



Molecular evidence for human population change associated with climate events in the Maya lowlands

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ABSTRACT

The analysis of faecal stanols in lake sediment cores offers a novel opportunity to reconstruct human population change, assuming that variability in faecal stanol concentration is a reliable proxy for relative human populations. The ancient lowland Maya of Mesoamerica represents an important ancient society whose demographic dynamics in many locations remain uncertain. We apply the faecal stanol proxy to a sediment core retrieved from a lake adjacent to the archaeological site of Itzan, an ancient population centre in the southwestern Maya lowlands. The sedimentary faecal stanol record from Laguna Itzan implies substantial centennial- and millennial-scale changes in local human populations from 3300 cal years BP to the present. Variability in faecal stanol concentrations is broadly consistent with archaeological evidence for regional societal change across the Maya lowlands, but also implies an earlier presence of humans at this site than is currently indicated in the Itzan archaeological record. We find evidence for high-frequency variability in coprostanol concentrations during the Maya Preclassic period, which we infer represents centennial-scale shifts in settlement patterns associated with changes in agricultural and land use patterns. Given Preclassic-period faecal peak stanol concentrations, we observe lower-than-expected Classic-period faecal peak stanol concentrations, and these may partly be a result of either use of human waste for fertiliser or reduced soil erosion or both. Three periods of inferred population decline are associated with palaeoclimate evidence for a drying climate, specifically during the Terminal Classic (1220–1050 cal yr BP) and the Protoclassic 2 (1860–1670 cal yr BP), as well as the less well-studied drought between 3330 and 2900 cal yr BP during the Early to Middle Preclassic periods. An additional decline and hiatus in coprostanol input coincides with a period of anomalously wet climate in the Late Preclassic. These linkages suggest that climatic change and variability could have played a role in demographic change at multiple points in the evolution of Maya civilisation. Our work shows that faecal stanols are valuable proxies for past human population dynamics, and their relation to climatic change, in Mesoamerica.

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1. Introduction

It is important to develop a clearer understanding of the complete history of how humans have altered and changed their environment, particularly in response to climate change. The lowland Maya of Mesoamerica are one example of a complex ancient society affected by multiple environmental and socio-political

stresses (Aimers, 2007), and who experienced a major demographic and political decline, or collapse, during a period of intense multidecadal drought, between approximately 750 and 900 CE (Hodell et al., 1995; Medina-Elizalde et al., 2016; Rosenmeier et al., 2002). The effects of climate change on ancient societies are generally not well understood, and remain incompletely constrained in the Maya lowlands (Douglas et al., 2016). Estimates of demographic change are important for understanding the impacts of past climate change on ancient societies, but are not always readily available. The objective of this paper is to apply an emerging proxy for reconstructing population change through time, faecal

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stanol concentrations in lake sediment cores, to better understand ancient Maya demographic change and its relationship to available palaeoclimate data. To accomplish this we analyse faecal stanols in a sediment core from Laguna Itzan, in Peten, Guatemala, that spans the past 3300 years.

Faecal stanols are lipid biomarkers found in animal faeces and their composition is determined by diet, the species of animal, and the presence and taxonomy of anaerobic bacteria in their digestive tracts. Coprostanol (5β -cholestan- 3β -ol) is produced during metabolic reduction of cholesterol in the intestinal tract of most mammals and is the major sterol present in human faeces (Bethell et al., 1994). It is therefore a potentially useful proxy for assessing the presence and concentration of human populations and has been used in both environmental pollution research (Adnan et al., 2012; Hussain et al., 2010; Reeves and Patton, 2005) as well as archaeological and palaeoenvironmental studies (Bethell et al., 1994; Bull et al., 2003; D'Anjou et al., 2012; Sistiaga et al., 2014; Vachula et al., 2019; White et al., 2018). Of the mammals capable of producing coprostanol, only pigs and sheep are known to produce sufficient concentrations to potentially mask the signal of human coprostanol (Prost et al., 2017; Zocatelli et al., 2017). The ancient lowland Maya domesticated dogs and turkeys, but cattle, pigs and sheep were only introduced to the Americas in the 16th century.

After production, coprostanol enters the environment as a component of faeces (Fig. 1). These lipid biomarkers are relatively resistant to degradation and accumulate in sediments within depositional settings such as marine and lacustrine basins (D'Anjou et al., 2012; Pratt et al., 2008). The conversion of coprostanol to epicoprostanol (5β -cholestan- 3α -ol), mediated by microbes, can occur in-situ in soils and sediments (Bull et al., 2003). The relative proportions of 5β -stanols can also be used as a means of determining the input of faecal matter from animals with different diets (Sistiaga et al., 2014). For example, faeces of herbivores contain the largest amounts of 5β -stigmastanol, followed by β -sitosterol or 5α -stigmastanol, because of the prevalence of plants in their diets (Prost et al., 2017). Therefore analysis of 5β -stigmastanol can help to control for contributions of coprostanol from grazing herbivores, which produce coprostanol in lower proportions than humans.

Ancient population estimates in the Maya lowlands have traditionally been obtained through ground inspection and excavation. To reconstruct Maya population dynamics, archaeologists

locate, map, and count residential structures, and they excavate them to establish dates of occupation (Johnston, 2002). To reveal temporal and spatial variations, archaeologists compare population trends at the site and regional levels. Carrying out comprehensive settlement surveys in forested regions is time consuming and labour intensive, and because site discovery is affected by surface visibility, surveys can produce biased samples (Johnston, 2002). In recent years, the detection of ancient Maya structures has been revolutionised by Lidar (Light Detection and Ranging), a remote sensing technique that use laser pulses to map settlements and infrastructure over large and often heavily forested areas. For example, the Pacunam Lidar Initiative covered 2144 km² of the Maya lowlands, identifying 61,480 ancient structures and inferring an average density of 80–120 persons/km² (Canuto et al., 2018). Lidar has also revealed an artificial plateau and causeways at the previously unknown site of Aguada Fénix in Tabasco, Mexico (Inomata et al., 2020). From Lidar data archaeologists can estimate the general, time-averaged, and potentially maximum population of sites and areas, but excavations are still needed to establish population trends at sites through time.

Another recently developed technique uses summed ¹⁴C-dates from burials and other archaeological sites as proxies for population trends. A greater proportion of dated material from a specific time period is thought to reveal a greater population increase, while fewer dates imply a smaller population (Torfing, 2015). A recent adaptation of this approach is the end-to-end Bayesian population modelling technique devised by Price et al. (2020). Applied to the Maya lowlands generally, the technique primarily reveals broad and smoothed demographic trends at large spatial scales, although at a few Maya sites with sufficient numbers of radiocarbon dates, such as Tikal, it can reveal site-specific trends at decadal scales (Price et al., 2020).

Finally, there are numerous techniques that can be used to construct palaeoecological change, such as pollen analyses, indicators of soil erosion into lake sediments, and plant wax isotopes (Anselmetti et al., 2007; Douglas et al., 2015; Leyden, 2002). These are proxies for environmental changes that can then be linked to population, land use and agricultural changes, which are signals that are often difficult to discern in the archaeological record. Faecal stanols can potentially complement the data produced by other palaeoecological methods, especially in that they are specifically

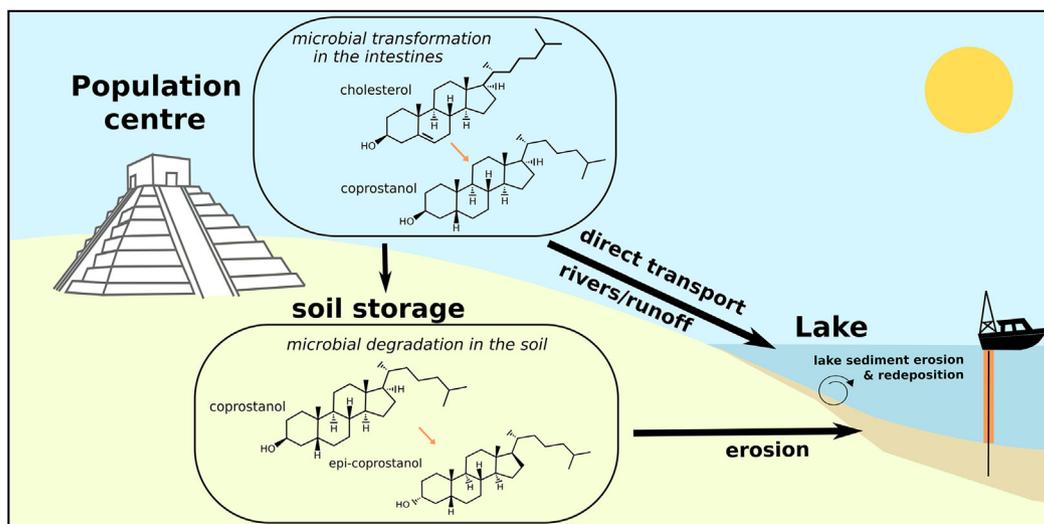


Fig. 1. Schematic diagram showing production of coprostanol in the gut of higher mammals, its input into the environment as a component of faeces, or via decomposition after death, and subsequent direct transport to lakes or storage in soils (modified from White et al., 2018). Possible groundwater transport is poorly constrained.

linked to human biological processes (D'Anjou et al., 2012; Battistel et al., 2007; White et al., 2019). We analyse faecal stanol concentrations in a lake sediment core that spans the past 3300 years from Laguna Itzan, adjacent to the ancient Maya centre of Itzan (Johnston, 2006) in southwest Peten, Guatemala. We then employ these concentrations as a proxy for population and land use change through time, and compare them with archaeological records of population change. We also compare these data with a synthetic climate record for the southern Maya lowlands (Douglas et al., 2016) and a pollen record from Lake Puerto Arturo that provides a record of changes in deforestation (Wahl et al., 2006) in order to evaluate how inferred changes in population relate to climate variability and vegetation cover change.

2. Methods and materials

2.1. Sediment core sampling

Sediment cores from Laguna Itzan in the southwest Maya lowlands were collected in 1997 and described by Breckenridge (2000). Two overlapping cores totalling 5.7 m of sediment were collected in 10.1 m water depth near the western shore of the lake, close to the deepest point of the lake (Fig. 2; 16.598° N, 90.4784° W). The upper 2.2 m of sediment is a massive, grayish brown (10 YR 5/2) sapropelic, clayey, carbonate mud with abundant plant macrofossils (Unit 1). Below this depth, the sediments transition to a darker (10 YR 4/2) calcareous clay (Unit 2), which alternates down core with beds of clayey, carbonate mud similar to the upper 2.2 m of sediment (Breckenridge, 2000; Douglas et al., 2014, 2018). We include a supplemental file with detailed lithostratigraphic data. The coring location is approximately 1 km from the Itzan site core, directly to the east of the ancient population centre (Johnston,

2006) and has a relatively small catchment of 1.51 km² (Douglas et al., 2014). The escarpment and lagoon system is part of a larger horst and graben formation, with Itzan sitting atop a horst, and the 7 km long escarpment crest and its eastern slope would have drained into the lagoon. The marshland lagoon system is fed by two large springs: one feeds into the cenote from where the core was retrieved, and the other feeds the pool immediately to the south of it; its few deep pools are relatively small and all are clustered along the laguna's western edge. During the dry season, the lagoon is fed entirely by the springs, and the water level, particularly in the large marshy area, can fall dramatically. The laguna is an open lake and is connected to and drains via a stream and marsh system into the nearby Río de la Pasión. The core has been stored refrigerated at the LacCore facility at the University of Minnesota Twin Cities. The core was sub-sampled to obtain a temporal resolution of approximately 50–100 year intervals, without reference to sediment lithology. Scatter plots of stanol concentrations vs. TOC, as well as dry bulk density and magnetic susceptibility (supplemental Figure S3) have a parabolic pattern and imply there was no sedimentological sampling bias. These samples were subsequently freeze-dried to remove water.

2.2. Age model

A core chronology was previously established based on radiocarbon measurements of plant fragments from the cores calibrated using IntCal13 (Reimer et al., 2013; Douglas et al., 2018) using a 4th-order polynomial fit with the Classical Age-Depth Modeling (CLAM) software in R (Blaauw, 2010), as shown in Fig. 3. The average age uncertainty (95% confidence interval) of the chronology is 100 ± 30 years. The ¹⁴C data used to develop the chronology can be found in supplemental Table S1.

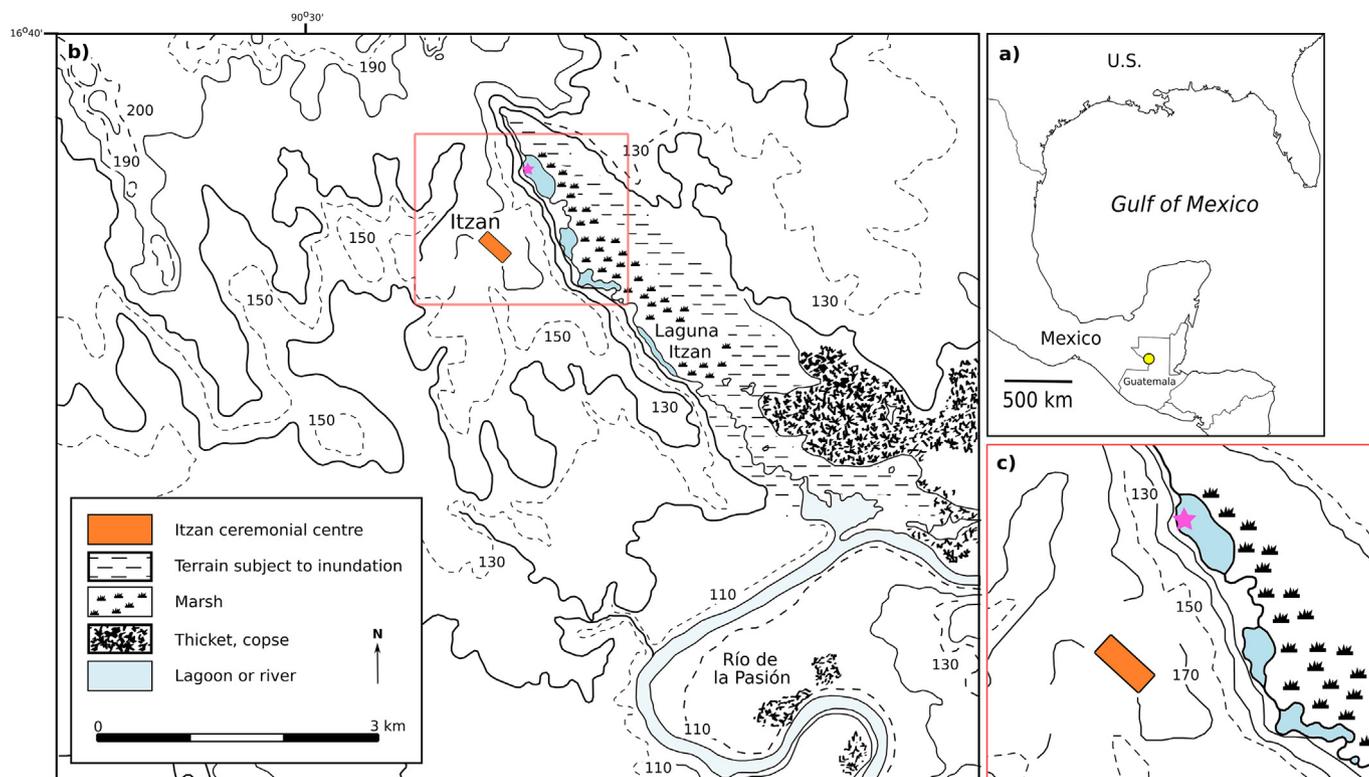


Fig. 2. Map showing the location of Itzan in a) Central America, b) in the Río de la Pasión area, and c) zoomed in to show the location of the core collected. b) and c) show the position of the ceremonial core of Itzan, which includes a substantial palace complex, and would have contributed a portion of the stanols in laguna sediments; the remainder can be traced back to the outlying farms to the east of the centre. Map modified from Johnston (2006).

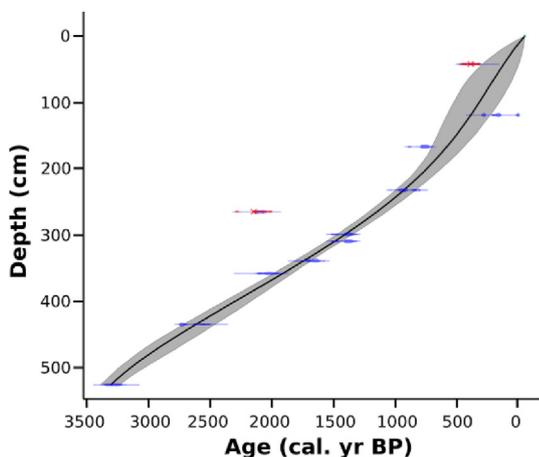


Fig. 3. ^{14}C based age-depth model for the Laguna Itzan sediment core. Probability distributions for individual calibrated ^{14}C ages are shown in blue. Two dates marked in red represented age-depth reversals and were not included in the model. The best-fit 4th-order polynomial age model is indicated by the black line, and the 95% confidence interval is shown in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.3. Stanol quantification

Dried sediment samples were ground, weighed, added to a PTFE tube and extracted using a CEM MARS 6 microwave extractor with 10 ml of 9:1 dichloromethane:methanol. This ratio of solvents was selected after testing various methods for their extraction efficiencies using lake sediment samples (Battistel et al., 2015; Kornilova and Rosell-Melé, 2003). The MARS 6 was heated to 80 °C and held at that temperature for 20 min. The contents of the PTFE tube were then transferred to a centrifuge vial, centrifuged and the Total Lipid Extract (TLE) was transferred to an evaporating vial. 9:1 DCM:Methanol was added twice more to the centrifuge tube in order to ensure complete removal of extracted material. The TLE was evaporated and split into 2 fractions (a non-polar fraction and a fraction containing the neutral and polar fractions) using silica gel chromatography. The pipette columns consisted of 5 cm of silica gel, and 1 cm of sodium sulphate. 15 ml of hexane was eluted to collect the non-polar hydrocarbon fraction and 15 ml of methanol was eluted to collect the remaining neutral and polar fractions. The solution containing the neutral and polar fractions was saponified using KOH (potassium hydroxide) and separated into a sterol and fatty acid fraction. The sterol fraction was then derivatised with BSTFA (bis-trimethyl silyl trifluoroacetamide) to replace the hydrogen with the less exchangeable trimethylsilyl (TMS) group.

The neutral (sterol) fraction was analysed using gas chromatography with a flame ionisation detector (GC-FID) with a TRACE TR-5 GC Column (60 m \times 0.25 mm) at McGill University in sequence with known standards for coprostanol, epicoprostanol, and stigmastanol (Sigma-Aldrich) in order to quantify these compounds. A set of representative samples were analysed using an Agilent 7890B GC with an Agilent 5977B MSD at Concordia University to confirm compound identification. Because of the similar retention time of coprostanol and epicoprostanol it was not possible to consistently resolve these molecules. We followed the approach of White et al. (2018) and reported the sum of these two compounds. This does not influence our interpretations since epicoprostanol is a transformation product of coprostanol, and therefore the summed concentration represents the net input of coprostanol to lake sediments. Stanol and TOC data are available at 10.6084/m9.figshare.14195222.

2.4. Total organic carbon

To measure total organic carbon concentration (TOC), dried and ground sediment samples were first weighed into an open silver cup, placed into a clean tray and fumigated in a closed glass container with a concentrated HCl for 24 h to remove inorganic carbon. The silver cup was then sealed with tweezers and wrapped in a tin cup, which is a better catalyst for flash combustion analysis, and analysed with a Carlo Erba NC2500 elemental analyser (Hélie, 2009). These analyses were performed at the GEOTOP light stable isotope laboratory at the Université du Québec à Montréal.

3. Results

3.1. Weight percent sedimentary organic carbon

Lake Itzan sediments have relatively high organic carbon content, with an average %Org C of 6.4 ± 1.8 (Fig. 4d). There is apparent centennial to millennial scale variability in %Org C, with highly variable measurements prior to 1500 cal yr BP. This variation may be related to increasing and highly variable soil erosion, as reflected in whole core, volume magnetic susceptibility measurements, which correlate with the non-calcareous, clastic fraction of the sediments (Fig. 4c; supplemental Figure S1; Douglas et al., 2018). Measurements are overall higher, and more stable after 1500 cal yr BP, possibly as a result of reduced rates of soil erosion.

3.2. Coprostanol concentrations

We present our faecal stanol concentration data as an absolute concentration relative to dry sediment weight ($\mu\text{g/g}$ dry sediment) (Fig. 4a) and normalised to total organic carbon ($\mu\text{g/g}$ OC) (Fig. 5a). For the interpretation of changes in stanol abundance we focus on concentrations normalised to TOC in order to account for the effects of mineral dilution as well as the potential effects of organic matter deposition and preservation on stanol concentrations (LeBlanc et al., 1992; Thienemann et al., 2017). Sedimentary concentrations of coprostanol (Fig. 4a) and coprostanol:TOC ratios (Fig. 5a) show very similar temporal patterns, and we infer that normalising to TOC has a minimal effect on the temporal signal.

Coprostanol concentrations exhibit marked variability on hundred-year timescales. We observe a large early peak at 3170 cal yr BP, followed by a period of stable low values. We then observe a ~1000 year period of large-amplitude, centennial-scale variability, from about 2770 to 1760 cal yr BP. This is followed by another period of stable, low concentrations, until concentrations begin increasing around 1410 cal yr BP. A period of relatively high stable concentrations persists until 1000 cal yr BP, at which point there is a rapid decrease. A set of further peaks in concentration occur through the remainder of the record, including the largest peak of the record at 235 cal yr BP.

3.3. Stigmastanol concentrations

As with coprostanol, we do not observe major differences in temporal patterns between sedimentary stigmastanol concentrations (Fig. 4a), and stigmastanol:TOC (Fig. 5a). Variability in stigmastanol is generally at a lower frequency than coprostanol. There is relatively minor variability in the early part of the record before 3000 cal yr BP. A set of four much larger-amplitude peaks in stigmastanol occur between 2590 and 1920 cal yr BP. This high-amplitude variation is coincident with high-amplitude variation in coprostanol, but the peaks in stigmastanol concentration are less frequent. Similar to coprostanol, there is a period of low

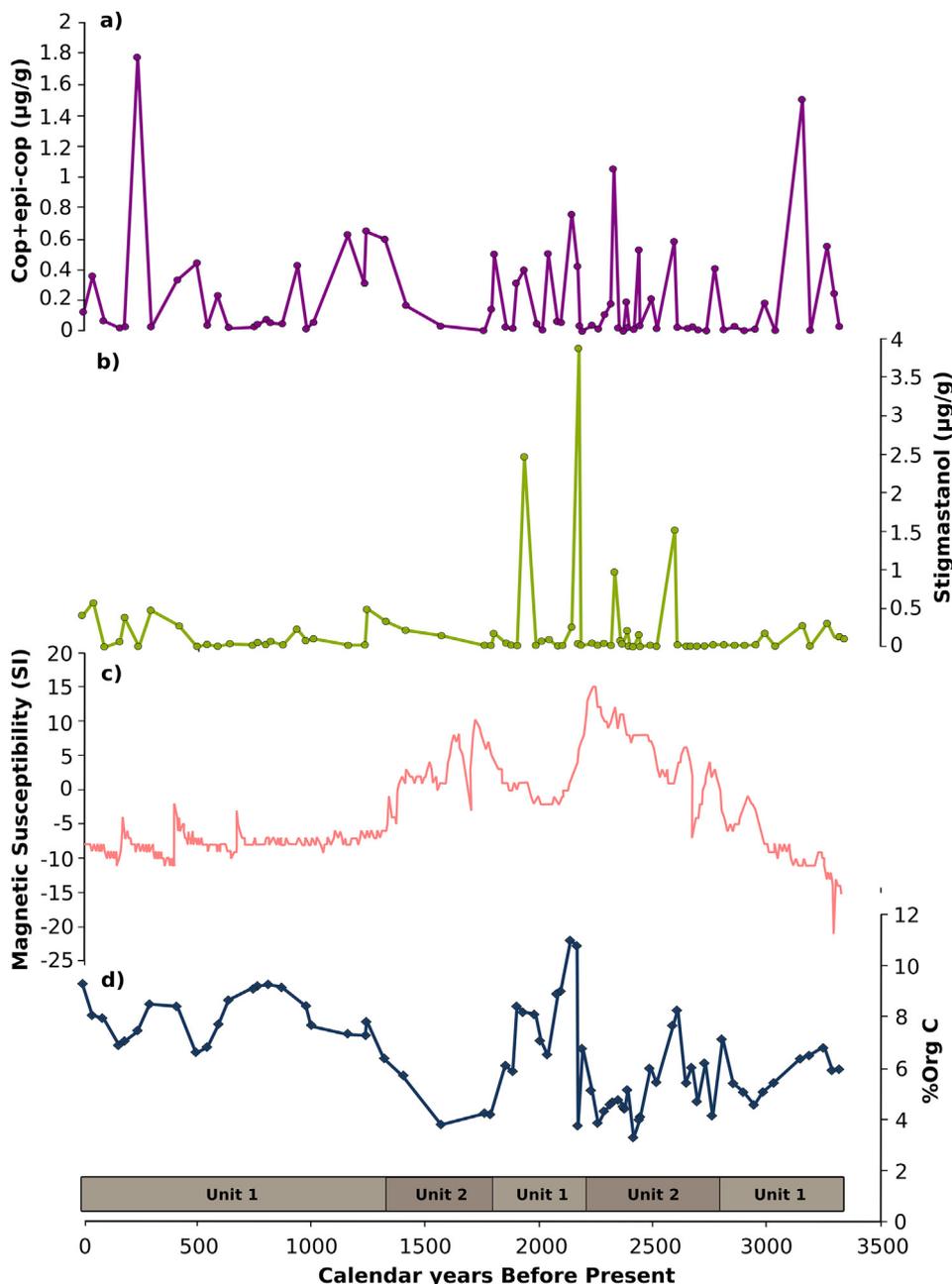


Fig. 4. Graphs showing a) concentration of coprostanol relative to dry sediment weight ($\mu\text{g/g}$ dry sediment), b) concentration of stigmastanol relative to dry sediment weight ($\mu\text{g/g}$ dry sediment), c) Magnetic susceptibility record from Itzan (Douglas et al., 2018), and d) weight percent organic carbon (%Org C) in sediments, and the generalised lithostratigraphy, which alternates between a sapropelic, clayey carbonate mud (unit 1), and a sapropelic, calcareous clay (unit 2).

concentrations from 1880 to 1760 cal yr BP, followed by a period of gradually increasing concentrations until 1240 cal yr BP, at which point there is an abrupt decline. Stigmastanol concentrations then remain relatively low until 490 cal yr BP, following which a series of peaks occurs until the present.

3.4. Stanol ratios

Ratios of faecal stanols are often used to infer the presence of humans or other animals (e.g. Prost et al., 2017; Vachula et al., 2019) given that there are different relative concentrations in the faeces of different mammals. We analysed the ratio

$(\text{coprostanol} + \text{epicoprostanol}) / [(\text{coprostanol} + \text{epicoprostanol}) + \text{stigmastanol}]$, and compared this to thresholds proposed in other studies to infer a human presence. We note that changes in this ratio do not necessarily indicate changes in human population density, but may be informative about the relative abundance of humans relative to other mammals, specifically grazing herbivores.

This ratio is highly variable throughout the Itzan record, with an average of 0.30 ± 0.33 . Using a threshold based on old-world grazing mammals (Prost et al., 2017) in order to define thresholds for human (0.73) and herbivore (0.29) input, 30% of timepoints indicate a definitive human presence, spanning time points across the

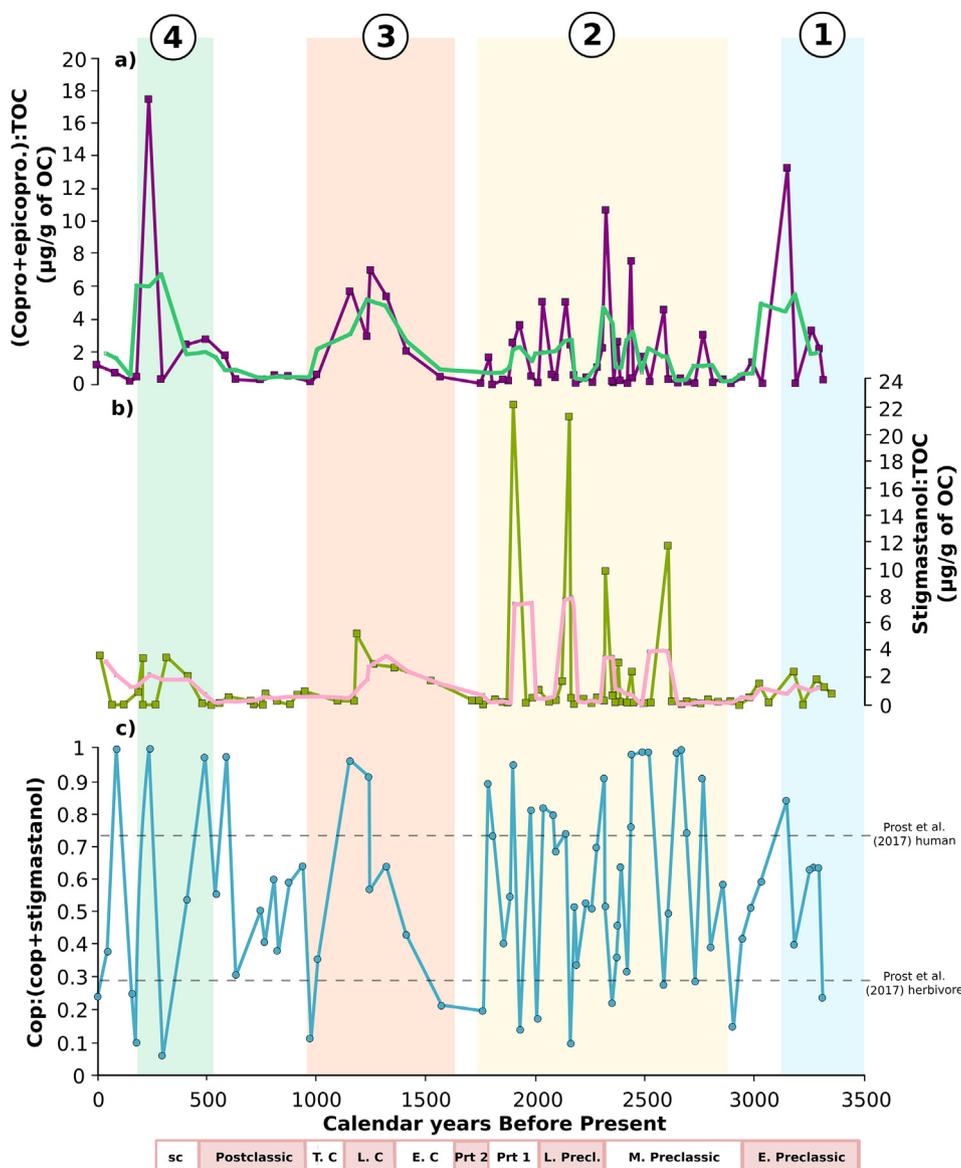


Fig. 5. Graphs showing a) coprostanol normalised to TOC from Itzan core (in purple). The mint green line show smoothed coprostanol:TOC records with a period set to 3 (approximately 100 years). b) stigmastanol normalised to TOC from Itzan core (green line). The pink lines show smoothed stigmastanol:TOC records as in a); c) the ratio of coprostanol to coprostanol + stigmastanol using the original data. The upper threshold for human presence is defined by Prost et al. (2017) and the lower threshold for herbivores defined by Prost et al. (2017). The numbered periods refer to periods of change correlated across proxy records, referred to in the discussion. A Maya archaeological timescale is show at the bottom of the plot. Prt = Protoclassic, E. C. = Early Classic, L. C. = Late Classic, T. C. = Terminal Classic and sc = Spanish Contact. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

entirety of the record, including the early coprostanol peak at 3170 cal yr BP.

4. Discussion

Given the proximity of the Laguna Itzan to the population centre of Itzan, we expect the dominant control on variation in faecal stanol concentrations to have been population size and the location of settlements relative to the lakeshore. However, as discussed below, other environmental and cultural processes may have influenced sedimentary stanol, including soil erosion rates, degradation of stanols in soils or sediments, population movements on the escarpment, and the waste management practices of ancient Maya populations. Zones refer to the coloured bands in Fig. 5 indicating time periods of interest.

4.1. Early occupation at Itzan

The cop:TOC record suggests that humans were present in the Itzan catchment during the Early Preclassic (3290 cal yr BP, Fig. 5a zone 1)—a date consistent with findings elsewhere in the Maya lowlands (Ebert et al., 2017). This date precedes the presence on the Itzan escarpment of Xe ceramics (Johnston, 2006) dated by Inomata et al. (2015) to around 2725 to 2650 cal yr BP, or the early Middle Preclassic period. In other words, the coprostanol record implies a human presence on the Itzan escarpment about 650 years before the archaeological record confirms it. Recent archaeological evidence reveals the periodic but temporary agglomeration of significant numbers of people from small local populations at the site of Ceibal, which is near Itzan, as well as at Aguada Fénix, located well to the north, during the early Middle Preclassic (Inomata et al.,

2019, 2020). Perhaps something comparable occurred on the Itzan escarpment as well. We cannot definitively discount the contribution of coprostanol derived from other large mammals living around the lake prior to human arrival, although we consider it unlikely that the assemblage of mammals in the region would have produced such high concentrations, as well as the high coprostanol:stigmastanol at 3170 cal yr BP.

A threshold based on Eurasian mammal assemblages (0.74; Prost et al., 2017), implies that humans are present at points across the record, including at 3170 cal yr BP. It is important to note that ratios below these thresholds do not rule out the presence of humans, but that higher values do indicate their presence based on our current understanding. Conversely, when the coprostanol:(coprostanol + stigmastanol) values are low it is possible that humans were absent, or at least were not major contributors to the sedimentary stanol input.

The coprostanol:TOC record suggests that human activity during this early period fluctuated over roughly 250 years, after which coprostanol concentrations declined towards the end of the Early Preclassic (3030 cal yr BP). Population reconstructions are complicated by the possibility that some Middle and Late Preclassic peoples inhabited residences that leave few recognisable archaeological traces (Hammond, 1991; Inomata et al., 2015).

4.2. Implications for Preclassic population dynamics

The Middle and Late Preclassic periods (2900–1880 cal yr BP) are characterised by high-amplitude variation in coprostanol:TOC ratios, with an average periodicity of 130 ± 53 years. This periodicity could reflect pulses of coprostanol to sediments derived from populations engaged in swidden agriculture, which potentially moved in and out of, and around, the Itzan catchment. This would lead to peaks of coprostanol as waste was washed into the lake during periods of occupation, even at relatively low population densities. This pattern could also possibly relate to changes in the transport and deposition in faecal stanols, although we do not observe major changes in sediment characteristics that match this periodicity (Fig. 4, Supplemental Figure S1). Each peak in coprostanol concentration from 2770 until 2320 cal yr BP gets larger, suggesting gradual but pulsed population growth over the course of 600–700 years. This possibility is independently suggested by large Middle Preclassic middens at Itzan (Johnston 2006). Intriguingly, comparable pulses in building and economic activity have been detected at roughly the same time at Ceibal (Inomata et al., 2017). Complicating matters, remains of later Classic buildings could bury evidence of Middle Preclassic architectural remains—a possibility highlighted by findings at nearby Ceibal (Inomata et al., 2019). We note that the size of the coprostanol peaks does not unambiguously reflect the size of the escarpment's population, as it may be influenced by other processes like transport, preservation, and waste management.

We observe a gap in coprostanol peaks between 2300 and 2140 BP in the Late Preclassic that coincides with a major decrease in grass pollen at L. Puerto Arturo, 85 km to the north of L. Itzan (Fig. 6c). While acknowledging that the timing and nature of vegetation changes at Puerto Arturo may not accurately reflect changes in the Itzan catchment, this association is intriguing, as it may imply that reduced regional populations led to a temporary reversal of regional deforestation. The Itzan Late Preclassic to Protoclassic 1 (2250–1850 cal yr BP) is characterised by variations in coprostanol concentrations that might be explained by the changing levels of activity at Itzan. The high-frequency peaks have similar coprostanol concentrations, and concentrations begin to decrease towards the end of the Protoclassic 1 (around 1880 cal yr BP). This contrasts with archaeological data that suggests that there

was steady population growth during the Middle and Late Preclassic (Johnston 2002). It is possible that this difference is caused by the low sampling resolution of faecal stanol concentrations, which may not fully capture population dynamics. Coinciding with a major population decline at nearby Ceibal (Inomata et al., 2017), coprostanol concentrations in Laguna Itzan decrease significantly around 1760 cal yr BP, suggesting a Protoclassic 2 and Early Classic (1760 to ca. 1570 cal yr BP) hiatus in Itzan settlement—a trend seen at other sites in the Río de la Pasión drainage system of Petén (Inomata et al., 2017).

4.3. Agreement with Classic Maya periods of growth and decline

Local political power is inferred to have shifted from the ceremonial centre of Chaak Ak'al, approximately 3 km north of Itzan, to Itzan between 1550 and 1350 cal yr BP (Johnston 2006). This is consistent with a sustained increase in coprostanol concentrations during this time (Fig. 5a zone 3), continuing into and peaking in the Late Classic (1240 cal yr BP). The coprostanol data suggests that population was more stable during the Late Classic and lacked the high-frequency variation seen in the Preclassic. However, the Early and Late Classic data are of relatively low resolution, and higher resolution analysis is needed to confirm this conjecture.

The Terminal Classic (ca. 1140 to 1000 cal yr BP) is marked by a relatively abrupt decline in coprostanol concentrations. The Terminal Classic was a time of major societal restructuring across the Maya lowlands, which has been hypothesised to be partly in response to dwindling water supplies as a result of climate change (Hodell et al., 1995; Medina-Elizalde et al., 2016; Rosenmeier et al., 2002). In line with the archaeological data (Johnston 1994), coprostanol concentrations are low during the late Terminal Classic–Early Postclassic transition (900 cal yr BP), suggesting that the area sustained only a small population after the Terminal Classic abandonment of the Itzan site core.

4.4. Population variability from the Postclassic to the present

The coprostanol record suggests a small increase in population at the end of the Late Postclassic (590–490 cal yr BP, Fig. 5a zone 4) followed by a decline shortly before or after European contact (470–300 cal yr BP). The record's largest single peak dates to 235 cal yr BP. Around the same time, in 253 cal yr BP, Spanish soldiers attacked and defeated the last Maya stronghold in the southern lowlands—Nojpeten, now known as Flores, in Lake Peten-Itza (Jones, 1998). The Maya inhabitants of now-subdued lakeside towns fled into the forest (Jones, 1998). It is possible that some of these refugees, or others displaced by them, migrated south, including to the shores of Laguna Itzan, causing a significant but short-term increase in population. Finally, the small peak at 80 cal yr BP is consistent with a Lacandon settlement at Itzan, which was visited by the naturalist Karl Sapper in 59 cal yr BP (Sapper, 1891).

4.5. Evidence for an association between climate change and demographic trends

At 3050 cal yr BP the early peak in coprostanol concentrations decreases at a time that coincides with evidence for a dry climate in the southern Maya lowlands (Fig. 6b, D-2; Douglas et al., 2016; Douglas et al., 2015; Kennett et al., 2012; Rosenmeier et al., 2002; Wahl et al., 2014). This period of drought and its effects are not as well studied as drought during the Terminal Classic, but the available data suggests it was of a similar magnitude in this region. Our data suggests that this period of a drier climate was associated with decreased coprostanol input and a reduction in Itzan's small

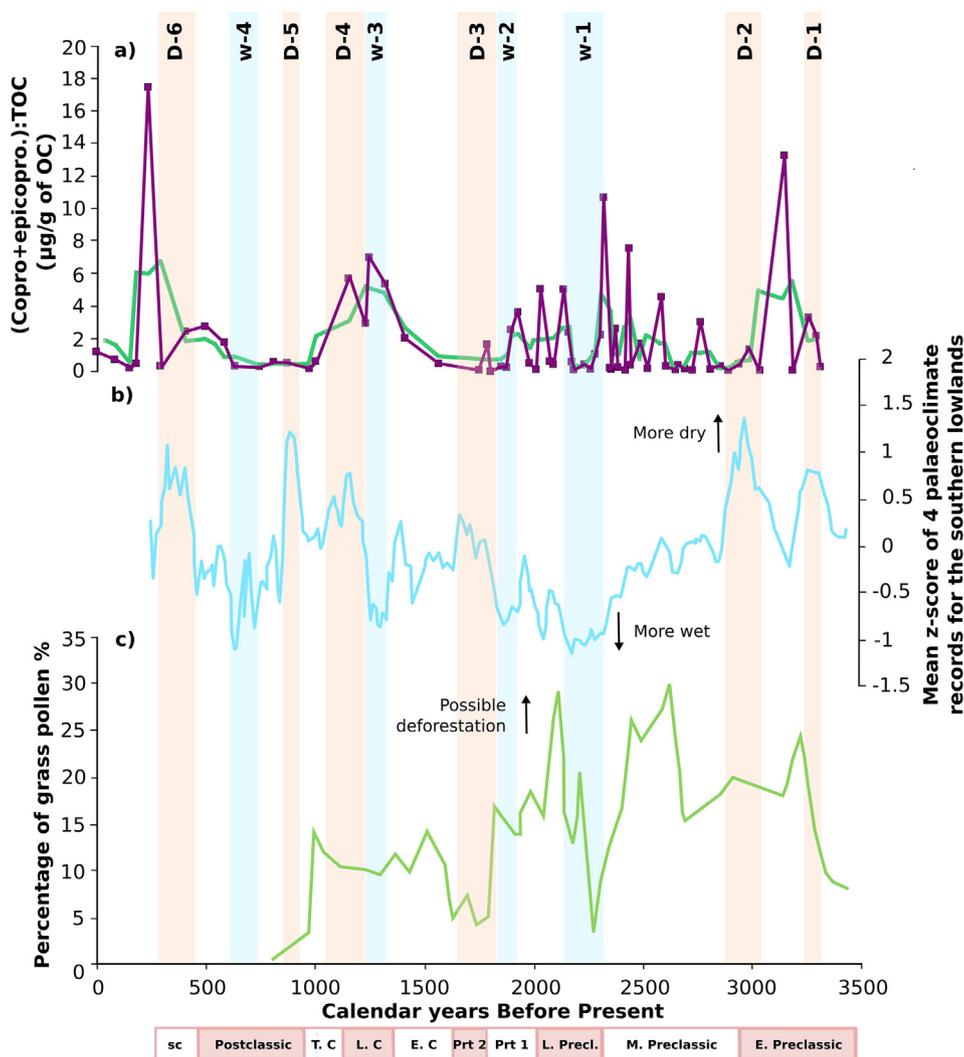


Fig. 6. Graphs showing a) coprostanol normalised to TOC from Itzan core (in purple). The mint green line show smoothed coprostanol:TOC records with a period set to 3 (approximately 100 years); b) mean z-score for the southern lowlands from Douglas et al. (2016) indicating variation in precipitation based on four palaeoclimate records (Douglas et al., 2015; Kennett et al., 2012; Rosenmeier et al., 2002; Wahl et al., 2014); and c) percentage of grass pollen (poaceae) at Puerto Arturo (Wahl et al., 2006). The coloured bars highlight the dry and wet periods across the records (D-1: dry period 1, w-1: wet period 1, etc.). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

population, possibly because early occupation of the catchment (even by low numbers of people) was negatively impacted by reduced rainfall. Given that the escarpment was likely populated only by a small number of mobile hunter-gatherers who cultivated some domesticates (Inomata et al., 2015), the relationship between the population dynamics impact on the coprostanol record is unclear.

As described above in Section 4.2 (Ebert et al., 2017), the relatively stable climate conditions that followed this period of drought set the stage for the later cultural developments, including rain-fed swidden agriculture and high-frequency variation in catchment population. However, a particularly wet period (w-1) between 2350 and 2160 cal yr BP is coeval with the co-occurrence of low abundances of grass pollen at L. Puerto Arturo (Fig. 6; Wahl et al., 2006) and ca. 150-year period of low coprostanol concentrations at L. Itzan. This period is puzzling because it coincides roughly with the growth of Itzan’s late Middle Preclassic and early Late Preclassic populations, as evidenced by the presence of abundant ceramics dating to this era at many locations. This association of an anomalously wet climate with a centennial scale reduction in regional

deforestation and decrease in coprostanol input is intriguing, and may be an indicator that Maya society was also sensitive to other climatic extremes such as with excess precipitation. Coprostanol input resumes later in the Late Preclassic when the climate starts to become relatively drier. Excess water was an issue at other lowland Maya sites where it interfered with agriculture and degraded water quality (Lucero et al., 2011). At Ucanal, in northern Guatemala, causeways and canals were constructed during the Classic to deal with excess water (Halperin et al., 2019).

Within the record we observe two additional linkages between evidence for drought and reduced coprostanol concentrations. An extended period of progressively drier conditions between 1860 and 1670 cal yr BP coincides with the reduction in coprostanol concentration beginning around 1760 cal yr BP. While the magnitude of this drying event is not as large as others identified in the record, it does represent substantial drying relative to the wet conditions of the Late Preclassic. The co-occurrence of low abundances of grass pollen at L. Puerto Arturo at this time suggest the potential regional recovery of forests due to population reductions or dispersals in many areas of the southern Maya lowlands (Fig. 6;

Wahl et al., 2006).

Finally, the Terminal Classic (ca. 1140 to 1000 cal yr BP) is marked by an abrupt decline in coprostanol concentrations that occurred in the context of a well-documented regional drought (Fig 6 D-4; Hodell et al., 1995; Rosenmeier et al., 2002). The Terminal Classic was a time of major societal restructuring across the Maya lowlands which has been hypothesised to be partly in response to dwindling water supplies as a result of climate change (Hodell et al., 1995; Medina-Elizalde et al., 2016; Rosenmeier et al., 2002). The decline in coprostanol concentrations in L. Itzan offers further evidence that the socio-political decline during the Terminal Classic drought was associated with depopulation, at least at some sites. Archaeological evidence from major population centres across the lowlands such as Tikal also indicates substantial depopulation at this time (Lentz et al., 2020). In line with the archaeological data, coprostanol concentrations are low during the late Terminal Classic-Early Postclassic transition (900 cal yr BP), suggesting that the Itzan area sustained only a very small population after the Terminal Classic abandonment of the Itzan site core. Dry conditions persisted in the southern Maya lowlands until ca. 800 cal yr BP, and may have contributed to the long-term absence of a major human population in the catchment during the early Postclassic.

The association of climatic change and coprostanol concentrations in the Postclassic and colonial periods is less apparent. The arrival of the Spanish in the region and associated diseases likely added additional drivers for population change that may have complicated the relationship between climatic change and human demographic dynamics.

4.6. Changes in stigmastanol concentrations

We interpret the stigmastanol:TOC record (Fig. 5b) as primarily reflecting the abundance of herbivorous grazing mammals, such as deer, in the Itzan catchment. However, the same environmental processes that may have influenced coprostanol concentrations also apply to stigmastanol. Furthermore, faecal stanol concentrations are not well studied in Neotropical mammals, and therefore direct inferences in terms of animal sources are not possible.

There are some key differences between the coprostanol:TOC record and the stigmastanol:TOC record. Stigmastanol peak concentrations tend to be much higher (up to 20 µg/g, versus ~3–5 µg/g for coprostanol; Fig. 4) during the Late Preclassic than the Early Preclassic. At the same time the frequency of stigmastanol peaks in the Preclassic is lower. The two stanols follow a similar pattern in the Classic period, but diverge at 1162 cal yr BP where stigmastanol exhibits an abrupt decline and input becomes zero and does not recover until 410 cal yr BP. Coprostanol input continues and does not decrease until 1080 cal yr BP. This could represent a decrease in herbivores living around the lagoon during the Postclassic.

The high stigmastanol:TOC values seen at several points in the Middle Preclassic do not re-occur in the record, even after the arrival of the Spanish and the late-1600s introduction of European livestock and modern agriculture. This suggests that despite these major ecological changes, herbivore populations never recovered to the peak levels of the Middle Preclassic. It is unclear what herbivores would have led to high stigmastanol concentrations in the Preclassic, although deer were often associated with Maya settlements (Sharpe et al., 2018). The putative association of elevated short-term herbivore populations with possible evidence for shifting or swidden agriculture in the Preclassic is interesting, and merits further exploration. The largest stigmastanol peaks generally occur after abrupt declines in coprostanol. One potential explanation for this pattern is that herbivores successfully moved into the open ecological space left by a human population decline within the catchment, with abandoned agricultural fields providing

good habitat for foraging. The short duration of these stigmastanol peaks may reflect that this large increase in herbivore populations was unsustainable.

4.7. Possible influences of land use practices on the Itzan coprostanol record

We see a relationship between faecal stanol concentrations and broader lowland Maya cultural trends, with sharp declines in faecal stanol input at the end of the Middle Preclassic, the end of the Late Preclassic, and the end of the Terminal Classic. These patterns are consistent with archaeological inferences of societal and demographic change at these times.

It is notable that peak coprostanol values during the Late Classic are lower than for the Middle and Late Preclassic, although the concentrations are more sustained. When a moving average is applied (Fig. 5a), the relative concentrations for these periods are more similar, and the Classic period presents overall higher time-averaged values. Lower peak concentrations during the Late Classic period are unexpected because dense populations are indicated by archaeological evidence. One possible explanation is that high peak values in the Preclassic were influenced by greater soil erosion, as indicated by the overall high magnetic susceptibility measurements during this time (Fig. 4c).

The relatively low coprostanol concentrations during the Late Classic could also indicate that during this period human waste was diverted from the Laguna Itzan catchment, perhaps because of shifts in land use, including agricultural strategies. Low magnetic susceptibility values during this time indicate low soil erosion rates, which could reflect a change in agricultural practices, and may have resulted in reduced transport of stanols to the lake. In addition, it is possible that human waste was used agriculturally to replace soil nutrients lost through deforestation, erosion and soil degradation. Nightsoil is human excrement collected to be used as fertiliser, and could have been used to boost soil productivity (Bacon, 1956; Kawa et al., 2019). Probable Late Classic nightsoil use in household agricultural plots at the site of Quim Chi Hilam, which is near Itzan, is indicated by phosphorous enrichment (Dunning et al., 1997). Evidence for the use of nightsoil as fertiliser is also well documented in archaeological sites elsewhere in Mesoamerica (Evans, 1990; Tuerehout and Weeks, 2005). Similar practices could have been used at Itzan to intensify agricultural production in response to population growth. Agricultural fields, inferred primarily through the presence of ancient houses, would have been found on the well-drained slopes that descend eastward from the escarpment crest to the lagoon. Fields to the west of the escarpment crest would have drained to the west and southwest. To account for the relatively low coprostanol in the sediment core during the Classic, nightsoil use could have included measures to prevent the flushing of faecal matter into the lake. The implementation of nightsoil as an agricultural strategy at Itzan, combined with other land use and waste management practices, could account for both the escarpment's ability to sustain high Late Classic population levels, as inferred from archaeological records, and the lagoon's coeval "missing" faecal stanol input relative to archaeological evidence for larger populations in the Classic relative to the Preclassic.

The archaeological work carried out at Itzan did not include the reconstruction of periodised population trends, although general trends can be surmised from the site's mapped and excavated architecture (Johnston, 2002). Thus, there are no fine-grained temporal population reconstructions to which to compare to the Itzan faecal stanol record. Yet the fact that the Early Preclassic faecal stanol record closely tracks magnetic susceptibility evidence of soil erosion, and, thus, probable forest clearing, and 19th century evidence of forest disturbance, attributable to Lacandon inhabitants,

indicates that the faecal stanol record can serve as a proxy for human presence, as well as the alteration of Maya forested landscapes when combined with palaeoenvironmental evidence. However, the relationship between the stanol concentrations and human population densities may not be constant or unambiguous over time, as is suggested by the discrepancy between archaeological evidence and coprostanol:TOC in terms of relative population between the Preclassic and Classic periods. Faecal stanols can therefore be useful in complementing the archaeological record (White et al., 2020; Skousen and Aiuvalasit, 2021; White et al., 2021).

Perhaps most intriguingly, coprostanol data implies the presence of human populations during periods for which there is no recognised archaeological evidence, implying populations who lived in archaeologically “invisible” residences (Johnston, 2004). For example, the Itzan coprostanol data suggest that remnant and presumably small Maya populations continued to reside in parts of the southern lowlands long after the Classic-period collapse—a phenomenon confirmed palaeoecologically elsewhere in the Pasión drainage (Johnston et al., 2001)—while not leaving behind a clear archaeological record. It also suggests the presence of humans during the Early Preclassic, well before any dated archaeological evidence in the area. Moreover, coprostanol data could aid in understanding ancient land use and waste management practices that leave few if any traces in the archaeological record.

5. Conclusions

This study shows that faecal stanols offer a new and exciting opportunity to determine past population and land use changes in the Maya lowlands. The Laguna Itzan sediment core record implies that an early human presence in the Lake Itzan catchment dates to the Early Preclassic. The coprostanol record provides evidence for declining human population associated with three periods of drought, during the Terminal Classic (1220–1050 cal yr BP), the Protoclassic 2 (1860–1670 cal yr BP), as well as less well-studied drought between 3300 and 2900 cal yr BP during the Early to Middle Preclassic periods. Our results also point to a 150-year decline in coprostanol input in the Late Preclassic that coincides with both an extreme wet period and evidence for a recovery of tropical forests from a regional pollen record.

High-frequency variability in coprostanol concentrations during the Middle and Late Preclassic is suggestive of centennial-scale changes in population dynamics, potentially related to shifting agricultural practices or population shifts atop the escarpment. In contrast, the data for the Classic period indicates less frequent variation, implying a more stable population. The stanol record during the Classic period is broadly consistent with archaeological evidence for the timing of population growth and decline. However, the relatively lower concentrations in the Classic period relative to the Preclassic may relate to Late Classic changes in land use practices. Specifically, we suggest that reduced soil erosion, alongside possible use of nightsoil in intensified agriculture, may have reduced the input of faecal stanols into lake sediments.

Overall these data imply that faecal stanols have a strong potential to serve as proxies for changes in human and animal populations in Mesoamerican landscapes, while also providing insights into land-use change. Therefore this is a promising method that can be used to complement archaeological datasets for demographic change, with the goal of linking human Mesoamerican population dynamics with palaeoenvironmental change.

Author statement

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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

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