at 2,414 m a.s.l

diversity studies in a global change scenario (Figure 1). The species accumulation curve from our study area shows that it took at least 6 years of surveys to find the baseline of plant biodiversity (>90% of observed species and curve saturation). As expected, precipitation seems to be the main driver of interannual productivity and plant species richness (Figure 1c). Although other factors, such as timing of precipitation and temperature changes, were also important for germination of some species. For instance, we observed an important annual bloom of *Lupinus* (reaching almost 50% of soil cover in some sites) only during one "La Niña" year in 2012 (Figure 1b). This species was not registered in other years with comparable precipitation (2017) or another "La Niña" year in 2018, indicating an interaction of environmental factors is required for major changes in *Lupinus* germination rates. These results underscore a surprising persistence of *Lupinus* seed banks, and also the importance of sustained efforts over time to understand biodiversity, particularly in extreme environments.

We compared the interannual distribution range of each plant species and discovered that perennial species did not change their distribution during the 8 years of surveys. In addition, perennial plants showed the same distribution in the last years as compared to a survey from 1998 (Villagrán et al., 1998). In contrast, annual herbaceous species contracted and expanded their distribution range each year (probably germinating from a persistent soil seed bank), according to interannual weather conditions (Figures 1c and 2b) and their permanence at each site was generally low (on average each annual was seen in three out of the 8 years). The outcome of our surveys underscores a surprising persistence of seed banks for some annuals (perhaps for decades, and not just years) in a hyperarid desert. These results indicate distribution of perennial plants is resilient to short-term (annual and decadal) environmental changes and annual plants showed more flexibility in their observed distribution according to environmental parameters, but probably also as a product of a resilient soil seed bank. These results also suggest that to produce significant community changes in perennial species, the magnitude of climate change should be more extensive in time or magnitude than what we have observed in the last two decades.

3.2 | Using DNA barcodes increases taxonomic resolution and identification of plant species in samples as old as 27,590 years

To understand diversity changes over longer periods of time than is possible to observe by standard field surveys, we obtained high-quality aDNA, amplified and sequenced the *trnL* barcode in fossil samples (dated 200–27,590 cal year BP) and from modern middens using Illumina technology (see Figure S1 for midden photographs and a workflow summary of aDNA recovery and analyses). Five low-quality samples were not included in posterior analyses (see Materials and Methods for details). For instance, the oldest midden (49,600 ^{14}C year BP) had 92% of reads identified as contamination (assigned as Pottiaceae), and due to the absence of Pottiaceae in the study region (and its presence in the negative control) we did not include this sample in the final analyses. For other samples, extraction and amplification success were independent of age. We obtained a mean of 115,000 reads per midden/sample for 21 middens (see details in Table S2). We compared replicates and different samples using the Bray–Curtis dissimilarity index (BCI) (Figure S2). Extraction replicates from two middens reveal similar identified taxa with a mean BCI of 0.3. PCR replicates for all samples yielded similar results (mean BCI of 0.3) and were significantly different compared to independent samples (mean BCI: 0.8). Therefore, we averaged and combined PCR replicates for posterior analysis. We sequenced three PCR negative controls, obtaining less than 70 reads (mostly Asteraceae, Pottiaceae, Pooideae), except for one sample from which 3,000 reads were obtained (mostly Triticeae tribe), and removed these taxa from posterior analyses. Of the sequences, 94% were taxonomically assigned using both plant databases. Of the total assigned reads, 99% were classified as Atacama taxa and 1% as contaminants (mainly from vegetation outside the laboratory or food, see Table S4). We identified a total of 73 Atacama plant taxa (25 species, 46 genera, and 26 families) (Table S3) (Figures 3 and 4).

We compared our aDNA record with pollen (De Porras et al., 2017) and macrofossil (Latorre et al., 2003) reconstructions previously analyzed in these same middens (Figure 3). The total number of taxa was highest for aDNA (n = 73), followed by macrofossils (n = 63) and finally pollen (n = 24). A total of 29 plant families were identified by all three methods, but nine were identified exclusively by aDNA (Figure 3). Using paleogenomics, we were able to identify 14 species that were not previously recorded in either pollen or macrofossil records. We believe the use of aDNA and barcode sequencing provides and accurate, complementary and, in some cases, more complete assessment of biodiversity over time in these fossil samples compared with what can be obtained with traditional experimental approaches alone.

3.3 | Major ecosystem responses to climate change in the past

To assess biodiversity in Atacama plant communities over the last 27,590 cal year BP, we used our barcode sequence data from aDNA and compared species occurrence to current plant distributions and plant assemblages from modern middens for which we used the same DNA barcodes. Figure 4 shows selected plant taxa (29 of 73) and their appearances over time. As plant tolerance defines the altitude at which we find a species, we present the results in relationship to existing vegetation belts (i.e., Prepuna, Puna or Steppe, Figures 2a and 5) to facilitate interpretations. We compared short-term distribution range of each plant species with long-term past distributions (Figure 2b,c). During the Last Glacial, at 27,590 cal year BP richness is very low and just a few Prepuna species were identified. The most dramatic changes occurred from 17,000 to 7,000 cal year BP, when Puna and Steppe plant species occurred almost 1,000 m lower than today (Figure 2b). Nevertheless, Prepuna species were also present during that period, generating mixed and novel plant communities (Figure 5b). During the last 7,000 cal year BP plant species richness is low and dominated by Prepuna species, except for the 200 cal year BP midden which has more Puna elements, indicative of more humid conditions than today.

At millennial timescales, our results show that large magnitude climate changes (in length or intensity) triggered both perennials and annuals to migrate downslope where water limitation and temperatures are extreme today.

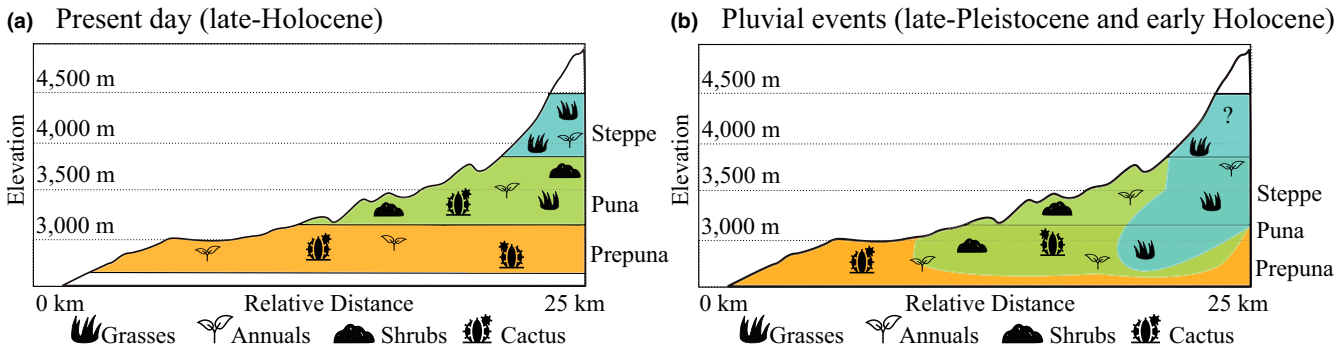

FIGURE 5 Present-day distribution vs. major past vegetation change in the Atacama Desert. (a) Current vegetation belts at 23°24′ lat. S, Atacama Desert, Chile. As elevation and precipitation increase, temperature decreases. White regions indicate absence of plants. (b) Past downslope plant movements during pluvial events provoked a mixing of different vegetation belts or "nonanalog communities" from 2,500 to 3,500 m. The question mark above 4,000 m represents our lack of middens from high altitudes (all midden sites are from 2,414 to 3,380 m, see Table S2 for details)

4 | DISCUSSION

Long-term paleoecological studies linked to short-term biodiversity studies are fundamental to understanding vegetation responses to ongoing (anthropogenically driven) climate change. In Andean arid ecosystems, we are severely data-limited on both of these timescales. Fossil rodent middens are unique archives of biodiversity and an important source of information from the past. Sequencing aDNA from middens is now possible (Kuch et al., 2002; Murray et al., 2012), and this approach offers great opportunities to address questions of changes in biodiversity over long periods of time. This area of research, at the intersection of genomics and paleobotany, should become an important area of discoveries to understand vegetation responses to climate change.

4.1 | An aDNA record from fossil rodent middens

We use radiocarbon dated fossil rodent middens and plant aDNA metabarcoding to describe plant community changes up to 27,590 years before present. Only one previous study (Kuch et al., 2002) has successfully extracted DNA from an Atacama rodent midden but used Sanger sequencing from individual clones. We compare our aDNA record with pollen and macrofossils reconstructions, and conclude that different experimental approaches have strengths and weaknesses and should therefore be considered as complementary (Figure 3) (Parducci et al., 2015; Wood et al., 2012, 2013). The number of genera was similar between aDNA and macrofossils, and both records are complementary in terms of identification. More than half of the genera were identified in just one type of record (aDNA or macrofossils). Plant macrofossils still provide the highest number of identified species (37), but aDNA was able to identify 14 new species (including some key indicators species from the Steppe belt, see taxa marked with an asterisk in Figure 4) not found as macrofossils or pollen (Figure 3). The combined use of pollen and macrofossils for midden analyses is time-consuming and involves a high level of expertise to identify local floras from the fragmentary plant remains found in middens. In contrast, aDNA requires specialized facilities for DNA extraction from fossil samples and PCR amplification, but if DNA is well-preserved taxonomic resolution only requires a local barcode database. The fact that our aDNA analyses were able to replicate and often expand on the total diversity indicates this technology as an important complement to standard midden analysis. Moreover, our study provides a reference database for future studies of these species.

As with other proxies, the use of aDNA to reconstruct biodiversity is limited by the resolution of the molecular markers or barcodes obtained from modern floras. The development of DNA barcodes from local floras is a fundamental step to improve the resolution of any aDNA record. These records can also be further biased by the unique taphonomic environment and age of the midden. Therefore, more than establishing comparisons with the entire modern plant communities we use specific plants as key indicators of past climate change. DNA originates from different sources, including pollen from both local and regional sources (De Porras, Maldonado, Zamora-Allendes, & Latorre, 2015), particularly in the case of wind-pollinated species. Since the ephemeral appearance of some species and the limitations mentioned before for identifying species using DNA barcodes, some local flora from the midden collection sites do not appear in the DNA from modern middens, but also DNA reveal species that we did not observe in the field. Nevertheless, DNA from modern middens shows that the reconstructed communities are similar to present-day communities, indicating that regional sources of DNA likely contribute very little to the record. Middens accumulated over a few years or a decade and could reflect a wider temporal frame than a one-time point survey.

4.2 | Integrating multiscale climate change with biodiversity dynamics

Plant communities and what has been defined as vegetation belts are temporary agglomerations of species constantly responding to climatic, biologic, and anthropogenic factors. Moreover, those current assemblages are ephemeral at the millennial timescale although their persistence at shorter timescales is still unknown. With pronounced rainfall seasonality and interannual variability, the desert vegetation of the Atacama can be challenging to study regarding short-term ecological responses. Annuals expand and contract their distributional ranges in response to precipitation in the short term (years to decades) and their permanence at each site is generally low. These species can respond quickly, and the seed banks are abundant and persistent enough to bridge across scarce precipitation events. Such strategies allow plants to remain sensitive to environmental change of relatively little magnitude, such as ENSO variations. In contrast, the elevational distribution of perennials did not change over the entire sampling period (2011–2018) and did not change even when we included a survey from 1998 (Villagrán et al., 1998). This absence of change is unmistakable as a reduction in mean annual precipitation (Sarricolea, Meseguer Ruiz, & Romero-Aravena, 2017) and an increase in mean annual temperature (Bennett, New, Marino, & Sillero-Zubiri, 2016) has occurred over the last decades in Atacama Desert. This suggests that neither ENSO variation nor recent climate change has been of sufficient magnitude over the last 20 years to induce any significant elevational migration in perennials or plant community changes. This resilience could be explained by the unique adaptations to variable arid and hyperarid Atacama conditions or could be the expression of an extinction debt or "extinction lag," as has been described in other high-altitude plant communities (Alexander et al., 2018; Dullinger et al., 2012).

In contrast, over longer timescales (thousands of years) stress-resistant perennial herbaceous and shrubs (e.g., Arroyo et al., 1988) appear to respond to larger or sustained climate change, such as experienced by plants during the Last Glacial period or the pluvial events at the Pleistocene-Holocene transition (e.g., CAPE I and II). The biggest changes compared to the present occurred from 17,000 to 7,000 cal BP, when plant species were found up to a 1,000 m below their current distribution (Figure 2c), likely driven by increases

in precipitation during major pluvial events (e.g., Latorre et al., 2002, 2003). The vegetation identified using aDNA reveals some important differences between CAPE I and CAPE II. Diverse perennial shrubs and few Steppe grasses are part of CAPE I assemblages, as found in our middens dated from 16,000 to 15,000 cal year BP. In contrast, middens dated between 10,500 and 8,500 cal year BP (CAPE II) show dominance of *Parastrephia*/*Baccharis* (both high elevation Puna shrubs) and Steppe grasses. These differences between the two wet phases of CAPE show that CAPE II was possibly wetter and likely warmer than CAPE I.

Such movements were not limited to the Pleistocene, however, and our data show that a few taxa descended ~800 m over the last 3,000 cal BP, indicating that plant distributions responded to shorter term climate variations during the late Holocene and on centennial timescales during the Historic Pluvial Event at the end of the Little Ice Age (c. 1,400–1,900 CE, see Lima, Christie, Santoro, & Latorre, 2016; Mujica et al., 2015). Since plant diversity and productivity have strong bottom-up effects (Scherber et al., 2010), major past plant changes would be felt throughout the entire Andean Atacama Desert ecosystem (Marquet et al., 1998). As interannual and seasonal climate variability have strong effects on short-lived herbivores, such as rodents (Meserve & Glanz, 1978), climate variability on lengthier timescales should impact long-lived herbivores and carnivores such as foxes and south American camelids (Marquet et al., 1998).

Although total species richness increased during pluvial events, vegetation belts were not completely displaced downslope. Instead, Prepuna communities were enriched by species moving in from higher elevations (Puna and Steppe) (Figure 5b), generating mixed novel plant communities or "nonanalog communities" (e.g., Williams & Jackson, 2007). Temperature changes, although important in explaining the presence of extreme xerophytes found at higher elevations where they grow today (Latorre, Betancourt, & Arroyo, 2006) were most likely not important in explaining these downslope shifts. Indeed, colder temperatures during the Pleistocene (~6° colder; Thompson, 2000) would have forced many Steppe species downslope from the Altiplano (where it would have been too cold; Mujica et al., 2015) but the presence of such species in what are today Prepuna environments, requires increases in precipitation as the current environment would have been too dry to support them (Latorre et al., 2006). Indeed, as previously shown in plant macrofossil records, aDNA flora during the very cold temperatures of the Last Glacial Maximum were mostly composed of just a few Prepuna species (Figure 4). Clearly, the higher elevation belt (Steppe) exhibits the largest elevational shifts. In part, this is due to where the middens were collected (in lower elevation belts) but another cause could reside in the fact that high Andean vegetation requires more frequent and larger amounts of water (rainfall) which in turn makes it much more sensitive to past and (most likely) future climate change.

Vegetation belts at our study sites (and in general for the Atacama Desert highlands) are highly dynamic and their composition appears to be mostly driven by past climate change although the extent of human impacts (both historical and in prehistory) has not been fully addressed in these ecosystems and remains an open question. Previous studies in the Atacama show how recent climate fluctuations over the past 600 years appear to be driving changes in richness (Mujica et al., 2015) and that even human populations respond to these same changes (Lima et al., 2016). Here, we evaluated the effect of recent climate change (over the last two decades) and observed that the perennial plant distributions have changed very little from 1998 to present. Although past human occupations in the Atacama peaked around 1,000 year ago (Gayo, Latorre, & Santoro, 2015), the hyperarid nature of the desert preserves past landscape use. Thus, human population density around our sites was likely very low in the past and is even lower today, thus minimizing impact on the natural landscape where we chose our sites. Furthermore, our sites are close to a small road, but away from any evident human perturbation such as settlements, major mining companies, farming terraces, fences, or garbage. The only major human impact today could be attributed to domesticated llamas roaming the landscape, but their population density is low and the pressure these herds currently exert could be of the same magnitude as the herbivorous pressures exerted by wild megafauna (including guanacos) in the past.

How vegetation adjusts to shifting environmental conditions, and the species richness and community diversity patterns that can arise from such restructuring, is relevant for the development of more comprehensive diversity studies and models (Whittaker et al., 2001). For example, regional climate models for northern Chile predict increases in temperature (4°C higher than today) and decreases in precipitation (10%–30% lower than today) over the next century (Minvielle & Garreaud, 2011; Thibeault, Seth, & Wang, 2012). Such potential future climate change is similar in magnitude to that which occurred during the Last Glacial–interglacial transition and over a timescale of several thousands of years. In a fast-changing world, Atacama plant communities will most likely undergo profound changes as perennials as well as annuals adjust accordingly. Such changes would see an upward shift of the upper boundary of the absolute desert as precipitation decreases, together with an invasion of high-altitude cold environments as temperature increases. A long-term perspective and widespread integration of paleoecology and conservation biology will need to be adopted if the vibrant Andean Atacama ecosystems are to be conserved for the future (Barnosky et al., 2017). Moreover, our results provide important elements to consider models of biodiversity to climate change in other desert or high mountain ecosystems.

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ORCID

Francisca P. Díaz https://orcid.org/0000-0002-1100-7801
Claudio Latorre https://orcid.org/0000-0003-4708-7599
Gabriela Carrasco-Puga https://orcid.org/0000-0001-7056-6235
Jamie R. Wood http://orcid.org/0000-0001-8008-6083
Janet M. Wilmshurst http://orcid.org/0000-0002-4474-8569
Daniela C. Soto http://orcid.org/0000-0002-6292-655X
Theresa L. Cole http://orcid.org/0000-0002-0197-286X
Rodrigo A. Gutiérrez http://orcid.org/0000-0002-5961-5005

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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