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The timing and ecological consequences of the late Pleistocene megafaunal declines on the Isthmus of Panama: Implications for trophic rewilding

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ABSTRACT

The timing and ecological consequences of Late Quaternary megafaunal extinctions in Central America remain poorly resolved, limiting our understanding of how the Pleistocene megafaunal declines may have influenced past and present ecosystem dynamics and ecological processes. To address this gap, we applied a high-resolution multiproxy palaeoecological approach to reconstruct the Pleistocene megafaunal presence in the Isthmus of Panama. Lake sediment cores from Lake La Yeguada were analysed for spores of coprophilous fungi (SCF) using a multi-genus SCF approach; an analytical advancement that significantly improves the resolution and reliability of megafaunal reconstructions compared to traditional reliance on *Sporormiella* alone. This multi-genus SCF approach was integrated with fossil pollen and charcoal analyses to establish the first detailed record of megafaunal presence/absence, vegetation dynamics, and fire activity, respectively, spanning the Late Pleistocene to the Holocene in Panama. Our SCF record indicates that megafauna were present in the region from at least 16,600 cal yr BP (calibrated years before present) and underwent three distinct phases of decline around 13,600, 10,000, and 8400 cal yr BP. These declines were followed by recoveries at 11,200, 9000 cal and 7600 cal yr BP, likely caused by changes in megafaunal community compositions. These transitions coincided with shifts in vegetation composition between forest and grasslands, marked by the loss of large-seeded, megafaunal-dispersed plant taxa and increased fire activity, suggesting that megafaunal decline had cascading impacts on ecosystem processes in Panama. Our findings highlight the ecological consequences of megafaunal declines on megafaunal-dispersed and large-seeded plants and suggest that future declines could have implications for vegetation and fire activity. These insights provide a valuable baseline to inform conservation strategies for extant species and offer guidance for future trophic rewilding efforts aimed at restoring ecological functions lost with the extinction of Pleistocene megafauna.

1. Introduction

Terrestrial large-bodied herbivores, or megafauna, are typically defined as species weighing over 44 kg (Martin and Wright, 1967). Through their behaviours, megafauna shape their environments and contribute to natural ecological processes (Mills et al., 1993). By directly altering the structure of both biotic and abiotic components, megafauna act as keystone ecosystem engineers (Owen-Smith, 1988; Svenning et al., 2024). Megafaunal foraging imposes a top-down trophic control on plant communities, directly influencing plant species assemblages (Asner and Levick, 2012; Coverdale et al., 2016), while their movement across landscapes facilitates the transport of nutrients and energy (Le Roux et al., 2020). This role extends to facilitating critical biotic interactions, such as the dispersal of large-seeded fleshy fruits essential for forest dynamics (Pizo, 2002; Tobler et al., 2010). Furthermore, their physical modifications of the terrain generate microhabitats for other organisms (Old et al., 2018).

At the end of the Late Quaternary, global extinctions led to declines in megafaunal diversity and abundance (Barnosky et al., 2004). While the causes of the Late Quaternary extinctions (LQE) remain debated, an

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Yeguada has a mean annual temperature of 23.4 °C and annual precipitation of 280.8 mm (Instituto de Meteorología e Hidrología de Panamá, 2025). Panama's tropical location and position on the Central American land bridge provide the area with a hotbed of biodiversity, a mosaic of vegetation, and a diversity of ecosystems (Palka, 2005). At La Yeguada, natural vegetation of the watershed is classified as pre-montane wet forest composed of low- and mid-elevation rainforest species (Bush et al., 1992). However, most of the natural vegetation has been replaced with large *Pinus* plantations and agricultural land (Autoridad Nacional de Panamá, 2009; Knutsen et al., 2013). In the Veraguas Province, extant megafauna includes the Baird's tapir (*Tapirus bairdii*) and white-tailed deer (*Odocoileus virginianus*), as well as smaller fauna such as the collard peccary (*Tayassu tajacu*) (Samudio and Pino, 2005; Donoso, 2010).

3. Material and methodology

3.1. Core collection and sampling

In 2009, a 16.35 m sediment core from La Yeguada was recovered using a modified Livingston piston corer (Colinvaux et al., 1999). The core was opened lengthwise, covered, and half of the core was transported in aluminium tubes to the Tropical Palaeoecology Laboratory at the University of Exeter. We used the Troels-Smith method and the Munsell colour chart to describe the stratigraphic units across the 16.35 m of sediment (Munsell Color, 1992; Kershaw, 1997). In 2010, six bulk sediment samples were extracted and sent to the Beta Analytic Carbon laboratory for radiocarbon (^{14}C) dating. Sixteen additional bulk sediment samples were extracted and sent to the Chrono Centre at Queen's University Belfast for ^{14}C dating in 2024 (Table 1). The intervals for ^{14}C dating were selected by visual analysis of stratigraphic changes across the whole core. A chronological model was built using the Bacon package v.2.3.9.1 (Blaauw and Christen, 2013) within R v.4.0.5 (R Core Team, 2021), applying priors of acc.mean = 10 yr/cm and acc.shape = 1.5 based on sedimentation rates and assessment of the cores stratigraphy (Fig. 2). Radiocarbon dates were calibrated using the IntCal20 calibration curve (Reimer et al., 2020).

To investigate the Late Pleistocene to Mid-Holocene, this study focused on sediment core depths 1635.3-730.6 cm (ca. 16,600-6000 cal yr BP) (Fig. 2). One hundred seventy-seven volumetric samples of 0.5 cm^3 were extracted for fungal spore, pollen and charcoal analysis at 200-year intervals from 1632 to 754 cm core depth. This temporal resolution was selected based on sedimentation rates, the timescale of ecological changes related to megafaunal declines, as well as practical laboratory considerations, including the time and resources required for sample processing and analysis. Three samples from 127.7 to 32.5 cm core depth and two from 227.2 to 127.7 cm were also extracted at 100-year intervals to cover the modern era.

3.2. Laboratory analysis

Fifty-nine fungal spore samples were prepared following a modified version of Fægri et al. (1989) (Pym et al., 2023). This modification removed acetolysis, which can cause spore deterioration in modern (van Asperen et al., 2016) and palaeo records (Pym et al., in prep). Per sample, a *Lycopodium* tablet (Batch 3862; 9666 ± 671 spores) was added for SCF and pollen analysis. The *Lycopodium* standard was used to calculate concentrations per volume (Stockmarr, 1971). SCF preparation included treatment in 10% HCl and 10% KOH, warming in a 90 °C ultrasonic bath, sieving at 180 μm, density separation using sodium metatungstate $Na_6(H_2W_{12}O_{40})$ at 1.9–2.0 g/litre, and suspending in glycerol (van Asperen et al., 2016). SCF and pollen were identified using a Zeiss Axioskop light microscope at 400× , 600× and 1000× magnification. All fungal spores were counted and identified until reaching a minimum of both 400 *lycopodium* spores and 300 pollen grains to calculate SCF concentrations and provide a percentage comparison with published studies (Etienne and Jouffroy-Bapicot, 2014; Raczka et al., 2016). The fungal spore quantification included all commonly identifiable spores of obligate-, facultative-, and non-coprophilous taxa. SCF identification was conducted using published descriptions, images and fungal keys (Bell, 1983; van Geel et al., 2003; Guarro et al., 2012; Richardson and Watling, 2018) and modern reference material archived at the University of Exeter. Fungal spore morphotypes were assigned to their primary substrate based on published associations (van Geel and Aptroot, 2006; Perrotti and van Asperen, 2019; van Asperen et al., 2020).

Fifty-nine pollen and fifty-nine charcoal samples were prepared following standard protocols (Fægri et al., 1989; Whitlock and Larsen,

Table 1
Radiocarbon ages (14C) from organic lake sediments and calibrated ages (cal yr BP, IntCal20, Reimer et al., 2020) from the La Yeguada core. Calibrated ages are rounded to the nearest 10 years. *Indicates ages identified as outliers.

Lab ID	Sample ID	Depth (cm)	Age (BP)	Error (±)	Age (calibrated range, 2σ)
Beta Analytic's Radiocarbon Dating					
B-282249	C2M3-98*	202	904*	40	840-970
B-201058	C2M3-365.37*	225.37*	1029*	40	970-1030
B-201059	C2M5-110:110.5	416.25	2255	40	2190-2260
B-201060	C2M6-115:115.5	521.75	3150	40	3080-3230
B-202061	C2M8-51:51.5	658.75	4598	40	4500-4740
Woods Hole Radicarbon Dating					
#76391	C2M11-109.8	1020.3	9560	50	9330-9560
The Chrono Centre Radiocarbon Dating					
UBA-53994	C2M1-115.5	26	584	24	−40 – 140
UBA-53995	C2M2-115.5	120.7	30	26	220 – 530
UBA-53996	C2M12-91.5	1044.6	4337	32	9950 – 10850
UBA-53997	C2M12-116	1069.1	9753	42	10790 – 11250
UBA-53998	C2M13-39	1087.8	2959	28	11030 – 11840
UBA-53999	C2M13-112	1160.8	11019	62	12760 – 13070
UBA-54000	C2M14-54	1202.5	11680	54	13310 – 13590
UBA-54001	C2M14-115.5	1264	12029	50	13790 – 14020
UBA-54002	C2M15-36	1284.7	12132	55	13890 – 14160
UBA-54003	C2M15-81.5	1330.2	12368	78	14150 – 14540
UBA-54004	C2M16-43	1391.3	10469	44	14530 – 14970
UBA-54005	C2M16-114	1462.3	12790	69	15010 – 15460
UBA-54006	C2M17-28.5	1478.1	12619	54	15090 – 15580
UBA-54007	C2M17-102	1551.6	13540	60	15670 – 16370
UBA-54008	C2M18-70	1584.6	4355	28	15870 – 16650
UBA-54009	C2M18-113	1627.6	13470	57	16180 - 17020

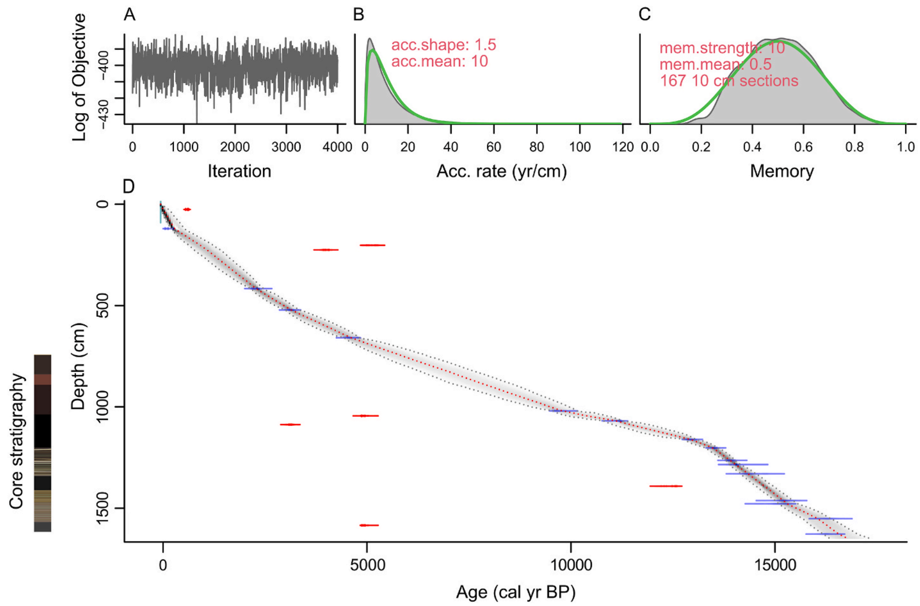

Fig. 2. Bayesian age-depth model and stratigraphic descriptions for the La Yeguada core. A prior outlier probability was assigned to each radiocarbon date, allowing the model to down-weight outliers that deviate from the expected accumulation history (Blaauw and Christen, 2013). Inset figures show: (**A**) Markov Chain Monte Carlo (MCMC) iterations; (**B**) posterior distributions of accumulation rates with prior assumption (green curve); (**C**) the memory (autocorrelation) of accumulation variability with prior assumption (green curve); and (**D**) calibrated radiocarbon dates (transparent blue) and the modelled age-depth curve. The red dotted line represents the weighted mean (best) model, and the shaded area represents 95% credible intervals. The loose red radiocarbon dates indicate outliers with low model influence. A stratigraphic diagram for drives 9-18 is included.

2002). Minimum pollen sums of 300 grains per sample were identified using published descriptions, keys and images for Neotropical pollen taxa (Roubik and Moreno, 1991; Colinvaux et al., 1999; Bush and Weng, 2006; Punyasena et al., 2022) and modern reference material archived at the University of Exeter. The ecological affinities of the pollen taxa were extracted from relevant literature (Bush et al., 1992; Bush and Colinvaux, 1990; Colinvaux et al., 1999; Marchant et al., 2002) and online resources (Royal Botanic Gardens Kew, 2025). Charcoal particles were sieved to retain fragments >125 μm (macroscopic) (Whitlock and Larsen, 2002; Brown and Power, 2013). The macroscopic particles were manually separated and photographed using a Zeiss Stemi stereo microscope at 50× magnification. Raw charcoal counts and charcoal area were extracted using image analysis in ImageJ software (Clark and Patterson, 1997; Abràmoff et al., 2004; Halsall et al., 2018). Stratigraphic diagrams of SCF, selected pollen taxa, and charcoal were plotted using C2 v.1.7.7 (Juggins, 2007) and edited using Affinity Designer V2.4 (Serif Europe Ltd, 2024).

3.3. Quantitative analysis

Pearson correlation coefficients were calculated using SPSS for all identified SCF at the 0.05 and 0.01 levels of significance (IBM Corp, 2020). SCF that positively and significantly correlated with *Sporormiella* were grouped into a multi-genus assemblage of key SCF associated with megafaunal presence (Johnson et al., 2015; Perrotti and van Asperen, 2019; Pym et al., 2023) (Table 2). Confidence limits were calculated for the key SCF concentrations to assess uncertainty linked to sampling effort (Maher, 1981). A sequential *t*-test analysis of regime shifts (STARS) (Rodionov, 2004) was performed using key SCF concentrations and accumulation to identify statistically significant deviations in megafaunal presence (Pym et al., 2023). The analysis utilised a regime shift index (RSI) to quantify the size of the regime shift (Rodionov and Overland, 2005) and was calculated using a cut-off length $l = 4$ and $l = 6$, respectively, probability level $p = 0.05$. STARS analysis was deployed in R v.4.0.5 (R Core Team, 2021) using the R package rshift (Room et al., 2024).

Pollen zones were assigned using a constrained hierarchical clustering analysis (chclust) using 'coniss' in the R package *rioja* (Grimm, 1987; Juggins and Juggins, 2019). The chclust included every pollen taxon quantified when their cumulative total was above 5% of an individual sample (Robinson et al., 2005). The 5% threshold was selected to avoid weakening the analysis due to the presence of rare taxa and to facilitate pollen diagram interpretation. Accordingly, taxa that never exceeded the 5% threshold were combined into broader groups: aquatics, herbs, arboreal, palms, lianas and mixed affinities (Bush and Colinvaux, 1990). A canonical correspondence analysis (CCA)

Table 2
Pearson correlation coefficients for all identified SCF. Statistical significance of correlations are highlighted at p-value <0.01 (**) and <0.05 (*) levels.

	Sporormiella	*Arnium*	*Arnium imitans*	*Cercophora*	*Chaetomium*	*Coniochaeta*	*Delitschia*	*Podospora*	*Saccobolus*	*Sordaria*
Arnium	0.237									
Arnium imitans	0.216	0.214								
Cercophora	0.510**	0.278*	0.530**							
Chaetomium	0.426**	0.424**	0.184	0.557**						
Coniochaeta	0.618**	0.230	0.260	0.639**	0.571**					
Delitschia	0.063	−0.094	0.368**	0.112	0.039	0.038				
Podospora	0.688**	0.402**	0.293*	0.707**	0.540**	0.611**	0.049			
Saccobolus	0.065	−0.059	−0.151	0.013	0.028	0.125	0.111	0.239		
Sordaria	0.568**	0.075	−0.052	0.374**	0.467**	0.750**	−0.072	0.438**	0.249	
Trichodelitschia	−0.021	0.217	−0.127	0.114	0.394**	0.170	−0.050	0.087	0.115	0.376**

(Oksanen, 2009) was performed using the R package *vegan* (Oksanen et al., 2007) to test the influence of constraining variables (key SCF and macroscopic charcoal concentration) on the pollen assemblage (percentages). The CCA included every pollen taxon when the cumulative total across the full temporal record (16,600-6000 cal yr BP) exceeded 5% of an individual sample. This pollen abundance filter was applied to match the cluster analysis and preserve the variability of the pollen dataset while reducing the weighting of rare taxa (Birks and Birks, 1980; Urrego et al., 2009). Both chclust and CCA were performed within R v.4.0.5 (R Core Team, 2021). To reconstruct local fire history at La Yeguada (Higuera et al., 2010), raw charcoal counts were converted into interpolated charcoal concentration (particles/cm^3) and peak magnitude and frequency (particles/cm^{-2}/peak^{-1}) using CharAnalysis v.1.1 at the 0.99 level of significance (Higuera, 2009; MathWorks, 2019). Charcoal concentrations were interpolated into 200-year intervals based on the median sample resolution. CHAR analysis could not be run on the charcoal data from zone LY5 due to the small number of samples.

4. Results

4.1. Chronology and stratigraphy

The 878-cm sediment section analysed provided a record of sediment accumulation from ca. 16,600-6000 cal yr BP (Table 1, Fig. 2). The deepest depositional unit between 1632 and 1572 cm (ca. 16,600–16,190 cal yr BP) consists of very dark grey homogenous clay (Munsell Color, 1992, 7.5YR 2/1) (Fig. 2, Supplementary Fig. 1). From 1572 to 1412.5 cm, dark greyish brown (10YR 4/2 dark) to grey (10YR 5/1) soil with some layers of reddish grey striations (5R 6/1) is overlain. The striations continue across a 79.5-cm layer of olive brown clay (2.5Y 4/3) up to 1412.5 cm (ca. 14,880 cal yr BP). The fourth depositional unit transitions to homogeneous very dark grey clay (7.5YR 3/1) from 1412.5 to 1357 cm (ca. 14,880 - 14,520 cal yr BP). From 1357 to 1203 cm (ca. 14,520–13,470 cal yr BP), homogenous greyish brown (2.5Y 5/2), very dark grey (7.5YR 3/1) and black clays (7.5YR 2.5/1) are interspersed with striated sediment layers between 4 and 30 cm in thickness (2.5Y 5/3 and 8/1). This unit is grouped due to the frequency of stratification. This unit is overlain with a homogeneous black (7.5YR 2.5/1) layer of clay up to 1034 cm (ca. 10,110 cal yr BP). Between 1034 and 891 cm (ca. 10,110-7900 cal yr BP), the sediments become homogenous and moist relative to the previous air-dry material (Kershaw, 1997). A gradual boundary is followed by homogeneous moist brown clay (10YR 4/3) with very dusky red tones (10R 2.5/2) up to 842 cm (7210 cal yr BP). The final depositional unit described between 842 and 758 cm (7210-6000 cal yr BP) consists of air-dry very dark greyish brown homogenous clay (10YR 3/2) and ends with a 6-cm layer of dark yellowish-brown clay (10YR 4/4).

4.2. SCF, pollen and charcoal records

The La Yeguada core record presented here comprised 29 identifiable fungal spore taxa, of which 11 were classified as SCF (Supplementary Fig. 2). The 18 other identifiable spore types included fire-indicative genera, including *Gelasinospora* and *Neurospora* (Supplementary Fig. 3) (van Geel and Aptroot, 2006). SCF were found in all 59 samples analysed for fungal spores (Pym and Urrego, 2026). *Ascobolus sacchariferus* was identified in the samples processed for pollen analysis, but not in the samples prepared for SCF and was therefore excluded from all quantitative analysis (Supplementary Fig. 4). The 95% confidence intervals calculated for key SCF showed that uncertainties linked to count size were minimal (Fig. 3). The pollen record comprised 186 identifiable families or genera (Supplementary Fig. 4) (Pym and Urrego, 2026). Of these, 93 were retained for interpretation after applying the 5% cumulative total pollen filter. The remaining taxa were grouped according to their ecological affinities. The SCF and pollen records were divided into five palynological zones based on sedimentological changes, key SCF regime shifts, and statistically significant changes in the pollen assemblage (Figs. 3 and 4). This includes zone LY5, dating between ca. 500–100 cal yr BP.

4.2.1. Zone LY1 (16,600–13,400 cal yr BP)

The concentration of key SCF increased from 2706 to 7152 spores/cm^3 (6.7-16%; 1140-3299 spores/cm^2/yr) by 13,600 cal yr BP, before decreasing to 1544 spores/cm^3 (16%; 1076 spores/cm^2/yr) at 13,400 cal yr BP (Fig. 3). *Sporormiella* peaked at 16,600 cal yr BP with a concentration of 677 spores/cm^3 (285 spores/cm^2/yr, 1.7%). STARS analysis identified a key SCF regime shift for both concentration and accumulation at 13,600 cal yr BP (1.10 and 0.41 RSI) (Fig. 3). Arboreal taxa, including *Eugenia* (3%), *Ilex* (10%), Myrtaceae (12%), *Myrsine* (4%), *Quercus* (17%), *Sloanea* (3%) and *Solanum* (6%) reached their peak abundance in zone LY1 (Fig. 4). *Alchornea* (1-7%) and Melastomataceae (1-6%) fluctuated whilst Moraceae/Urticaceae (25-5%) and *Symplocos* (10-0%) declined across the zone. Herbs, including *Alternanthera* (2%) and *Iresine* (3%), increased to their highest recorded abundance at 14,200 cal yr BP, whilst *Acalypha* (0-3%) and *Pilea* (1-4%) fluctuated across the zone. Poaceae was relatively low (1-6%). Cyperaceae

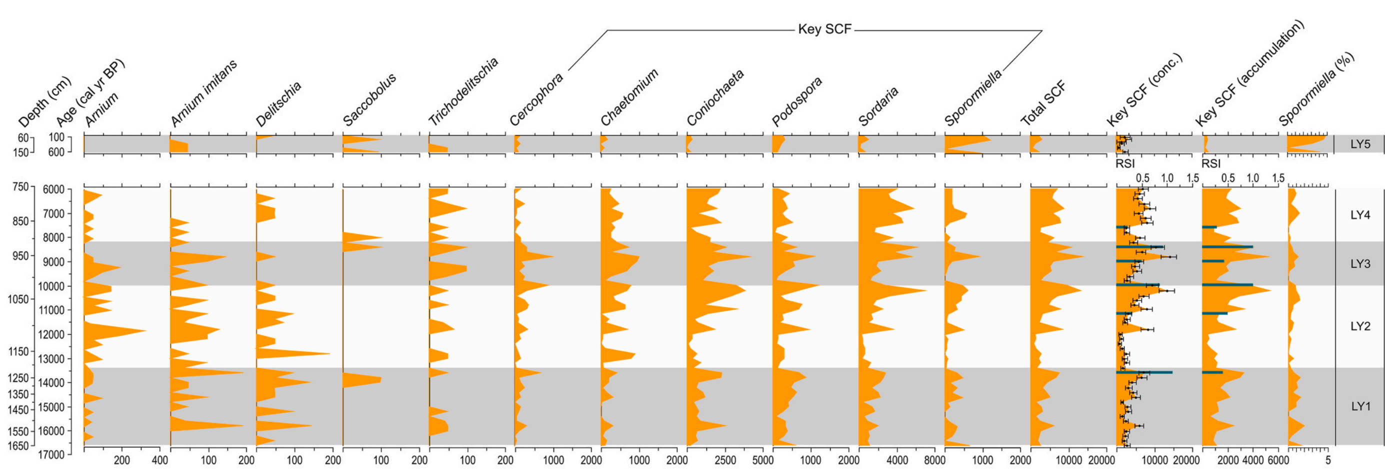

Fig. 3. Summary diagram of the spores of coprophilous fungi (SCF) recorded from the sediments of Lake La Yeguada. All SCF taxa are expressed as a concentration per cubic centimetre (spores/cm^3). The Key SCF concentration includes 0.95 confidence intervals represented as whiskers (Maher, 1981). The key SCF RSI (tomb blue bars) shows the regime shift analysis and plots the statistically significant shifts in key SCF concentration and accumulation (p-value <0.05). *Sporormiella* is also expressed as a percentage of the total pollen assemblage (%TPA). Total SCF is expressed as concentration (spores/cm^3).

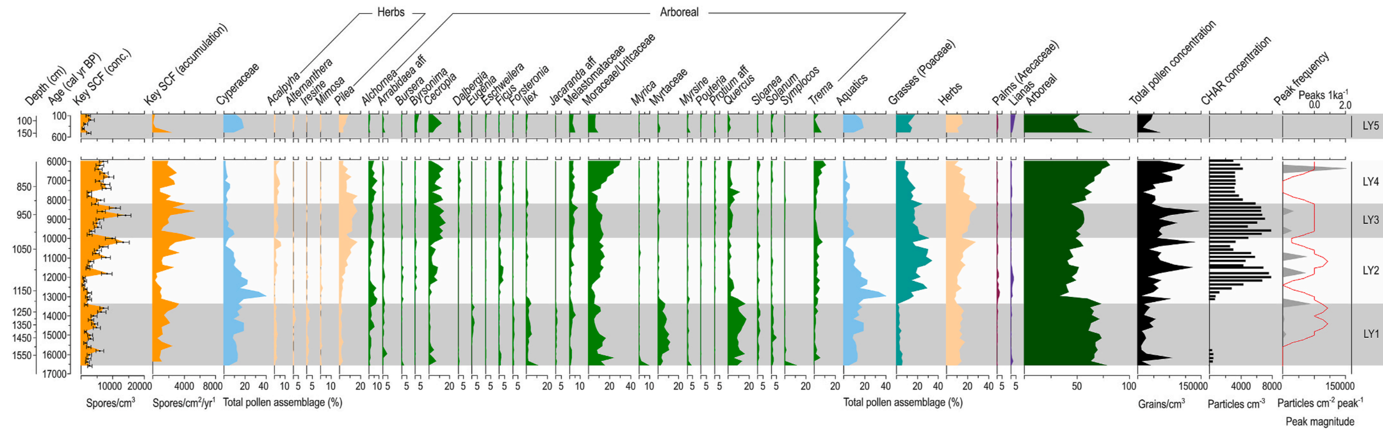

Fig. 4. Summary diagram of selected pollen taxa from Lake La Yeguada. The selected taxa are grouped and coloured by their ecological affinity: aquatics (light blue), herbs (light peach), arboreal (medium green), grasses (teal green), palms (reddish-brown), and lianas (purple). The full pollen record (see Supplementary Fig. 4) is condensed into their ecological affinities. Key SCF are shown as both concentration (spores/cm^3) and accumulation (spores/cm^2/yr). Macroscopic charcoal is presented as concentration (black bars; particles/cm^3), peak frequency (red line; peaks/ka), and peak magnitude (grey silhouettes; particles/cm^2/peak).

increased to 13% by 16,400 cal yr BP and remained present (9-19%) until 13,400 cal yr BP. The chclust analysis identified a statistically significant zone at 13,400 cal yr BP (Supplementary Fig. 5). Macroscopic charcoal concentrations were lowest during zone LY1 (0-559 particles/cm^3) (Fig. 4). The rise to 559 particles/cm^3 occurred at 16200 cal yr BP and was followed by a lagged increase in peak frequency to 1.43 particles cm^{-2} peak^{-1} at 15,400 cal yr BP (Pym and Urrego, 2026).

4.2.2. Zone LY2 (13,400–10,000 cal yr BP)

Key SCF fluctuated between 677 and 2507 spores/cm^3 (2-7%; 305-1153 spores/cm^2/yr) up to 12,000 cal yr BP (Fig. 3). At 10,200 cal yr BP, the key SCF record increased from 7991 to 13,146 (22.4%; 2931 spores/cm^2/yr) spores/cm^3. *Sporormiella* peaked at 628 spores/cm^3 (1.4%, 2932 spores/cm^2/yr) at 10,200 cal yr BP. The SCF record also included the highest concentration of *Podospora* (773 spores/cm^3) and *Sordaria* (7008 spores/cm^3). *Gelasinospora*- and *Neurospora*-type spores both increased to a record high at 11,800 cal yr BP (1289 and 322 spores/cm^3) (Supplementary Fig. 3). STARS analysis identified a key SCF concentration regime shift at 12,000 cal yr BP (0.82 RSI). A regime shift for both SCF quantifications was identified at 11,200 cal yr BP (0.31 and 0.50 RSI) and 10,000 cal yr BP (0.86 and 1.01 RSI). Arboreal taxa declined from 73 to 33% at the start of Zone LY2 (Fig. 4). *Alchornea* (8%) peaked in the record at 13,200 cal yr BP before declining. *Eugenia* (2-0%), *Ilex* (3-0%), Myrtaceae (2-0%) and *Quercus* (9-2%) decline across the zone. Moraceae/Urticaceae declines to 3% at 12,600 BP but rises to 14% by 10,400 BP. *Forsternoia and Parahancornia* become absent from the record. *Cecropia* (9%) and *Ficus* (4%) increase in abundance up to 11,400 cal yr BP, then decline to <1% by 10,200 cal yr BP. Melastomataceae (0-4%) and *Trema* (0-7%) fluctuate across the zone. *Acalypha* (0-6%) and *Pilea* (2-17%) increase across the zone. *Mimosa* (>2%) peaks in the record at 12,600 cal yr BP. Arecaceae (3%) peaks at 12,600 BP before declining to 0% by 10,200 cal yr BP. Poaceae increased from 0 to 33% by 11,200 cal yr BP, followed by a decline to 15% by 10,200 cal yr BP. Cyperaceae increased from 19 to 40% by 13,000 cal yr BP, before declining to 2% by 10,200 cal yr BP. In zone LY2, chclust analysis detected statistically significant zones at 11,400 and 10,400 cal yr BP (Supplementary Fig. 5). Macroscopic charcoal increased from 8 to 7963 particles/cm^3 by 12,000 cal yr BP (Fig. 4). Charcoal remained present but declined from 7666 to 3373 particles/cm^3 by 10,000 cal yr BP. Peak frequency fluctuated from 0 to 1.43 particles cm^{-2} peak^{-1}. Peak magnitude increased to 51,200 and 54,436 particles cm^{-2} peak^{-1} at 11,800 and 10,900 cal yr BP, respectively.

4.2.3. Zone LY3 (10,000-8200 cal yr BP)

The highest abundance of key SCF for all quantifications was observed in zone LY3 (Fig. 3). Key SCF increased from 2603 to 13,629 spores/cm^3 (41%; 5209 spores/cm^2/yr) by 8800 cal yr BP, before declining to 4301 spores/cm^3 (<15%; 1602 spores/cm^2/yr) at 8200 cal yr BP. The highest abundance of *Sporormiella* concentration and accumulation across the Late Pleistocene and Early Holocene was observed in zone LY3 at 8800 cal yr BP (918 spores/cm^2; 351 spores/cm^2/yr). Two regime shifts were identified for key SCF concentration and accumulation at 9000 cal yr BP (0.50 and 0.44 RSI), and 8400 cal yr BP (0.92 and 1.01 RSI). Arboreal taxa increased from 49 to 57% in zone LY3 (Fig. 4). Melastomataceae increased from 4 to 8% by 8400 cal yr BP. *Alchornea* (2-5%), *Myrtaceae* (1-2%) and *Quercus* (1-4%) fluctuate across the zone. *Cecropia* fluctuates across the zone (10-16%) but declines to 4% at 8200 cal yr BP. *Trema* (5-0%), Moraceae/Urticaceae (13-7%) decline across the zone. Herbs, including *Acalypha* (0-4%) and *Pilea* (9-17%), increase across zone LY3. Poaceae declines from 31 to 15% by 9200 cal yr BP, remains relatively stable, then increases to 25% at 8200 cal yr BP. Cyperaceae peaks to 10% at 9800 cal yr BP, then remains between 2 and 4%. The chclust analysis detected no statistically significant zones in LY3. Macroscopic charcoal increased to 7943 particles/cm^3 by 9610 cal yr BP (Fig. 4). Charcoal remained present across zone LY3 but declined to 5943 particles/cm^3 by 8200 cal yr BP. Peak frequency increased to 1.00 particles cm^{-2} peak^{-1} by 9610 cal yr BP and remained constant throughout the zone. Peak magnitude was low but increased at 9610 and 8600 cal yr BP (between 20,289 and 23,135 particles cm^{-2} peak^{-1}).

4.2.4. Zone LY4 (8200-6000 cal yr BP)

The SCF record declined at the beginning of zone LY4 (Fig. 3). Key SCF declined to 2513 spores/cm^3 (9%; 909 spores/cm^2/yr) by 7800 cal yr BP. Between 7400 and 6000 cal yr BP, the key SCF record remained relatively constant (7975-6960 spores/cm^3). *Sporormiella* became absent in the record at 7800 cal yr BP but rebounded to levels comparable to those recorded in previous zones by 7000 BP. STARS identified a final key SCF regime shift for both quantifications at 7600 cal yr BP (0.22 and 0.29 RSI). Arboreal taxa increase from 44 to 82% within zone LY4 (Fig. 4). *Ficus* (1-3%), Moraceae/Urticaceae (9-30%), *Pterocarpus* (0-3%), *Trema* (2-10%) and *Sloanea* (1-3%) increase across the zone. *Alchornea* (7%), Melastomataceae (3%) and *Quercus* (11%) increase to a zonal peak at 7600 cal yr BP and fluctuate between 3 and 8%, 1-3% and 0-4%, respectively, thereafter. *Cecropia* declines from 13 to 3% by 7600 cal yr BP and fluctuates between 2 and 14% across the rest of the zone. *Acalypha* rises to 4% at 7600 cal yr BP, then fluctuates between 2 and 5% across the rest of zone LY4. *Pilea* declines from 13 to 4% across the zone. Poaceae declines from 14 to 9% across the zone. Cyperaceae was present but relatively low (1-6%). The chclust analysis identified a statistically significant zone at 7600 cal yr BP (Supplementary Fig. 5). Macroscopic charcoal steadily decreased from 4532 to 3137 particles/cm^3 by 6000 cal yr BP (Fig. 4). Peak frequency decreased from 0.29 to 0.00 particles cm^{-2} peak^{-1} by 7000 cal yr BP, but then increased to 1.00 particles cm^{-2} peak^{-1} by 6000 cal yr BP. The largest macroscopic peak magnitude was recorded at 6400 cal yr BP (159,393 particles cm^{-2} peak^{-1}).

4.2.5. Zone LY5 (500-100 cal yr BP)

Key SCF peaked at 3190 spores/cm^3 (12%; 284 spores/cm^2/yr) ca. 200 cal yr BP in zone LY5 (Fig. 3). *Sporormiella* concentration reached a record high of 1208 spores/cm^2 (4.8%); however, the associated accumulation rate (284 spores/cm^2/yr) was lower than that recorded in Zone LY3. *Byrsonima* (2-3%), *Pterandra* (2-3%) and *Pinus* (1-5%) increased within zone LY5 (Fig. 4, Supplementary Fig. 4). *Dalbergia* (2-3%), Melastomataceae (3-5%), Moraceae/Urtiaceae (6-9%) and *Quercus* (1-3%) fluctuated throughout the zone. *Cecropia* increased to 12% at 300 cal yr BP, before declining to 4% by 100 cal yr BP. *Trema* (6-1%) and Myrtaceae (2-0%) also declined. *Mimosa* (1-5%) and *Pilea* (3-8%) increased across the zone. Poaceae increased from 13 to 18% by 100 cal yr BP. Cyperaceae increased from 9 to 18% by 400 cal yr BP but declined to 12% by 100 cal yr BP. The concentration of macroscopic charcoal was low (5-41 particles/cm^3) in zone LY5 (Fig. 3).

4.3. SCF correlations

Sporormiella concentration positively correlates with *Cercophora*, *Chaetomium*, *Coniochaeta*, *Podospora* and *Sordaria* in the La Yeguada record ($p < 0.01$) (Table 2). This includes high positive correlations with *Coniochaeta* ($R^2 = 0.618$, $p < 0.01$), *Podospora* ($R^2 = 0.688$, $p < 0.01$) and *Sordaria* ($R^2 = 0.568$, $p < 0.01$). There was no significant correlation at the 0.05 or 0.01 level of significance between *Sporormiella* and *Arnium*, *Arnium imitans, Delitschia, Trichodelitschia* or *Saccobolus*. SCF that showed positive and significant correlations with *Sporormiella* were grouped to create a multi-species key SCF sum (Pym et al., 2023) (Fig. 3).

4.4. Multivariate ordinations

The CCA analysis indicated that a total of 14.3% of the total variance in the pollen record is explained by the two environmental variables: key SCF (megafauna) and macroscopic charcoal (local fire activity) (Fig. 5). CCA axis 1 explained 71% of the variance (eigenvalue: 0.1113) of the pollen data, and CCA axis 2 explained 29% of the variance (eigenvalue: 0.04528). CCA axis 1 ordinated taxa, including species of *Mimosa*, *Jacaranda*, *Parahancornia*, Myrtaceae, and *Eugenia* at the positive end of axis 1, and *Spondias*, *Protium*, *Pilea*, *Trema*, and *Ficus* at the negative end (Fig. 5a). CCA axis 2 ordinated taxa, including *Spondias* and *Poaceae* towards the positive end of axis 2, and *Eugenia*, species of Apocynaceae and *Symplocos* at the negative end. Pollen taxa in the centre of the ordination are not strongly grouped with any of the environmental variables. The CCA ordination displayed a clear separation between samples from the Late Pleistocene on the positive end from the Holocene samples on the negative end of CCA axis 1 (Fig. 5b). The CCA also showed that the key SCF variable covaried with the negative ends of axis 1 and axis 2, explaining 69% and 31% of the variance along these axes, respectively. Macroscopic charcoal covaried with the negative end of axis 1 and the positive end of axis 2, explaining 86% and 12% of the variance along these axes, respectively.

5. Discussion

5.1. Megafaunal dynamics

We found robust evidence for continuous megafaunal presence at La Yeguada, but with several phases of decline and recovery (Fig. 3). In our Late Pleistocene and Holocene record, *Sporormiella* reached the 2% megafaunal extinction threshold (Davis, 1987; Davis and Shafer, 2006). However, this threshold may not be directly applicable to all environments (Perrotti and van Asperen, 2019). Reporting *Sporormiella* abundance as concentration and accumulation is recommended (Perrotti, 2018; Perrotti and van Asperen, 2019) and has been effective in inferring Pleistocene megafaunal declines (Pym et al., 2023). In the Neotropics, *Sporormiella* concentrations between 0 and 339 spores/cm^3 indicate low to medium cattle use around lakes (Raczka et al., 2016). At La Yeguada, we recovered *Sporormiella* concentrations between 0 and 1208 spores/cm^3 (Fig. 3). Despite *Sporormiella*'s strong preference for dung substrates and dominant use in LQE reconstructions (Bell, 1983; Gill et al., 2009), reliance on *Sporormiella* alone warrants caution (van Asperen et al., 2021). The germination and dispersal of SCF are influenced by complex biotic and abiotic interactions (Bell, 1983), yet research on these influences in Neotropical environments is notably limited. Environmental and microenvironmental factors are impactful; however, the generality of effects is not fully understood (Bell, 1975; Lee et al., 2022; Jakob et al., 2024). SCF recovery can also be affected by the species of vegetation (Zhang et al., 2025) and herbivores present (van Asperen et al., 2020; Basumatary et al., 2024). By incorporating full SCF assemblages, megafaunal reconstructions can be more robust (Johnson et al., 2015; Conroy et al., 2020; Pym et al., 2023). At La Yeguada, we found statistically significant positive correlations between *Sporormiella* concentrations and those of six other SCF (Table 2). This includes *Sordaria* and *Podospora*, two reliable indicators of megafauna activity (van Geel et al., 2003; Baker et al., 2013). We used these correlations to create a multi-genus key SCF group to mitigate the challenges of using *Sporormiella* alone. Our multi-SCF approach improved the resolution and reliability of estimating megafaunal presence and tracking declines. We also calculated concentration and accumulation to increase the robustness of our interpretations. In Guatemala and Colombia, SCF concentrations between 0 and 4500 spores/cm^3 have been used to reconstruct Pleistocene megafaunal declines (Rozas-Dávila et al., 202; Pym et al., 2023). Our minimum recovered key SCF concentration at La Yeguada was 532 spores/cm^3, which we interpret may be a result of continuous local megafaunal activity in the area. In Europe and Australia, SCF accumulation rates between 0 and 160 spores/cm^{-2} yr^{-1} and 0-400 spores/cm^{-2} yr^{-1} have been used to infer livestock abundance and the LQE, respectively (Etienne et al., 2013; Johnson et al., 2015). At La Yeguada, we recovered key SCF accumulation rates between 65 and 5350 spores/cm^{-2} yr^{-1} (Fig. 3). In comparison, these rates suggest continual megafaunal activity, with the combined multi-genus SCF record and interpretation of concentration and accumulation rates providing more robust evidence than using *Sporormiella* alone to reconstruct megafaunal presence and declines.

Our key SCF record indicated three phases of megafaunal decline and recovery at La Yeguada (Fig. 3). The first phase of decline occurred at ca. 13,600 cal yr BP and was followed by a recovery at 11,200 cal yr BP. The second phase of decline occurred at 10,000 cal yr BP and was followed by a recovery at 9000 cal yr BP. The third megafaunal decline occurred at 8400 cal yr BP, and the final recovery was recorded at 7600 cal yr BP. These declines were identified as statistically significant regime shifts (Fig. 3). Our SCF record shows similarities with records in the Neotropics, which found continuous megafaunal presence during the Pleistocene in Guatemala (Rozas-Dávila et al., 2021) and phases of decline and recovery in Guatemala, Colombia and Peru (Rozas-Dávila et al., 2016, 2021; Pym et al., 2023). However, the precise timings of the onset and phases of these declines show variability between 30,000 and 10,900 cal yr BP. Notably, the extinction of Pleistocene megafauna in the lowlands of Guatemala is recorded at 13,600 cal yr BP (Rozas-Dávila et al., 2021), coinciding with the initial decline observed in our lowland record. However, the recoveries of SCF in our record suggest that a local extinction of all megafaunal species at La Yeguada did not occur simultaneously. Across other sites in the Neotropics, including Ecuador and Brazil, there is no evidence of megafaunal recovery (Raczka et al., 2018, 2019). These differences highlight the habitat specificity of the

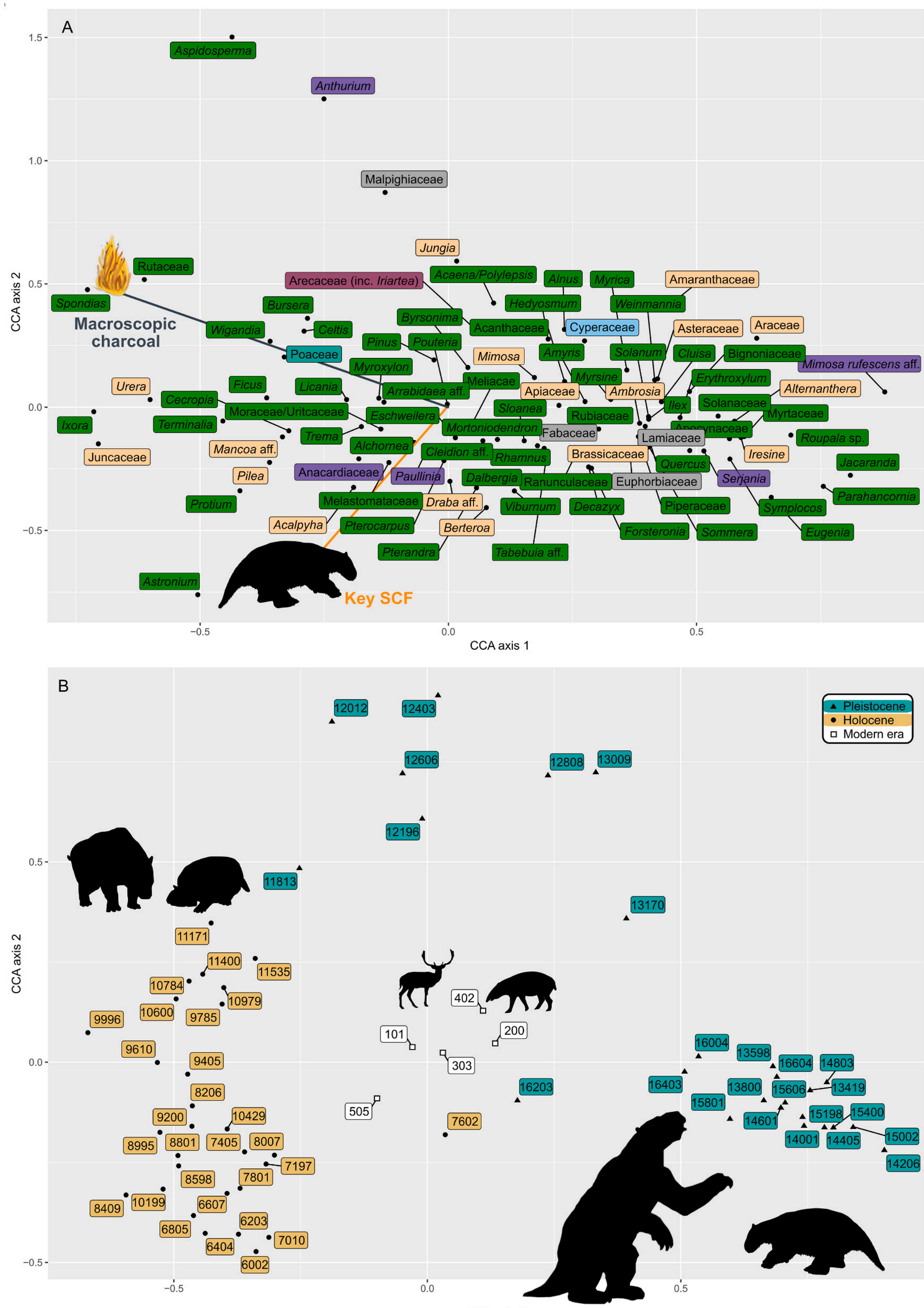

Fig. 5. Canonical correspondence analysis (CCA) results for the pollen record from La Yeguada. (**A**) Species scores for the pollen record using key SCF and macroscopic charcoal as constraining variables. Pollen labels are coloured according to their ecological affinities (aquatics: light blue, herbs: light peach, arboreal: medium green, grasses: teal green, palms: reddish-brown, lianas: purple, mixed affinities: grey). (**B**) Sample scores labelled with corresponding ages (cal yr BP). The sample labels are coloured Pleistocene (teal), Holocene (sand) and Modern era (white). Megafauna are depicted by the *Eremotherium laurillardi* representation (based on a *Megatherium americanum* by Anton, n.d.), *Mixotoxodon larensis* (Zimices, 2015a) *Paramylodon harlani* (Zimices, 2015b), *Odocoileus virginianus* (Michaud, 2021), *Tapirus bairdii* (Palomo-Munoz, 2021) and *Glyptotherium floridanum* representation (based on a *Glyptotherium arizonae* by Zimices, 2016).

Pleistocene megafaunal declines, suggesting that extinction dynamics varied across the Neotropics.

SCF are yet to be directly linked to extinct megafaunal species in palaeoecological records, limiting our ability to reconstruct the specific megafaunal assemblage at La Yeguada. Recovered fossilised bone evidence for extinct megafauna in Panama is concentrated in the Herrera Province, ca. 100 km from La Yeguada. Megafaunal bone records include a mixed assemblage of grazers, browsers and mixed feeders (Gazin, 1957; Pearson, 2005; Lucas, 2014). This includes browsers to mixed feeders that favoured forest habitats such as giant ground sloths (*Eremotherium laurillardi* and *Paramylodon harlani*) and gomphotheriids (*Cuvieronius hyodon*) (Prado et al., 2005; de Andrade et al., 2024), as well as grazers to mixed feeders that favoured open vegetation such as glyptodonts (*Glyptotherium floridanum*), toxodons (*Mixotoxodon larensis*) and horses (*Equus conversidens*) (MacFadden, 2005; Pérez-Crespo et al., 2012; Pérez-Crespo et al., 2022). However, some Pleistocene megafauna exhibited variable diets and habitat use (MacFadden, 2005; França et al., 2015; Pérez-Crespo et al., 2022). In the Late Pleistocene, our pollen record showed a high abundance of arboreal and some cold-adapted montane taxa (Fig. 4). If browsers and mixed feeders were inhabitants, they would likely have favoured the Late Pleistocene forest structure (Fig. 6). Notably, the first megafaunal decline at 13,600 cal yr BP coincided with a reduction in arboreal plants. Consequently, the vegetation structure was likely no longer favourable to forest-adapted megafauna. After the first megafaunal decline, we record a rise of Poaceae, suggesting a grassland expansion at La Yeguada (Fig. 4). Once grasslands were established, we recorded a rise in key SCF (Fig. 4). This includes the highest concentration of *Podospora* throughout the record, which may be the most reliable indicator of changes in grazing intensity (Wei et al., 2024; Zhang et al., 2025). If grazers were present, the key SCF recovery and regime shift we detected at 11,200 cal yr BP may indicate a rise in open grassland-adapted megafauna (Fig. 6).

Our key SCF record may reflect variability in the timing of megafaunal declines across the Neotropics; however, it also likely indicates shifts in community composition and dietary preferences (Fig. 6). For example, a possible replacement in the primary contributors to the SCF record from extinct to extant megafauna, including tapir and white-tailed deer during the Holocene (Samudio and Pino, 2005; Donoso, 2010) (Fig. 5b). This pattern may explain why our record shows multiple phases of SCF recovery. These recoveries contrast with other palaeoecological records, including Brazil and Ecuador, where megafauna ceased imposing a signal on the landscape (Raczka et al., 2018, 2019). In Brazil, bone records indicate an increase in brocket deer (*Subulo gouazoubira*) abundance alongside the decline in Pleistocene megafauna between the Late Pleistocene and Holocene (Hubbe et al., 2025). These patterns could be attributed to vegetation changes or increased resource availability following the LQE (Hubbe et al., 2025). Notably, we recovered *Ascobolus sacchariferus* in our pollen record from 12, 200–6200 cal yr BP (Supplementary Fig. 4). To date, *Ascobolus sacchariferus* is only known to grow on extant deer dung (Richardson and Watling, 2018). Taken together, the trends found at La Yeguada could suggest a gradual reorganisation of megafaunal community compositions over time, likely driven by the variable timing of extinction among different Pleistocene megafauna, with smaller extant species such as deer having a greater contribution to the key SCF record in the Holocene.

Key SCF were recovered at a lower concentration during the modern era at La Yeguada (Fig. 3). SCF can germinate on the dung of herbivores weighing less than 44 kg; however, smaller herbivores typically produce less dung, which influences the succession and abundance of SCF (Lee et al., 2022). In contrast, megafauna tend to contribute a larger amount to SCF records (Feranec et al., 2011; Gill et al., 2012). The reduction in key SCF at La Yeguada in the modern era relative to the Pleistocene may reflect the loss of large dung substrates. Our record does not cover the onset and intensification of agriculture in the area, which dates to ca. 4000 cal yr BP (Bush et al., 1992). However, our SCF record and the notably high concentration of *Sporormiella* in the modern era are also likely influenced by domestic megafauna. *Sporormiella* is a reliable indicator of modern livestock presence in the Neotropics (Raczka et al., 2016). However, *Sporormiella* is also sensitive to abiotic conditions (Asina et al., 1977; Kuthubutheen and Webster, 1986). This could suggest that environmental factors favoured *Sporormiella* germination and preservation in the modern era record. Our data is insufficient to determine the exact cause of the high *Sporormiella* concentration relative to the other SCF. Therefore, future studies could investigate the influences of livestock, wild herbivores and climate on modern Neotropical SCF records.

5.2. Vegetation and fire responses

Our pollen record showed that some palatable taxa responded to the presence and absence of megafauna (Fig. 4). Myrtaceae, *Eugenia* and *Solanum* are genera with large fleshy fruits and seeds often distributed by megafauna (Lughadha and Proença, 1996; Guimarães et al., 2008). These all declined following the initial megafaunal decline ca. 13,600 cal yr BP (Fig. 4). Birds and monkeys are the primary dispersers of Myrtaceae in modern ecosystems. However, extant megafauna are also influential in their dispersal (Pizo, 2002). Other taxa, such as *Eugenia* and *Solanum*, produce fruits too large to be consumed or transported by small animals and are termed megafauna fruits (Yumoto et al., 1995; Guimarães et al., 2008). These require large-bodied animals for dispersal. Several such megafaunal fruit species have seen extinctions and range contractions following the LQE (Galetti et al., 2018; Onstein et al., 2018). These patterns may have occurred in our record, as plants such as Myrtaceae did not recover relative to their Late Pleistocene abundance. This could indicate that the Late Pleistocene megafauna declines and the resulting loss of seed dispersal resulted in sustained ecological consequences and profound changes in ecosystem structure and function at La Yeguada.

After the wave of megafaunal recovery at ca. 11,800 cal yr BP, we also record an increase in some palatable plant taxa (Fig. 4; Supplementary Fig. 4). Some of these plants, including *Ficus*, *Spondias and Iriartea,* also rely on medium to large-bodied animals for seed dispersal (Bodmer, 1989; Foster, 1982; Janzen, 1985; Beck, 2006; Tobler et al., 2010; Sampaio et al., 2019). In our CCA analysis, *Spondias* groups towards the key SCF and macroscopic charcoal variables (Fig. 5). This pattern may highlight a historic account of the modern relationship between grazing, fire and seed dispersal of *Spondias* in the Neotropics (Janzen, 1985). Similarly, *Iriartea* can be megafauna-dispersed (Bodmer, 1989), suggesting a close link that is corroborated by our CCA analysis (Fig. 5). Other plants, including *Spondias*, *Pouteria* and *Licania*, are also capable of producing megafaunal fruits (Guimarães et al., 2008). The correlation between key SCF recovery and the increased abundance of some palatable plants, with large, megafauna-dispersed fruits and seeds in our Mid-Holocene record, could reflect dispersal by extant megafauna such as tapirs at La Yeguada.

La Yeguada supported megafauna and a forest rich in *Ilex*, Myrtaceae and *Quercus* during the Late Pleistocene (Fig. 4). *Quercus* typically grows in open to semi-open forests and in areas subject to disturbance (Nixon, 2006). The high abundance of *Quercus*, coupled with the absence of fire during the Late Pleistocene, suggests the maintenance of a semi-open forest landscape shaped by the continued presence of megafauna. The presence of *Myrsine*, a typical gap species alongside *Alternanthera* and *Iresine*, herbs that are typically found in open vegetation, forest gaps and disturbed sites, provides further support for an open forested disturbed landscape (Gentry, 1993; Marchant et al., 2002). After the first phase of megafaunal decline, *Jacaranda* became absent from our record (Fig. 4). *Jacaranda* regeneration requires high-light availability, commonly associated with large treefall gaps (Brokaw, 1985). Treefall gaps are part of the natural disturbance cycle of *Jacaranda* in Panama (Jones et al., 2005). Given the influential role of megafauna in promoting treefall (Asner and Levick, 2012), the observed decline in *Jacaranda* may be

Fig. 6. A reconstructed conceptual landscape illustration depicting changes in megafaunal presence, vegetation, and fire activity at La Yeguada throughout the Late Pleistocene, Holocene and Modern era. Produced using Affinity Designer 2 (Affinity Designer, 2022). The five titled panels represent the five temporal zones (LY1 - LY5) described in the results. The sediment shown in the lake coring illustration represents the sediment descriptions from core depths 730.6 – 1635.3 cm (ca. 6000 – 16,600 cal yr BP) (Supplementary Fig. 1). Megafauna are depicted by the *Eremotherium laurillardi* representation (based on a *Megatherium americanum* by Anton, n.d.), *Cuvieronius hyodon* (Osborn, 2016), *Tapirus bairdii* (Palomo-Munoz, 2021), *Mixotoxodon larensis* (Zimices, 2015a), *Glyptotherium floridanum* representation (based on a *Glyptotherium arizonae* by Zimices, 2016), *Odocoileus virginianus* (Michaud, 2021; Wilson, 2022), *Equus conversidens* representation (based on *Equus neogeus* by Zimices, 2017), and livestock representation (based on *Bos primigenius taurus* by Palomo-Munoz, 2022a). Smaller fauna are depicted by a two-toed sloth representation (based on a *Choloepus didactylus* by Caspar, 2022) and collard peccary representation (based on a *Tayassu tajacu* by Palomo-Munoz, 2022b). Local fire activity is represented by the number of flames. Human silhouettes indicate hunter-gatherer groups and the manipulation of fire (Vlasiuk, 2020). Lake coring illustration in the top panel is based on a photograph taken by AmazInk in 2022.

attributed to reduced disturbance resulting from the decline of Pleistocene megafauna at La Yeguada. Our Late Pleistocene record is consistent with megafaunal disturbance-linked vegetation types in Europe (Pearce et al., 2023) and the Neotropics (Doughty et al., 2016a). If forest-associated browsers and mixed feeders such as *Eremotherium* and *Cuvieronius* were present at La Yeguada during the Late Pleistocene, the relative stability observed in the pollen record may partly reflect their ecological influence.

Our pollen record indicates the expansion of grassland around 13,600 cal yr BP (Fig. 4). If the first megafaunal decline resulted from a decline of browsers and mixed feeders, a concurrent forest expansion could be expected, as megafauna can influence the density, composition and growth of woody vegetation (Gordon et al., 2021). However, the shift from arboreal to grass-dominated taxa may reflect a reduction in the long-distance dispersal and subsequent range declines of large-seeded plants facilitated by megafauna (Janzen and Martin, 1982; Doughty et al., 2016b). This could give a competitive advantage to wind-pollinated plants, including *Poaceae* and *Cecropia,* which can also indicate open and disturbed environments (Berg et al., 2005). At ca. 14, 000 cal yr BP, La Yeguada experienced a warmer climate (Bush et al., 1992) and increasing fire activity (Fig. 4). This burning of plant biomass may have functionally replaced megafaunal disturbance, likely contributing to the spread of grasslands. The decline of *Forsternoia* and *Parahancornia*, the peak of *Alchornea* and the increase of *Celtis* and *Cecropia* from 13,200 cal yr BP further suggest disturbance to the forest canopy and vegetation succession to secondary forest (Gentry, 1993; Piperno et al., 1990; Bush and Rivera, 1998) (Supplementary Fig. 4). These patterns are reflected in our CCA analysis as *Forsternoia* and *Parahancornia* cluster at the opposite end of the key SCF and macroscopic charcoal variables (Fig. 5). In contrast, *Celtis* and *Cecropia* are more closely associated with macroscopic charcoal, indicating the likelihood of a strong correlation between fire activity and the presence of these plants (Fig. 5). In the absence of human disturbance at La Yeguada until at least 11,000 cal yr BP (Bush et al., 1992), this vegetation shift was likely driven by the combined effects of megafaunal decline and rising fire activity. Based on our record, we infer that the first decline of megafauna contributed to grassland expansion by reducing the maintenance of a previously stable natural forest habitat. The decline of megafauna and warmer climate in the Late Pleistocene was therefore likely influential in a rapid, fundamental change in ecosystem structure and function.

The second and third phases of megafaunal decline at ca. 10,000 and 8200 cal yr BP overlapped with the decline of grasslands and a rise in arboreal plants (Fig. 4). Herbaceous plants, including *Pilea,* also increased. Lowland and secondary forest taxa, including *Cecropia*, Melastomataceae, Moraceae/Urticaceae, and *Mortoniodendron,* were present. The rise of lowland and disturbance taxa at La Yeguada is likely influenced by the contribution of warming at the onset of the Holocene (Bush et al., 1992) (see discussion in section 5.3). However, this change could also reflect a contributing role of megafauna. The Holocene megafaunal declines could have promoted the expansion of arboreal and herbaceous plants due to the removal of top-down browsing and natural grazing. For example, in modern African savannas, megafauna can outweigh geology and climate in regulating tree turnover (Asner and Levick, 2012). Reduced browsing can also reduce the pressure on saplings, promoting sapling recruitment and wood regeneration (Walters et al., 2020). However, this relationship is complex as megafaunal presence can facilitate understory growth by creating herbivory refuges from other species (Coverdale et al., 2016). The rise in herbaceous plants from 9800 to 8400 cal yr BP may reflect this pattern, as we record a megafaunal recovery at La Yeguada between the two Holocene declines. Megafauna exhibit different traits, life history strategies and behaviours, and therefore have different ecological functions in ecosystems. If the recovery of key SCF at 9000 cal yr BP resulted from a different assemblage of megafaunal species, then the species present likely had different ecological influences compared to those in the Pleistocene. From 8400 to 7600 cal yr BP, we record an increase in some secondary forest pioneer plants, including Melastomataceae, *Cecropia*, and *Alchornea* (Fig. 4). The increase in these plants could suggest vegetation succession because of megafaunal loss and an available niche resulting from reduced natural grazing. This change reinforces the interpretation that the Late Pleistocene megafaunal decline had a sustained impact on vegetation composition and ecosystem structure and function at La Yeguada.

Macroscopic charcoal was absent from the record until 13,200 cal yr BP (Fig. 4). In the Pleistocene, fire activity could be expected in La Yeguada's semi-open forest canopy, as this structure allows greater sunlight and airflow to reach the forest floor. This could result in warmer, drier, and windier conditions, increasing fire activity (Cochrane and Schulz, 1997). The absence of fire, coinciding with the presence of Pleistocene megafauna, may suggest that herbivory and trampling prevented the accumulation of the fuel necessary for fire activity. Our charcoal record shows a transition to increased local fire activity during the Late Pleistocene and Early Holocene megafaunal declines. The reduction in megafaunal presence at ca. 13,600 and 10, 000 cal yr BP alongside the warmer climate probably influenced the build-up of grass biomass and led to increased local fire activity (Fig. 6). In our CCA analysis, *Poaceae* groups towards the macroscopic charcoal variable reflecting the relationship between grasslands and increased fire activity in the absence of megafauna (Fig. 5). In the record, *Gelasinospora* and *Neurospora* spores increased alongside the rise in macroscopic charcoal (Supplementary Fig. 3). While *Gelasinospora* can be recovered from dung (Cain, 1950), its dependence on fire-related germination has led to its primary interpretation as a fire indicator (Wicklow, 1975; van Geel and Aptroot, 2006). *Neurospora* primarily grows on charcoal (Wicklow and Zak, 1979). At La Yeguada, we suggest the presence of *Gelasinospora* and *Neurospora* supports increased fire activity after the first megafaunal decline. However, human land management practices (Bush et al., 1992), coinciding with megafaunal decline at 8400 cal yr BP, may have mitigated increases in fire frequency. In our CCA analysis, megafaunal presence and fire activity both show a negative relationship along CCA axis 1 but an opposite relationship along CCA axis 2 (Fig. 5). This could highlight a complex relationship between megafauna, humans and fire activity at La Yeguada. Our Late Pleistocene and Early Holocene record is consistent with increased fire activity after megafaunal collapse, as has been reported across the continents (Burney et al., 2003; Gill et al., 2009; Rule et al., 2012; Pym et al., 2023). At La Yeguada, megafauna likely influenced natural fire activity in the Late Pleistocene. However, the complexity of Neotropical ecosystems and the changes in the presence and diversity of megafauna across different habitats likely resulted in heterogeneous relationships between Pleistocene megafaunal declines and variations in fire activity that were also influenced by climate and anthropogenic drivers.

5.3. Climatic and anthropogenic drivers

Arboreal plants dominated the vegetation record between 16,500 and 13,400 cal yr BP (Fig. 4). In the Pleistocene, *Quercus* was the dominant plant genus, accompanied by other montane forest taxa such as *Ilex* and *Symplocos*. The presence of these plants suggests cooler temperatures at La Yeguada. However, these genera were recorded alongside warm-adapted lowland taxa, including Arecaceae (Palms), Myrtaceae, *Solanum* and *Sloanea* (Fig. 4). The mixing of montane and warm lowland taxa could suggest a transitional vegetation zone or a period of climatic instability. However, as these species persisted during the Late Pleistocene, glacial cooling was likely strongly seasonal (Bush and Colinvaux, 1990). These conditions may have created a relatively stable habitat that supported megafaunal presence under minimal environmental change during the Late Pleistocene. The lack of an increase in secondary forest and/or disturbance taxa such as *Alchornea* and *Cecropia* until after ca. 13,200 BP further supports this interpretation. Evidence from our key SCF and pollen records indicates that the semi-open forest structure was particularly favourable for sustaining megafaunal presence during this period.

In the Late Pleistocene, Melastomataceae peaked at 14,000 cal yr BP when fire was absent (Fig. 4). Melastomataceae is associated with warmer conditions and secondary forest disturbance (Piperno et al., 1990; Colinvaux, 1996). In Panama, evidence of Palaeoindian presence and human occupation is limited but suggested as early as 13,000 cal yr BP (Cooke et al., 2013). However, higher sea levels may mean that any older coastal archaeological sites are inaccessible (Redwood, 2020). At La Yeguada, no indicators of human activity are recorded until 11,000 cal yr BP (Piperno et al., 1990; Bush et al., 1992). In the absence of fire and anthropogenic disturbance, but under continuous megafaunal presence, the secondary forest disturbance and peak of Melastomataceae appear to have been driven by warmer conditions. This warming could relate to the Bølling-Allerød interstadial (14,700–12,800 cal yr BP) (Hodell et al., 2008) and suggests a period of climatic seasonality in the Late Pleistocene. However, the presence of montane taxa suggests that these warmer temperatures at La Yeguada were likely brief (Bush et al., 1992). Our key SCF record shows that the first megafaunal decline occurred ca. 400 years after the brief warming. Comparable cases of megafaunal declines have been recorded in Mexico during the Bølling-Allerød interstadial (Solís-Torres et al., 2025). We do not have evidence to estimate exact temperatures or the longevity of changes; however, it appears that the first megafaunal decline occurred after a temperature fluctuation. Nevertheless, the short duration of the temperature changes likely contributed to the continuity of the key SCF record we recovered during the Late Pleistocene.

At La Yeguada, we record a decrease in some arboreal plants and a rise in grassland vegetation from 13,600 cal yr BP, suggesting novel vegetation assemblages (Figs. 4 and 5). Cyperaceae continued to rise between 13,400 and 13,000 cal yr BP, suggesting moist conditions and an expansion of lake levels as Cyperaceae is largely aquatic and typically found in wetland and moist environments (Marchant et al., 2002). However, from 12,600–10,200 cal yr BP, Cyperaceae declines, which could explain the maintained presence of *Eschweilera*, a genus commonly found in lowland forests free from flooding (Mori and Prance, 1990). Alongside grassland dominance, we record a higher abundance of Arecaceae and *Mimosa* from 13,400 cal yr BP. These lowland and disturbance taxa suggest a warm environment (Bush et al., 1992; Marchant et al., 2002). In Central America, the Younger Dryas stadial is recorded in multiple regions between 12,000 and 10,300 cal yr BP; however, Panama may have been unaffected by this temperature decline (Bush et al., 1992; Leyden, 1995). This absence is supported in our record between 12,200 and 10,000 cal yr BP by the decline of montane taxa such as *Ilex*, a peak in *Acalypha* (a weedy herb found in dry vegetation) and the rise in lowland vegetation including *Pilea*, *Arrabidaea*, *Trema*, *Cecropia*, and *Byrsonima* (Marchant et al., 2002) (Fig. 4). The key SCF recovery at 12,000 cal yr BP suggests the environmental conditions and novel vegetation at the end of the Late Pleistocene once again provided a favourable habitat for megafauna at La Yeguada.

Lowland tropical tree taxa, including *Dalbergia*, *Protium* and *Mortoniodendron*, document the presence of lowland moist tropical forests during the Early Holocene (Fig. 4). Alongside the arboreal record, the rise of herbaceous taxa and decline of grassland vegetation suggest another transition in the structure of vegetation at La Yeguada. Reduced disturbance and forest regeneration could be due to a reduction in natural grazing as indicated by our key SCF record (Fig. 4). However, the rise in lowland plant taxa likely resulted from the warming that marked the end of the deglacial period and the onset of the Holocene. This warming is also reflected by the sustained presence of *Acalypha* and *Quercus*, which declined in the Early Holocene and may have retreated upslope. However, *Quercus* persisted at a consistent, low-level presence, which may reflect the resilience of the genus (Bush and Colinvaux, 1990). From 11,000 cal yr BP, the effects of human activities, including burning and deforestation, are observable at La Yeguada (Piperno et al., 1990; Bush et al., 1992), coeval with stone tool culture (Cooke et al., 2013). Palaeoindian stone tool kits allude to hunting in Panama; however, radiocarbon-dated deposits are limited, and their remains have not been found in stratigraphic association with megafaunal bones (Cooke et al., 2013). We have limited evidence to explore the causes of the megafaunal declines. Nevertheless, human occupation and disturbance are also likely to have been influential in the structure of vegetation and the second decline of megafauna from 11,000 cal yr BP. The combined effects of vegetation change, Early Holocene warming and anthropogenic activity likely made the habitat at La Yeguada unfavourable for megafaunal presence, at least periodically. However, the forthcoming phases of megafaunal recovery suggest that these pressures were not equally strong through time, allowing megafauna to recover in periods of reduced pressure.

Our key SCF record indicates an increase in megafauna at 9000 and 7600 cal yr BP, either side of a decline at 8400 cal yr BP (Fig. 3). These recoveries were likely caused by major changes in megafaunal community compositions rather than the recovery of previous megafaunal species. The decline at 8400 cal yr BP is likely related to human disturbance. However, the forthcoming SCF recovery suggests local megafaunal activity despite human presence. These findings contribute to the broader debate on human involvement in the LQE. The LQE have been widely linked to anthropogenic impacts (Barnosky et al., 2004; Svenning et al., 2024), with humans considered major extinction drivers in regions such as North America (Stuart, 1991; Gill et al., 2009). In the Neotropics, the human role remains less certain due to the paucity of records (Becerra-Valdivia, 2025). Nevertheless, megafaunal kill sites (Sanchez et al., 2014; Bampi et al., 2022) and the temporal overlap with human occupation (Raczka et al., 2018) and activity (Raczka et al., 2019) suggest a correlation. In contrast, evidence from Peru indicates megafaunal decline occurred before human arrival (Rozas-Dávila et al., 2016). In Colombia, megafauna declined in two waves, the first during climatic instability and the second coinciding with the presence of hunter-gatherers (Pym et al., 2023). These contrasting records suggest local and regional-based variability in the causes of the LQE. The possible coexistence of humans and megafauna at La Yeguada in the Early to Mid-Holocene implies that human impact after the second phase of megafaunal decline may have been more nuanced than in other regions. This may have enabled the recovery and persistence of some megafaunal populations, as indicated by our key SCF record (Fig. 3). These recoveries likely resulted from changes in megafaunal community compositions. This scenario could relate to broader Neotropical behavioural trends where human populations adopted more diverse dietary strategies, incorporating both plant resources (Chen et al., 2024) and extant fauna (Davis and Rindel, 2024). Our results suggest a complex narrative of human-megafauna interactions in the Holocene. Nevertheless, the precise timing and extent of anthropogenic impact cannot be concluded due to limited archaeological evidence and the lack of recovered megafaunal bones from La Yeguada.

At La Yeguada, we record a grassland to arboreal turnover and fluctuation in herbaceous plants from 8200 cal yr BP, indicating secondary forest succession (Fig. 4). This change in vegetation may reflect reduced light levels resulting from a closure of the canopy as indicated by the presence of canopy genera such as *Licania* and open area indicators including Amaranthaceae (Gentry, 1993; Marchant et al., 2002) (Supplementary Fig. 4). The megafaunal decline at 8400 cal yr BP may have contributed to the canopy closure and rise in arboreal taxa during the Mid-Holocene; however, the peak of *Cecropia* at 6000 cal yr BP likely reflects secondary forest succession caused by anthropogenic disturbance. Moreover, the appearance of forest taxa such as *Pterocarpus*, *Trema*, *Alchornea*, *Dalbergia*, and *Sloanea*, along with fluctuations in Moraceae/Urticaceae, was also likely influenced by both anthropogenic land management and warmer Holocene temperatures that promoted forest regrowth (Bush et al., 1992). However, in the absence of megafauna, these changes in vegetation structure may in part relate to the lost functions resulting from the Late Pleistocene and Early Holocene phases of megafaunal decline.

Across the modern era, arboreal taxa declined whilst open grassland vegetation increased (Fig. 4). This change in vegetation structure suggests further forest disturbance and land-use change, likely caused by modern deforestation by humans for agriculture. At La Yeguada, *Pinus* increased in the last 200 years, highlighting the recent *Pinus* plantations found near the lake (Bush et al., 1992). The near absence of plants that were dominant in the Late Pleistocene, including Myrtaceae and *Ficus,* could suggest an ecological legacy resulting from the loss of Pleistocene megafauna and possibly the loss of a natural ecological process (Fig. 6). An open water body has persisted at La Yeguada over the modern era, but declining Cyperaceae abundance suggests a possible reduction in lake levels (Fig. 4). The lake was likely more extensive in the Pleistocene but has declined due to human management and precipitation changes (Bush et al., 1992). The vegetation cover and environmental conditions at La Yeguada likely continue to provide habitat, water, and food for extant megafauna and smaller herbivores. However, continued land-use changes could contribute to future local extinctions. Across the Late Pleistocene and Holocene, fire activity was regular (Fig. 4). In comparison, fire activity has been relatively low across the past 500 years. As a result of reduced megafaunal activity, the fire record could suggest that humans may have replaced the suppressive effect megafauna had on fire activity in the Pleistocene.

5.4. *Interpretations and limitations*

It is important to consider the spatial scale and inferences that can be made from microfossils when drawing palaeoecological inferences. For pollen assemblages, the spatial scale of vegetation representation is often related to the lake size (Davis, 2000; Smith and Mayle, 2018). Quantitative methods of classifying vegetation and the source of pollen from palaeoecological records are available (Sugita, 2007a; 2007b). However, these models depend on pollen data and parameters, including assumptions about pollination that remain untested for all taxa in Neotropical settings (Smith and Mayle, 2018). The selection of these model parameters is therefore influential in vegetation classifications (Hellman et al., 2008). Given these limitations, modelling was not employed to distinguish between the local and regional pollen. Instead, our interpretations are informed by relevant literature (Marchant et al., 2002). To infer fire activity, we analysed macroscopic charcoal particles. It is widely accepted in palynology that the spatial scale of macroscopic charcoal represents local fire activity (Whitlock and Larsen, 2002; Brown and Power, 2013). Our inferences of fire activity are therefore local to La Yeguada.

SCF are typically a local indicator of megafaunal activity due to their short dispersal distance from their fruiting body (Yafetto et al., 2008). Although some SCF can travel further through airborne dispersal, most are likely deposited within 100 m of the dung source (Gill et al., 2013). Hydrological dispersal of SCF also occurs, yet these mechanisms are poorly understood (van Asperen et al., 2021). Lake La Yeguada has a closed basin fed by streams that drain the small catchment area (Bush et al., 1992). We therefore interpret our SCF record as a proxy for local megafaunal activity. Within a lake, hydrological conditions, including changes in water depth and size, can influence the preservation of SCF in palaeoecological records (Dickinson and Underhay, 1977; Raper and Bush, 2009; Wood and Wilmshurst, 2013). However, fluctuations in SCF have not been consistently correlated with water level changes across sites, indicating that they may more accurately reflect variations in megafaunal activity than hydrological conditions (Wood and Wilmshurst, 2013). La Yeguada may have experienced low or fluctuating water levels up to 12,800 cal yr BP (Fig. 4). However, the lake has retained continuous open water since at least 14,300 cal yr BP (Bush et al., 1992). This hydrological stability likely provided attractive habitat conditions for local megafauna. Cyperaceae increases in our record following the initial megafaunal decline, declines across the Pleistocene–Holocene transition, and shows relative stability throughout the Holocene (Fig. 4). Our multi-SCF assemblage does not track the record of Cyperaceae, reducing the likelihood that water-level changes influenced changes in the key SCF record. Moreover, *Sporormiella* and *Coniochaeta*, considered drought-tolerant genera (Kuthubutheen and Webster, 1986; Gelorini et al., 2012), declined during a peak in Cyperaceae around 13,000 cal yr BP. Taken together, these patterns suggest that hydrological factors did not significantly influence SCF preservation at Lake La Yeguada. Instead, the key SCF record more likely reflects megafaunal activity marked by distinct phases of megafaunal decline and recovery.

Our multiproxy palaeoecological approach is the first to reconstruct the timing and ecological consequences of the Pleistocene megafaunal declines in Panama. However, the study is based on a single site and uses microfossils that are best interpreted as local proxies for megafaunal activity and fire, rather than indicators of regional trends. These limitations highlight the need for further investigations across Panama to establish broader spatial patterns and to validate and compare our interpretations of the timing and ecological consequences of the Pleistocene megafaunal declines. Moreover, our record is focused between 16,600 and 6000 cal yr BP and 500-100 cal yr BP. Our interpretations of changes in megafaunal presence, vegetation and fire activity are therefore constrained by chronological gaps across the Holocene. It is therefore possible that additional phases of megafaunal decline and recovery may have occurred at La Yeguada, as, although debated, some megafauna persisted into the Mid-Late Holocene in other Neotropical regions (Faria et al., 2025). In addition, we infer sustained ecological consequences, including the loss of megafaunal fruits and large-seeded plants resulting from the Late Pleistocene megafaunal decline. It is possible that plants such as Myrtaceae recovered in the Late Holocene to abundances found in the Late Pleistocene. Nevertheless, Holocene pollen records from La Yeguada suggest otherwise (Bush et al., 1992). Despite these limitations, our findings provide important insights into the timing and consequences of the Late Pleistocene megafaunal declines in Panama, highlight opportunities for future investigations and provide crucial implications for present-day conservation and rewilding.

5.5. *Implications and rewilding*

Our multi-proxy record from La Yeguada was highly sensitive to the loss of Pleistocene megafauna and to fluctuations in megafaunal presence (Fig. 4). For example, the loss of certain megafauna-dispersed fruits and large-seeded plants suggests that the decline of megafauna during the Late Pleistocene may have resulted in the loss of important ecological functions. Moreover, the increase in fire activity following the megafaunal decline at 13,600 cal yr BP suggests that Pleistocene megafauna may have had an influential role in suppressing fire activity. The ecological consequences observed at La Yeguada resonate with current discussions on trophic rewilding. By documenting how megafauna once regulated dispersal networks, forest/grassland mosaics, and fire activity, our reconstructions aim to provide a baseline for future assessments of the potential of reintroducing ecological analogues via trophic rewilding to restore lost functions in Neotropical landscapes. However, implementing trophic rewilding in the Neotropics is complex due to the severity of the LQE and practical challenges, including modern human-animal conflicts. To design effective rewilding strategies, palaeoecological records are essential for reconstructing the structure of past ecosystems and clarifying the ecological functions once performed by megafauna (Gillson, 2015; Jeffers et al., 2015). Our record also emphasises the need to integrate Neotropical evidence into global models of historic, present, and future megafaunal declines to inform contemporary conservation and rewilding strategies.

6. Conclusions

This study is the first in Panama on the timing and ecological consequences of Pleistocene megafaunal declines. At La Yeguada, we showed that a combined multi-genus SCF record, along with the analysis of key SCF concentrations and accumulation, offers stronger and more reliable evidence for reconstructing megafaunal presence and declines in Central America than relying solely on *Sporormiella* percentage. The key SCF record indicated the presence of Pleistocene megafauna since at least 16,600 cal yr BP. At La Yeguada, megafaunal populations showed three phases of decline and recovery. The first megafaunal decline was reached by 13,600 cal yr BP and overlapped with a short period of warming in the Late Pleistocene. The second and third phases of decline occurred at 10,000 and 8400 cal yr BP, coinciding with the earliest evidence for human occupation and disturbance at La Yeguada. The key SCF record and phases of recovery suggest favourable habitats for different megafaunal communities throughout the Late Pleistocene and until at least the Mid-Holocene. Our pollen record indicates that La Yeguada experienced changes in the structure of vegetation, which could suggest that these SCF recoveries resulted from changes in the assemblage of megafaunal species with varying dietary preferences.

The decline of Pleistocene megafauna in Panama likely led to the loss of megafauna-dispersed fruits and large-seeded plants at La Yeguada. This ecological consequence of megafaunal decline highlights the role Pleistocene megafauna had in seed dispersal in Panama and how their decline resulted in the loss of a natural ecological process at La Yeguada. The phases of megafaunal decline and recovery likely influenced vegetation turnover, contributing to shifts between arboreal and grassland-dominated structures. In the Late Pleistocene, megafauna also had roles in suppressing fire activity. The decline of herbivory and trampling at 13,600 cal yr BP, before known evidence of anthropogenic impact, suggests that megafaunal loss likely contributed to increased fire activity at La Yeguada.

Of major significance, this research reveals that ecosystems in Panama were highly sensitive to the loss of Pleistocene megafauna and to fluctuations in megafaunal presence. Trophic rewilding with megafauna in the Neotropics could help to restore some of the lost ecological functions that we recorded at La Yeguada. With extant megafauna and smaller herbivores at risk due to the current biodiversity crisis, our understanding of the ecological consequences of megafaunal declines suggests that future extinctions in Panama could have profound consequences for biodiversity, which in turn will have negative consequences for humans. The knowledge presented here is therefore crucial not only for contemporary conservation and the prevention of future extinctions, but also for forward-thinking rewilding initiatives and efforts to restore ecological functions lost from the decline of Pleistocene megafauna in Panama.

Author contributions (CRediT)

Felix Pym: Conceptualisation, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualisation, Writing – original draft, Writing – review & editing. Felipe Franco-Gaviria: Data curation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review &; editing. Marco Raczka: Methodology, Project administration, Supervision, Validation, Writing– review & editing. Gbotemi A. Adediran: Funding acquisition, Project administration, Supervision, Writing – review & editing. Stephen Sitch: Project administration, Supervision, Validation, Writing – review & editing. Dunia H. Urrego: Conceptualisation, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.quascirev.2026.109887.

Data availability

The data supporting this publication are openly available from the NERC National Geoscience Data Centre repository (https://doi.org/10.5285/2cdbc8e5-c766-47a3-8301-0bec8d83e3e2) or can be accessed by contacting the corresponding author(s).

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