An Organic Geochemical Investigation of the Practice of Manuring at a Minoan Site on Pseira Island, Crete

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Lipid components in a soil profile developed in an agricultural terrace at a Minoan site on Pseira Island, Crete, were analysed to determine whether the practice of manuring in antiquity, as inferred by distributional and temporal sherd scatter, could be confirmed through the use of biomarker compounds as proxies for manuring. Analysis of total organic carbon and the abundance of n-alkyl lipids (n-alkanols and fatty acids) demonstrated that while the upper part of the soil profile had received more recent inputs of vegetation-derived organic matter, the deeper archaeological strata remained essentially undisturbed. Further analysis of 5β-steranes, steroid components which may be utilized as fecal biomarkers, revealed a signal indicative of manuring, with human or porcine-derived fecal material, in the lower 15 cm of the profile. Additional appraisal of epi-prostanol abundance inferred the possible practice of composting in later periods. This study represents a detection of manuring, in the oldest samples to date, using organic geochemical methods.

INTRODUCTION

The detection of organic matter derived from feces, in soils and sediments of archaeological interest, greatly enhances the reconstruction of ancient settlements in terms of temporal and spatial features, for example, stables, latrines, field systems. Furthermore, it can provide information about local resource utilization from which inferences concerning settlement economies may be drawn and as such represents a useful tool to be used in archaeological investigations. Many methodologies have been adopted to identify manured zones within the catchment area of archaeological sites, for example, micromorphology (Limbrey, 1975; Macphail et al., 1990; Macphail, 1998), magnetic susceptibility (Mullins, 1977), and phosphorus concentration (Eidt, 1984; Prösch-Danielsen and Simonsen, 1988; van de Wetering, 1988). Moreover, the reliability of methodologies utilizing inorganic chemical proxies to detect the deposition of fecally-derived manure (e.g., Al2O3, CaO, Fe2O3, Mn, and P2O5) has been questioned by Evershed et al. (1997), who observed no significant differences in the concentration of these proxies between strips of manured and unmanured soil.
One method, pioneered by Wilkinson (1982) involving the analysis of large spatial scatterings of sherds, has been readily adopted by the geoarchaeological community for assessing ancient field systems and manuring regimes. Wilkinson observed the occurrence of thin carpets of worn and degraded sherds scattered three to six km around three sites in Iran and Oman. It was suggested that the artifacts were transported as a component of urban domestic waste which was used to fertilize surrounding fields. The interpretation of sherd scatter was consolidated and strengthened by Bintliff and Snodgrass (1988), who advanced four possible models to explain the occurrence of such scatter; the most probable primary factor was taken to be manuring. Additional work by Wilkinson (1988) used sherd scatter in soil profiles to obtain information relating to post-depositional soil processes, for example, ploughing, deflations, sedimentation, and erosion. The relationship between sherd scatter and soil phosphate concentrations was investigated enabling a tentative differentiation between the general composition of potential manures to be made.

A comparatively more recent approach, involving the detection of diagnostic organic chemical compounds in the soil environment, has been used with success to assess the systematic deposition of fecal matter in antiquity (Evershed and Beethell, 1996; Evershed et al., 1997; Bullock et al., 1999; Simpson et al., 1999). 5β-stanols are organic compounds produced by a microbiologically mediated alteration (reduction) of the sterol cholesterol (and several structurally related compounds) in the intestinal tracts of most higher animals, making them ideal molecular markers (biomarkers) to be used in the detection of fecal deposition. The major 5β-stanol produced by humans is 5β-cholestan-3β-ol (coprostanol), a reduction product of cholest-5-en-3β-ol (cholesterol; Murtaugh and Bunch, 1967; Hatcher and Mcgilivray, 1979). Figure 1 is a schematic depicting the formation of coprostanol and its stereoisomers: 5β-cholestan-3α-ol (epicoprostanol; a product of anaerobic microbial reworking) and 5α-cholestanol (a product of cholesterol reduction in the natural environment).

STUDY AREA

Situated at the eastern end of the Gulf of Mirabello, approximately 2 km off the northeastern coast of Crete, Pseira is a small, barren island measuring about 2 km in length (Figure 2). Topographically, the island possesses a rugged terrain composed of steep hills and sheer, precipitous cliffs which support an extremely limited cover of vegetation including only a few trees (Betancourt and Davaras, 1988). Investigations by Richard H. Seager from 1906 to 1907 established the existence of a settlement in Late Minoan I (ca. 1675–1650 to 1490–1470 B.C.; chronology from Manning, 1995) for which Pseira is now best known (Seager, 1910). More recent excavations at Pseira have expanded the known history of habitation from the establishment of a small settlement during the Final Neolithic (ca. 4000–3000 B.C.) to a population peak in Late Minoan IB (ca. 1600–1550 to 1490–1470 B.C.). After this period of occupation, which ended with the destruction of the settlement be-
Between 1550 and 1450 B.C., the island remained mostly uninhabited until the Byzantine period (395–1453 A.D.), although evidence does suggest the possible presence of a small trader/pirate community during Late Minoan IIIC (ca. 1190 to 1125–1110 B.C.; Floyd, 1997). Occupation after the Byzantine period has been minimal (Betancourt and Davaras, 1988).

Soils on Pseira are calcareous and generally quite thin. In the Minoan period, agricultural use of land in this arid, marginal environment was augmented through the construction of a series of terraces. Such terraces are considered to have increased gradually between the Final Neolithic and Middle Bronze Age; the final terracing of available agricultural land coinciding with a large population increase during the Middle Minoan period (ca. 2050–2000 to 1700–1680 B.C.; Betancourt and Hope-Simpson, 1992). Full subsistence agriculture continued until Late Minoan I, and a final bout of limited terrace farming occurred in Late Minoan III (ca. 1445–1405 to 1125–1110 B.C.). Byzantine agriculture was confined mainly to walled compounds, leaving the terrace system as an abandoned form of farming (Hope-Simpson and Betancourt, 1990). Excavation of an agricultural terrace on Pseira (Figure 2—P1) has revealed the presence of ceramic sherds and general settlement-related debris occurring in two distinct levels of the soil profile with an archaeological stratum containing Late Minoan (ca. 1675–1650 to 1125–1110 B.C.) sherds overlying an older soil stratum containing only sherds from the Middle Minoan period (Figure 3; Betancourt and Hope-Simpson, 1992). As mentioned above, it has been
Figure 2. Map of Pseira and its geographical location relative to Crete. The sample site is labeled P1. Map by Julie Clark, courtesy of the Pseira project, Philip P. Betancourt and Costis Davaras, directors.
proposed that such distributional scatters arise from the systematic spreading of household wastes (broken artifacts, food, excrement, etc.) across the cultivated landscape as fertilizer (Wilkinson, 1982; Bintliff and Snodgrass, 1988). An analogous interpretation of the terrace sherd deposits indicates the action of a highly structured and carefully maintained system of manuring in operation on Pseira during the Minoan period.

Our study was instigated to test the hypothesis that a Minoan agricultural manuring regime, as inferred by sherd scatter, would leave a persistent and diagnostic signature in the biogeochemical component of terrace soils, and that such an effect can be detected through the use of biomarkers such as 5β-stanol.

METHODS

Sample Site
Most of the samples were taken from a profile exposed in a trench dug in soil (red/brown Mediterranean lithosols) retained behind an ancient terrace wall located to the north of the main archaeological site (Figure 2—P1). Inspection of the soil profile revealed three distinct layers comprising a mixed modern surface and two lower archaeological strata (Figure 3). The upper archaeological stratum...
contained quantities of sherds dated to Late Minoan I and earlier while the deepest stratum exhibited sherds dating from Early (ca. 3100–3000 to 2850–2000 B.C.) to Middle Minoan. The occurrence of sherds and other village debris near bedrock indicates that tillage was deep and that all soil within the lower profiles had, at some point, been subjected to some form of agrarian practice. The terrace is on a 25° slope, 90 m above sea level; vegetation was scarce and the soil very dry when sampled.

Soil was collected at defined depths from the whole of the soil profile (0–5, 5–15, 15–25, 25–35, 35–40, 40–50, 50–60, 60–70, 70–80, 80–85, 85–95 cm), and a control surface soil sample was taken from the site of a Minoan building, as unlikely to contain manure as any other control sample that could be collected on the island.

Sample Preparation and Solvent Extraction

All soil samples were crushed with a pestle and mortar and subsequently passed through a 2 mm and 75 μm sieve. About 30 g of each soil sample was Soxhlet extracted for 24 h using 200 ml dichloromethane (DCM)/acetone (9:1 v/v) to obtain a total lipid extract (TLE). 2-hexadecanol, 10-nonadecanone, heptadecanoic acid, 5β-pregn-3-one, 5β-pregn-3-ol, 5α-cholestane, and hexadecyloctadecanoate were added as internal standards before extraction. Solvent was removed by evaporation under reduced pressure.

Initial Fractionation of the Total Lipid Extract (TLE)

TLEs were separated into two fractions, “acid” and “neutral,” using an extraction cartridge with a bonded aminoethyl solid phase (500 mg sorbent, 2.8 ml eluent capacity; Varian). Extracts dissolved in DCM/isopropanol (2:1 v/v) were slowly flushed through a cartridge preeluted with hexane. After further elution with DCM/isopropanol (2:1 v/v, 8 ml) a “neutral” fraction was removed and the cartridge flushed with 2% v/v acetic acid in diethylether (8 ml) thereby eluting an “acid” fraction. Solvent was removed from both fractions under a gentle stream of nitrogen. Acid fractions were derivatized and analyzed by GC and GC/MS.

Column Chromatography of Neutral Lipids

Columns were packed with dried activated silica gel 60 (100°C, > 24 h; Fluka) and preeluted with hexane. Samples were applied to the column as a mixture of dissolved and finely suspended particulates in hexane. Gradient elution was performed under positive pressure supplied by a stream of nitrogen providing an elution rate of ~15 ml min⁻¹. The eluents used comprised five separate solvent systems: hexane, hexane/DCM (9.1 v/v), DCM, DCM/methanol (1:1 v/v), and methanol, applied in elutropic order to give five fractions: “hydrocarbon,” “aromatic,” “ketone/wax-ester,” “alcohol,” and “polar,” respectively. The relative volumes of solvents applied were determined by the ratio 2:1:3:2:2 following the above elutropic series, and the size of the column being used for a particular separation. Column fractions
were collected and dried in an identical manner to fractions from the acid/neutral separation.

**Urea Adduction of the Alcohol Fraction**

Samples (~10 mg), dissolved in hexane/acetone (2:1 v/v, 1.5 ml) in a Pyrex test tube, were agitated with a vortex mixer whilst a warm, saturated solution of urea in methanol (1 ml) was added dropwise, thereby forming a dense white precipitate of urea. Solvent was removed under a stream of nitrogen (ensuring the complete evaporation of methanol), and 3 ml of DCM were added to resuspend the urea precipitate. After centrifugation (2500 rpm, 15 min), the nonadduct (containing purified sterols) in DCM was removed by pipette and passed through a small plug of preextracted cotton wool (ensuring complete removal of urea crystals). The adduction procedure was then repeated, and the second nonadduct solution combined with the first. The adduct (containing purified n-alkanols) was recovered by washing the urea crystals with DCM (10 ml) and then dissolving them in double distilled water (10 ml) and extracting with DCM (2 ml). The urea-saturated aqueous layer was removed and a further aliquot of double distilled water (10 ml), mixed with the DCM, and left to partition (ensuring complete removal of urea from the organic layer). The organic layer was then removed and passed through a small column of anhydrous sodium sulfate to remove any residual water. Solvent was removed from the elutes by evaporation under a gentle stream of nitrogen.

**Derivatization**

Fractions containing functional and polyfunctional compounds were derivatized to form their respective trimethylsilyl (TMS) ethers and/or esters by adding 30 \( \mu l \) of \( N,O\)-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane (BSTFA + 1% TMCS; Sigma) to sample aliquots and heating for 30 min at 70°C. Excess derivatization agent was removed under a gentle stream of nitrogen and samples redissolved in 50 \( \mu l \) hexane.

**Elemental Analysis**

Elemental analyses were performed using a Perkin Elmer 240C elemental analyzer to determine total carbon content of the soils. Inorganic carbon content was determined using a Strichlein Instruments Coulomat 702 carbon analyzer adapted to analyze CO\(_2\) liberated from H\(_3\)PO\(_4\) digestion; the total organic carbon (TOC) value was then calculated as the difference between total carbon and total inorganic carbon. Each sample was analyzed four times, and a mean TOC value calculated; values typically exhibited standard errors < ±0.1% soil dry wt.

**Gas Chromatography (GC)**

Derivatized fractions were analyzed using a Hewlett-Packard 5890 series II gas chromatograph equipped with a fused-silica capillary column (Chrompack CPSil-
**Table I.** Diagnostic fragment ions used to detect target stanols and stenols by GC/MS with SIM.

<table>
<thead>
<tr>
<th>m/z</th>
<th>Parent Compounds</th>
<th>Fragment Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>129</td>
<td>5α-sterols</td>
<td>[M-SideChain-216]</td>
</tr>
<tr>
<td>213</td>
<td>5α-sterols</td>
<td>[M-SideChain-TMSOH(90)-42]</td>
</tr>
<tr>
<td>215</td>
<td>5α and 5β stanols</td>
<td>[M-SideChain-TMSOH(90)-42]</td>
</tr>
<tr>
<td>216</td>
<td>cholest-5-en-3β-ol</td>
<td>[M-TMSOH(90)]</td>
</tr>
<tr>
<td>368</td>
<td>24-methylcholest-5-en-3β-ol</td>
<td>[M-TMSOH(90)]</td>
</tr>
<tr>
<td>369</td>
<td>24-ethylcholest-5-en-3β-ol</td>
<td>[M-TMSOH(90)]</td>
</tr>
<tr>
<td>370</td>
<td>5α and 5β-cholestanols</td>
<td>[M-TMSOH(90)]</td>
</tr>
<tr>
<td>382</td>
<td>24-methyl-5α and 24-methyl-5β-cholestanols</td>
<td>[M-TMSOH(90)]</td>
</tr>
<tr>
<td>384</td>
<td>24-ethyl-5α and 24-ethyl-5β-cholestanols</td>
<td>[M-TMSOH(90)]</td>
</tr>
<tr>
<td>396</td>
<td>24-ethylcholest-5-en-3β-ol</td>
<td>[M-TMSOH(90)]</td>
</tr>
<tr>
<td>398</td>
<td>24-ethyl-5α and 24-ethyl-5β-cholestanols</td>
<td>[M-TMSOH(90)]</td>
</tr>
</tbody>
</table>

5CB, 50 m length × 0.32 mm i.d. × 0.12 µm film thickness). Samples in hexane were injected (1.0 µl) on-column. The temperature was programmed from 40°C (1 min isotherm) to 200°C at a rate of 10°C min⁻¹ and finally to 300°C at 3°C min⁻¹ (20 min isotherm). The detector temperature was kept at 320°C. Hydrogen was used as carrier gas (10 psi head pressure).

**Gas Chromatography–Mass Spectrometry (GC/MS)**

GC/MS analyses were made using a Carlo Erba 5160 GC equipped with on-column injection coupled, via a heated transfer line (320°C), to a Finnigan MAT 4500 quadrupole mass spectrometer operating in the selected ion mode (SIM) monitoring (m/z 129, 213, 215, 368, 370, 382, 384, 396, and 398) with a scan time of 0.1 s per ion (for assignments see Table I). The current was maintained at 300 µA with an ion source temperature of 190°C and an electron energy of 70 eV. GC separation was achieved using a fused silica capillary column (Chrompack CPSil-5CB, 50 m length × 0.32 mm i.d. × 0.12 µm film thickness) and the temperature was programmed from 40°C (1 min isotherm) to 200°C at a rate of 10°C min⁻¹ and finally to 300°C at 3°C min⁻¹ (20 min isotherm), helium was used as carrier gas (10 psi head pressure).

**RESULTS AND DISCUSSION**

**Total Organic Carbon (TOC) Analysis**

TOC measurements were made to identify any high incursions of organic material, possibly indicative of manuring or burned organic soils, in the soil profile. Figure 4 shows the percentage TOC determined for each of the soils excised from the profile of the terrace. Organic carbon content fluctuates with depth, maximizing at 2.3% at 5–15 cm having risen from a surficial level of 1.9%. At depths of more than 25 cm, all levels fall within a narrow 0.3% range (0.6–0.9%). Recent work has shown that the TOC of a modern, continually manured soil need not be high (i.e., 3%) and that this will quickly drop upon cessation of manuring (Bull et al., 1998). The high values observed in the shallowest samples can be rationalized as an input of modern-day plant detritus; sloughed material, and exudates arising from root...
material may account for the peak in TOC observed at 5–15 cm depth. The rate of
decomposition is lower in samples below 25 cm in direct accordance with pseudo
first-order kinetics (i.e., degradation rates infer a directly proportional dependence
on absolute concentration; Pincket et al., 1950; Floate, 1970). The low levels of TOC
in the deeper samples is most likely the result of organic matter loss through in-
tensive cropping and oxidative degradation (Bull et al., 1998). Overall, these results
demonstrate little evidence for recent disturbance of the archaeological profile
since major bioturbation (be it natural or anthropogenic mediated) would probably
have resulted in an increase of TOC levels in the lower layers of the terrace profile.
However, despite this reassuring result, TOC measurements cannot provide any
detailed information with regard to the source and/or age of specific inputs.

**n-Alkyl Lipids**

Two classes of aliphatic chemical components, n-alkanols and fatty acids, were
analyzed so that any disturbances or irregularities in the soil profile resulting from
vegetation, that is, root inputs, might be detected. Analysis of fatty acids was re-
stricted to homologues generally considered indicative of higher plant inputs, that
is, $\geq \text{C}_{20}$.

Figure 5(a) depicts the homologous series of n-alkanols determined for the ma-
roidy of samples from the terrace profile. Analyses show n-alkanols to be the most
abundant class of free compounds and the overall trend in their total absolute
abundance parallels that of TOC. Homologues occur at highest concentration in
the 5–15 cm sample and exhibit a general decrease in abundance with depth until
a minimal background level in the deepest soils is reached. Similar decreases with
depth, in a hydromorphic forest-podzol, were reported by Jambu et al. (1993) de-
spite distinct differences in soil type. An increase is observed in the sample taken
from 40–50 cm depth which may result from the penetration to and/or proliferation

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**Figure 4.** Percent TOC determined for the soil profile.
Figure 5. Histograms summarizing the absolute abundance distributions of: (a) n-alkanols and (b) fatty acids derived from the soil profile.
of plant roots at this depth. Samples down to 50 cm depth generally exhibit bimodal distributions centered about the C_{22} and C_{26} or C_{28} homologues while, in lower samples (60–70 and 80–85 cm), this bimodality is replaced by a monomodal distribution, although there is little difference in abundance between the C_{26}, C_{28}, and C_{30} homologues. The latter 80–85 cm distribution is also strikingly similar to the control sample, lacking only the more dominant central C_{28} component. As noted (Figure 3), roots from modern-day vegetation infiltrate depths of up to 50 cm and are probably responsible for the dominant C_{28} and C_{30} homologues in the shallower soils, with the sample at 5–15 cm depth being the best example of this. The similarities between the 80–85 cm sample and the control probably arise from a combination of long-term oxidative degradation and lack of any recent input, although the slightly enhanced C_{30} homologue of the control is most likely the result of minor incorporation of vegetation, probably in the form of wind-blown detritus.

Figure 5(b) depicts the homologous series of fatty acids (≤ C_{30}) obtained from all soil samples. There is a general decrease in the overall abundance of fatty acids with depth, the highest abundances being associated with the surficial soil sample rather than that from 5–15 cm depth. The three shallowest samples (0–5, 5–15, 15–25 cm) all exhibit significantly more abundant distributions than deeper samples strongly paralleling the trends observed for TOC. Additionally, the distribution at 0–5 cm is monomodal (C_{26} maximum) while those associated with 5–15 and 15–25 cm soils are bimodal (C_{24} and C_{30} maxima). This probably results from the differential incorporation of various types of vegetation and/or release of bound fatty acids. Distributions in samples of 25–35 cm depth or deeper decrease in abundance, and nearly all possess monomodal distributions centered about a C_{24} maximum. As observed for the n-alkanols, similarities with the control sample suggest that fatty acids of deeper soils have been subjected to long-term oxidation with no recent input of lipid material.

The results obtained by the analysis of n-alkanol and fatty acid components generally parallel those of the TOC, demonstrating that while shallow soils have received a minor lipid input from aerial plant detritus and the incursion of roots, lower sediments exhibit no similar evidence of recent disturbance and/or input. Having established this, analysis of fecal biomarkers may be made with little concern regarding recent, anomalous inputs to the soil profile from extraneous sources.

Steroidal Lipids

In many of the samples from site P1, the abundance of free sterols was too low to utilize GC as a viable method for detection. However, this problem was resolved by using SIM GC/MS. Table I lists the specific ions used for the analyses and their mass spectral origin.

Figure 6 is a schematic of the total reconstructed sterol ion trace for the fraction separated from the soil at 80–85 cm depth. The method reveals a simple series of steroidal components, thereby facilitating relative quantification. Each distribution
Figure 6. The steroid profile of the soil taken at 80–85 cm depth represented as a total reconstructed ion current, and six mass chromatograms recording the occurrence of diagnostic ions arising from specific steroid fragments. For ion assignments, see Table I.
generally comprises cholesterol and its phytosterol congeners campesterol, stig- 
masterol, and sitosterol. A number of saturated reduction products also occur: 
namely, coprostanol, epicoprostanol, 5α-cholestanol, 5β-stigmastanol, and 5α-stig-
mastanol. Figure 7 summarizes the abundance of the most common sterols in each 
sample relative to sitosterol. This is the dominant component in every terrace soil 
sample except that taken from 80–85 cm depth, the next most abundant com-
pounds being the C27 and C28 homologues, cholesterol, and campesterol, in that 
order. The soil sample from 80–85 cm yields a dominant peak corresponding to 
cholesterol with that of the C27 homologue residing at a slightly lower abundance. 
Interestingly, this is also the type of sterol distribution exhibited by the control 
sample, although the difference in component abundance is much higher. On this 
evidence alone, it may be surmised that the soil at 80–85 cm contains a lower 
relative proportion of plant derived material, given the lower concentration of si-
tosterol (a sterol commonly found in terrestrial higher plants) that is observed 
(Goad, 1991). Additionally, the highest relative proportion of coprostanol, a biom-
arker of fecal matter (Hatcher and McGilivray, 1979; Knights et al., 1983; Evershed 
and Bethell, 1986; Evershed et al., 1987; Bull et al., 1989) occurs in this sample. 
Values obtained for the same component in the soil at 35–40, 50–60, and 65–95 
cm are also relatively high but not to such a great extent. Such observations are 
promising with respect to the detection of ancient fecal deposition. However, more 
reliable criteria, independent of concentration and unbiased by background levels 
of 5β-stanols, must be satisfied in order to affirm these tentative conclusions.

The ratio of stanol epimers [equation (i)] has been proposed and further used as 
a more reliable parameter for fecal deposition than mere concentration:

$$\frac{\text{coprostanol}}{\text{coprostanol} + 5\alpha\text{-cholestanol}}.$$  (i)

Ratio values $> 0.7$ are generally considered to indicate sewage pollution (Grimault 
et al., 1990). However, it should be appreciated that this theoretical threshold was 
determined from modern-day sewage investigations and similar studies of ancient 
archeological soils may well have a lower threshold because of possible dispro-
portionate degradation of coprostanol compared with 5α-cholestanol, over time, 
giving a ratio which erroneously indicates a lower level of fecal input. Significantly, 
significant inspection of the 5β-stanol distributions associated with the shallower soils reveals 
samples with disproportionately high concentrations of epicoprostanol relative to 
its more common epimer coprostanol. The possibility of conversion of coprostanol 
to epicoprostanol under anaerobic conditions has already been mentioned above, 
and we would, therefore, propose a modified ratio [equation (ii)] as a more accurate 
parameter for ascertaining fecal matter inputs:

$$\frac{\text{coprostanol} + \text{epicoprostanol}}{\text{coprostanol} + \text{epicoprostanol} + 5\alpha\text{-cholestanol}}.$$  (ii)

On applying ratio (ii) to the terrace stanol data, several high values may be
Figure 7. Histograms summarizing the abundance of sterol components, relative to sitosterol, derived from the soil profile. Numbers refer to the compound structures depicted in Figure 6.
observed, with samples from depths of 35–40, 50–60, 80–85, and 85–95 cm, exceeding the threshold for fecal pollution (0.73, 0.76, 0.77, and 0.71, respectively), thereby providing evidence of manuring episodes correlating with all of the shed scatter results (Figure 8(a)). Figure 8(b) depicts the ratio of coprostanol to epicoprostanol with depth. It can be observed that values associated with shallower soils representing the Early to Late Minoan all yield fairly low ratios (< 1.6), and in some cases, the quantity of epicoprostanol actually exceeds that of the coprostanol (25–35 and 35–40 cm; 0.6 and 0.7, respectively). Conversely, ratios generated by the surficial and deepest soils are all higher (> 2.1), especially that of the 80–85 cm sample (3.8). While McCalley et al. (1981) ascribed epicoprostanol formation to bacterial reworking under anaerobic conditions in sewage sludge, it is difficult to envisage this process existing to any significant degree in the dry, arid environments which constitute the soil formations of Pseira Island. However, it is conceivable that increases in the abundance of epicoprostanol may arise from transformation during predepositional accretion of the fecal matter. For example, long-term composting of fecal matter could generate the conditions necessary for epimer conversion. Hence, information about the predepositional use of fecal material may be tentatively derived from soil sterol distributions. Samples associated with the very deepest soils (80–85 and 85–95 cm) do not appear to have received the same predepositional treatment. Likewise, the ratio associated with the surficial soil indicates an aerobic environment as might be expected for a modern-day background of fecal steroids. Similar analysis of the cholesterol:5α-cholestanol ratio, an indicator of natural microbial reduction reveals that while there is initial rapid reduction, it decreases to a low rate for all soils below 5 cm depth (Figure 8(c); Mermoud et al., 1984; Peakman and Maxwell, 1988). The absence of any sudden increase in microbial reduction at intermediate depths would appear to support the action of predepositional treatment of fecal matter in increasing the relative content of epicoprostanol.

Additional study of the relative abundance of 5β-stanols in the 80–85 cm sample can provide further useful information concerning the ancient fecal source. Bethell et al. (1984), and more recently Evershed and Bethell (1996), proposed criteria to enable identification of human fecal deposition:

- higher absolute levels of 5β-stanols,
- generally, higher absolute abundances of 5α-stanols,
- a percentage of coprostanol, relative to the total 5α-stanol content, of 55% or more,
- a ratio of coprostanol: 5β-stigmastanol of 1.5:1 or greater (the ratio for human feces is 5.5:1 whilst that of ruminants [sheep and cows] is 1:4).

Fecal inputs of samples meeting these criteria were ascribed to human or porcine sources while samples failing the latter criteria were considered to have received a fecal input from a ruminant animal (large quantities of plant derived sitosterol ingested in plants, producing a dominant 5β,3β-sitostanol gut reduction product). Inspection of the 80–85 cm sterol distribution reveals that criteria are met which
Figure 8. Plots of (a) the ratio (coprostanol + epicoprostanol)/(coprostanol + epicoprostanol + 5α-cholestanol), (b) the ratio coprostanol:epicoprostanol, and (c) the ratio cholesterol:5α-cholestanol for soils from the profile.
indicate a probable human or porcine-derived fecal input to the lower terrace soils. Such criteria were not matched by the control sample.

Archaeological Implications
Analysis of steroidal lipid data obtained from the terrace soils reveals a signal, indicative of manuring, at various depths below 35 cm; most notably that at 35–40, 50–60, 80–85, and 85–95 cm. This represents, in the deepest samples, the oldest manuring regime ever detected through the use of biomarker techniques. The manuring signal correlates well with the occurrence of sherds which, as well as strengthening the argument for the deposition of manure, act as a temporal reference. Hence, we propose that a highly organized agricultural strategy, involving deliberate and systematic application of manures, was being applied at Pseira between the Early to Late Minoan I periods. Manure formed from human excrement (there exists no evidence of pig husbandry) and household waste would have been transported from the main site of occupation and spread over agricultural terraces, thereby fertilizing them. The high relative abundance of 5β-stanols in the 80–85 cm sample indicates that a higher proportion of fecal material was incorporated in the former part of the manuring period. As the population grew, the rate of household manure formation would have increased accordingly, probably leading to the formation of long-term piles of composting manure which could be applied to terraces as needed. This is concordant with the recognized need to either dilute, compost, or allow human excrement to rot before its agricultural utilization (Alcock et al., 1994 and references therein). It is possible that such heaps of composting matter would provide the conditions required for the anaerobic reworking of coprostanol to produce epicoprostanol, thereby explaining the abundance of this compound observed in the shallower manured soils. The co-occurrence of both chemical (5β-stanols) and artifactual evidence (sherds) of manuring is extremely encouraging and supports the future use of both techniques. Certainly, given the location of the terrace above the associated settlement site, it is unlikely that the sherd scatter arose as a result of postdepositional disturbance of site artifacts.

CONCLUSIONS
The objective of this study was to test the hypothesis that a Minoan agricultural manuring regime, as inferred by sherd scatter, would have a significant and persistent effect on the organic chemistry of terrace soils and that such an effect could be detected through the use of biomarkers such as 5β-stanols. While it is accepted that the limited sample set constrains this study to a more preliminary nature, observations made have enabled a number of tentative conclusions to be drawn. Analysis of TOC levels and the abundances of aliphatic compounds (n-alkanols and fatty acids) demonstrated that the terrace was relatively undisturbed since its use in antiquity and, at deeper levels, was not in receipt of significant amounts of modern-day organic material. Subsequent analysis of steroidal lipids revealed several interesting points, namely:
1. Using the modified ratio (ii), episodes of fecal deposition were confirmed in the two lower archaeological strata, indicating manuring of the terrace from Early to Late Minoan I and thereby supporting the same inference drawn from the observed distribution of sherds.

2. The applied manure was ascribed as containing a human fecal component; a porcine origin being ruled out due to an absence of evidence for pig husbandry on Pseira.

3. Analysis of relative epicoprostanol abundance provided insights into the pre-depositional treatment of manure, indicating the possible long-term accretion of midden heaps in the latter stages of the Minoan period.

Despite the limited sample set, the detection of a structured manuring regime in Minoan times, using fecal biomarkers (5α-stanols), represents a positive identification of this type made on the oldest samples (4500–3500 yr B.P.) studied using organic geochemical methods, which further validates their use in investigating ancient agricultural practices in other archaeological investigations.

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REFERENCES


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standard


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