Fecal stanols show simultaneous flooding and seasonal precipitation change correlate with Cahokia's population decline

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A number of competing hypotheses, including hydroclimatic variations, environmental degradation and disturbance, and sociopolitical disintegration, have emerged to explain the dissolution of Cahokia, the largest prehistoric population center in the United States. Because it is likely that Cahokia's decline was precipitated by multiple factors, some environmental and some societal, a robust understanding of this phenomenon will require multiple lines of evidence along with a refined chronology. Here, we use fecal stanol data from Horseshoe Lake, Illinois, as a population proxy for Cahokia and the broader Horseshoe Lake watershed. We directly compare the fecal stanol data with oxygen stable-isotope and paleoenvironmental data from the same sediment cores to evaluate the role of flooding, drought, and environmental degradation in Cahokia's demographic decline and sociopolitical reorganization. We find that Mississippi River flooding and warm season droughts detrimental to agriculture occurred circa (ca.) 1150 CE and possibly generated significant stress for Cahokia's inhabitants. Our findings implicate climate change during the Medieval Climatic Anomaly to Little Ice Age transition as an important component of population and sociopolitical transformations at Cahokia, and demonstrate how climate transitions can simultaneously influence multiple environmental processes to produce significant challenges to society.

Cahokia | fecal stanols | paleodemography | paleoclimate

The Cahokia archaeological complex became the largest prehistoric population center in the United States early in the second millennium CE (Common Era), but the site was principally abandoned within several centuries (1, 2). Explanations for the exodus from the region largely focus on environmental and sociopolitical factors and draw from a wide range of geological, paleoclimatological, and archaeological evidence. Environmental explanations include multidecadal drought (3, 4), changes in seasonal precipitation (5), changes in the magnitude and frequency of Mississippi River flooding (6), environmental degradation via deforestation (7, 8) and resource overexploitation (9). Social explanations primarily focus on political collapse, internal factionalization, and economic decline (2, 10–13).

Despite decades of work, the relationship between the environment and Cahokia's decline has remained unclear. Previous studies could not directly compare environmental stressors and environmental degradation with Cahokia-region population because the population reconstructions were based on separate studies that relied on ceramic chronologies and archaeological data sets, whereas the paleoenvironmental analyses were tied to dendrochronology (3, 4) or calibrated radiocarbon dates that have significant uncertainties (5, 6). Chronological uncertainties between population and environmental reconstructions derived from independent age models, in addition to geographical differences between study sites, limit the confidence in such comparisons and render potential conclusions equivocal.

Pauketat and Lopinot (14) estimated population for Cahokia by extrapolating from excavations in two centrally located areas of the site, and Milner (2) formed models of Cahokia-region population change using architectural data derived from excavations in more than five centrally located areas within the site. Although the population sizes estimated by these two studies differ, both studies concluded that the highest population at Cahokia occurred during the Lohmann phase, c. 1050–1100 CE. Furthermore, Pauketat and Lopinot (14) estimated that the population at Cahokia during the Lohmann phase was five to 10 times higher than in the preceding century and a half, and that it did not decline to pre-Mississippian levels until the 14th century.

This study directly compares reconstructions of Cahokia-region population change inferred from fecal stanol concentrations (15) with changes in hydroclimate inferred from oxygen-isotopic analysis and grain size within two cores, HORM12 and 15HSL, from Horseshoe Lake, an oxbow lake on the Mississippi River floodplain in southern Illinois (Fig. 1). The lake contains within its watershed the Cahokia Mounds Historic Site (UNESCO No. 198), a massive mound complex and major prehistoric population center. By comparing demographic reconstructions and paleoenvironmental data from the same core, changes in the region's population size and environment can be evaluated on the basis of stratigraphic information, eliminating timing uncertainty related to age dating techniques.

Significance

Our article examines the relationship between the population size of Cahokia, one of the most significant archaeological sites in North America, and evidence for major flooding and drought events. We use changes in the concentrations of fecal molecules contained in lake sediment as a proxy of population change and directly compare these variations with paleoenvironmental data from the same sediment core. These data show that a shift to decreased summer precipitation and a Mississippi River flood occurred circa 1150 CE, coinciding with a decline in the region's population and a major climate transition. Our study highlights the importance of multiple, concurrent environmental stressors in combination with societal tensions as contributors to sociopolitical change.

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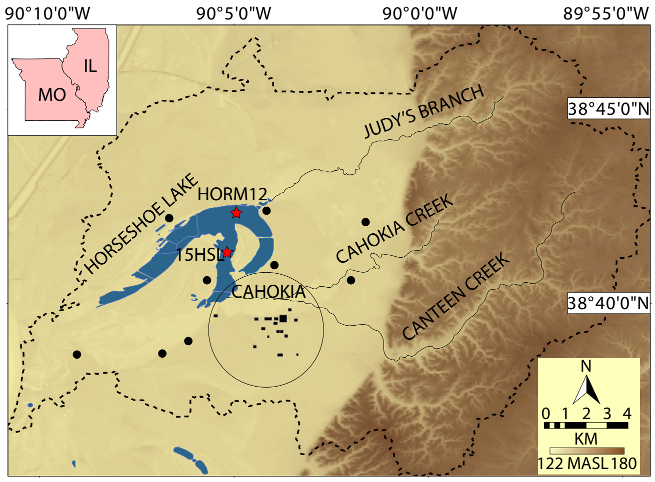

Fig. 1. Cahokia region and Horseshoe Lake watershed, shown as the black dashed line. Dark brown colors indicate higher topography, principally the river bluffs, and the yellow indicates the Mississippi River floodplain. Coring sites are indicated by red stars. The Cahokia complex is approximated by the large circle around black rectangles showing the position of some of the mounds at the site. Black dots show the locations of other sites with mounds within the Horseshoe Lake watershed that were occupied contemporaneously with Cahokia [~1000–1400 CE (2)]. Base map elevation data are derived from the National Elevation Dataset (57).

Environmental Hypotheses for Cahokia's Population Decline

Multiple hypotheses for Cahokia's sociopolitical reorganization have been presented that focus on environmental changes. The environmental degradation hypothesis argues that wood overexploitation and agricultural ground clearing increased watershed erosion and possibly led to flooding, standing water, and saturated soil conditions through higher sedimentation rates (8, 16). In the flooding hypothesis, Munoz et al. (6) identified multiple Mississippi River flood events from HORM12 grain size data and proposed that an increase in the frequency of high-magnitude floods after several centuries without major floods played a synergistic role in Cahokia's decline in concert with economic, social, and political changes. One of the largest events, flood event V, has a modeled median age of ca. 1160 CE, and Munoz et al. (6) postulated that this flood occurred at the onset of Cahokia's depopulation and sociopolitical reorganization.

Other hypotheses suggest that changes in hydroclimate, including multidecadal droughts (4, 17) and shifts in the seasonal distribution of precipitation (5), made agricultural untenable for a large population. Benson et al. (4, 17) used two tree-ring inferred Palmer Drought Severity Index (PDSI) reconstruction records from Illinois and Missouri to postulate that a series of long and severe droughts affected prehistoric North American populations. They contend that Cahokia emerged during an unusually wet 11th century, but suffered from several long and intense droughts that began at ~1150 CE (4).

In the seasonality of precipitation hypothesis (5), variations in the isotopic composition of endogenic carbonate from Martin Lake in northeastern Indiana are interpreted as responding to changing precipitation source and seasonality as the region transitioned out of the Medieval Climatic Anomaly (MCA) and into Little Ice Age (LIA) conditions. In this hypothesis, high δ^{18}O values of the 11th and 12th centuries indicate regional rainfall dominated by summer moisture advected from the Gulf of Mexico. Lower δ^{18}O values of the early 13th century represent a shift to a Pacific moisture source, with precipitation advected from the northwest across the Rocky Mountains during winter storms, and thus having a signal depleted in ^{18}O (5). As summer precipitation is crucial to maize agriculture, on which Mississippian cultures depended, Bird et al. (5) argued that the higher summer precipitation in the 11th and early 12th centuries was conducive to population growth, whereas a reduction of summer rainfall in the late 12th and 13th centuries led to warm season droughts that limited agricultural production. However, Martin Lake is 500 km to the northeast of Cahokia, and may have a different climatic history, making extrapolation from that isotopic record to Cahokia tenuous.

Methodological Background

Fecal stanols, notably coprostanol and epicoprostanol, are organic molecules that originate in the guts of humans and may persist in sediment for hundreds to thousands of years (18). Fecal stanols deposited in sediment provide evidence of trace human waste products, and are used as a proxy for measuring population change over time (15, 19). Although other mammals, including dogs, donkeys, seals, horses, goats, and cattle, produce coprostanol, only sheep and pigs are known to generate sufficient quantities that could mask changes in human stanol concentration (20–23), and neither domesticate was present in the Cahokia area before Euroamerican settlement (24). White et al. (15) demonstrated the validity of this approach by producing a 1,200-y record of Cahokia region population change through fecal stanol analysis of Horseshoe Lake sediment that parallels population trends identified by previous demographic reconstructions derived from archaeological evidence (2, 14). Because smaller sites contemporary with Cahokia's occupation are present in the watershed (2), and the proximity of a population to drainages and the lake may affect the amount of fecal stanols at each coring site, the Horseshoe Lake fecal stanol record (15) captures population change at the watershed level. However, Cahokia is the most likely candidate for controlling the lake's fecal stanol signature because HORM12 and 15HSL show parallel trends; the stanol trends in both cores closely track the Cahokia population estimates of previous demographic reconstructions (2, 14); the footprint of Cahokia's inhabitants on the landscape likely expanded throughout much of the watershed through activities such as hunting, fishing, and gathering; modern hunter-gatherers (25) and rural farmers (26) without sewage systems frequently practice open defecation away from their homes, where much of their daily activities take place; archaeological surveys around Horseshoe Lake show fewer Mississippian Lohmann phase sites, c. 1050–1100 CE, compared with sites from the preceding century and a half, whereas within the watershed, the numbers of Lohmann phase sites and sites from the preceding phase are similar, which is evidence of shifting settlement locations associated with the rise of Cahokia (27, 28); and Cahokia is the largest archaeological site in the watershed, followed by the East St. Louis Mound precinct near the watershed's southwest corner (29).

To account for variations in degradation rate and low stanol abundance, the concentrations of coprostanol are reported in comparison with 5α-cholestanol, following Grimalt et al. (30), as a ratio of coprostanol to coprostanol and 5α-cholestanol. 5α-cholestanol is formed from the degradation of cholesterol by soil microbial communities (21). By relating coprostanol to 5α-cholestanol, we make a comparison of stanol input and preservation in a specific environment (5α-cholestanol) with stanol input from feces (coprostanol). Thus, high values of the stanol ratio indicate a large presence of humans in the region, and low values indicate a small human presence. Stanol data from two Horseshoe Lake cores, HORM12 and HSL15 (Fig. 1), indicate that the regional population rose rapidly in the 10th century CE to a maximum early in the 11th century (15). The watershed's population was in decline by the 12th century, and dwindled to a population minimum at ~1400 CE.

Reconstructions of hydroclimate from the lake sediments are based on δ^{18}O values of endogenic carbonates ($\delta^{18}O_c$), which in turn reflect changes in the δ^{18}O value of the lake water (*SI Appendix*, Figs. S1 and S2). Decreases in $\delta^{18}O_c$ values are often attributed to increases in effective moisture (precipitation minus evapotranspiration), which may be related to either increased precipitation and/or decreased evapotranspiration (31). However, in the midcontinental United States, intra-annual variations in the isotopic value of precipitation result in winter snows having more negative δ^{18}O and summer rains having more positive values. This difference is partly a result of variations in temperature (32–34) and partly variations in source region, with Pacific and Arctic air masses contributing to negative isotopic values in winter (35–37). Because the δ^{18}O value of river and lake water is determined by the weighted annual means of precipitation (38), shifts in the seasonal distribution of precipitation can also affect the $\delta^{18}O_c$ (5, 39). The $\delta^{18}O_c$ can also be affected by the residence time of the lake water. Long residence time, commonly associated with hydrologically closed basins, can amplify the evaporative concentration of ^{18}O. Thus, changes between open/closed status can influence the isotopic record (40).

Variations of sediment grain size in flood plain lakes can be linked to overbank flood events that transport and deposit the suspended load of the river into the lake, leaving an event bed with distinct compositional and geochemical properties (41, 42). Munoz et al. (6) identified a series of Mississippi River flood events through grain size distribution in sediment cores from Horseshoe Lake and another oxbow lake further downstream. We compare these paleoenvironmental data (5, 6) and fecal stanol data (15) within the same stratigraphic profile to produce interpretations that are independent of absolute chronology and temporal uncertainties associated with radiocarbon dating.

Results and Discussion

The sedimentary evidence for environmental and climatic changes in Horseshoe Lake, when combined with the stanol record from the same sediment archive, permits robust tests of environmental hypotheses proposed for sociopolitical and demographic changes at Cahokia. These hypotheses focus on environmental degradation and disturbance, through increased watershed erosion or flooding, and hydroclimate variation, through multidecadal droughts or changes in the seasonality of precipitation.

Environmental Degradation and Disturbance. Lopinot and Woods (16) and Woods (8) proposed that environmental degradation played a major role in Cahokia's population decline, through deforestation and subsequent increased watershed erosion. However, palynological data indicate that much of the native forest was cleared at ∼450 CE (43), well before Cahokia's population maximum in the 11th century. Although increased soil erosion is not the only cause of increased detrital mineral flux, it is anticipated that mineral content and mass accumulation rates would be higher during periods of maximum catchment disturbance related to mound building and agriculture. Mass accumulation rates of detrital minerals in HORM12 (43) are highest, predating the population maximum as defined by the fecal stanols, and drop abruptly in the 10th century. Mass accumulation rates are actually lowest during the population maximum and increase slowly as population declined (Fig. 2). Thus, increased soil erosion seems to occur as the region is depopulated, rather than immediately before. Although not definitive, this relationship suggests that if a link exists between anthropogenic disturbance and population dynamics, it is complex.

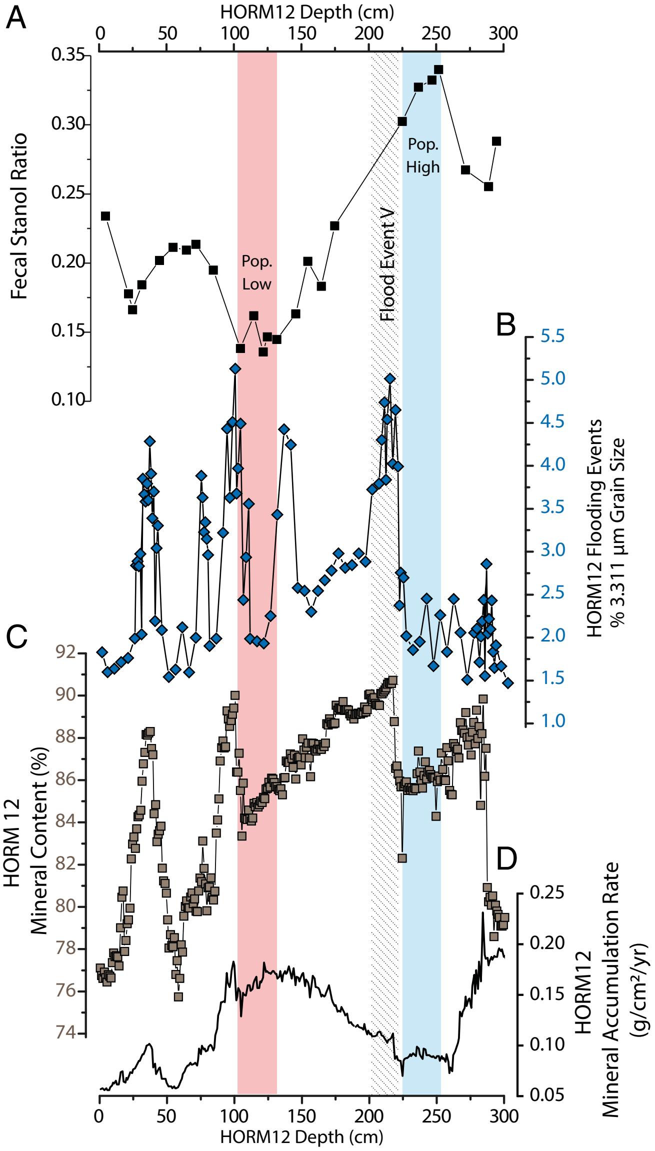

Fig. 2. HORM12 fecal stanol data (*A*) in relation to HORM12 flood events (6) (*B*), HORM12 loss-on-ignition-derived mineral content (43) (*C*), and HORM12 mass accumulation rates (56) (*D*).

Natural disturbance caused by Mississippi River floods may have contributed to Cahokia's social reorganization and depopulation. HORM12 fecal stanol values indicate a population maximum before flood event V of Munoz et al. (6), whereas after the flood, the fecal stanol ratio is lower than at any preceding time in the record (Fig. 2). The stratigraphic position of the decline in fecal stanols relative to the flood supports Munoz et al.'s (6) association between the timing of massive flooding of the Mississippi River and late 12th century sociopolitical changes in the Cahokia area that are indicated by evidence for regional population decrease, agricultural contraction, palisade construction, a significant reduction in the creation of new mounds, final capping of some mounds, catastrophic abandonment of some sites, and the reorganization of residential space at Cahokia (2, 11, 13, 29, 43). The majority (∼70%) of historical flood crests on the Mississippi River at Saint Louis (adjacent to Cahokia) occur in April–June, when crops are particularly sensitive to moisture and temperature (44, 45). Floodwaters on low-gradient rivers such as the Mississippi recede gradually over days or weeks, amplifying the effect of inundation on croplands. Although an increase in the fecal stanol ratio from 100 to 50 cm indicates the population may have increased in the mid-16th to mid-18th centuries, it is unlikely that floods had a significant effect on these later populations because protohistoric and early historic groups in the region, such as the Tamaroas, were semisedentary; maize agriculture was less central to their subsistence than Mississippian groups; and they used the landscape in a different way than Mississippians (46, 47).

Hydroclimate Variation. Despite the detailed information provided by both tree ring reconstructed PDSI measurements (4, 17) and δ^{18}O values of endogenic carbonate from Martin Lake in Indiana (5), the direct relationship between hydroclimate and Cahokia's regional population change has been obscured by chronological uncertainties of the associated age models and, in the case of Lake Martin, by distance to Cahokia. We present $\delta^{18}O_c$ data from 15HSL as a proxy of hydroclimate to evaluate Benson et al.'s (4, 17) and Bird et al.'s (5) climate change hypotheses in relation to changes in Cahokia region population, as interpreted from fecal stanol data. We measured $\delta^{18}O_c$ values on 44 15HSL sediment samples with multiple replicates. $\delta^{18}O$ values ranged from −9.3‰ to −6.05‰ (*SI Appendix*, Table S1). Carbonates are not present in sufficient concentration for isotope analysis between 12 and 82 cm in 15HSL; thus, $\delta^{18}O$ values provide only a partial picture of regional hydroclimatic change. $\delta^{18}O$ values are moderately high during Cahokia's population maximum and the initial drop from its peak (230–240 cm), alternating between approximately −6.5‰ and −7.5‰ (Fig. 3). $\delta^{18}O$ values then rapidly decline to approximately −8.0‰ to −9.0‰ during Cahokia's depopulation. The low $\delta^{18}O$ values are interrupted by a positive excursion between 217 and 220 cm in core depth at the base of flood event V, and may signal the influx of river water with a different isotopic composition. Values remain low (average, approximately −8.5‰) from 217 to 187 cm (ca. 1150–1250 CE) before increasing steadily to a maximum value of −6.05‰ during Cahokia's regional population low.

The low values of $\delta^{18}O_c$ during the population decline at Cahokia can be interpreted in multiple ways: as an increase in effective moisture; reduction in hydrologic residence time of the lake water, and hence a decrease in the evaporative modification of the lake water; or a shift in the mean weighted isotopic value of the lake water, resulting from a shift to more negative winter precipitation values. All these can work independently or in combination; however, any viable interpretation of the HSL $\delta^{18}O_c$ record must be consistent with other proxy data. Tree ring reconstructions of PDSI from north and south of Cahokia show three multidecadal droughts from ∼1125 to 1250 CE (4). Modern tree growth in southern Illinois occurs primarily in the late spring and summer (48). The tree ring records that form the basis of PDSI reconstructions also reflect late spring and summer seasonality (4). We expect droughts of the magnitude described by Benson et al. (4) to be recorded as more positive $\delta^{18}O$ values in the carbonates of Horseshoe Lake during Cahokia's population decline, as evaporative modification of the lake water preferentially removes ^{16}O.

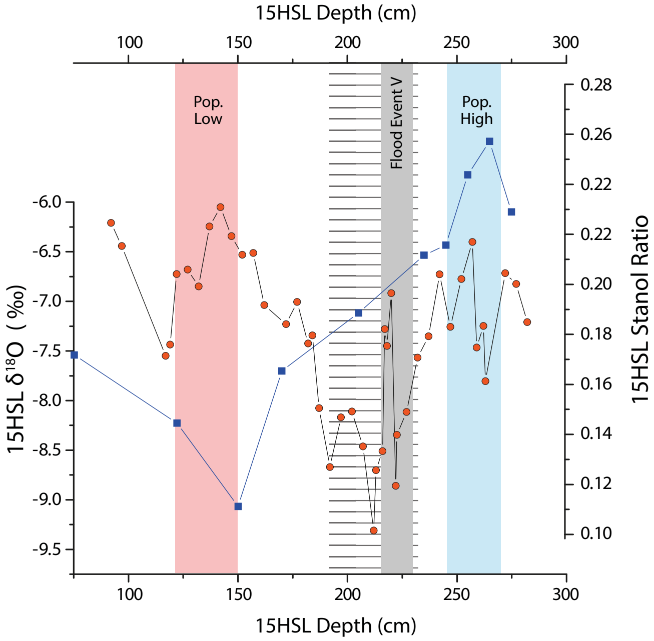

Fig. 3. 15HSL fecal stanol data (blue) plotted against 15HSL $\delta^{18}O$ data (orange). The horizontal lines mark a period of limited warm season precipitation, as interpreted from negative $\delta^{18}O$ values.

However, the droughts are coincident with a marked decrease and sustained low $\delta^{18}O_c$ values. To sustain low $\delta^{18}O$ values during droughts requires either that the lake became hydrologically open to the Mississippi River or that the weighted $\delta^{18}O$ value of the lake water decreased substantially. Maps reconstructed by Milner (2) indicate that in the early 19th century, Horseshoe Lake was connected by a tie channel to the Mississippi River and to other lakes in the floodplain. An isotopic measurement from the 19th century (*SI Appendix*, Table S1), when the lake was open to the Mississippi River, is similar to isotopic values from the peak population in the 11th century and the population minimum in the 15th century, suggesting that if a tie channel was present through the 19th century, it did not reduce the residence time of the lake water enough to decrease the $\delta^{18}O$ value to that seen in the period of the population decline.

Alternatively, a shift in the relative contribution of winter precipitation is compatible with the model proposed by Bird et al. (5). The low $\delta^{18}O_c$ values during population decline are similar in timing and duration to an initial decrease in $\delta^{18}O_c$ at Martin Lake (Fig. 4). While acknowledging uncertainties from different chronologies, $\delta^{18}O$ values from both sites are relatively high in the 11th and early 12th centuries CE, but reach a minimum in the early 13th century. $\delta^{18}O$ values increase out of the 13th century and reach higher values in the 14th century, after which the two records decouple.

The coincidence of decreasing stanol values (i.e., diminishing population size) with lower 15HSL $\delta^{18}O_c$ values is consistent with Bird et al.'s (5) and Benson et al.'s (4) hypotheses that growing season drought was detrimental to maize agriculture, and thus the Cahokia population (Fig. 4). Seasonal drought documented in the PDSI reconstruction is consistent with changes to the seasonality of precipitation outlined by Bird et al. (5); drought in reconstructed PDSI reconstructed from tree rings coincides with a dominance of winter precipitation and loss of spring/summer rains inferred from the isotope data. The correlation of high fecal stanol and $\delta^{18}O$ values at Horseshoe Lake supports Bird et al.'s (5) hypothesis that more positive $\delta^{18}O$ values during the MCA represent climatic conditions conducive to supporting large Mississippian populations in the midcontinent.

The transition of 15HSL $\delta^{18}O$ to more negative values occurred at ∼1150 CE (Fig. 4). The timing of this shift is significant because Benson et al.'s negative PDSI excursion and Munoz et al.'s (6) flood event V also took place at that time. After lows in the 13th century, $\delta^{18}O$ values increase to a maximum at ∼1400 CE, despite a continued decline of fecal stanols. The increase in $\delta^{18}O$ values may indicate a return to enhanced summer precipitation. Although this initially corresponds to a slight increase in $\delta^{18}O$ values at Martin Lake, the lake shows a second century-long decrease (∼1375–1425 CE) that is delayed by several decades in the 15HSL record. The decoupling of the records can be explained by regional climatic variation, uncertainties in chronologies, or evaporative enrichment of Horseshoe Lake carbonates from a potential closure of the basin overpowering the effect of precipitation source.

MCA to LIA Transition Hypothesis. The effect of climate change on human populations during the MCA and LIA is well documented worldwide (49–52). Previous studies (5, 6) noted that Cahokia developed during the warm MCA, but did not compare temperature reconstructions with a robust population proxy and other paleoenvironmental indicators. Cahokia's population maximum coincided with warm reconstructed North American mean temperatures (53) during the 11th century. Temperature and fecal stanol values then decrease in unison until the 13th century (Fig. 4). The correlation of reconstructed North American mean temperatures and Horseshoe Lake fecal stanol trends implies that large-scale climate variability is an important contributor to population change at Cahokia.

Notably, the transition appears to have occurred rapidly and uniformly, as $\delta^{18}O$ values, grain size, and tree-ring data all underwent major changes at ∼1150 CE. Thus, a major flood and reduced warm season precipitation occurred at the same time. Perhaps

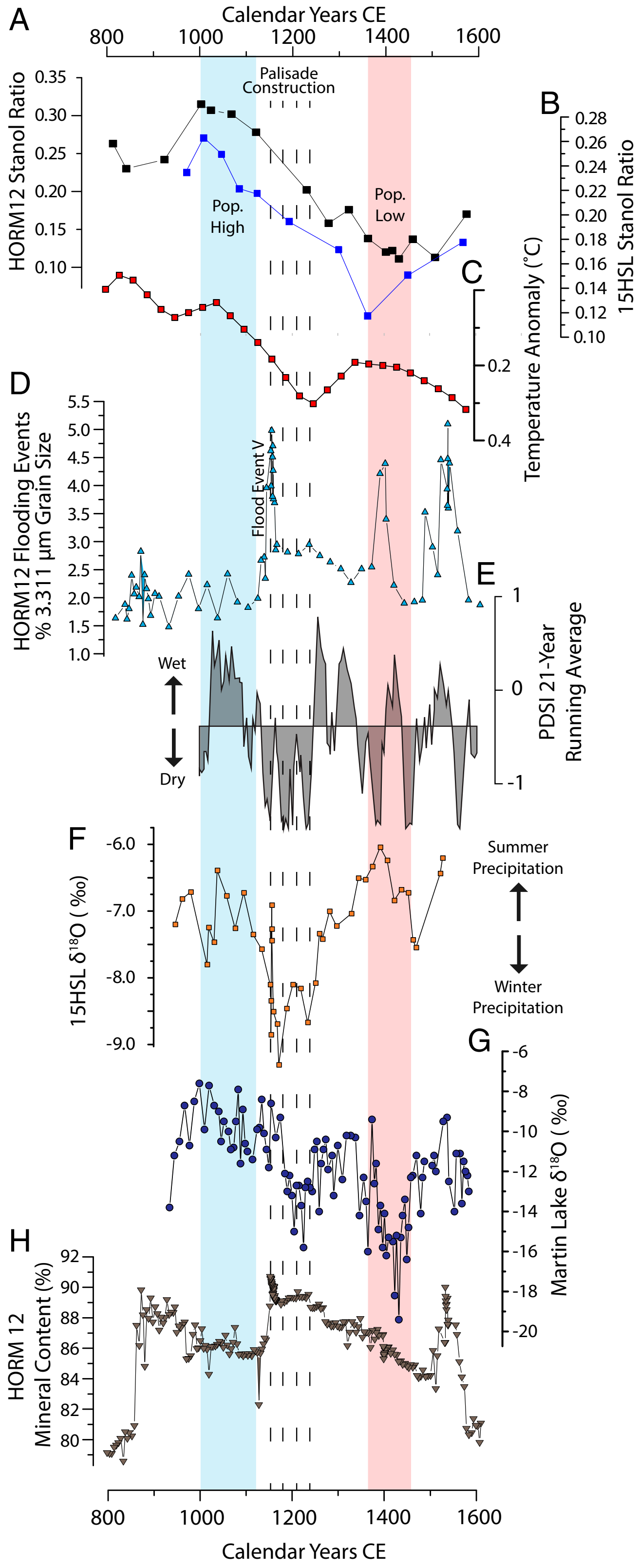

Fig. 4. Fecal stanol data (*A* and *B*) plotted against North American temperature reconstruction (*C*) (53), HORM12 flooding events (*D*) (6), regional PDSI reconstruction (*E*) (4), δ^{18}O data from 15HSL (*F*) and Martin Lake (*G*) (5), and HORM12 mineral content (*H*) (43). The chronology was established by Munoz et al. (43); time in calendar years (CE) is plotted from older (*Left*) to younger (*Right*). The erections of palisades are marked by dashed vertical lines (4, 13).

one environmental change would not have been consequential, but a combination of two or more changes would have presented significant challenges for a centralized, agrarian social system. Around this time, the inhabitants of Cahokia constructed a series of palisades that have been interpreted as indicators of societal stress (13), and several other lines of evidence indicate that there was a reorganization of Cahokia's sociopolitical structure, including the destruction of outlying population centers, decline in construction of earthen monuments at Cahokia, shifts in the prestige goods economy, and a contraction of agriculture (2, 10, 13, 29, 43, 54). The construction of the first palisade at the time of flood event V, followed by repeated rebuilding during the period of decreased summer season precipitation, along with evidence for considerable change in other aspects of Cahokian society, implies that environmental events were significant factors synergistically associated with Cahokia's population decline and reorganization.

Conclusions

Dry conditions during the growing season and a large flood event were major factors in the onset of Cahokia's population decline ca. 1150 CE, as interpreted from the stratigraphic relationships between δ^{18}O values of carbonates, sedimentology, and Horseshoe Lake fecal stanols. It is noteworthy that this result is independent of chronology. However, the findings are strengthened by integrating the work of Benson et al. (4), Munoz et al. (6), and Bird et al. (5), and outline a complex narrative of Cahokia's demographic decline. The agricultural implications of decreased warm season precipitation, coupled with a natural disaster in the form of a Mississippi River flood around 1150 CE, would have created stress for the Cahokia system atop cultural problems such as economic decline and political strife that cannot be measured through sedimentary analysis. We posit that climate change during the transition from MCA to LIA conditions is an important factor to consider in concert with social, political, and economic aspects of the occupation history at Cahokia and the surrounding area.

Methods

Core Selection and External Data. Two cores from Horseshoe Lake, an oxbow lake that includes the site of Cahokia within its watershed, provide the material for analysis. The first core, HORM12, was collected by researchers from the University of Wisconsin–Madison in 2012 and is the basis for two papers on environmental conditions at Cahokia (6, 43). A second core, 15HSL, was collected in 2015 by the lead author ~2 km south of the HORM12 site and closer to the input of Cahokia Creek (Fig. 1). Both cores were recovered using a modified Livingstone piston corer at water depths of ~1 m. The purpose of the second core was to test the robustness of the fecal stanol record (15) and to provide sufficient material for stable isotope analysis. The two cores are readily correlated on the basis of stratigraphic variations, particularly layers interpreted as representing flood deposits (6), and variation in loss-on-ignition data (*SI Appendix*, Fig. S3 and Table S2).

Munoz et al. (43) used Clam 2.2 (55) and dates from nine terrestrial accelerator mass spectrometry samples (*SI Appendix*, Table S3) to build an age model for HORM12. This age model also provided the basis for our analyses of Core 15HSL. In this study, we incorporate grain size distribution and mineral content data (6) and fecal stanol data (15) from core HORM12 (43) with fecal stanol data (15) and new stable oxygen isotope data from 15HSL. We used HORM12 mineral content data and the Clam 2.2 age model (56) to show HORM12 mass accumulation rates (*SI Appendix*, Table S4). White et al. (15) analyzed 29 HORM12 sediment samples and 13 15HSL sediment samples for fecal stanol content through overnight soxhlet extraction with 200 mL dichloromethane, derivatization into trimethylsilyl ethers through a reaction with N,O-bistrifluoroacetamide, and gas chromatography/mass spectrometry analysis. White et al. (15) identified stanol compounds by comparing characteristic mass spectra fragmentation patterns and gas chromatographic retention times of samples and blank spikes with the chemical standard solutions of these compounds, and quantified samples by comparing peak areas with a calibration curve and the relative response factor of an internal standard.

Oxygen Isotopes. We measured δ^{18}O values on endogenic carbonate from 44 15HSL sediment samples (*SI Appendix*, Figs. S1 and S2). We sampled historic occupation levels (2–112 cm) at 10-cm intervals, and prehistoric occupation levels (117–282 cm) at 5-cm intervals. All sediment was pretreated with 2% reagent-grade bleach to oxidize reactive organic matter. Sediment was then sieved at 65 μm to remove any ostracods and shell material that could influence the isotope values. We rinsed each sample four times with high-purity deionized water to remove the bleach before freeze-drying. Samples were analyzed on a Finnigan MAT delta-XP isotope-ratio mass spectrometer coupled to a GasBench II autosampler at California State University, Long Beach. We calibrated values with NBS-18, NBS-19, and an internal standard. Analytical precision is 0.12‰ for δ^{18}O; however, 10 replicates show greater variation within the sediment. Replicates can vary up to 0.5‰, likely because of the low concentration of carbonate in the sediment. Regardless of the low precision of individual samples, the general pattern of isotopic variation is not affected.

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