Journal of Archaeological Science: Reports 17 (2018) 640–649

Contents lists available at ScienceDirect

Journal of Archaeological Science: Reports

journal homepage: www.elsevier.com/locate/jasrep

Residue analysis of smoking pipe fragments from the Feltus archaeological site, Southeastern North America

Stephen B. Carmody[a,*], Megan C. Kassabaum[b], Ryan K. Hunt[c], Natalie Prodanovich[c], Hope Elliott[c], Jon Russ[c]

[a] Department of Social Science, Troy University 132d MSCX, Troy, AL, 36081, United States
[b] Department of Anthropology, University of Pennsylvania, 3260 South Street, Room 325, Philadelphia, PA 19104, United States
[c] Department of Chemistry, Rhodes College, 2000 North Parkway, Memphis, TN 38104, United States

ARTICLE INFO

Keywords:
Tobacco
Nicotine
GC/MS
Residue analysis
Feltus
Smoking pipe

ABSTRACT

The practice of pipe smoking was commonplace among indigenous cultures of the Eastern Woodlands of North America. However, many questions remain concerning what materials were smoked and when tobacco first became a part of this smoking tradition. Chemical analysis of organic residues extracted from archaeological smoking pipes is an encouraging avenue of research into answering questions regarding the development of a smoking complex within indigenous cultures of the Eastern Woodlands. In the right environmental conditions, absorbed organic compounds within artifacts can remain structurally stable for millennia, allowing analyses of organic matter to be performed on relics of advanced age. In this study, organic matter from six pipe fragments derived from the prehistoric Feltus site in Mississippi was extracted and analyzed via GC-MS, a process that allows for the identification of compounds in a complex mixture. Preliminary experiments tested the effects of pH on the efficacy of our extraction solvent to maximize the detectability of alkaloids such as nicotine. Several notable compounds were identified, including nicotine, which serves as a biomarker for tobacco.

1. Introduction

Tobacco (*Nicotiana* spp.) is widely recognized as the most sacred plant used by native North American groups, both past and present. At the time of European contact, Native Americans living in the Eastern Woodlands reportedly cultivated tobacco as widely as maize (Asch 1994; Dunavan and Jones 2011; Ford 1981; Winter 2000). However, despite ethnohistorical accounts of widespread tobacco use, archaeological evidence is seriously lacking, leaving these claims largely unsubstantiated. Tobacco seeds are infrequently recovered from archaeological sites while pipes are generally deemed to be tobacco pipes too frequently without physical evidence. Recently, chemical analyses of pipe residues have provided a new path for archaeologists to explore tobacco use from sites where tobacco seeds are absent (Eerkens et al. 2012; Rafferty 2002, 2006; Rafferty et al. 2012; Tushingham and Eerkens 2016; Tushingham et al. 2013). Here, we briefly discuss factors that may limit the identification of tobacco in archaeological contexts. We then report new data from Feltus (22Je500), a prehistoric mound site dating to the Late Woodland period (AD 400–1100) in Jefferson County, Mississippi (Fig. 1). We analyzed fragments from six ceramic pipes from Feltus using gas chromatography – mass spectrometry (GC-MS) to determine whether they contained nicotine, a biomarker for tobacco.

1.1. Site and samples

Feltus is a prehistoric Native American mound site located in the Lower Mississippi River Valley (see Fig. 1). The site sits on the edge of the aeolian bluffs overlooking the Mississippi alluvial plain and originally consisted of four mounds surrounding an open plaza (Fig. 2). The site was constructed and used during the Late Woodland period. A ring of midden defining a central plaza area indicates that much of the activity at the site took place during Baytown and early Coles Creek times (AD 400–850), before the mounds were built. The mounds were then constructed and used during middle Coles Creek times (AD 850–1100) before the eventual abandonment of the site. Three mounds stand today (A, B, and C), while the fourth (D) is no longer visible. Its location has been reconstructed based on historical documents (Wailes 1852; Steponaitis, 2012). Mounds A and B are flat-topped platform mounds that served either as foundations for structures or stages for activity. Mounds C and D were burial mounds.

Data from excavations undertaken by the Feltus Archaeological

* Corresponding author.
E-mail address: scarmody@troy.edu (S.B. Carmody).

https://doi.org/10.1016/j.jasrep.2017.12.011
Received 20 August 2017; Received in revised form 29 November 2017; Accepted 8 December 2017

Fig. 1. Map showing the location of the Feltus site.

Project from 2006 to 2012 support the conclusion that Coles Creek people inhabited Feltus episodically for some 400 years (Fig. 3), with little evidence of permanent habitation (Steponaitis et al. 2012, 2014). More specifically, analysis of the ceramic, floral, and faunal assemblages suggest that Feltus provided a location for periodic ritual events that included feasting, the setting and use of ceremonial posts, mound building, and burial of the dead (Kassabaum 2014). Kassabaum (2014, in press b) analyzed over 40,000 sherds from these excavations. Vessel form was identifiable on only a subset of these (n = 1131), and included beakers, bowls, jars, restricted bowls, and pipes. While pipes are quite rare compared to other vessel forms, they are more common at Feltus than at other Coles Creek sites, making up about 1.5% of the identifiable vessel assemblage (n = 18). Their presence has been interpreted as evidence for the ritual use of plants (Kassabaum, in press a; Kassabaum and Nelson 2016; Nelson and Kassabaum 2014); however, the identities of the plant species smoked in the Feltus pipes have remained a mystery. Paleobotanical analysis of plant remains recovered through flotation of feature fill and midden deposits at Feltus has identified potentially important ritual plants (e.g., nightshade, morning glory, sumac, pokeweed, and maygrass), but no tobacco seeds have been recovered (Kassabaum, 2014, in press a).

1.2. Issues with tobacco identification

Several factors potentially affect the identification of tobacco in archaeological contexts. Traditionally, identification of tobacco has relied on the recovery of small (0.5–1.1 mm) carbonized seeds. Carbonization affects plants differentially, based on the temperature of the fire, length of exposure of the plant material to fire, moisture content of the plant tissue, and the plant part's surface area (Braadbaart and Wright 2007; Lentz et al. 2001; Wright 2008, 2010). During the carbonization process, bioorganic compounds are converted to more stable substances, making them more durable and protecting the remains from elemental decay and microbial activity (Lopinot 1984; Miksicek 1987; Hastorf and Popper 1988:57). While this process makes seeds more stable, it can also distort or damage them beyond recognition, and they are still susceptible to post-depositional mechanical damage as well as damage during recovery and processing (Miksicek 1987). The particularly small size of tobacco seeds amplifies these challenges.

In addition to these general issues with the identification of carbonized remains, tobacco poses added challenges because leaves and flowers are the mostly commonly used portions of the plant. Aside from being smoked, leaves and flowers were chewed, turned into a drink, snuffed as a powder, and burned as incense. In some ceremonies, tobacco was buried or cast into the air, rock crevices, water, or onto the ground (Kroeber 1941; McGuire 1897; Paper 1988). Due to these practices, it is likely that tobacco seeds never entered the archaeological record at all, while small bits of leaves and flowers would be more common. However, preservation depends largely upon the physical properties of the plant parts, such as their size and density (Dennell 1976; Popper and Hastorf, 1988), and carbonization tends to favor parts that are denser, for example nutshell and corncobs, and exclude non-dense parts, such as leaves, flowers, tubers, and fruits (Dennell, 1976; Fritz 1994; Miksicek 1987). Because of their high water and/or sugar content, exposure to fire would make the archaeological identification of these parts difficult, further hampering our ability to find evidence of tobacco.

Finally, the recovery of tobacco is further complicated by the plants primary use in rituals and ceremonies as opposed to more typically domestic contexts. Whereas the small seeds of plants that were used for subsistence were usually parched, toasted, or boiled, increasing their chances for carbonization and preservation, tobacco seeds were not. Subsistence activities are more easily recognizable at archaeological sites (hearths and pits) and are more often the focus of collection during excavations. The remains from ritual and ceremonial events are often treated differently during disposal, and may not occur in these traditional midden deposits.

1.3. Alternative methods to identify tobacco

In lieu of paleobotanical evidence of tobacco, another strategy for ascertaining which plants were smoked in pipes – including *Nicotiana* species – is through chemical analyses of organic residues from the artifacts (Tushingham and Eerkens 2016). The realization that organic matter associated with buried archaeological artifacts can persist for thousands of years has revolutionized the study of excavated cultural materials (Evershed 2008a). Most buried organic substances decompose rather quickly, generally in less than a century, with the rate of decomposition governed primarily by environmental (soil type, moisture, temperature, etc.) and biological conditions (Schmidt et al. 2011). However, if there is no influx of fresh bioorganic matter from plant decay, which is vital for microbial communities, buried organic compounds can become structurally stable for millennia (Fontaine et al. 2007; Chaopricha and Marin-Spiotta 2013). This is especially pertinent for bioorganic substances absorbed into ceramics, as organic matter does not readily migrate into ceramic fabrics (Heron et al. 1991); thus, substances can be preserved within the artifact. Such persistent organic substances are providing new sources of information on human activities in the past based on the extraction and analysis of bioorganic residues from excavated artifacts (Evershed 2008a, 2008b; Steele 2013).

There are a variety of strategies for establishing information from archaeological organic residues (Roffet-Salque et al. 2017). For

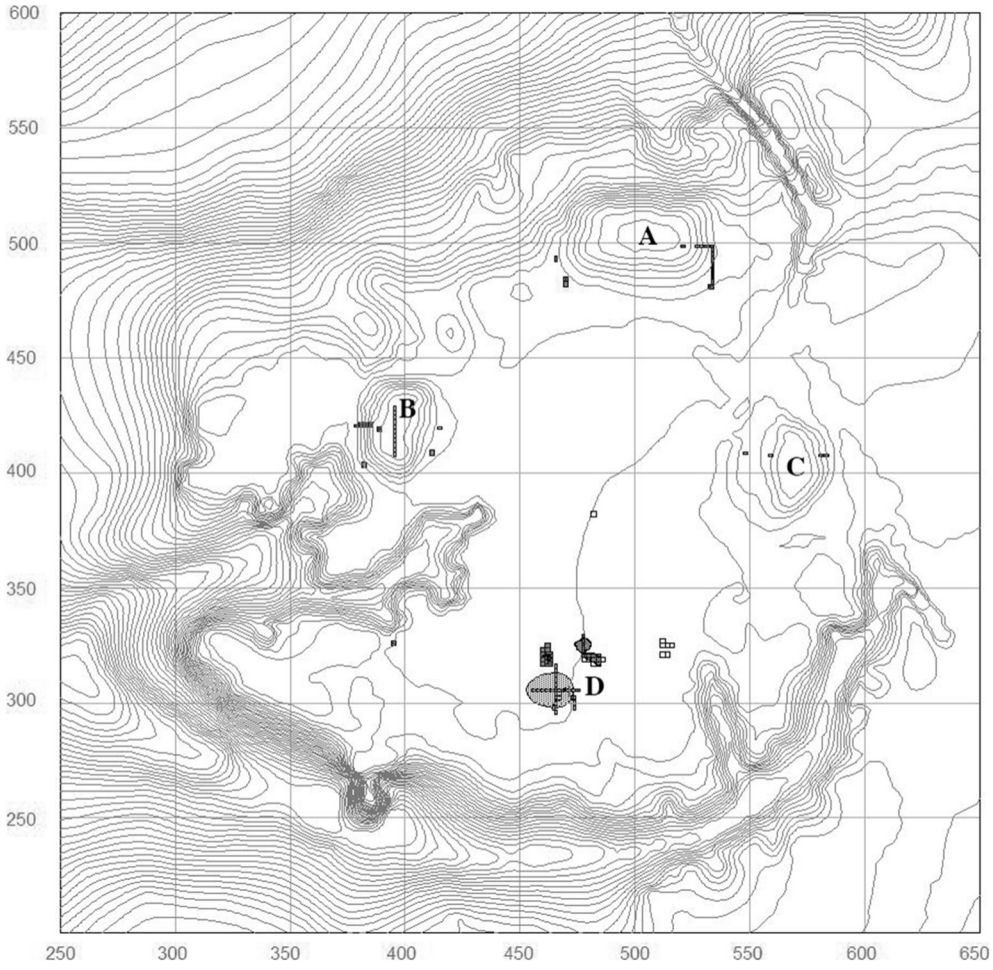

Fig. 2. Topographic map of Feltus on a 50 m grid, contour interval, 1 m. Excavation blocks from are shown as shaded rectangles. Hypothesized location of Mound D is shown as a shaded oval (from Kassabaum 2014: Fig. 2.7).

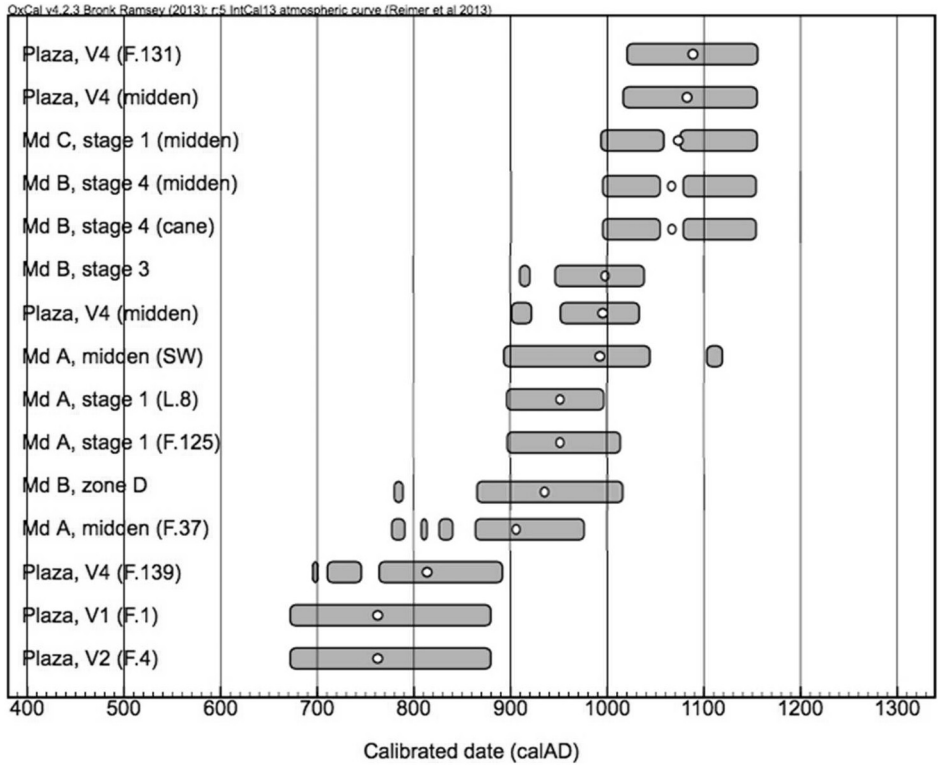

Fig. 3. Plot of all Feltus radiocarbon dates showing clustering, indicating episodic use of the landscape.

example, stable carbon isotope ratios of milk fats extracted from pottery vessels have provided evidence for the earliest exploitation of milk from domesticated animals (Evershed et al. 2008). The relative proportions of fatty acids with various carbon chain lengths also provide clues to residue provenance, whether plant, animal, freshwater, or marine origins (Eerkens 2005; Heron et al. 2010). Biomarker compounds – specific organic molecules that can be traced to unique biological processes – have also been used to establish residue origins, such as cholesterol and phytosterols for animal and plant sources, respectively.

2. Materials and methods

We used GC-MS to analyze fragments from six clay smoking pipes from the Feltus site. The primary goal was to determine whether nicotine, a biomarker for *N. tabacum* and *N. rustica*, was present in the artifacts. We also identified other compounds in solvent extracts from the pipes that could provide evidence of other substances that were smoked in the pipes. In addition to the analysis of the pipe fragments, we evaluated the effect of adding a base (potassium hydroxide) to the extraction solvent on the recovery nicotine from clay. We also analyzed dried leaves from recently harvested *N. rustica* plants, as well as the residues produced by smoking the plants in the laboratory using a vacuum pump connected to a stone pipe.

2.1. Archaeological sample descriptions

The Feltus Archaeological Project excavated in four major site areas at Feltus — Mound A, Mound B, Mound C, and the area surrounding the former location of Mound D in the southern end of the plaza (Steponaitis et al. 2012, 2014) (see Fig. 2). Within these site areas, excavations were divided into blocks defined by spatial proximity, such that each excavation within a given block shared at least a portion of its profile with the others, providing a clear stratigraphic sequence. Each block was composed of between one and fifteen units, which corresponded to the horizontal extent (generally 1 × 2 m) of excavations as they were dug in the field. Units were identified using northing and easting grid coordinates with reference to an arbitrary site datum (e.g., N500E500) and named according to their southwest corner. Within units, excavations were divided vertically by arbitrary levels or stratigraphic zones and features were identified and excavated separately whenever possible. All fill from levels and zones consisting of primary deposits was screened through 1/4″ mesh. A subsample of these deposits and all feature fill was water screened through 1/16″ mesh and 10 L flotation samples were collected and processed.

Fragments from six individual smoking pipes from the Feltus excavations were selected for organic residue analysis. The pipes were made from clay and most showed evidence of black sooty material (Table 1). The sample numbers were assigned based on the bag numbers created in the field. Two samples were collected from the early Coles Creek deposits in the southern plaza (ca. AD 700–850). Sample 988 came from a large midden-filled pit just north of Mound D's former location. This pit was 6 m in diameter, 1.6 m deep and full of animal bone and ceramic refuse. The character of this refuse suggested rapid dumping, with pot breaks and partly articulated deer bones, and likely represents feasting residue. In addition to the pipe fragments included in this study, three figurine fragments were recovered from this feature, further indicating the inclusion of ritual refuse. The source of Sample 2827/2830 was a sheet midden deposited over this pit and the surrounding area. This midden also appeared to have been rapidly deposited as primary refuse associated with feasting, as it contained intact portions of animal skeletons and showed no stratigraphic evidence of breaks during its formation.

Dating to the subsequent phase (ca. AD 850–950), Sample 1266 came from the ashy-fill surrounding a free-standing post set amidst an expansive pre-mound midden identified beneath the eastern flank of Mound A. After this post was set, a layer of dense refuse accumulated rapidly around it. Before this debris weathered, the post was pulled and the first 2 m of Mound A were immediately constructed atop the remaining void. This final deposit of refuse appears to represent a feasting event associated with the mound construction process. Sample 1019 was recovered from the first zone of fill in Mound A and was likely deposited or lost during the earliest episode of mound-building activity. The only direct evidence of summit use at Mound A is in the form of large bathtub-shaped barbeque pits, likely associated with continued feasting (Kassabaum 2014; Kassabaum et al. 2014). A flank midden that accumulated off the southwestern corner of the mound ca. 950–1050 CE further suggests that feasting activity took place on the mound summits. This midden showed no internal differentiation and sherds from the surface and base of the midden were refit, indicating that it was rapidly deposited in a single event. Sample 644 was recovered from this context.

Finally, Sample 367 was recovered from a post hole on the penultimate summit of Mound B. Excavations in Mound B revealed a construction history that differed from Mound A (Kassabaum et al. 2014). Five stages of construction were evident and each was capped with a clearly defined floor showing evidence of intensive use. The

Table 1
Sample information including the sample numbers, year excavated, source information and photographs.

Sample #	Year excavated	Source information and description	Photograph
988	2012	South Plaza/N320E483/ Feature 4, Level 2/Pit ca. AD 700–850 Segment of mid-bowl (3.5 cm inside diameter) Inside charred black Outside reddish yellow 7.5R 7/6	
2827/2830	2012	South Plaza/ N318E481S/Feature 148, Zone B/Midden ca. AD 700–850 Upper rim, decorated (4.0 cm inside diameter) 10YR 5/4 Yellowish brown	
1266	2007	Mound A, East/ N488E533S/Feature 37/ Posthole ca. AD 850–950 Lower bowl/stem 10YR 7/4 Very pale brown	
1019	2012	Mound A, East/ N488E533/Level 1/ Mound Fill ca. AD 850–950 Unknown section of pipe 5YR 6/6 Reddish yellow	
644	2007	Mound A, Southwest/ N483E470/Level 3/ Midden ca. AD 950–1050 Upper rim and bowl, decorated (3.0 cm inside diameter) 7.5 YR 6/6 Reddish yellow	
367	2006	Mound B, Summit/ N418E388/Zone F/ Posthole 2 ca. AD 1050–1150 Segment of rim (5.8 cm inside diameter) 10YR 5/4 Yellowish brown	

surface of the penultimate summit is the most completely excavated summit context at Feltus; however, its interpretation is complicated. The surface is made up of a series of stacked floors with uneven veneering and burning that took place ca. AD 1050–1150. This may represent variable use of the summit over time, or variable treatment at the time of its decommission. Features identified on this floor include small hemispherical pits and large posts, though no structural patterns could be identified. It is possible that these posts, including the one from which Sample 367 was recovered, represent standing posts like those identified in other site areas.

2.2. Reagents and instrumentation

All solvents used for the extraction experiments were pesticide residue analysis (PRA) grade purchased from Sigma-Aldrich. Standards, including nicotine (> 99%), were also purchased from Sigma Aldrich. The glassware used was first washed with Alconox™, rinsed thoroughly with water, and baked in a muffle furnace (~550 °C) overnight to remove residual organic matter. Prior to use, the glassware was triple rinsed with solvent (2:1 chloroform:methanol) and allowed to air dry.

Two gas chromatography (GC) systems were used for this study. For the organic residue analyses we used a Varian 3900 GC coupled to a Saturn 2100T mass spectrometer (MS) and a Varian 8410 autosampler. The GC-MS column was a DB-1 ms (100% dimethylpolysiloxane; 25 m in length, 0.25 mm I.D., and 0.25 μm film thickness). We also used a Varian 430 GC with a flame ionization detector (GC-FID) to evaluate the solvent extraction efficiency of nicotine from clay standards. The GC column was a VF-1 ms (100% dimethylpolysiloxane; 15 m in length, 0.25 mm I.D., and 0.25 μm film thickness).

Because camphor was identified in three of the six samples we opted to analyze the relative concentrations of the two enantiomers (R- and S-) of this compound in the residue extracts. This provided evidence of the original source of the compound, whether from natural substances that might have been smoked in the pipes or from contamination (Ravid et al. 1993; Buonasera 2007). For this we used the GC-FID with an enantioselective column (Rt-bDEXsa, 30 m in length, 0.32 mm ID, and 0.25 μm film thickness). The experimental GC parameters for the three types of analyses are given in Table 2.

All extractions were made using a Fisher Scientific FS140 ultrasonicator with a 40 kHz frequency and 185 W ultrasonicating power.

2.3. Solvent extraction optimization

Because we were interested in extracting as many classes of lipid compounds as possible from the archaeological pipes, we employed a modified Folch extraction method using a 2:1 chloroform:methanol solvent mixture (Reber et al. 2010; Kałużna-Czaplińska et al. 2016). However, to enhance the extraction of nicotine we evaluated the effect of adding methanolic hydroxide (MeOH·OH) to the solvent mixture. The aim was not to derivatize nicotine, and under the mild conditions of the extraction we would not expect derivatization reactions to readily occur; but the goal was to prevent free protons from binding to one or both of the two tertiary nitrogen functional groups in the nicotine molecule. If one or both of these amine groups becomes protonated the molecule becomes polar and the solubility in the semi-polar solvent would be decreased dramatically.

We prepared a 10% methanolic·OH solution by dissolving 40.0 g of KOH in 500 mL of anhydrous methanol. The solution was magnetically stirred overnight and then decanted into a clean glass storage bottle. A nicotine clay standard was prepared by adding 5.00 mL of 3.35 mg/mL standard nicotine in hexane into a small unglazed, fired stoneware ceramic bowl and the solution allowed to absorb into the ceramic. The hexane solvent was evaporated and the bowl dried in a 60 °C oven overnight. The bowl was ground to a powder using an agate mortar and pestle and sieved through a 150-μm screen to produce a fine powder of a homogenous nicotine/clay standard. A second bowl without nicotine was also processed in the same manner and used as a control.

Aliquots (200 mg) of the clay standard were weighed into 10-mL glass centrifuge tubes and varying ratios of the methanolic·OH and pure methanol were pipetted into the tubes such that the total volume of the two solutions was 1.00 mL (Table 3). A 2.00 mL aliquot of chloroform was added to each tube to bring the total volume of solvent to 3.00 mL for each experiment. The centrifuge tubes were ultrasonicated for 1 h in repeated 20-min cycles with a 10-min rest between. The tubes were centrifuged for 15 min and the supernatant separated from the solid using a Pasteur pipette. The solutions were filtered through sodium sulfate (Na_2SO_4) to remove water and particulate matter and collected in 5.0-mL glass reaction vials. The solvent volumes were evaporated to dryness using a gentle stream of ultrahigh purity (UHP) nitrogen gas and mild heat. The residues were reconstituted in 1.00 mL of 2:1 chloroform:methanol and analyzed directly using GC-FID. The relative efficiency of each extraction was determined based on the nicotine peak area on the chromatogram.

The results of these experiments demonstrated that the addition of methanolic hydroxide to the solvent extraction solution substantially increased the recovery of nicotine from the nicotine/clay standards. The most effective MeOH:MeOH·OH ratio was 3:1 (Fig. 4). For the remainder of the experiments we used a solvent mixture comprised of 8

Table 3
Volumes of methanol and methanolic·OH used in the standard nicotine clay extraction experiments to establish the most effective ratios of solvents.

Sample	Volume MeOH (mL)	Volume MeOH·OH (mL)	MeOH:MeOH·OH ratio	Average peak area × 10^4
1	1.000	0.000	1:0	6.2
2	0.875	0.125	7:1	25.4
3	0.750	0.250	3:1	26.7
4	0.625	0.375	1.7:1	2.4
5	0.500	0.500	1:1	6.1
6	0.000	1.000	0:1	4.0

Table 2
GC-MS operating parameters.

Instrument Component	Nicotine (GC-MS)	Solvent efficiency (GC-FID)	Camphor (GC-FID)
Injector	Temperature = 240 °C Volume = 1.0 μL Injector split = Splitless	Temperature = 280 °C Volume = 1.0 μL Injector split = Splitless	Temperature = 230 °C Volume = 1.0 μL Injector = split 1:20
Column	Initial temperature = 50 °C, hold for 3 min Ramp 10 °C/min to 270 °C, hold for 2 min Ramp 20 °C/min to 300 °C, hold for 10 min Carrier gas UHP He at 1.0 mL/min	Initial temperature = 50 °C, hold for 3 min Ramp 10 °C/min to 270 °C, hold for 2 min Ramp 20 °C/min to 300 °C, hold for 10 min Carrier gas UHP He at 1.0 mL/min	Initial temperature = 80 °C hold for 5 min Ramp 4.0 °C/min to 174 °C hold for 10 min Carrier gas UHP He at 1.0 mL/min
Detector	Mass Spectrometer Ion trap temperature = 220 °C Transfer line = 280 °C Mass scan from 40 m/z to 500 m/z	Flame ionization detector Temperature = 300 °C	Flame ionization detector Temperature = 230 °C

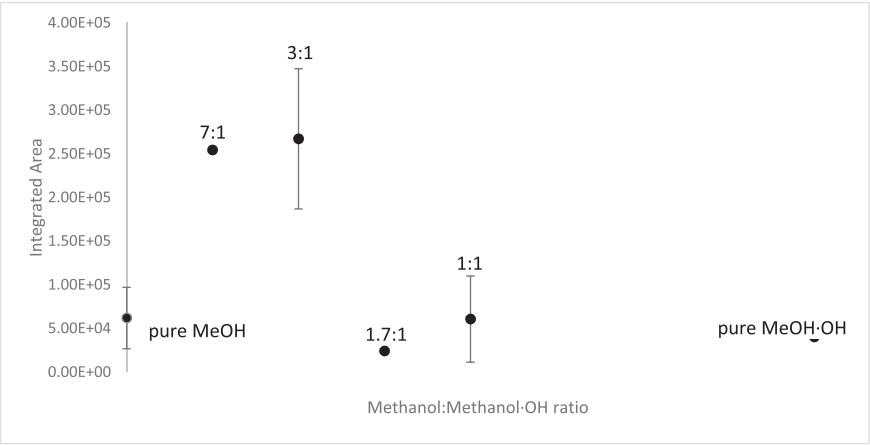

Fig. 4. Plot of the recovery efficiency of nicotine from a clay standard versus the ratio of methanol:methanolic·OH in the extraction solution. Error bars show 1σ of samples with multiple extractions.

Table 4
List of compounds systematically searched for in the sample chromatograms, including the common names, peaks amplified, molecular formulae, molecular weights, IUPAC names and CAS numbers.

Compound	Base/parent	Formula	MW	IUPAC name	CAS
Menthol	81/156	$C_{10}H_{20}O$	156	5-Methyl-2-(propan-2-yl)cyclohexan-1-ol	1490-04-6
Cinnamaldehyde	131/132	C_9H_8O	132	(2*E*)-3-Phenylprop-2-enal	14371-10-9
Hydroquinone	110/81	$C_6H_6O_2$	110	Benzene-1,4-diol	123-31-9
Vanillin	152/152	$C_8H_8O_3$	152	4-Hydroxy-3-methoxybenzaldehyde	121-33-5
Safrole	162/162	$C_{10}H_{10}O_2$	162	5-(Prop-2-en-1-yl)-2*H*-1,3-benzodioxole	94-59-7
Eugenol	164/164	$C_{10}H_{12}O_2$	164	2-Methoxy-4-(prop-2-en-1-yl)phenol	97-53-0
Camphor	95/152	$C_{17}H_{23}NO_3$	289	1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one	101-31-5
Tropine	124/289	$C_8H_{15}NO$	141	(*RS*)-(8-Methyl-8-azabicyclo[3.2.1]oct-3-yl) 3-hydroxy-2-phenylpropanoate	51-55-8
Nicotine	84/162	$C_{10}H_{14}N_2$	162	(*S*)-3-[1-Methylpyrrolidin-2-yl]pyridine	54-11-5
Atropine	82/141	$C_{17}H_{23}NO_3$	169	(*RS*)-(8-Methyl-8-azabicyclo[3.2.1]oct-3-yl) 3-hydroxy-2-phenylpropanoate	51-55-8

parts chloroform, 3 parts methanol, and 1 part methanolic hydroxide.

2.4. Analyses of modern N. rustica and combustion byproducts

We analyzed solvent extracts from freshly grown *N. rustica* leaves harvested in July 2016. The leaves were dried in a 60 °C oven for 24 h and ground to a powder using an agate mortar and pestle. Aliquots (0.5 mg) of the powdered plant leaves were placed in 15.0-mL centrifuge tubes and 3.0 mL of the 8:3:1 extraction solvent added. We followed the method described above for the extraction and analysis.

We also combusted the plant leaves in a stone smoking pipe with air drawn through the pipe using a vacuum pump. The combustion residues in the pipe were extracted using ~50 mL of extraction solvent in a beaker that was ultrasonicated 1 h in repeated 20-min cycles with a 10-min rest between. The solvent volume was reduced using a vacuum rotary evaporator to a final volume of ~5 mL, and then prepared for that analysis as described above. For the analysis we used GC-MS with parameters given in Table 2.

2.5. Analysis of archaeological smoking pipe sherds

We analyzed fragments from six different clay pipes (see Table 1). Although grinding ceramics to a fine powder for the extraction releases more absorbed organic matter (Copley et al. 2005; Evershed 2008b), our intent was to preserve the artifacts so we kept the pipe fragments intact for the extraction. The sherds were placed into 50-mL beakers and 100 μL of internal standard (hexatriacontane in hexane) was added to the sample surfaces and allowed to dry. Approximately 25 mL of extraction solvent (8:3:1 chloroform:methanol:methanolic·OH) was added such that the fragment was completely submersed in the solvent. The beaker was covered and ultrasonicated for a period of 60 min at 20-min intervals with 10-min rests between. The extraction solution was reduced to ~3 mL by evaporation using a gentle stream of UHP N_2 with mild heat. The solutions were filtered through sodium sulfate to remove water and particulate matter and collected in 5.0-mL reaction vials. The solvent volumes were evaporated to dryness then 250 μL of ethyl acetate added and the vial vortexed to solvate the extracted analytes. The reconstituted solutions were transferred to GC vials with 250-μL silanized glass GC inserts. The samples were analyzed with GC-MS with the conditions shown in Table 2.

The identification of nicotine in the extracts was based on retention times of nicotine standards and mass spectra. To increase the detectability of nicotine in the analyses we displayed selected ions *m/z* 84 and 162 to enhance the signal-to-noise ratio. We also undertook a systematic search by displaying the parent and base peak for specific compounds associated with other plants that might have been smoked. For example, jimson weed (*Datura stramonium*) contains the alkaloids atropine and tropine. We displayed *m/z* 289 and 124 to search for atropine and *m/z* 141 and 82 to search for tropine. Other compounds searched for in the sample chromatograms are listed in Table 4.

3. Results

3.1. Analysis of modern N. rustica and combustion byproducts

Previous studies have shown that commercial tobacco contains > 2500 chemical compounds, while the combustion byproducts contain at least 3800 chemical compounds (Baker 1987). Results of the analyses of the *N. rustica* plant and the combustion byproducts showed nicotine as the predominate compound present in the extracts; minor tobacco alkaloids were also detected in the combustion byproducts including nornicotine and cotinine (Fig. 5).

3.2. Analysis of Feltus pipe sherds

Nicotine was detected in all six of the pipe fragments (Table 5)

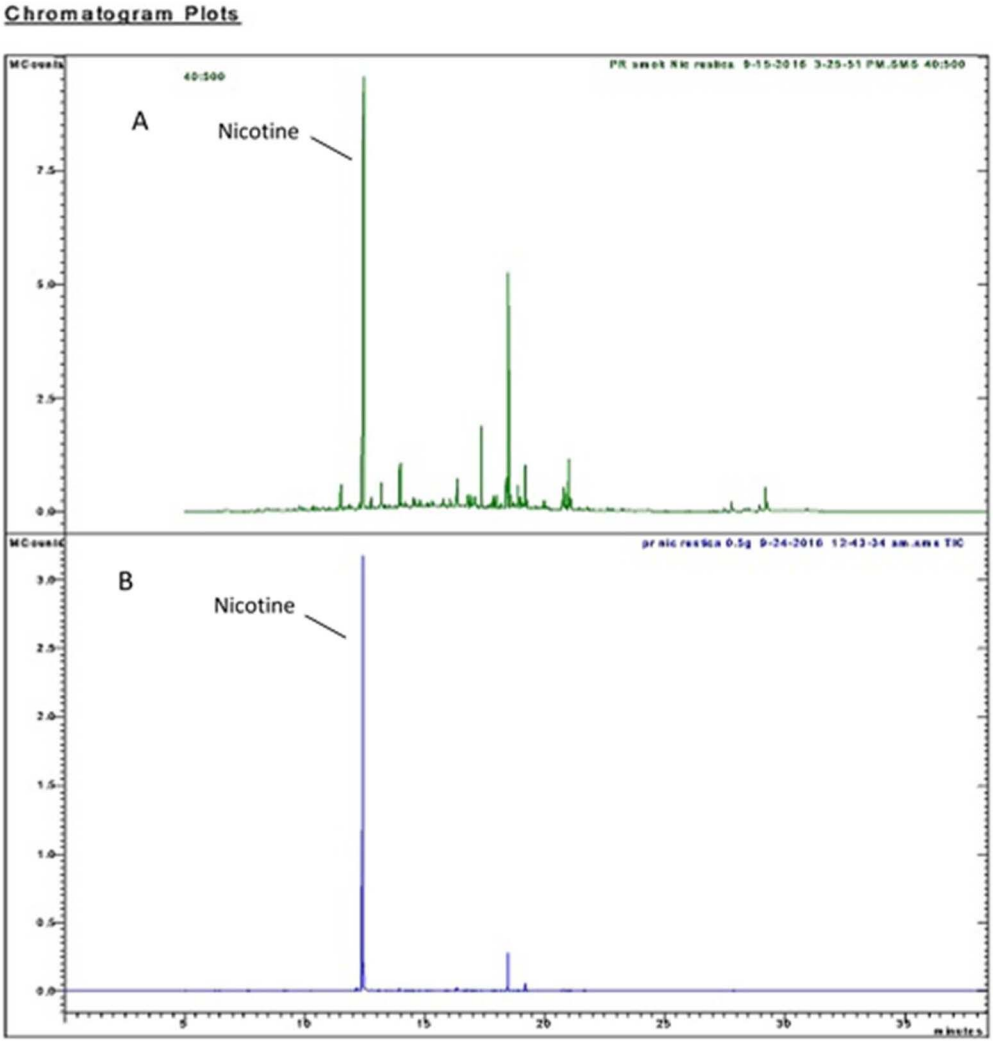

Fig. 5. Chromatograms from the analysis of recently harvested *N. rustica* leaves. The top chromatogram (A) is from the combustion by-products produced by smoking the leaves in a stone pipe. The bottom chromatogram (B) is from the unprocessed leaves. In both cases, nicotine is the predominate compound in the extracts.

Table 5
List of compounds detected in each pipe fragment.

Sample #	Nicotine	Camphor	Vanillin	Oleic acid	Menthol	Straight chain FA
1266	Yes	Yes	No	Yes	No	Yes
644	Yes	No	No	No	Yes	Yes
2827	Yes	No	No	Yes	No	Yes
988	Yes	Yes	No	Yes	Yes	Yes
367	Yes	No	Yes	Yes	Yes	Yes
1019	Yes	Yes	No	Yes	No	Yes

based on retention times and mass spectra (Fig. 6 and Fig. 7). Also notable was that saturated fatty acids, mainly palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids were identified in all samples. The monounsaturated fatty acid (oleic acid, $C_{18:1}$) occurred in all but one sample (644). Camphor (1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one) was present in three of six samples and vanillin (4-Hydroxy-3-methoxy-benzaldehyde) in one.

Camphor and vanillin have been identified in analyses of other excavated pipes, for example, from historic pipes from England (Thackeray et al. 2001) and pipes from southeastern North America (Hunt et al. 2017). Most natural sources of camphor contain unequal concentrations of the two enantiomers, although there are exceptions (Ravid et al. 1993). Synthetic camphor, however, has equal concentrations of these two stereoisomers (racemic mixture). To test whether the camphor was from contamination or natural we analyzed the extracts using GC-FID with an enantioselective column. The results demonstrated that the two enantiomers were equal in concentration; thus, it is probable that this compound is from post-excavation contamination (Buonasera 2007).

The presence of palmitic, stearic, and oleic acids in the extracts, but lacking short chain fatty acids, suggests the former were part of the original smoked material and from not contamination or degradation of higher molecular weight compounds. Indicators of contamination and degradation are shorter carbon chain compounds, including fatty acids, and hydrolysis of the double bond in oleic acid (Regert et al. 1998). We did not identify other compounds that could be associated with other plants that might have been smoked in the pipes.

4. Discussion

Aside from one seed tentatively identified as tobacco, macrobotanical evidence of the plant is absent from Feltus (Kassabaum 2014). This, however, is not an uncommon occurrence across the greater Eastern Woodlands where tobacco seeds are underrepresented in flotation samples collected from archaeological sites, especially from Late Woodland and Emergent Mississippian period deposits (Wagner 2000). Additionally, the majority of identified tobacco seeds come from domestic contexts (Wagner 2000), possibly reflecting the preparation of the plant for smoking in the home, whereas evidence from Feltus comes from ritual deposits (Kassabaum 2014). While the absence of tobacco seeds from archaeological sites is usually attributed to their small size

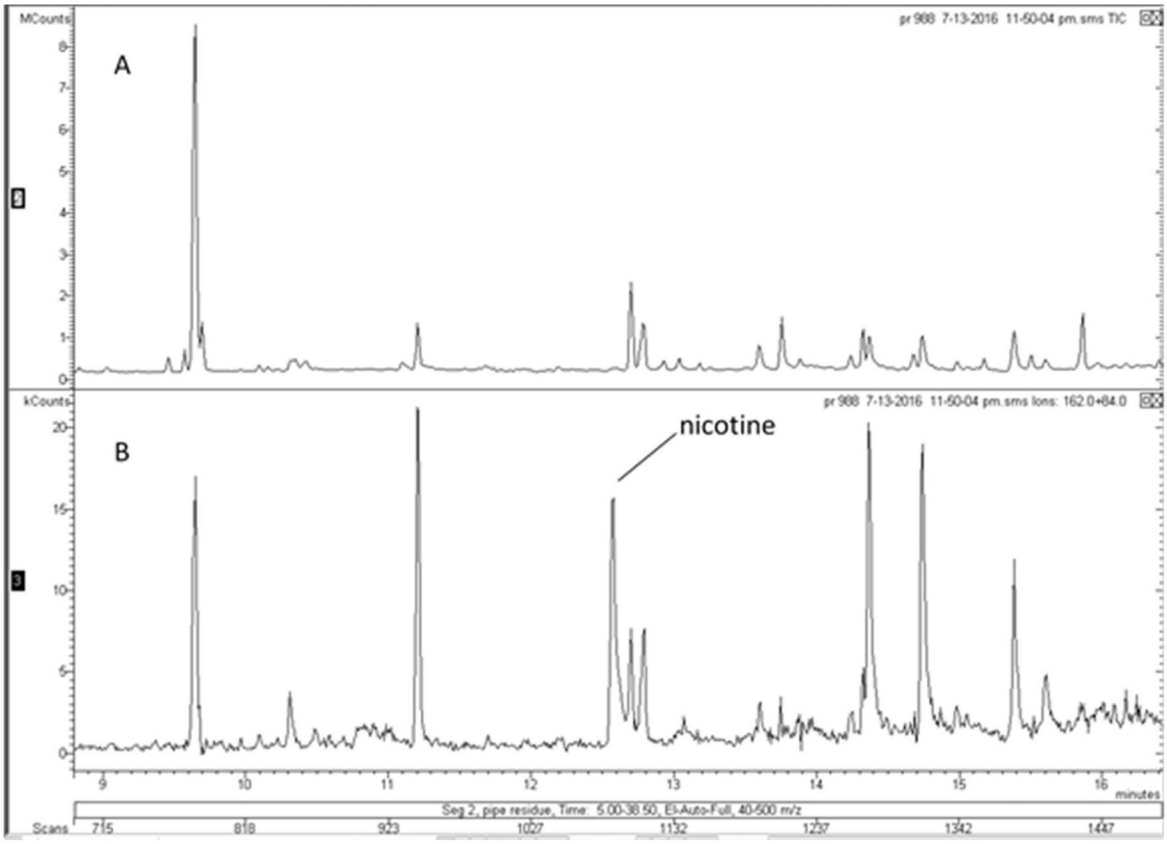

Fig. 6. Partial chromatograms from the analysis of Sample 988. The top chromatogram (A) shows the total ion current from the mass spectrometer. The bottom chromatogram (B) shows only selected ions with *m/z*, which enhances the detectability of the compound.

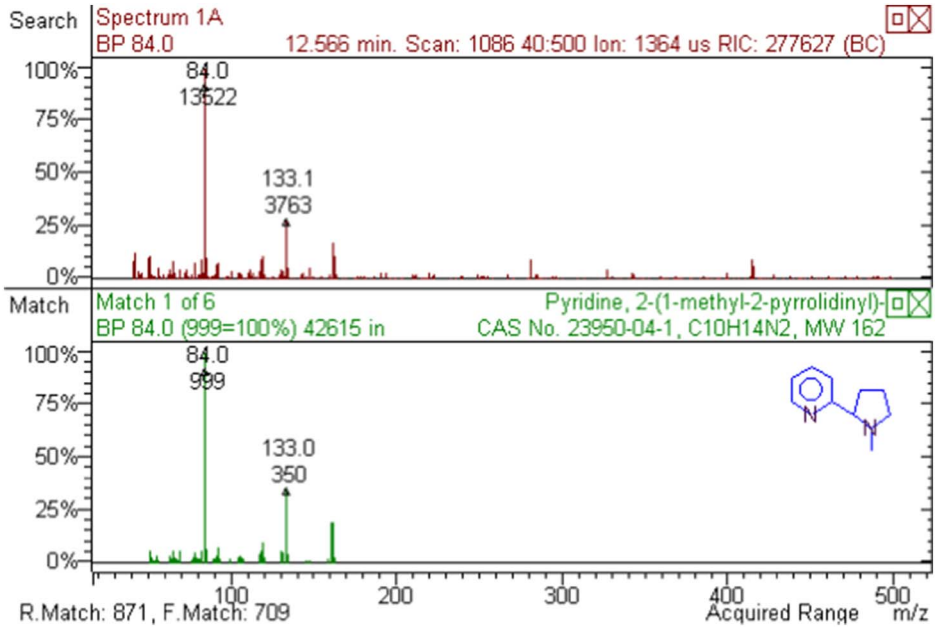

Fig. 7. (A) Mass spectrum of the peak at 12.566 min retention time from the analysis of Sample 988 and (B) the matching NIST library mass spectrum of pyridine(*S*)-3-[1-Methylpyrrolidin-2-yl]pyridine (nicotine).

(0.05 mm) and preservation issues, it is possible that their absence reflects differing cultural treatments of the plant. As with any plant, collecting and saving seeds was critical to ensure future crops. Moreover, historical documents suggest that, in an effort to increase overall yield, tobacco flowers were removed from the plant before they bloomed to ensure that the plant channeled their energy into leaf production (Li et al. 2016).

Whether the absence of seeds at archaeological sites is a result of cultural practices, preservational processes, or a combination of the two, residue analysis provides an alternative approach to exploring prehistoric tobacco use. In this case, the identification of nicotine in six pipe fragments excavated from Feltus indicates that tobacco was part of the ritually charged activities that took place at the site. These findings compliment previous interpretations of Feltus as a ritual landscape. The archaeological record from Feltus suggests a repeated ritual cycle that included the construction and use of monumental architecture, the

erection and removal of standing posts, ritual feasting, and burial of the dead. These activities have been previously interpreted as focused on gathering the Coles Creek community to strengthen social relationships and networks between living, non-living, and fictive kin. When understood through the lens of the ethnographic and ethnohistoric record, the high frequency of both pipe fragments and black bear remains included in feasting deposits and alongside human burials supports this community-focused interpretation of the Feltus rituals (Kassabaum 2014; Kassabaum and Nelson 2016; Nelson and Kassabaum 2014).

In ethnohistoric and ethnographic accounts of Native groups in the Eastern Woodlands, the act of smoking together builds connections between communities and facilitates inter-group interaction by concealing apparent differences and making strangers into temporary kin (Rafferty and Mann, 2004; Springer, 1981; Steinmetz, 1984). Given the dispersed nature of Late Woodland settlement in the Lower Mississippi River Valley, tobacco smoking would have been an important aspect of the Feltus rituals, allowing for the creation and maintenance of social bonds. This interpretation is further supported by the exceptionally high number of identified black bear remains from this site. Ethnographic and ethnohistoric accounts suggest a longstanding connection between humans and bears as kin or ancestors and imply that tobacco may have mediated these relationships. For example, after shooting a female bear, Alexander Henry (1921) witnessed Native Americans speaking to her as their grandmother and begging her forgiveness. His account further ties in tobacco as a part of the rituals associated with the death and consumption of bear as the hunter was later forced to blow smoke into the deceased bear's nostrils to appease her anger. Before the group feasted on the meat, an elder preformed a speech to the souls of his deceased ancestors. The study presented here demonstrates that, despite the lack of macrobotanical evidence, this connection between bear, tobacco use, and communal feasting materializes in the archaeological deposits at Feltus.

5. Conclusion

The use of tobacco in prehistoric eastern North America is poorly understood. Although we know that it was the preeminent ritual plant used in the Eastern Woodlands, its underrepresentation at archaeological sites forces many inferences regarding its use. The recent application of residue analysis has helped to change our understanding of the plant's use, including its entry into and diffusion across the region. It also provides additional support for previous interpretations of archaeological sites, as is the case with Feltus. Our data strongly suggests that tobacco played an important role in Late Woodland rituals in the Lower Mississippi River Valley, where paleobotanical evidence is lacking. Finally, this study demonstrates the importance of chemical analysis even at archaeological sites where flotation samples are systematically recovered to more fully explore prehistoric plant use, especially regarding the role of plants in prehistoric rituals.

Acknowledgements

We are grateful to Vincas P. Steponaitis and John W. O'Hear, co-directors of the Feltus Archaeological Project, for allowing us to analyze the pipe fragments and for their leadership on the excavation project.

Funding

This work was supported by the Eastern Band of Cherokee Indians Tribal Historic Preservation Office, the Rhodes Fellowship Program, and Rhodes Faculty Development Program.

References

Asch, D.L., 1994. Aboriginal specialty-plant cultivation in Eastern North America: Illinois prehistory and a post-contact perspective. In: Winter, J.C. (Ed.), Agricultural Developments and Origins in the Midcontinent. University of Iowa Press, Iowa City, pp. 25–86.

Baker, R.R., 1987. A review of pyrolysis studies to unravel reaction steps in burning tobacco. J. Anal. Appl. Pyrolysis 11, 555–573.

Braadbaart, F., Wright, P.J., 2007. Changes in mass and dimension of sunflower achenes and seeds due to carbonization. Econ. Bot. 61, 137–163.

Buonasera, T., 2007. Investigating the presence of ancient absorbed organic residues in groundstone using GC-MS and other analytical techniques: a residue study of several prehistoric milling tools from central California. J. Archaeol. Sci. 34 (9), 1379–1390.

Chaopricha, N.T., Marin-Spiotta, E., 2013. Soil burial contributes to deep soil organic carbon storage. Soil Bio. Biochemist 69, 251–264.

Copley, M.S., Bland, H.A., Rose, P., Horton, M., Evershed, R.P., 2005. Gas chromatographic, mass spectrometric and stable carbon isotopic investigations of organic residues of plant oils and animal fats employed as illuminants in archaeological lamps from Egypt. Analyst 130, 860–871.

Dennell, R.W., 1976. The economic importance of plant resources represented on archaeological site. J. Archaeol. Sci. 3, 229–247.

Dunavan, S.L., Jones, V.H., 2011. Tobacco and smoking in native North America. In: Smith, B.D. (Ed.), Subsistence Economies of Indigenous North American Societies: A Handbook. Smithsonian Press, Washington D.C., pp. 517–525.

Eerkens, J.W., 2005. GC-MS analysis and fatty acid ratios of archaeological potsherds from the Western Great Basin of North America. Archaeometry 47 (1), 83–102.

Eerkens, J.W., Tushingham, S., Lentz, K., Blake, J., Ardura, D., Palazoglu, M., Fiehn, O., 2012. GC/MS analysis of residues reveals nicotine in to late prehistoric pipes from CA-ALA-554. Proceedings of the Society for California. Archaeology 26, 212–219.

Evershed, R.P., 2008a. Organic residue analysis in archaeology: the archaeological biomarker revolution. Archaeometry 50 (6), 895–924.

Evershed, R.P., 2008b. Experimental approaches to the interpretation of absorbed organic residues in archaeological ceramics. World Archaeol. 40 (1), 1–12.

Evershed, R.P., Payne, S., Sherratt, A.G., Copley, M.S., Coolidge, J., Urem-Kotsu, D., Kotsakis, K., Özdoğan, M., Özdoğan, A.E., Nieuwenhuyse, O., Akkermans, P.M.M.G., Bailey, D., Andeescu, R., Campbell, S., Farid, S., Hodder, I., Yalman, N., Özbaşaran, M., Biçakci, E., Garfinkel, Y., Levy, T., Burton, M.M., 2008. Earliest date for milk use in the near east and southeastern Europe linked to cattle herding. Nature 455, 528–531.

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277–280.

Ford, R.I., 1981. Gardening and farming before A.D. 1,000: patterns of prehistoric cultivation north of Mexico. J. Ethnobiol. 1, 6–27.

Fritz, G.J., 1994. The value of archaeological plant remains for paleodietary reconstruction. In: Sobolik, K. (Ed.), Paleonutrition: The Diet and Health of Prehistoric Americans. Center for Archaeological Investigations Occasional Paper No. 23, Carbondale, pp. 21–33.

Hastorf, C.A., Popper, V.S., 1988. Current Paleoethnobotany: Analytical Methods and Cultural Interpretations of Archaeological Plant Remains. University of Chicago Press, Chicago.

Henry, A., 1921. Alexander Henry's Travels and Adventures in the Years 1760–1776. Lakeside Press, Chicago.

Heron, C., Evershed, R.P., Goad, L.J., 1991. Effects of migration of soil lipids on organic residues associated with buried potsherds. J. Archaeol. Sci. 18 (6), 641–659.

Heron, C., Nilsen, G., Stern, B., Craig, O., Nordby, C., 2010. Application of lipid biomarker analysis to evaluate the function of 'slab-lined pits' in Arctic Norway. J. Archaeol. Sci. 37 (9), 2188–2197.

Hunt, R., Prodanovich, N., Davis, J., Carmody, S.B., Russ, J., 2017. Organic Analysis of Smoking Pipe Fragments and Residue Scrapings. Poster presentation at the 82nd Annual Meeting Society for American Archaeology, Vancouver, Canada.

Kałużna-Czaplińska, J., Rosiak, A., Kwapińska, M., Kwapińska, W., 2016. Different analytical procedures for the study of organic residues in archaeological ceramic samples with the use of gas chromatography – mass spectrometry. Crit. Rev. Anal. Chem. 46 (1), 67–81.

Kassabaum, M.C., 2014. Feasting and Communal Ritual in the Lower Mississippi Valley, AD 700–1000. Ph.D. diss. University of North Carolina, Chapel Hill.

Kassabaum, M.C., 2017a. Social subsistence: integrating analyses of ceramic, plant, and animal remains at Feltus. In: Peres, T.M., Deter-Wolf, A. (Eds.), Eating the Past: Foodways Archaeology in the Southeastern United States. University of Alabama Press, Tuscaloosa in press.

Kassabaum, M.C., 2017b. Getting vessels from sherds: the utility of archaeological illustrations in reconstructing assemblages. Mississippi Archaeology in press.

Kassabaum, M.C., Nelson, E.S., 2016. Standing posts and special substances: ritual deposition at Feltus, Jefferson County, Mississippi. Southeast. Archaeol. 35 (2), 134–154.

Kassabaum, M.C., Henry, E.R., Steponaitis, V.P., O'Hear, J.W., 2014. Between surface and summit: the process of mound construction at Feltus. Archaeol. Prospect. 21 (1), 27–37.

Kroeber, A.L., 1941. Culture Element Distributions: XV Salt, Dogs, Tobacco. Anthropological Records VI(1):1–20. University of California Press, Berkeley.

Lentz, D.L., Pohl, M.E.D., Pope, K.O., Wyatt, A.R., 2001. Prehistoric sunflower (*Helianthus annuus* L.) domestication in Mexico. Econ. Bot. 55, 370–376.

Li, F., Zhang, H., Wang, S., Xiao, W., Ding, C., Weiqun, L., Hongxiang, G., 2016. Identification of topping responsive proteins in tobacco roots. Front. Plant Sci. 7, 582–593.

Lopinot, N.H., 1984. Archaeobotanical Formation Processes and Late Archaic Human-plant Interrelationships in the Midcontinental U.S.A. Ph.D. dissertation. Department of Anthropology, Southern Illinois University, Carbondale. University Microfilms, Ann Arbor.

McGuire, J.D., 1897. Pipes and Smoking Customs of the American Aborigines, Based on Material in the U. S. National Museum. Report of U. S. National Museum. 1897. pp. 351–645.
Miksicek, C.H., 1987. Formation processes of the archaeobotanical record. Advances in Archaeological Method and Theory 10, 211–247.
Nelson, E.S., Kassabaum, M.C., 2014. Expanding social networks through ritual deposition. Archaeological Review from Cambridge 29 (1), 103–128.
Paper, J., 1988. The sacred pipe: the historical context of contemporary Pan-Indian religion. J. Am. Acad. Relig. 56 (4), 643–665.
Popper, V., Hastorf, C., 1988. Introduction to Current Paleoethnobotany.
Rafferty, S.M., 2002. Identification of nicotine by gas chromatography/mass spectroscopy analysis of smoking pipe residue. J. Archaeol. Sci. 29 (8), 897–907.
Rafferty, S.M., 2006. Evidence of early tobacco in Northeastern North America? J. Archaeol. Sci. 33 (4), 453–458.
Rafferty, S.M., Mann, J., 2004. Smoking & Culture: Archaeology Tobacco Pipes Eastern North America.
Rafferty, S.M., Lednev, I., Virkler, K., Chovanec, Z., 2012. Current research on smoking pipe residues. J. Archaeol. Sci. 39 (7), 1951–1959.
Ravid, U., Putievsky, E., Katzir, I., 1993. Determination of the Enantiomeric composition of (1R) (+)- and (18) (−)-camphor in essential oils of some Lamiaceae and Compositae herbs. Flavour Frag. J. 8, 225–228.
Reber, E.A., Blitz, J.H., Thompson, C.E., 2010. Direct determination of the contents of a ceramic bottle from the Moundville site, Alabama. Midcont. J. Archaeol. 35 (1), 37–55.
Regert, M., Bland, H.A., Dudd, S.N., van Bergen, P.F., Evershed, R.P., 1998. Free and bound fatty acid oxidation products in archaeological ceramic vessels. Proc. R. Soc. Lond. B 265, 2032–2072.
Roffet-Salque, M., Dunne, J., Altoft, D.T., Casanova, E., Cramp, L.J., Smyth, J., Whelton, H., Evershed, R.P., 2017. From the inside out: Upscaling organic residue analyses of archaeological ceramics. J. Archaeol. Sci. Rep. 16, 627–640 (December).
Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56.
Springer, J.W., 1981. An Ethnohistoric study of the smoking complex in Eastern North America. Ethnohistory 28 (3), 217–235.
Steele, V., 2013. Organic residues in archaeology - the highs and lows of recent research. In: Armitage, R.A., Burton, J.H. (Eds.), Archaeological Chemistry VIII. ACS Symposium Series 1147. American Chemical Society. Chapter Vol. 5. pp. 89–108.
Steinmetz, P.B., 1984. The sacred pipe in American Indian religion. Am. Indian Cult. Res. J. 8 (3), 27–80.
Steponaitis, V.P., Kassabaum, M.C., O'Hear, J.W., 2012. Coles Creek Earthworks and Ritual at the Feltus Mounds in Southwest Mississippi, AD 700-1100. Poster Presented at the Annual Meeting of the Society for American Archaeology, Memphis, Tennessee.
Steponaitis, V.P., Kassabaum, M.C., O'Hear, J.W., 2014. Coles creek predecessors. In: Alt, S.M., Pauketat, T.R. (Eds.), Medieval Mississippians: The Cahokian World. SAR Press, Albuquerque, New Mexico, pp. 13–19.
Steponiaits, V.P., 2012. Notes on B. L. C. Wailes's survey of the Feltus Mounds in 1852. Manuscript on file, Research Laboratories of Archaeology. University of North Carolina, Chapel Hill.
Thackeray, J.F., van der Merwe, N.J., van der Merwe, T.A., 2001. Chemical analysis of residues from seventeenth-century clay pipes from Stratford-upon-Avon and environs. S. Afr. J. Sci. 97, 19–21.
Tushingham, S., Eerkens, J.W., 2016. Hunter-gatherer tobacco smoking in ancient North America: Current chemical evidence and a framework for future studies. In: Bollwerk, E.A., Tushingham, S. (Eds.), Prospectives on the Archaeology of Pipes, Tobacco and Other Smoke Plants in Ancient Americas. Springer International Publishing, pp. 211–230 Chapter 12.
Tushingham, S., Ardurab, D., Eerkens, J.W., Palazoglu, M., Shahbaz, S., Fiehn, O., 2013. Hunter-gatherer tobacco smoking: earliest evidence from the Pacific Northwest Coast of North America. J. Archaeol. Sci. 40 (2), 1397–1407.
Wagner, G., 2000. Tobacco in prehistoric Eastern North America. In: Winter, J.C. (Ed.), Tobacco Use by Native North Americans: Sacred Smoke and Silent Killer. University of Oklahoma Press, Norman, pp. 185–201.
Wailes, B.L.C., 1852. Notes in the Field, March 11–April 22, 1852. Field diary. Mississippi Department of Archives and History, Archives and Library, Wailes papers, Z/0076.000/S, Box 2, Folder 2.
Winter, J.C., 2000. Tobacco Use by Native Americans: Sacred Smoke Silent Killer. University of Oklahoma Press, Norman.
Wright, P.J., 2008. Understanding the carbonization and preservation of sunflower and sumpweed remains. Midcont. J. Archaeol. 33, 139–153.
Wright, P.J., 2010. Methodological issues in paleoethnobotany: a consideration of issues, methods, and cases. In: Van Der Warker, Amber M., Peres, Tanya M. (Eds.), Integrating Zooarchaeology and Paleoethnobotany. Springer, New York, pp. 37–64.