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Title: Reconstruction of prehistoric pottery use from fatty acid carbon isotope signatures using Bayesian inference

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Abstract: Carbon isotope measurements of individual fatty acids (C16:0 and C18:0) recovered from archaeological pottery vessels are widely used in archaeology to investigate past culinary and economic practices. Typically, such isotope measurements are matched with reference food sources for straightforward source identification, or simple linear models are used to investigate mixing of contents. However, in cases where multiple food sources were processed in the same vessel, these approaches result in many equivocal solutions. To address this issue, we tested the use of a Bayesian mixing model to determine the proportional contribution of different food sources to a series of different mixed food compositions, using data generated both by simulation and experimentally. The model was then applied to previously published fatty acid isotope datasets from pottery from two prehistoric sites; Durrington Walls, near Stonehenge in Southern Britain and Neustadt in Northern Germany. We show that the Bayesian approach to the reconstruction of pottery use offers a reliable probabilistic interpretation of source contributions although the analysis also highlights the relatively low precision achievable in quantifying pottery contents from datasets of this nature. We suggest that, with some refinement, the approach outlined should become standard practice in organic residue analysis, and also has potential application to a wide range of geological and geochemical investigations.

Fatty acid (FA) stable carbon isotopes were used to investigate ancient pottery use

A Bayesian approach was applied to these data to estimate source contributions

This approach was tested using simulated, experimental, and archaeological data

Results obtained illustrate the advantages of a Bayesian approach

A probabilistic approach to the reconstruction of pottery use is recommended

1 Reconstruction of prehistoric pottery use from fatty acid carbon isotope signatures using Bayesian  
2 inference

3

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## 1 **ABSTRACT**

2 Carbon isotope measurements of individual fatty acids ( $C_{16:0}$  and  $C_{18:0}$ ) recovered from  
3 archaeological pottery vessels are widely used in archaeology to investigate past culinary and  
4 economic practices. Typically, such isotope measurements are matched with reference to food  
5 sources for straightforward source identification, or simple linear models are used to investigate  
6 mixing of contents. However, in cases where multiple food sources were processed in the same  
7 vessel, these approaches result in equivocal solutions. To address this issue, we tested the use of a  
8 Bayesian mixing model to determine the proportional contribution of different food sources to a  
9 series of different mixed food compositions, using data generated both by simulation and by  
10 experiment. The model was then applied to previously published fatty acid isotope datasets from  
11 pottery from two prehistoric sites: Durrington Walls, near Stonehenge in southern Britain and  
12 Neustadt in northern Germany. We show that the Bayesian approach to the reconstruction of  
13 pottery use offers a reliable probabilistic interpretation of source contributions although the  
14 analysis also highlights the relatively low precision achievable in quantifying pottery contents  
15 from datasets of this nature. We suggest that, with some refinement, the approach outlined should  
16 become standard practice in organic residue analysis, and also has potential application to a wide  
17 range of geological and geochemical investigations.

18

19 *Keywords:* Fatty acids; carbon isotopes; pottery use; Bayesian mixing models; FRUITS

20

## 21 **1. Introduction**

22 Organic residue analysis is a well-established method for determining the contents of  
23 archaeological pottery. This approach has been particularly important for establishing major

1 changes in prehistoric economic (e.g., Evershed, 2008; Cramp et al., 2014) and culinary practices  
2 (Craig et al., 2011), as well as understanding the origins of ceramic technology itself (Craig et al.,  
3 2013). Many of these studies have relied on measurements of the stable carbon isotope ratios  
4 ( $\delta^{13}\text{C}$ ) of saturated fatty (*n*-alkanoic) acids (e.g.,  $\text{C}_{16:0}$  and  $\text{C}_{18:0}$ ) to distinguish different products  
5 (Regert, 2011; Craig et al., 2012). These fatty acids are commonly preserved in archaeological  
6 pottery and their isotope ratios have been well characterised in a range of authentic modern food  
7 products. Occasionally, vessels dedicated for specific uses can be discerned using this approach  
8 due to their clear and distinctive isotope composition (Salque et al., 2013). Yet for most situations  
9 in the past, it is likely that pots were used for preparing a range of foodstuffs, either as a result of  
10 these items being cooked together, or through sequential use of the pot over time. These  
11 processes add complexity when making inferences about the relative proportions of food types  
12 processed in the vessel.

13 Determining the ratio of different foods contributing to mixtures is difficult, since different  
14 foodstuffs not only vary isotopically, but also contain different amounts of  $\text{C}_{16:0}$  and  $\text{C}_{18:0}$  acids.  
15 Attempts have been made to resolve mixtures of foods in pottery quantitatively, using simple two  
16 end-member models which consider the concentration of each fatty acid determined from  
17 authentic reference samples (Mukherjee et al., 2008; Craig et al., 2011). However, in cases where  
18 multiple foods were potentially processed, this approach can result in equivocal solutions. A  
19 more robust method for resolving mixtures in archaeological pottery is therefore needed, both to  
20 confirm product identification, but also to identify ancient culinary practices that may involve the  
21 combination or separation of foods.

22 The primary goal of this study was to employ a Bayesian approach to quantify the  
23 proportions of different foodstuffs in archaeological pottery based on previously published

1 carbon isotope analysis of fatty acids from two prehistoric sites; the inland Late Neolithic henge  
2 monument of Durrington Walls, near Stonehenge in southern Britain, (Craig et al., 2015) and the  
3 coastal Late Mesolithic/Early Neolithic site of Neustadt on the Baltic coast of Germany (Craig et  
4 al., 2011). The performance of this Bayesian approach was first tested using both simulated  
5 examples and isotopic data from experimental pots, where known mixtures of three different  
6 foods were cooked in a controlled experiment.

7

## 8 **2. Model specification**

9 A model instance, represented by Equation 1, is defined here with the following characteristics:

10 (i) Lipid groups are defined by the  $\delta^{13}\text{C}$  measurements of multiple fatty acids (for the present  
11 study these are  $\text{C}_{16:0}$  and  $\text{C}_{18:0}$ ) in modern authentic foodstuffs (i.e. reference samples); (ii) Non-  
12 weighted model: excluding taphonomic effects it is assumed that the source of carbon for a  
13 particular fatty acid extracted from the ceramic matrix can only be the same fatty acid found in  
14 the lipid sources; (iii) Offset model: since modern reference isotopic values are employed it is  
15 necessary to include an offset quantifying the difference between modern and past stable carbon  
16 isotopes ratios due to fluctuations in atmospheric  $\delta^{13}\text{C}$  values; (iv) Concentration-dependent  
17 model: the concentration of each fatty acid within each lipid group is included in the model.

18 The model for the observed value of the k-th isotope signal:

19  $H_k \sim N(\mu_{H,k}, \sigma_{H,k}^2)$  (Equation 1)

20 where:

21 
$$\mu_{H,k} = \frac{\sum_{i=1}^{N_k} \alpha_i C_{ik} (T_{ik} + I_{ik})}{\sum_{i=1}^{N_k} \alpha_i C_{ik}}$$

1 and:  $H_k$  represents the  $k$ -th isotopic signal measured in the pottery lipid extracts. This  
2 corresponds to  $\delta^{13}\text{C}$  measurements on fatty acids (in the present study  $\text{C}_{16:0}$  and  $\text{C}_{18:0}$ ) extracted  
3 from the archaeological ceramic extracts;  $\alpha_i$  represents the contribution from the  $i$ -th lipid group.  
4 The  $\alpha_i$ 's are unknown and their estimation, together with estimation of their uncertainties,  
5 represents the ultimate analytical goal. Physical restrictions apply:  $0 \leq \alpha_i \leq 1$  for  $i = 1, \dots, n$   
6 and  $\sum_{i=1}^n \alpha_i = 1$  where  $n$  represents the number of lipid groups;  $I_{ik}$  is the isotopic signal (e.g.,  
7  $\delta^{13}\text{C}_{16:0}$  and  $\delta^{13}\text{C}_{18:0}$ ) measured for the  $i$ -th lipid group contributing to the  $k$ -th isotopic signal  
8 measured in the pot. Due to the presence of measurement errors (and inter-individual  
9 heterogeneity), it is assumed to behave as a random variable which is modelled by a multivariate  
10 normal distribution,  $I_i \sim \text{N}(\mu_I, \Sigma_I)$  with an average vector  $\mu_I$  and a  $\Sigma_I$  variance-covariance matrix;  
11  $T_{ik}$  is the offset for the  $k$ -th isotopic signal in the  $i$ -th lipid group due to fluctuations in  
12 atmospheric  $\delta^{13}\text{C}$  values. This is modelled as a normal variable,  $T_{ik} \sim \text{N}(\mu_{T,ik}, \sigma_{T,ik}^2)$ ;  $C_{ik}$  is the  
13 concentration of the  $k$ -th fatty acid in the  $i$ -th lipid group. This is modelled by a multivariate  
14 normal distribution,  $C_i \sim \text{N}(\mu_C, \Sigma_C)$  with an average vector  $\mu_C$  and a  $\Sigma_C$  variance-covariance  
15 matrix.

16

### 17 *2.1. Adding prior information*

18 A simple approach was developed for incorporating a priori constraints of non-standard  
19 types into the expanded version of the model in Equation 1. Prior expert opinion is incorporated  
20 through user-defined algebraic expressions  $y(\alpha_i, I_{ik}, C_{ik})$  that serve to express relationships of  
21 equality or inequality between model parameters (e.g., when prior knowledge allows imposing  
22 that certain lipid groups contribute more than others).

1 To link a relationship of equality into the model a parameter  $p$  (Equation 2) is assigned a  
2 normal distribution with a mean given by  $y(\alpha_i, I_{ik}, C_{ik})$  and a user-defined uncertainty,  $r$ . The  
3 equality constraint is imposed by having an ‘observed’ value of zero for  $p$ .

4 
$$p \sim N(y(\alpha_i, I_{ik}, C_{ik}), r^2) \text{ (Equation 2)}$$

5 To link an inequality relationship, a parameter  $l$  (Equation 3) is assigned a Bernoulli  
6 distribution  $Bernoulli(k)$  where  $k$  is a Heaviside function,  $H(y(\alpha_i, I_{ik}, C_{ik}))$ , which provides a  
7 value of one or zero depending on whether  $y(\alpha_i, I_{ik}, C_{ik})$  is positive or negative. The parameter  $l$   
8 may also include an additional error term  $\varepsilon$  modelled as a normal distribution,  $\varepsilon \sim N(0, r^2)$  with 0  
9 average and a user-defined uncertainty,  $r$ . The inequality constraint is then imposed by having  
10 the ‘observed’ value of one for  $l$ .

11 
$$l \sim Bernoulli(H(y(\alpha_i, I_{ik}, C_{ik}) + N(0, r^2))) \text{ (Equation 3)}$$

12

## 13 2.2. Bayesian inference

14 Modelling was carried out using the 3.0 Beta version (available at  
15 <http://sourceforge.net/projects/fruits/>) of the Bayesian mixing model FRUITS (Fernandes et al.,  
16 2014a). Although FRUITS has mainly been used for the reconstruction of ancient human diets  
17 (e.g., Fernandes et al., 2012), the model is also applicable to any problems that aim at estimating  
18 the contributions from different sources to a given mixture, using quantitative signals (e.g.,  
19 elemental or isotopic profiles) as input data. The FRUITS generic model (Fernandes et al., 2014a)  
20 includes a weight parameter that allows building model instances in which different food  
21 fractions (e.g., food nutrients, single compounds) contribute in varying proportions to a single  
22 target signal (here,  $\delta^{13}\text{C}$  measured in fatty acids extracted from archaeological potsherds).  
23 However, since it is assumed that there is a one-to-one correspondence between pot and food



1 fatty acids the weight parameter has a value of one when target and source fatty acid match and  
2 zero otherwise resulting in the simplified model representation (Equation 1).

3 Numerical Bayesian inference was performed using the BUGS software, a Markov chain  
4 Monte Carlo (MCMC) method that employs Gibbs sampling and the Metropolis-Hastings  
5 algorithm (Gilks et al., 1996). The first 5,000 iterations of the MCMC chains were discarded  
6 (burn-in steps) and these were then run for an additional 10,000 iterations. Model convergence  
7 for the different  $\alpha_i$ 's was checked by inspecting if the trace plots of the respective posterior  
8 chains exhibited an asymptotic behaviour. Trace autocorrelation plots were also inspected to  
9 assess convergence.

10

### 11 **3. Model implementation**

12 The first stage of model building is to identify the lipid groups (i.e. foodstuffs) that  
13 potentially contributed to the ceramic organic residue. This is usually based on locally available  
14 archaeological and historical evidence. For example, at Durrington Walls (see Section 5.1), the  
15 composition of the faunal assemblage and the near absence of plant remains (Craig et al., 2015),  
16 indicates that pottery was likely only used for the processing of cattle (meat and/or milk) and  
17 porcine products. At Neustadt (see Section 5.1) a broader range of foods was available including  
18 marine fish and mammals and wild ruminants, both of which made up a significant proportion of  
19 the faunal assemblage (Glykou, 2014). Freshwater fish were much sparser and consequently were  
20 not considered. Reference  $\delta^{13}\text{C}_{16:0}$  and  $\delta^{13}\text{C}_{18:0}$  values for these products (Table 1) were obtained  
21 from modern fats obtained from southern Britain (Copley et al., 2003) Denmark (Craig et al.,  
22 2011) and Poland (Craig et al., 2012).

1 An additional source of uncertainty is the so-called Suess effect that describes the  $^{13}\text{C}$   
2 depletion of atmospheric  $\text{CO}_2$  as a result of the burning of fossil fuels during the 19<sup>th</sup> and 20<sup>th</sup>  
3 centuries (Friedli et al., 1986). Under the defined model (Equation 1), through the offset  
4 parameter  $T_{ik}$ , it is assumed that each lipid group has a specific  $\delta^{13}\text{C}$  offset between modern and  
5 ancient values. This is expected since modern references were likely sampled at different times.  
6 However, since collection times are not exactly known an approximation was made and the same  
7 offset value was used for all terrestrial lipids. Modern terrestrial lipid references were sampled  
8 during the 1990s and 2000s and an atmospheric  $\delta^{13}\text{C}$  value of  $-8.1\text{‰} \pm 0.2\text{‰}$  was taken as  
9 reference for this time period (Hellevang and Aagaard, 2015). During the second half of the  
10 Holocene, which includes the archaeological periods under study, atmospheric  $\delta^{13}\text{C}$  values were  
11 ca.  $-6.35\text{‰} \pm 0.1\text{‰}$  (Schmitt et al., 2012). Thus, the offset included in model ( $T_{ik}$ ) to account for  
12 the differences in  $\delta^{13}\text{C}_{16:0}$  and  $\delta^{13}\text{C}_{18:0}$  between modern and archaeological values was  
13 conservatively estimated to be  $1.75\text{‰} \pm 0.2\text{‰}$ . For lipid references from modern marine  
14 organisms, the extent of mixing of  $\text{CO}_2$  between the atmosphere and the Baltic Sea needs to be  
15 considered. Global estimates for the full oceanic  $^{13}\text{C}$  Suess effect since pre-industrial times are  
16 estimated to be as high as 40% ( $\pm 10\%$ ) of the atmospheric, depending on the depth and extent of  
17 mixing (Eide et al., 2017). The marine fish and mammals used as references here (Craig et al.,  
18 2011), and those available to prehistoric fisher-hunter-gatherers were likely to have fed both in  
19 the shallow Baltic and deeper North Atlantic waters (Craig et al., 2006). For the modelling  
20 purposes here, we used an estimate of 50% of the atmospheric Suess effect (i.e.  $0.875\text{‰} \pm 0.2\text{‰}$ ).

21 The concentration of  $\text{C}_{16:0}$  and  $\text{C}_{18:0}$  fatty acids (Table 1) as a proportion of total fatty acids  
22 in the modern authentic fats were either measured and reported with the  $\delta^{13}\text{C}$  values or, where  
23 these were unavailable, obtained from previously published values. Whilst the proportion of  $\text{C}_{16:0}$

1 and C<sub>18:0</sub> in total fatty acids are generally well reported for different food classes (Table 1), the  
2 amount of fat by weight varies greatly between species and between tissues. This represents the  
3 greatest source of uncertainty in reconstructing pottery use in terms of relative weights of  
4 different foods. In the majority of cases, the outputs are given in weight percentage of total fatty  
5 acid. Fatty acids account for < 90% of the fat present in most animal tissues (Weihrauch et al.,  
6 1977), so the output values are a reasonable approximation for the relative weight of different  
7 animal fats and oils processed in the pottery vessels.

8 For the different lipid groups, the  $\delta^{13}\text{C}_{16:0}$  and  $\delta^{13}\text{C}_{18:0}$  values are highly correlated (Fig. 1);  
9 this is the motivation for allowing the correlations between isotopic values. Therefore, isotopic,  
10 and also concentration, values of fatty acids from the lipid groups are described within the model  
11 by multivariate normal distributions defined by a mean vector (Table 1) and a variance-  
12 covariance matrix. The observed isotopic distributions are naturally randomly distributed around  
13 a central value. Thus, with longer periods of pottery use it becomes more likely that a wide range  
14 of isotopic values would have been sampled and the overall combined value would tend towards  
15 the mean. In this case, as an approximation, the standard error of the mean (SEM) of multivariate  
16 normal distributions would be an appropriate reference. As with many prehistoric pots, the  
17 vessels from both Durrington Walls and Neustadt had built up accumulations of soot on their  
18 exteriors and had other signs of use-wear consistent with repeated use. In a study of pottery use  
19 from a Danish Early Neolithic site, a low median value (6 months) was taken as the reference for  
20 the usage of ceramic pots although compiled ethnographic data for the same study showed that  
21 the duration of use could actually be as high as several years (Madsen and Jensen, 1982).  
22 Nonetheless, the number of samples employed to define isotopic and concentration values of  
23 lipid groups is still relatively small (Table 1). Thus, we have examined two models to estimate

1 source contribution to the archaeological pottery: a conservative model with, and a non-  
2 conservative model without, elements of covariance matrices divided by number of samples.

3 Where several potential lipid groups exhibit considerable overlap in their distribution of  
4  $\delta^{13}\text{C}_{16:0}$  and  $\delta^{13}\text{C}_{18:0}$ , it may not be possible to generate unambiguous estimates of proportions.  
5 For these cases, individual lipid groups may be aggregated into a new, enlarged, group. This is  
6 illustrated in Table 1 where the lipid group “combined ruminant” corresponds to the aggregation  
7 of the lipid groups “wild ruminant adipose”, “domesticated ruminant adipose”, and “dairy fats”.  
8 However, when data are combined in this manner the assumption about the natural randomness  
9 and symmetry of isotopic values is no longer valid.

10 For our experimental material, replicate measurements corresponding to extracts of different  
11 portions of the same vessel showed a reproducibility equal or better than 0.2‰ for  $\delta^{13}\text{C}_{16:0}$  and  
12 typically equal or better than 0.4‰ for  $\delta^{13}\text{C}_{18:0}$ . However, besides measurement uncertainties  
13 there are also other sources of uncertainty. These include potentially unknown contributing lipid  
14 groups that were not taken into account, degradation processes, cooking and lipid absorption  
15 processes. For instance, previous cooking experiments have shown that different cooking  
16 methods could result in isotopic differences between cooked and raw food which are larger than  
17 the measurement uncertainty (Fernandes et al., 2014b). In contrast, there is good evidence that  
18 fatty acids absorbed to pottery retain their  $\delta^{13}\text{C}$  values despite extensive degradation under oxic  
19 and anoxic conditions (Evershed, 2008). Here, an uncertainty of 0.6‰ was taken as reference for  
20 measurements of  $\delta^{13}\text{C}_{16:0}$  and  $\delta^{13}\text{C}_{18:0}$  from ceramic containers. We believe this to be a  
21 conservative uncertainty since it represents the double of the value (0.3‰) commonly used for  
22 measurement reproducibility.

23

## 1 **4. Model evaluation**

### 2 *4.1. Testing the model using simulated data*

3 To test model performance we first employed simulated scenarios. A large variety of  
4 scenarios could have been selected but we chose four similar to those that are likely to occur in  
5 actual archaeological case studies. These were defined as: scenario 1 consisting of two lipid  
6 groups (“wild ruminant adipose” and “marine oils”); scenario 2 consisting of three lipid groups  
7 (“dairy fats”, “domesticated ruminant adipose”, and “porcine adipose”); scenario 3 in which the  
8 different ruminant fats are combined into a single group (“combined ruminant”) and “porcine  
9 adipose” as a separate lipid group; scenario 4, as with scenario 2, but with additional prior  
10 information added to the model. For each scenario of available lipid groups, three different cases  
11 were simulated. Fig. 2 shows expected values and model outputs for the different scenarios.  
12 Expected mixed isotopic values were calculated using the average isotope and concentration  
13 values of C<sub>16:0</sub> and C<sub>18:0</sub> fatty acids given in Table 1 and represented in Fig. 1.

14 For scenario 1, consisting of two well-separated food groups, model outputs were in  
15 excellent agreement with simulated values (Fig. 2). For scenario 2, simulated contributions are  
16 contained within the 68% credible intervals. However, credible intervals for scenario 2 are larger  
17 than those observed for scenario 1. The ambiguity in model estimates is a consequence of the  
18 distribution of isotopic values for the three lipid groups (Fig. 1). The “domestic ruminant  
19 adipose” values are relatively close to the mixing line between “porcine adipose” and “dairy fat”.  
20 Thus, contributions from “domestic ruminant adipose” may also be interpreted as the mixing of  
21 “porcine adipose” and “dairy fat”.

22 In order to reduce the uncertainties different strategies may be adopted: (1) adding other  
23 isotopic or elemental proxies (e.g., bulk  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  or %C or %N, if available); (2) including

1 additional prior information in the model; or, (3) grouping ranges of lipids that have similar  
2 values. In scenario 3, “combined ruminant” corresponds to the aggregation of the lipid groups  
3 “domesticated ruminant adipose”, “dairy fats”, and also “wild ruminant adipose” that was not  
4 included in scenario 2. The estimates of the contributions from “combined ruminant” vs  
5 “porcine” show that for the former the credible intervals are narrower than those observed for  
6 “wild ruminant adipose” or “dairy fats” in scenario 2. Scenario 4 is similar to scenario 2, however  
7 prior information was added to constrain model estimates (see Section 2.1). Introduced  
8 constraints impose relationships of inequality on the contributions from the different lipids  
9 groups or of equality with an uncertainty of 20% (Fig. 2). These types of relationships may be  
10 employed, for example, when palaeodietary evidence can be independently and safely assessed  
11 from the archaeological evidence. This could rely on the study of well-preserved faunal or  
12 botanical assemblages, but may also require the taphonomic analysis of assemblages to determine  
13 employed cooking methods (Roberts et al., 2002; Koon et al., 2003). In cases where robust  
14 archaeological information is lacking, and taphonomic effects may be unaccounted for, different  
15 scenarios should be tested to verify the sensitivity of model estimates. The presence of lipid  
16 biomarkers for specific products could also be employed to impose threshold values on the  
17 contribution from certain foods, although experimental work is still required to establish these  
18 values. One has to be aware of the fact that while imposing correct relationships can help  
19 efficiency, imposing wrong relationships can be entirely detrimental to the whole model (results  
20 can be easily biased heavily).

21

#### 1 *4.2. Reconstructing mixtures from pots with known contents*

2 Eleven replica ceramic vessels, of approximately 9 cm diameter and fired at 750 °C, were  
3 used to prepare ten different mixtures of whole wild salmon, ground chestnut flour and wild red  
4 deer, plus a blank control containing only distilled water. These were chosen due to their  
5 expected distinct isotopic values. They are also products typically exploited by northern Eurasian  
6 Holocene hunter-gatherers. None of the animals are protected and they were not killed for the  
7 purpose of this research. All animals were pre-killed by license holders using the appropriate  
8 methods of killing as outlined in Appendix D of the Animals (Scientific Procedures) Act 1986.  
9 The salmon and deer tissue samples were homogenised in a food blender, and each product was  
10 weighed in preparation for addition to the pots in proportions shown in Table 2.

11 The total amount of each of the individual wet tissue mixtures was added to the experimental  
12 pots, which were then boiled continuously for 4 h over an open wood-fuelled fire in the York  
13 Experimental Archaeology (YEAR) Centre. Distilled water was added to each pot throughout the  
14 experiment to maintain a level up to the rim. Once completed, the remaining cooked foodstuffs  
15 were removed from each pot. The pots were placed in a drying oven at 40 °C for 48 h until dry.  
16 All pots were then wrapped in aluminium foil and stored in a cool, dry environment until needed  
17 for further analysis.

18 Approximately 1 g portions of ceramic powder were obtained from each pot by drilling to a  
19 depth of 2–5 mm from the interior surface. Separate portions of each pot were extracted at least  
20 in duplicate and methylated in one-step with acidified methanol as described previously (Craig et  
21 al., 2013; Papakosta et al., 2015). Briefly, HPLC grade methanol was added to each sample (4  
22 mL/g). Each sample was sonicated for 15 min, and then acidified with concentrated sulphuric  
23 acid (800 µl). The acidified suspension was heated in sealed tubes for 4 h at 70 °C before being

1 allowed to cool. Lipids were extracted with *n*-hexane ( $3 \times 2$  ml), and quantified using a GC-FID  
2 equipped with a DB23 column ( $60 \text{ m} \times 0.25 \text{ mm} \times 0.25 \text{ }\mu\text{m}$ ; J&W Scientific, Folsom, CA, USA).  
3 Untreated samples of the three foodstuffs (ca. 300–900 mg) used in this experiment, chestnut  
4 flour, deer and salmon, were freeze dried and then extracted in the same manner as described for  
5 the ceramic powder.

6 Carbon isotope measurements were determined on two fatty acid methyl esters (FAMES),  
7  $\text{C}_{16:0}$  and  $\text{C}_{18:0}$  from sample aliquots that had been cleaned using  $\text{AgNO}_3$ -impregnated silica gel  
8 columns. The fraction containing saturated fatty acids, eluted from the silica gel columns using *n*-  
9 hexane:DCM (1:1, v:v), were dried under  $\text{N}_2$ , re-dissolved in *n*-hexane and directly analysed by  
10 GC–C–IRMS using standard conditions and protocols (Craig et al., 2007, 2012). Analysis was  
11 performed on an Isoprime 100 (Isoprime, Cheadle, UK) linked to a Hewlett Packard 7890B series  
12 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) with an Isoprime GC5  
13 interface (Isoprime, Cheadle, UK). The gases eluting from the GC were directed through the GC5  
14 furnace held at  $850 \text{ }^\circ\text{C}$  to oxidise all carbon species to  $\text{CO}_2$ . All  $\delta^{13}\text{C}$  values are expressed relative  
15 to Vienna Pee Dee Belemnite (VPDB), based on in-house reference gases ( $\text{CO}_2$ , BOC) adjusted  
16 daily using an international standard mixture of *n*- $\text{C}_{16}$  to *n*- $\text{C}_{30}$  alkanes (the isotope ratios of  
17 which were measured offline by A. Schimmelmann, Biogeochemical Laboratories, Indiana  
18 University). Sample values were also corrected to account for the methylation of the carboxyl  
19 group that occurs during acid extraction, based on comparisons with an in-house standard  
20 containing  $\text{C}_{16:0}$  and  $\text{C}_{18:0}$  fatty acids of known isotopic composition processed in the same way as  
21 the experimental pottery samples.

22 The  $\delta^{13}\text{C}$  values of fatty acids from each pot are shown in Table 2. No lipids could be  
23 identified in the blank control containing only water. Modelling was carried out using these as



1 target values and the lipid group isotope information and the concentration data (Table 3). No  
2 additional prior information was added to the model.

3 The model output is shown in Fig. 3 as the contribution by wet weight of each product to  
4 each pot. Results from the experimental vessels show that the model can predict the salmon  
5 component of the mixture with relatively small credible intervals (Fig. 3). However, the credible  
6 intervals for the chestnut and deer are often considerably larger given the proximity in  $\delta^{13}\text{C}_{16:0}$   
7 and  $\delta^{13}\text{C}_{18:0}$  values for these two foodstuffs. The known foodstuff contributions are contained  
8 within the 95% credible intervals except in two instances where they are just outside of the 95%  
9 interval (pots 2 and 3), while for pot 6 the model output for deer contribution is clearly distant  
10 from the known contribution.

11 In a separate model, deer and chestnut were aggregated as a single 'terrestrial' lipid group.  
12 The  $\delta^{13}\text{C}$  values of the aggregated lipid group were calculated to reflect the different fatty acid  
13 concentrations of each single food group. The outputs for a two-source model are shown in Fig. 4  
14 and predictably, the credible intervals for the terrestrial aggregated lipid group are narrower (Fig.  
15 4) than those observed in the previous model output for deer and chestnut lipid groups (Fig. 3). In  
16 the new model instance the 95% credible intervals always contain the known foodstuff  
17 contribution. The model performance suggests that the uncertainty adopted for pot isotopic values  
18 is suitable (i.e. 0.6‰). In addition, the measured values of the pots containing single sources  
19 (pots 1–3) correspond very well with measurements of the raw foodstuffs (Tables 2 and 3),  
20 suggesting that the process of cooking and absorption does not cause any isotopic fractionation of  
21 these compounds. In contrast, the observed discrepancies between the actual contents and the  
22 content predicated by the model may be attributable to differential rates of absorption of lipids  
23 from different foodstuffs when cooked together.

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**5. Archaeological case studies**

*5.1. Sites and materials available*

Durrington Walls is a Late Neolithic henge monument in Southern Britain located near the site of Stonehenge. The main occupation of the site dates to 2,535–2,475 cal BCE and includes large amounts of animal bones, broken ‘Grooved Ware’ ceramics and other food-related debris, and has been interpreted as a place of feasting (Craig et al., 2015). Faunal remains at the site are dominated by domesticated pigs and, to a lesser extent, cattle. Other wild and domesticated animals and plants are extremely sparse. Lipids extracted from 151 potsherds were previously classified into three food categories (dairy, ruminant adipose, and non-ruminant) based on the difference stable carbon isotope ratios ( $\Delta^{13}\text{C}$ ) of their  $\text{C}_{16:0}$  and  $\text{C}_{18:0}$  *n*-alkanoic acids (Craig et al., 2015, Fig. 5A), using the criteria of Copley et al. (2003), although substantial mixing, particularly of ruminant adipose (presumably beef) and non-ruminant (presumably pork) was thought to have occurred (Craig et al., 2015).

Neustadt is a submerged coastal site on the Baltic coast of northern Germany that was occupied from ca. 4,600 to 3,800 cal BCE. The pottery at the sites includes both Late Mesolithic Ertebølle (EBK) and Early Neolithic Funnel Beaker (TRB) typologies (Saul et al., 2013). Exploitation of marine resources was very important at this site, indicated by thousands of fish bones and a particularly high frequency of marine mammals (Glykou, 2014). Terrestrial fauna are also well represented, including a small number of domesticates in the Early Neolithic layers. Organic residue analysis of 46 vessels has identified the presence of marine foods throughout the sequence, but also the presence of dairy and other ruminant products (Craig et al., 2011), which could also include wild ruminants such as deer, aurochs or moose. Five vessels have been

1 interpreted as oil lamps thought to have been used exclusively for burning marine oil (Heron et al.,  
2 2013). The presence of aquatic biomarkers (Hansel et al., 2004), including  $\omega$ -(*o*-alkylphenyl)  
3 alkanolic acids and isoprenoid fatty acids, on a number of vessels shows that fish or marine  
4 mammal oils made a contribution to the residue and may be used to constrain the model based on  
5 C<sub>16:0</sub> and C<sub>18:0</sub> measurements.

6

### 7 *5.2. Model estimates for pottery residues from Durrington Walls*

8 Source estimates were obtained for 121 sherds from Durrington Walls using the reference  
9 ranges for ‘dairy fat’, ‘porcine fat’ and ‘domesticated ruminant adipose’ as defined in Table 1.  
10 Credible intervals (68%) for the contributions from food sources to each vessel were estimated  
11 using conservative and non-conservative (standard error of the mean) models. The posterior  
12 distributions generated by the model are represented by ternary plots of the median values with  
13 marginal 68% credible intervals represented by error bars (Fig. 5B and 5C) allowing large  
14 number of samples to be visually compared. It should be noted, however, that the intervals in the  
15 three directions are not independent, and therefore the bars do not correspond to the true 68%  
16 credible area around the point, but they give a simple representation of the scale of the  
17 uncertainty. Overall, estimates provided by the model shows that for about two-thirds of pots, the  
18 main contributions are either from dairy or domesticated ruminant adipose. Under a conservative  
19 model credible intervals for the two lipid groups are relatively broad and in most cases it is not  
20 possible to exclude a significant contribution (ca. 20%) of either of these products in each pot.  
21 This ambiguity is slightly ameliorated when a non-conservative model is employed and the  
22 estimates typically indicate higher contributions from ‘dairy fat’. There are also several pots (ca.  
23 35%) showing large contributions from ‘porcine fat’ with more confidence.

1       The overlap in credible intervals for different products is attributable to the isotopic  
2 proximity of fatty acids from ruminant adipose and dairy. Previous studies (e.g. Evershed, 2008;  
3 Cramp et al., 2014) have used the difference between the  $\delta^{13}\text{C}$  values of  $\text{C}_{16:0}$  and  $\text{C}_{18:0}$  fatty acids  
4 ( $\Delta^{13}\text{C}$ ) to distinguish these products. It has been demonstrated that the  $\text{C}_{18:0}$  acid is depleted in  $^{13}\text{C}$   
5 compared to the  $\text{C}_{16:0}$  component in ruminant tissues and to an even greater extent in ruminant  
6 milk, due to physiological differences in the biosynthesis of these lipids (Copley et al., 2003).  
7 The established  $\Delta^{13}\text{C}$  ranges for non-ruminants, ruminants and dairy products are approximately  
8 1‰ to -1‰, -1‰ to -3.3‰ and -3.3‰ to -7‰ respectively (Fig. 5A). To evaluate the utility of  
9 this approach, the mean contributions of each of these products to the 121 vessels, as estimated  
10 through the conservative model, are compared with the  $\Delta^{13}\text{C}$  values for each vessel in Fig. 5D–F.

11       Vessels interpreted as dairy and porcine based on their  $\Delta^{13}\text{C}$  values were estimated to have  
12 contained at least 50% of fatty acids from these sources, showing the value of this proxy.  
13 However, many vessels with  $\Delta^{13}\text{C}$  values in the ruminant adipose fat range were estimated to  
14 have contained less than half their fatty acids from this source (Fig. 5E). This discrepancy serves  
15 as a note of caution when interpreting data of this nature. Mixing of sources with distinct isotope  
16 end-members, in this case dairy and porcine products, produces similar isotope values to sources  
17 with intermediate values, i.e. ruminant adipose. It is difficult to distinguish, for example, an  
18 absence of dairy fats when there is a possibility that this product was mixed with other non-  
19 ruminant sources. Furthermore, even pots that fall clearly within the  $\Delta^{13}\text{C}$  ranges for dairy and  
20 porcine fats could credibly also contain substantial amounts of other products. The issue of  
21 equifinality is often overlooked in residue analysis studies, but becomes clear when more refined  
22 estimations including uncertainties are provided using the Bayesian approach we describe here.

23

### 1 5.3. Model estimates for pottery residues from Neustadt

2 Source estimates were obtained for previously reported data from 46 sherds from the site  
3 of Neustadt (Craig et al., 2011). The vessels were typologically classified as belonging to the  
4 Late Mesolithic Ertebølle culture (EBK) or Early Neolithic Funnel Beaker culture (TRB).  
5 Ertebølle vessels were sub-divided into two distinct forms; cooking pots (EBK), or lamps (EBK  
6 lamps). The defined model contained three sources: combined ruminant (including dairy and all  
7 adipose tissues), porcine and marine as described in Section 4.1. In this case, the model estimates  
8 have relatively narrow credible intervals (Fig. 6). Furthermore, the credible intervals are  
9 considerably reduced when the non-conservative model is used (Fig. 6b).

10 Biomarkers for aquatic products, including isoprenoid fatty acids and long-chain (C<sub>18</sub>–  
11 C<sub>22</sub>) ω-(*o*-alkylphenyl) alkanolic acids (APAAs: Hansel et al., 2004; Craig et al., 2011), were  
12 observed in 13 of the samples analysed (Fig. 6). These are only formed by protracted or repeated  
13 heating of the fish, shellfish or marine mammal oils. Seven of these samples were estimated to  
14 have lipids derived predominantly from a marine source (Fig. 6), but four samples were estimated  
15 to only have contained at most low amounts of marine-derived lipid. Unfortunately, without  
16 further information regarding the conditions required for their formation, the presence or absence  
17 of such aquatic biomarkers is unable to provide quantitative information on source contributions  
18 and therefore constrain the model. Experiments to ascertain such quantified limits would be  
19 useful for future studies. In addition, the use of other quantitative measures of aquatic product  
20 content, such as the ratio of diastereomers of phytanic acid (Lucquin et al., 2016) or elemental  
21 analysis-IRMS of the nitrogen in carbonised deposits (Heron and Craig, 2015) could be easily  
22 incorporated as additional proxies or priors into the model.

1 Even without incorporating further proxies or prior information, the analysis of mixtures  
2 shows some interesting patterning in pottery use that are difficult to infer from plotting isotope  
3 data alone. Firstly, the model estimations reveal that marine fats were extensively mixed with  
4 ruminant products in the Neolithic (TRB), but less so in the Mesolithic pots (EBK). Conversely,  
5 porcine and ruminant products were mixed in late Mesolithic vessels, whilst porcine products  
6 were much less common in the Neolithic vessels (Fig. 6). These data contribute to the debate  
7 regarding the changing values and culinary roles of specific foods across the transition to farming  
8 (Saul et al., 2013). At Neustadt, pottery use does not reflect the proportion of different animals  
9 exploited, as determined by the well preserved faunal assemblage, indicating that very deliberate  
10 culinary choices were made on what to include or exclude from pottery, and perhaps what foods  
11 were allowed to be mixed together, and that the value of these foods changed following the  
12 introduction of domesticated species. Secondly, it is estimated that the six Ertebølle lamps,  
13 believed to be used for burning marine oils, may have had a more variable use than previously  
14 thought (Heron et al., 2013). The estimates show that the isotope data supports substantial use of  
15 porcine fat in addition to marine oils (Fig. 7). For one vessel (N1009) there is also a discrepancy  
16 between lipids absorbed into the ceramic (N1009i) and those recovered from a carbonised surface  
17 deposit (N1009s), suggesting that terrestrial fats and marine oils were burnt during different  
18 episodes in this lamp.

19

#### 20 *5.4. Future developments and applications*

21 As a next step, we suggest incorporating additional datasets to improve model performance.  
22 This includes the use of independent prior information, such as data from the presence of lipid  
23 biomarkers. These data could be used to broadly constrain the model parameters but require

1 understanding of the bounding conditions (e.g., minimum relative source concentrations)  
2 necessary for their formation. Further quantitative information on relative source contributions  
3 can also be obtained by employing additional proxies. These could include ratios of different  
4 stable isotopes (e.g.,  $\delta^2\text{H}$  or  $\delta^{15}\text{N}$ ), isotopic measurement of a wider range of compounds and the  
5 relative concentrations of different individual compounds, provided these are preserved during  
6 diagenesis (e.g., phytanic acid diastereomers). Furthermore, it is necessary to establish the  
7 parameter values of food sources with greater confidence through measurement of a greater  
8 number of authentic reference samples. Notably, the uncertainty due to geographical effects,  
9 Suess effect and other temporal effects could be reduced by establishing in situ reference ranges  
10 directly from archaeological bones of known species that are contemporary with the artefacts to  
11 be analysed (Colonese et al., 2015).

12 Finally, we note that this approach may have applications beyond archaeological science and  
13 could be used to more accurately quantify source contributions to mixtures in a range of  
14 environmental and geological settings using different forms of geochemical data. Such  
15 applications might include the sourcing of sediment inputs in fluvial systems using, for example,  
16 a combination of organic molecular (e.g., Chen et al., 2017) and inorganic elemental data  
17 (Haddadchi et al., 2014), alongside compound-specific radiocarbon measurements (Pearson and  
18 Eglinton, 2000; Feng et al., 2015; Fernandes et al., 2017). Other applications, include estimating  
19 the contributions of different end members (plants, microorganisms) to modern (e.g., Eley et al.,  
20 2012) or ancient sediments, based on the molecular and isotopic properties of their biomarkers  
21 (Newman et al., 2016).

22

## 1 **6. Conclusions**

2 In this study, we provide a new approach for assessing the contribution of different  
3 foodstuffs to archaeological pottery vessels based on fatty acid stable carbon isotope  
4 measurements, using a Bayesian mixing model. These estimates, expressed as posterior  
5 probability distributions, were determined by taking into account both the uncertainties  
6 associated with the measurement of isotope values in fatty acids extracted from potsherds and the  
7 uncertainties associated with the concentration and carbon isotope composition of reference food  
8 groups. We contend that this approach provides a more nuanced interpretation of vessel use than  
9 has been achieved before. By testing the model under different simulated scenarios and by  
10 applying it to data from pots used in controlled cooking experiments, we are also able assess the  
11 degree of ambiguity when estimating contents based solely on fatty acid carbon isotope values. In  
12 particular, we highlight the problem of equifinality, i.e. where many possible solutions account  
13 for the observed the data, which is due to the proximity of isotope signals from different food  
14 reference groups, and co-linearity among these values.

15 In an attempt to circumvent this problem, we improved the model's performance by using  
16 the standard error of the mean (SEM) to represent the error describing the distribution of the  
17 source data or by aggregating reference food groups, albeit at the loss of source resolution. When  
18 applied to archaeological data, we show that the mixing model could identify patterns in pottery  
19 use that were hitherto unknown, although the precision of the estimations was generally low with  
20 generally broad credibility intervals for each product. However, these data highlight the real  
21 precision achievable with this approach, a fact that needs to be considered when assigning pottery  
22 use to a single or mixture of source(s) and when making archaeological interpretations.

23



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10

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12

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- 15

Figure 1

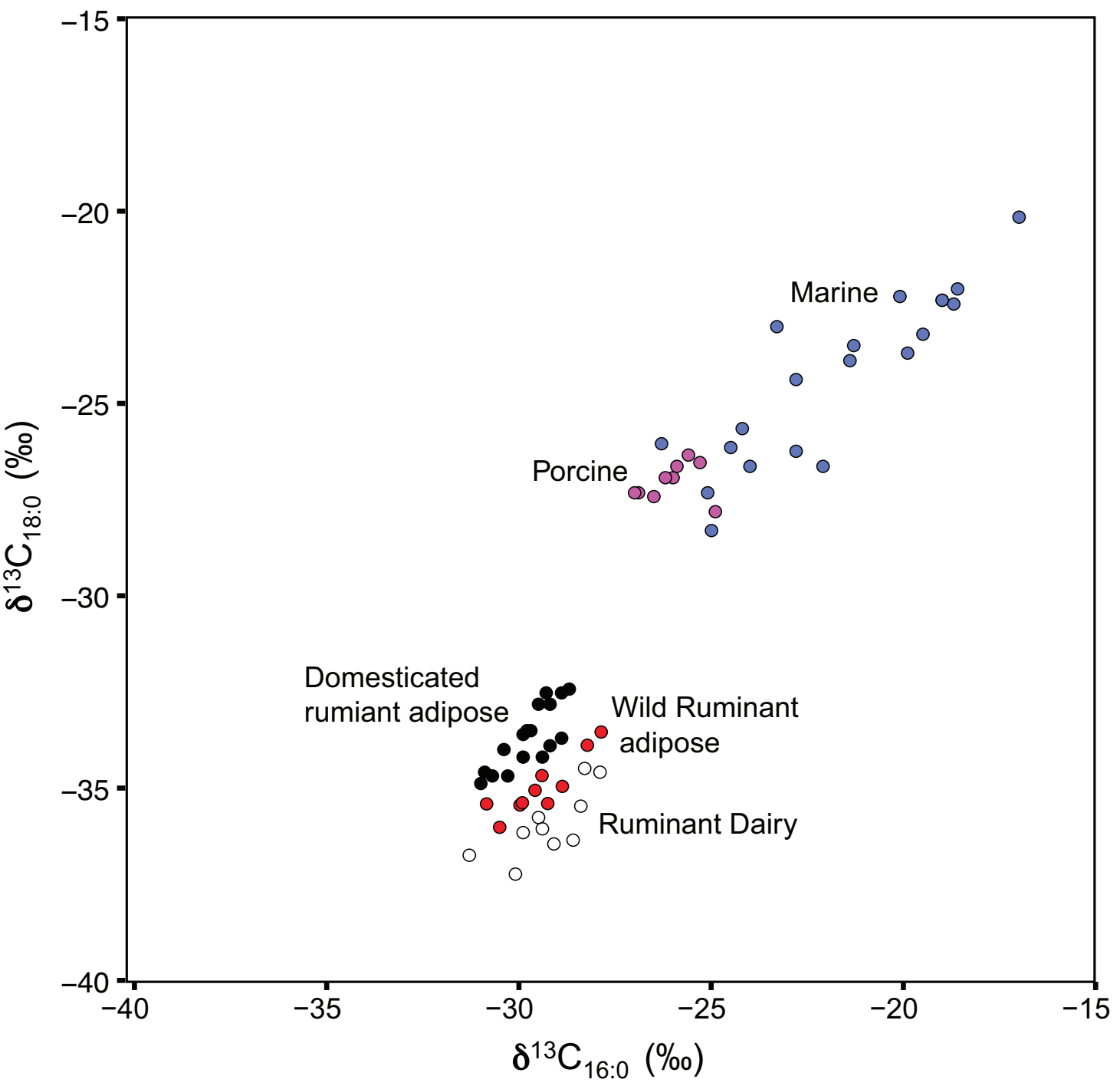


Figure 2

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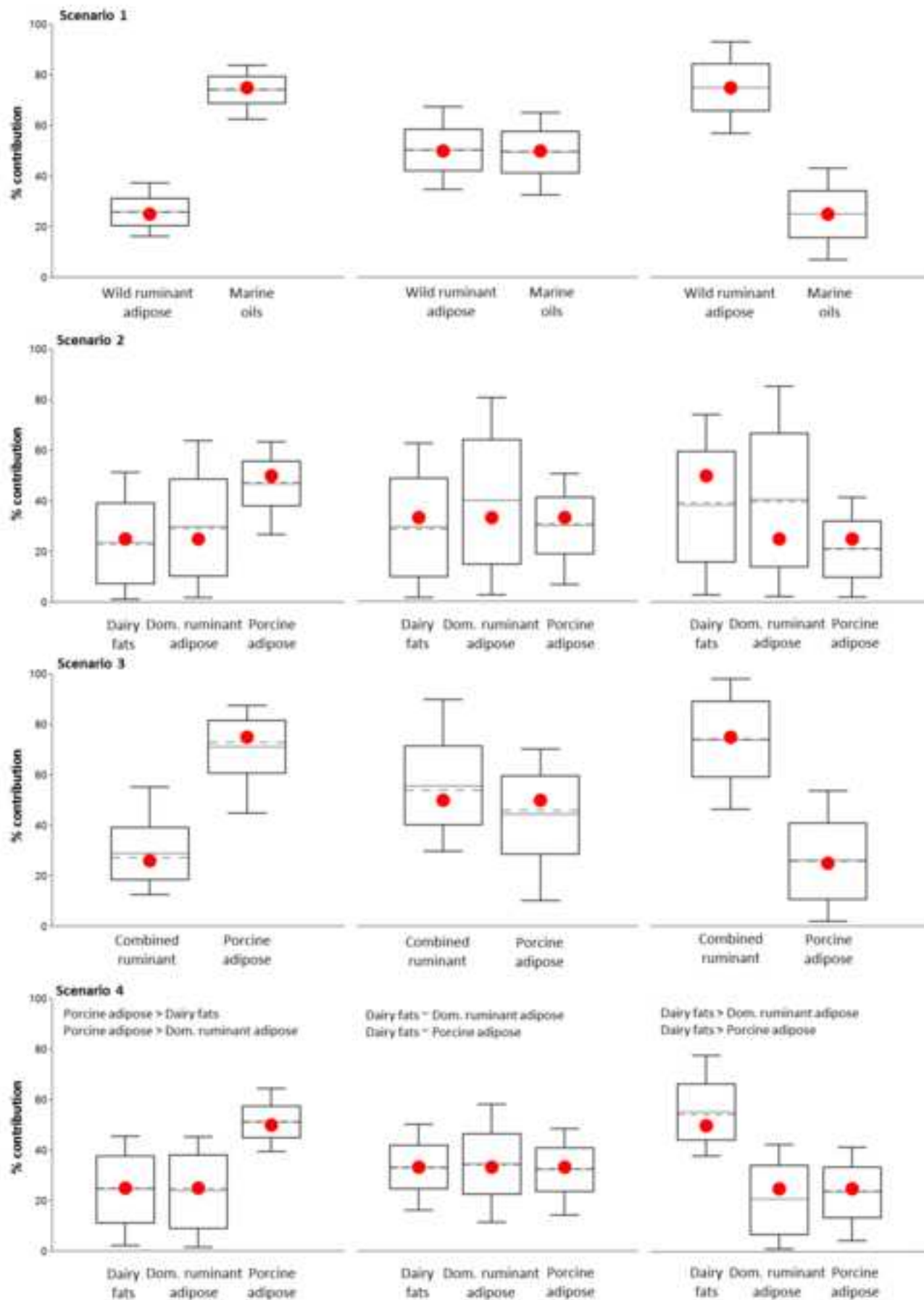




Figure 3

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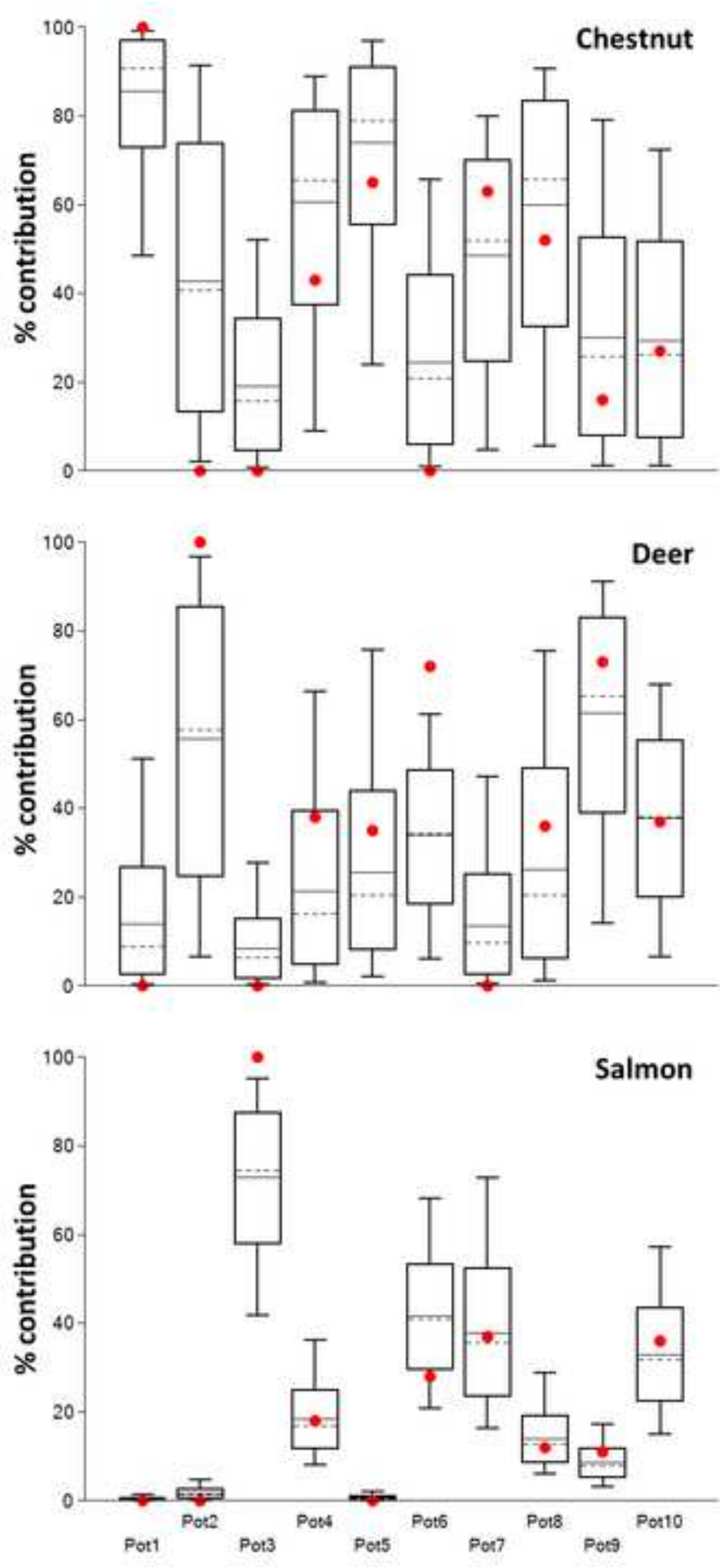
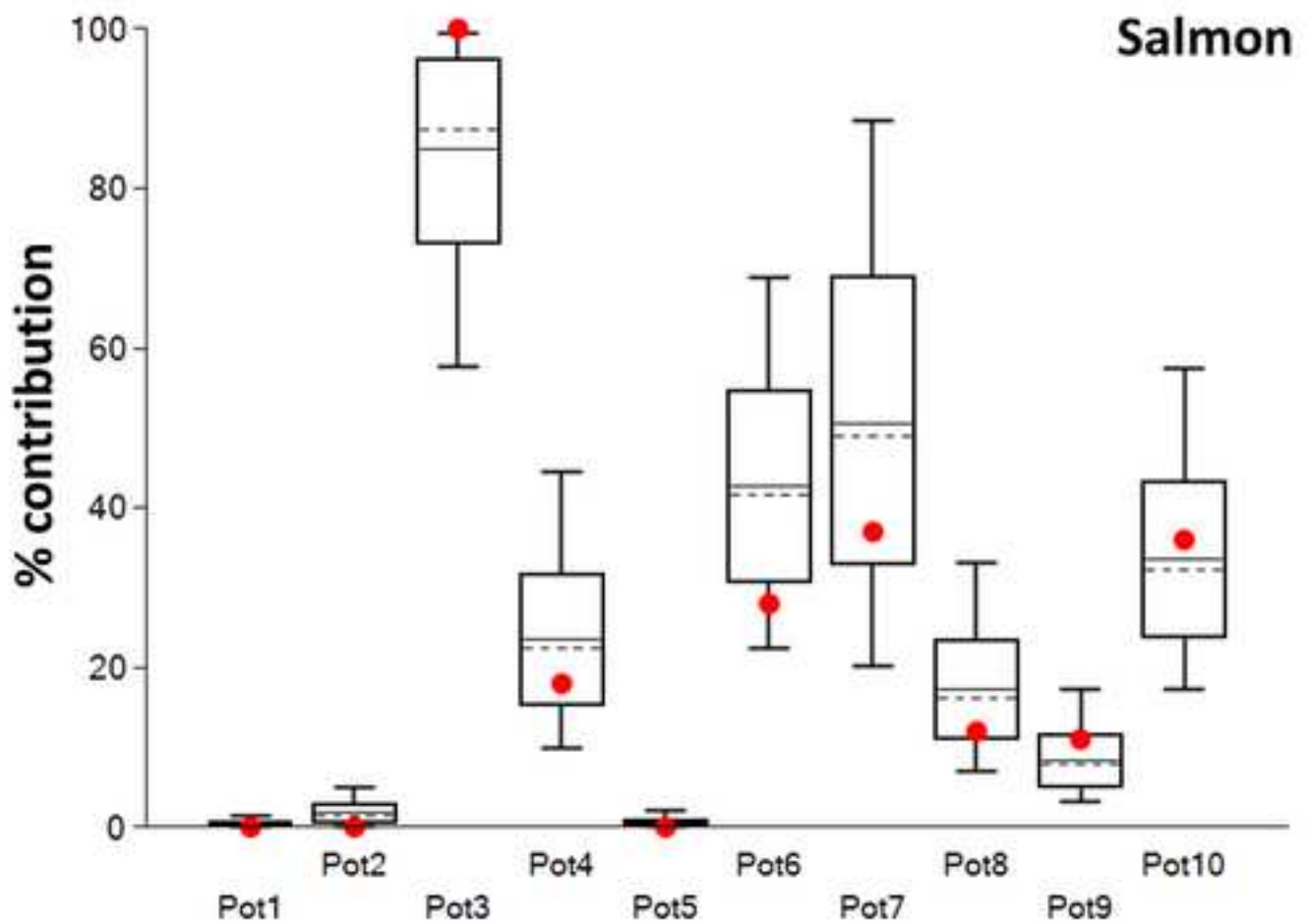
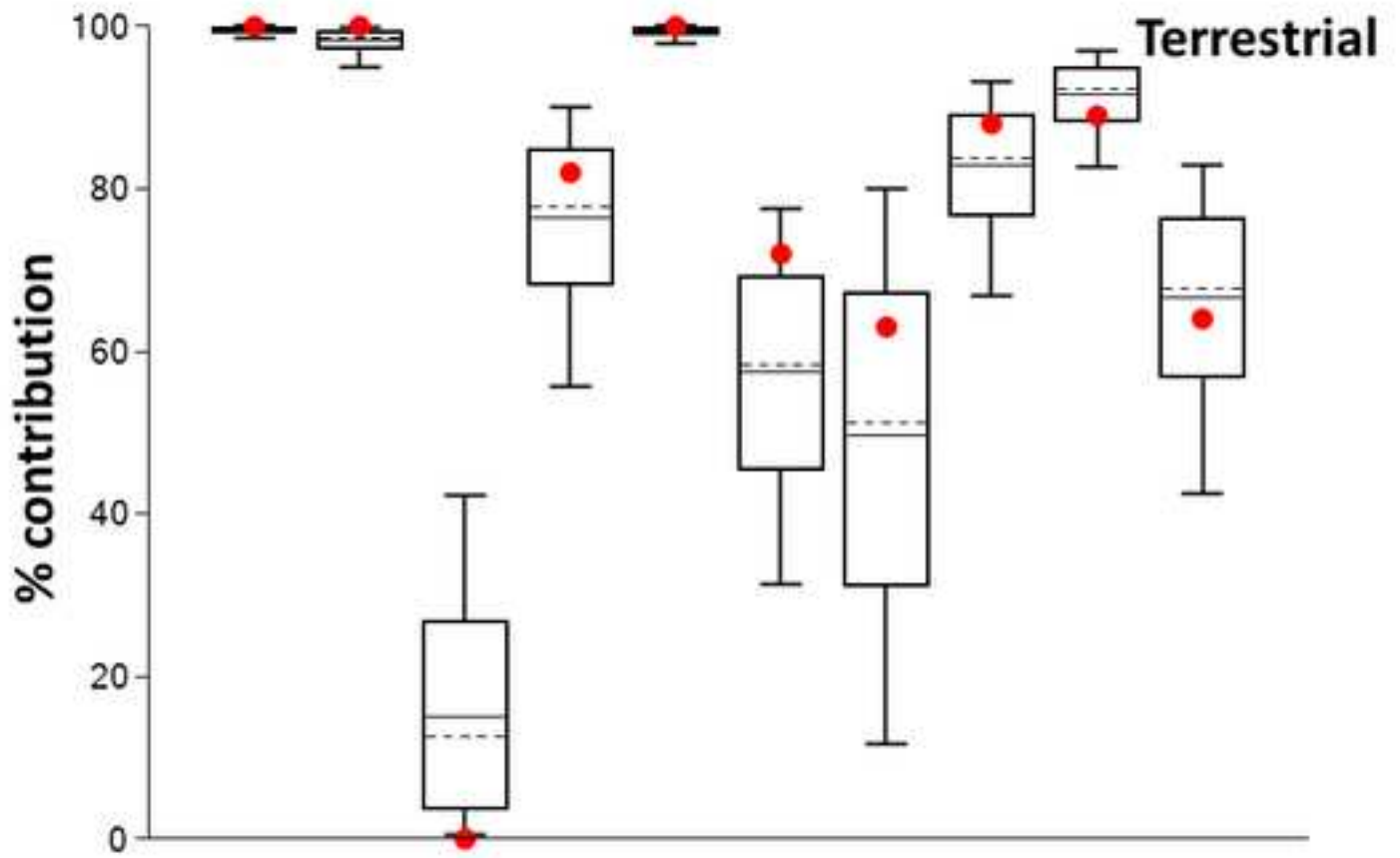


Figure 4  
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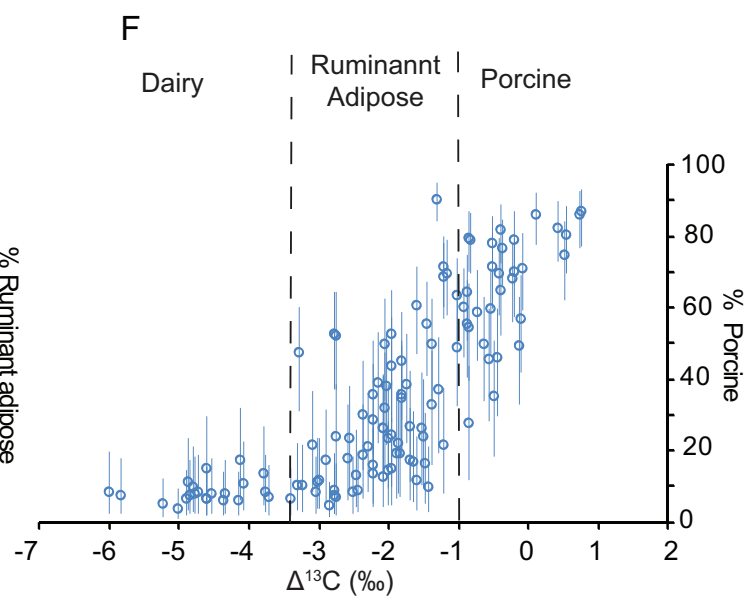
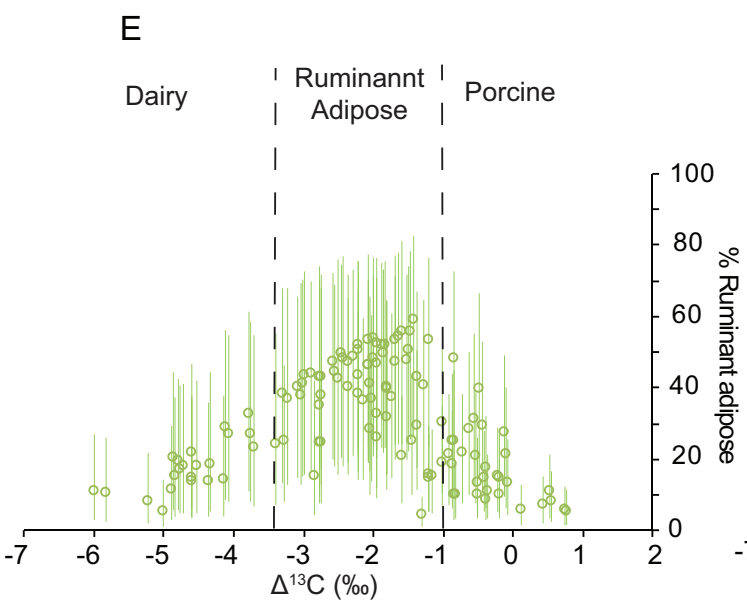
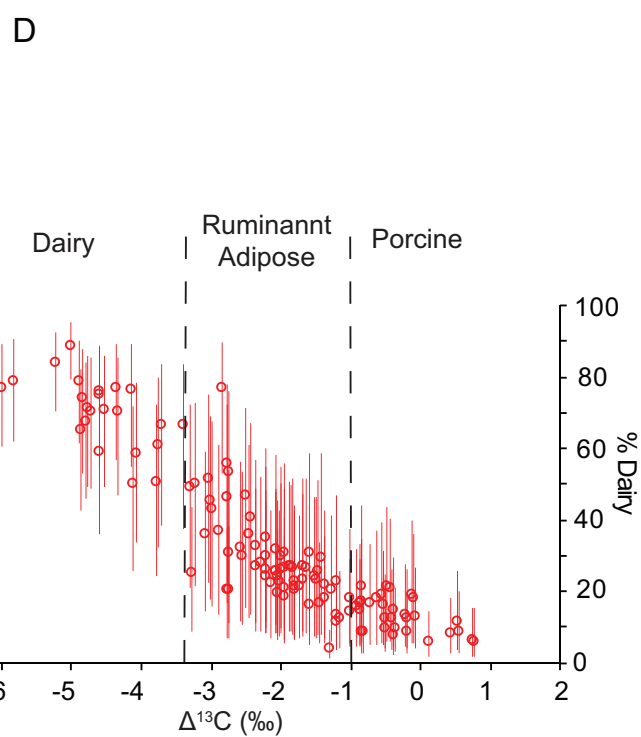
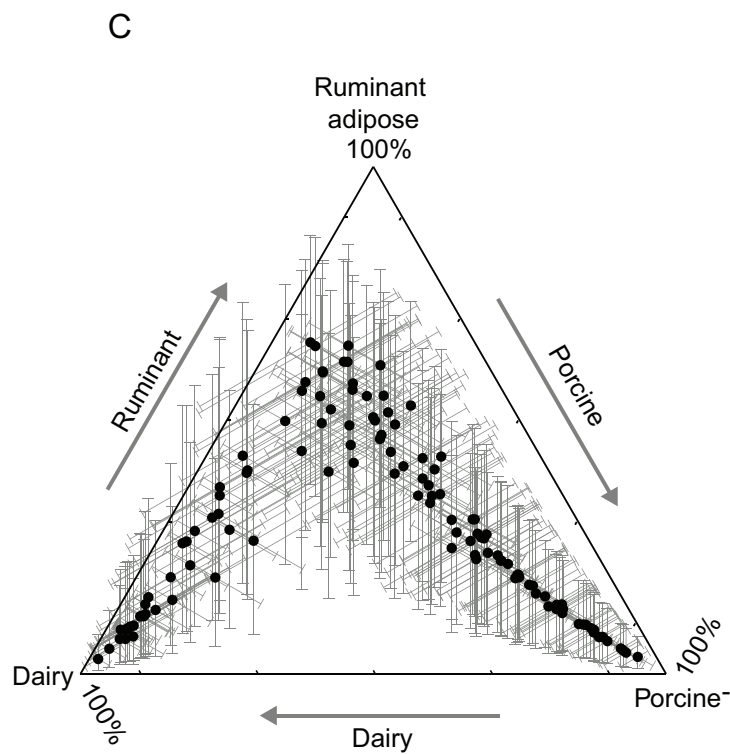
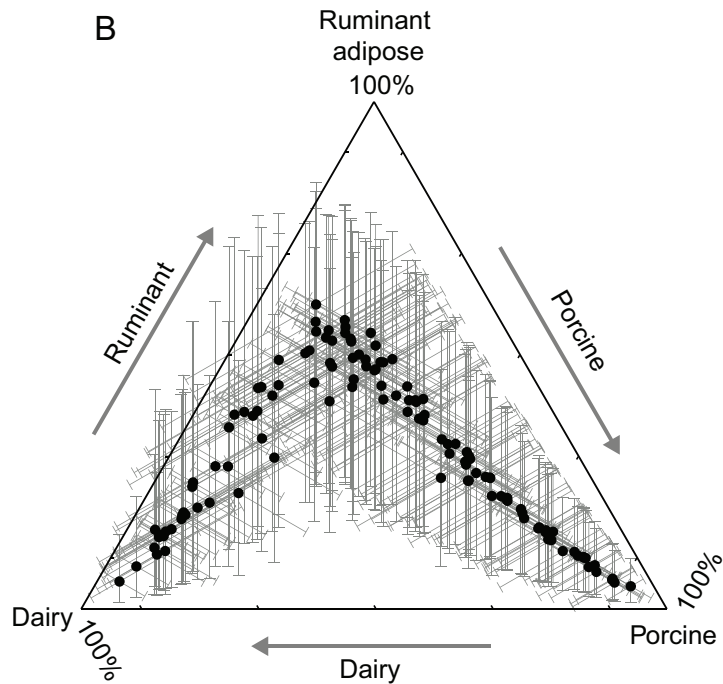
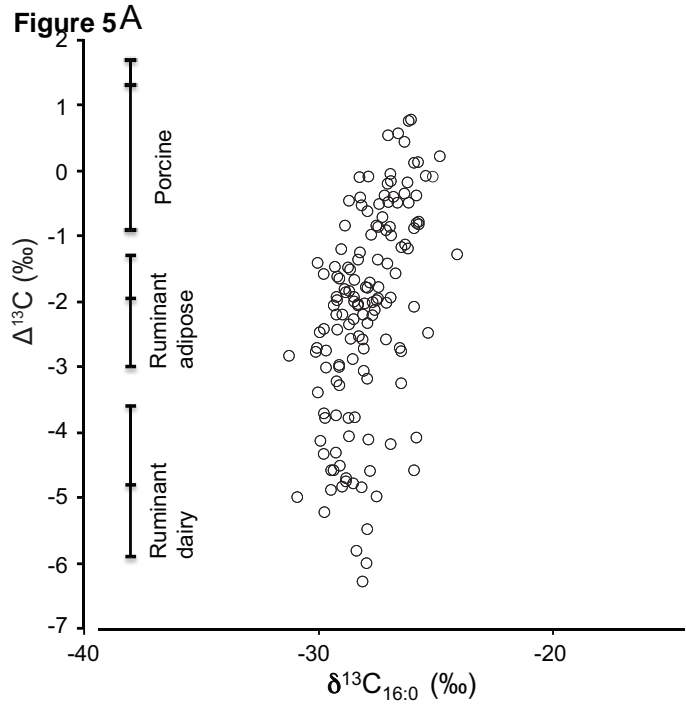


Figure 6

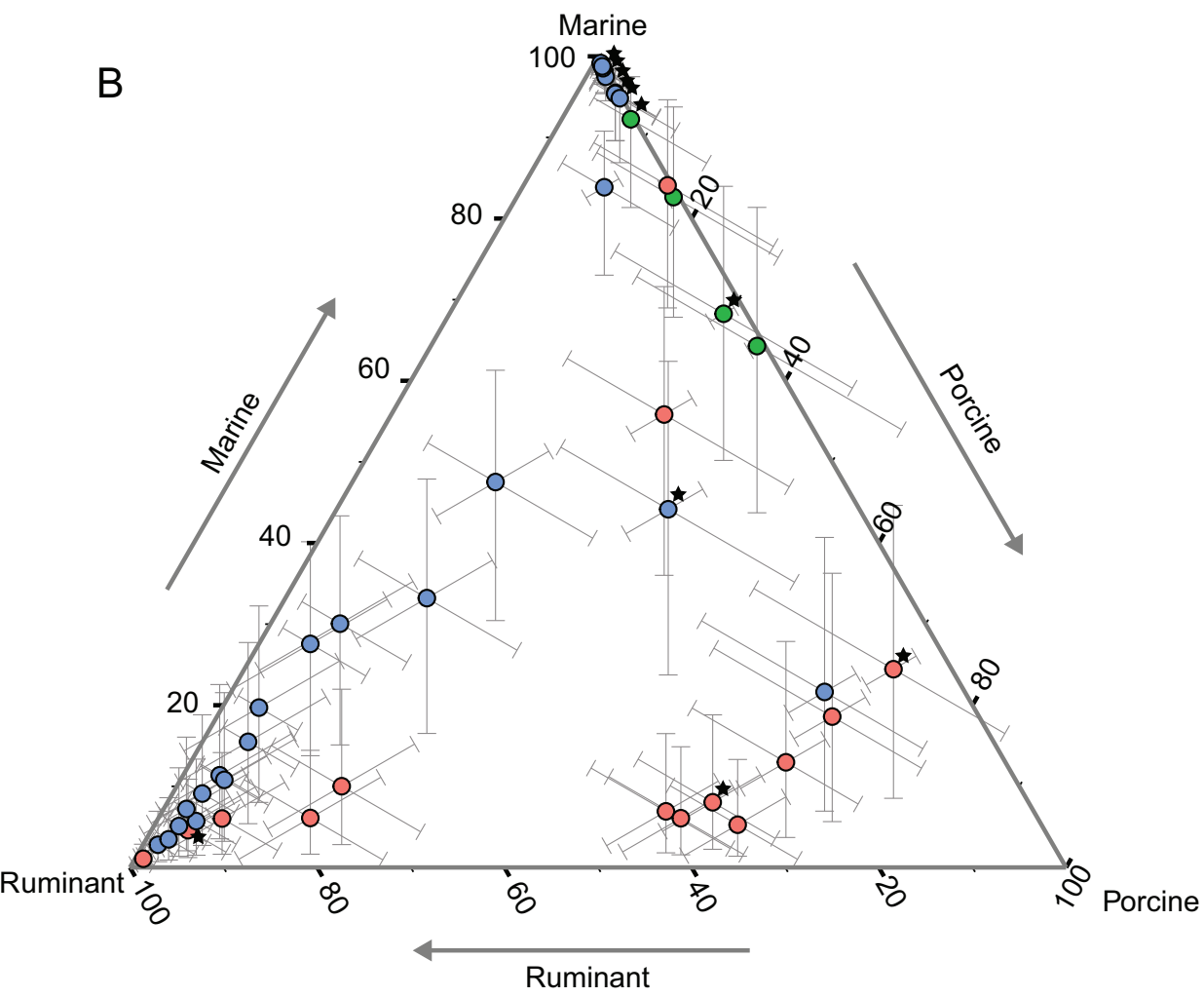
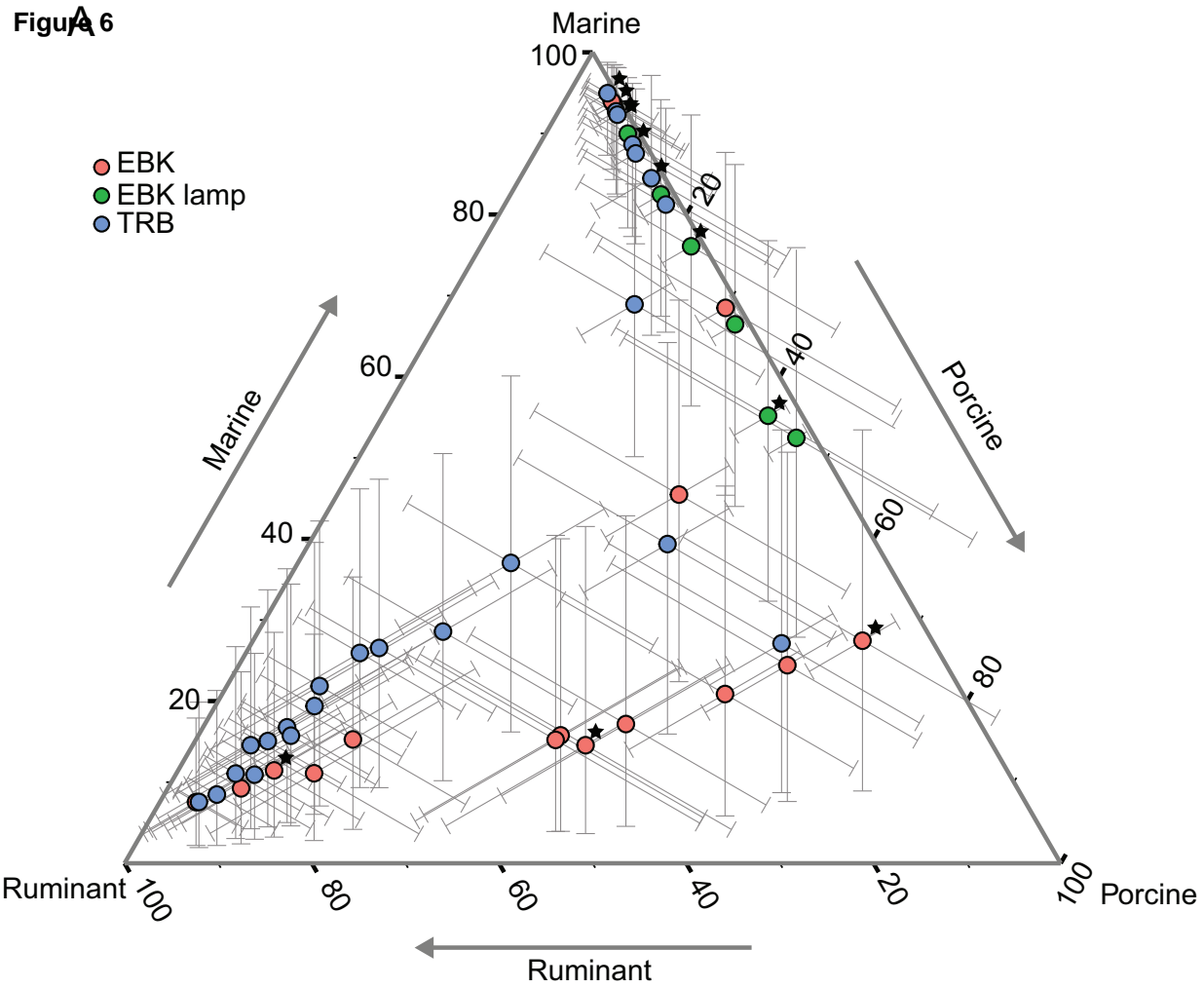
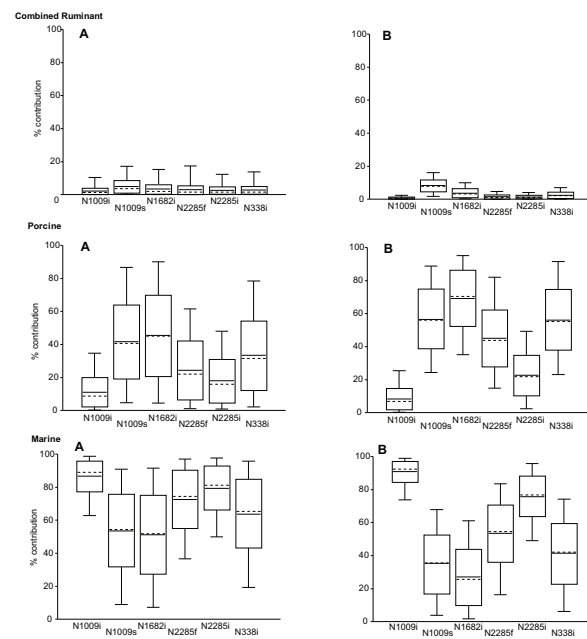


Figure 7



## Figure captions

**Fig. 1.** Distribution of stable carbon isotopic values ( $\delta^{13}\text{C}$ ) of fatty acids from different lipid groups. The data have not been corrected for Suess effect (see text).

**Fig. 2.** Comparison of simulated contributions and model estimates. Box plots show model output, while filled circles show simulated contribution. The boxes represent a 68% credible interval while the whiskers represent a 95% credible interval. The horizontal continuous line indicates the mean while the horizontal discontinuous line indicates the median.

**Fig. 3.** Modelled versus actual percentage of each foodstuff added to the experimental pots. Box plots show model output, while filled circles show actual content. The boxes represent a 68% credible interval while the whiskers represent a 95% credible interval. The horizontal continuous line indicates the mean while the horizontal discontinuous line indicates the median. All estimations are reported as percentage contribution by wet weight to each pot.

**Fig. 4.** Modelled versus actual percentage of aggregated terrestrial and salmon input to the experimental pots. Box plots show FRUITS output, while filled circles show actual content. The boxes represent a 68% credible interval while the whiskers represent a 95% credible interval. The horizontal continuous line indicates the mean while the horizontal discontinuous line indicates the median. All estimations are reported as percentage contribution by wet weight to each pot.

**Fig. 5.** (:) Plot of  $\delta^{13}\text{C}_{16:0}$  and  $\Delta^{13}\text{C}$  values of fatty acids extracted from individual vessels at Durrington Walls against the ranges (median, max, min) in  $\Delta^{13}\text{C}$  from authentic reference fats. (B and C): Ternary plots with points representing the median contributions from each source using conservative (B) and non-conservative (C) model parameters. Whiskers represent the 68% credible intervals of marginal distributions. For each lipid group whiskers are parallel to a bisector axis of the triangular plot (lipid group identified at the vertices of the triangular plot) and constrained by the length of the bisector they lie parallel to. As such, whiskers may extend beyond the boundaries of the triangular plot. (D–F): Plots showing medians and 68% credible intervals representing the contributions of each source to each potsherd, as estimated from non-conservative mixing model, against their  $\Delta^{13}\text{C}$  values.

**Fig. 6.** Ternary plots with points representing the median source contributions to vessels from Neustadt, Germany using conservative (A) and non-conservative model parameters (B). Whiskers represent the 68% credible intervals of marginal distributions. For each lipid group whiskers are parallel to a bisector axis of the triangular plot (lipid group identified at the vertices of the triangular plot) and constrained by the length of the bisector they lie parallel to. As such, whiskers may extend beyond the boundaries of the triangular plot. All estimations are reported as % fatty acid of each source in total fatty acid. All estimations are reported as % fatty acid of each source in total fatty acid. The presence of aquatic biomarkers (isoprenoid fatty acids and  $\text{C}_{20}$ ,  $\text{C}_{18}$  APAAs) is marked with a star.

**Fig. 7.** Estimates for contributions of different sources to samples of Late Mesolithic ‘lamps’ from Neustadt using conservative (A) and non-conservative (B) model parameters. The boxes represent a 68% credible interval while the whiskers represent a 95% credible interval. The horizontal continuous line indicates the mean while the horizontal discontinuous line indicates the median. All estimations are reported as % fatty acid of each source in total fatty acid. Pot sample number are shown. i indicates interior drilled residue, s and f are carbonised surface deposits.



## Tables

**Table 1.** Mean plus standard deviation fatty acid isotopic and concentration values for different lipid groups.

	Marine fats	Dairy fats	Domesticated ruminant adipose	Porcine adipose	Wild ruminant adipose	Combined ruminant
$\delta^{13}\text{C}_{16:0}$ (‰)	-21.6±2.7	-29.2±1.0	-29.6±0.7	-25.9±0.7	-29.4±0.9	-29.4±0.9
$\delta^{13}\text{C}_{18:0}$ (‰)	-22.1±2.2	-34±0.9	-31.7±0.8	-24.9±0.5	-33±0.8	-32.7±1.3
n (isotopes)	20	10	17	9	10	37
$\text{C}_{16:0}$ (% total FA)	13.6±3.9	34.6±7.2	24.2±5.0	24.1±2.1	17.2±3.6	26.3±8.6
$\text{C}_{18:0}$ (% total FA)	1.9±1.2	18.4±8.4	28.5±6.5	11.5±2.4	15.7±1.7	22.4±8.6
n (concentration)	15	10	13	9	6	29

**Table 2.** Known mixtures of foodstuffs added to the experimental pots as part of the YEAR cooking experiment.

Mass wet tissue added (g)	Experimental number										
	1	2	3	4	5	6	7	8	9	10	11*
Chestnut	22.5	0.0	0.0	14.2	13.8	0.0	14.2	13.0	3.9	6.5	0.0
Deer	0.0	21.3	0.0	12.7	7.5	18.6	0.0	9.2	17.0	9.1	0.0
Salmon	0.0	0.0	19.8	6.1	0.0	7.0	8.4	3.1	2.6	8.8	0.0
Total	22.5	21.3	19.8	33.0	21.3	25.6	22.6	25.2	23.4	24.4	0.0
% by mass in each pot											
Chestnut	100%	0%	0%	43%	65%	0%	63%	52%	16%	27%	0%
Deer	0%	100%	0%	38%	35%	72%	0%	36%	73%	37%	0%
Salmon	0%	0%	100%	18%	0%	28%	37%	12%	11%	36%	0%
Measured isotope value (‰)											
$\delta^{13}\text{C}_{16:0}$	-35.8	-33.5	-26.4	-30.6	-35.7	-27.2	-29.3	-31.1	-30.4	-27.9	n/a
$\delta^{13}\text{C}_{18:0}$	-37.9	-35.7	-26.3	-29.9	-36.5	-30.2	-27.6	-31.1	-34.6	-30.6	n/a

\*Pot processed as a control containing only water

**Table 3.** Carbon isotope values and concentration of C<sub>16:0</sub> and C<sub>18:0</sub> fatty acids from the foodstuffs used in the cooking experiments.

Foodstuff	Carbon stable isotope value $\delta^{13}\text{C}$ (‰)		Fatty acid concentration (mg/g of wet mass)	
	C <sub>16:0</sub>	C <sub>18:0</sub>	C <sub>16:0</sub>	C <sub>18:0</sub>
Chestnut	-35.8	-38.1	0.631	0.095
Deer	-33.6	-35.3	0.839	0.872
Salmon	-26.6	-26.6	7.314	1.640

Dear Organic Geochemistry Editors,

We hereby submit for your consideration a revised version of our manuscript titled “Quantitative reconstruction of prehistoric pottery use from fatty acid carbon isotope signatures using Bayesian inference”. We have addressed all of the issues raised by the editors and reviewers (see below remarks in red). We have also submitted two manuscript files (with and without track changes).

We are looking forward to receiving your feedback!

Best wishes,

Ricardo Fernandes (on behalf of all authors)

Please remove the space before the per mil sign throughout the text

Done!

replace the dash in GC-MS with an n-dash.

Done!

Organic Geochemistry also encourages authors to include an acknowledgement of the time and effort provided by the reviewers, whether they are anonymous or have been identified.

Done!

AE's comments:

Page 18, line 1: Change to "Model estimates for pottery residues from Neustadt"

Done!

Conclusions should be condensed to half of its current length by generating a model achievement/outlook section (5.4) at the end of the discussion.

We followed this suggestion and added section 5.4 titled "Future developments and applications"

Reviewer #1: Manuscript Number: OG3503

Comments:

The paper explores the utility of using a Bayesian model (FRUITS) to quantify the proportions of different foodstuff in archaeological pottery. As such, the topic of the paper fits the scope of the journal. While organic residue analysis is a well-established field in archaeology, challenges associated to quantify contributions of the various constituents to the residues still exist. In this paper, the authors introduced a novel approach (the use of a Bayesian model: FRUITS) to predict the different proportions in which different food sources contributed to a given mixture. The performance of the model was tested first by simulating four different scenarios combining lipid groups of known C16:0 and C18:0 isotope and concentration dependencies values. Then, the model was used to reconstruct mixtures from ten ceramic vessels of known contents and to analyze in which extent the model reflects the actual proportions of the food items added to the residues during the controlled experiment. It is important to highlight that the authors properly discussed the main sources of uncertainties associated to the proposed approach,

although I would suggest to include more arguments on how these uncertainties may affect the conclusions reached when analyzing archaeological data. Please find below additional comments/suggestions to improve the manuscript:

1. I would suggest to include all information regarding the model specifications, model performance (simulated examples and controlled experiment to test the model) and model implementation (archaeological data) in a clearly separated section ("Materials and Methods"). Then, I would suggest to discuss the results in a different section.

We think that it is important to structure the article in this way as the results of each stage inform the next. That is, first we demonstrate model performance using simulated data, then we introduce real experimental data, and finally we apply the model to archaeological studies. By grouping everything under "Materials and Methods" followed by "Results" we feel that this logical sequence would be lost and that readability reduced.

2. In Model Evaluation (p. 10), the authors tested model performance by simulating four scenarios. It would suggest to describe the rationale for the design of the different scenarios and to explain the different cases simulated.

There would be a large number of combinations that could be tested and so we choose four that are likely to occur in archaeological case studies. We modified the beginning of the section to read: "To test model performance we first employed four simulated scenarios. A large variety of scenarios could have been selected but we chose four that could be similar to those that are likely to occur in actual archaeological case studies. These were defined as were defined:..."

3. In addition, the scenario 4 was described in page 10 (line 10) as similar to scenario 2, but with additional prior information added to the model. Then, in page 11, line 8, the text says that scenario 4 is similar to scenario 3, please correct.

Corrected!

4. In table 1 you showed the mean fatty acid isotopic and concentration values for different lipid foods. How variable were the values among technical replicates? (I would suggest to include the SD).

We have added SD values to the table!

5. In section 4.2, you stated that eleven replica ceramic vessels were used to prepare different mixtures. However, in table 2, you said that sample 11 is a control containing only water. Please indicate this clearly in the text and provide information of results obtained from the blank.

Corrected! No lipids could be identified on the control containing only water. We have inserted a sentence in the text to confirm this.

6. In page 14 (lines 17-19), you stated that the observed discrepancies between the actual contents and the contents predicated by the model may be attributable to differential rates of absorption of lipids from different foodstuff when cooked together. While this can certainly be a possibility, how would you explain discrepancies showed in pot 3 (Figure 4) where a single source is present? It is interesting that there is a certain tendency to decrease discrepancies in pots with lower portions of salmon (Figure 4), why would be a possible reason?

For pot 3 (Figure 4) the actual value is within the estimated 95% credible interval although out of this interval in Figure 3. The model performs excellently for salmon. Given the comparatively large uncertainty that we employ the lower estimate precision reflects a possible mixed contribution from the two terrestrial sources. When there is a single terrestrial source, or almost, the estimates will be more precise given the proximity of isotopic values for the two terrestrial sources. The estimates will be again less precise when there are more mixed contributions from the three sources.

7. The model was implemented by including lipids groups that had been identified in previous studies. For the FRUIT model to provide good estimates it is important that the proposed dietary scenarios approximate the real scenario (Fernandes et al. 2014). Taking into account that taphonomic factors can prevent the preservation of organic material in archaeological contexts, and that it is unlikely to find all plants and animal consumed by the population in the archaeological assemblages, I would suggest to discuss how this possibility would affect interpretations resulting from the model.

This is an extremely valid point, also mentioned by reviewer 2, and we believe that we have reliable archaeological information for the case studies that we present. We have added the following to the text: "In cases where robust archaeological information is lacking and taphonomic effects may be unaccounted for different scenarios should be tested to verify the sensitivity of model estimates."

8. Figure 5E and 5F are mentioned in the text before Figure 5C and 5D.

Figure corrected, text corrected and figure captions amended.

Reviewer #2: This paper offers a much-needed model for the interpretation of compound specific isotope values in the field of organic residue analysis. Equifinality, as the authors rightly point out, is unfortunately a significant problem in this field of research, caused by mixtures of products becoming absorbed within the ceramic matrix during single or multiple uses of vessels. Researchers working in this field turn to faunal and botanical sources recovered from the archaeological context, and biomarkers identified by Gas Chromatography-Mass Spectrometry in an effort to identify the source products, as well as mixing models, which however take into consideration only few parameters. This prevents a full understanding of the data generated.

Many thanks for the encouraging remarks!

While I cannot specifically comment on the mathematical analysis behind the model, it is clear from

reading the paper that the authors have taken into considerations the relevant factors influencing the isotopic values of the targeted palmitic and stearic fatty acids, and have included them as parameters for the model they propose. The paper presents experimental and archaeological data. The results presented from the experimental work carried out are promising, although it is clear that the model needs further refinement in distinguishing between products having similar isotopic values. Combining sources is not effective when trying to distinguish the different products making up the mixture, however it does make the point that when the mixture comprises products with distinct isotopic values, the model can distinguish the different contributions. The archaeological data shows the potential behind the use of this model, and also a good correlation with the biomarker evidence in the Neustadt example.

We fully agree with the reviewer! And one of the points we wish to make with this paper is the inability of making definite statements on source contributions when the isotopic signals for these are similar. In these cases, additional isotopic proxies are needed or prior information is added to the model.

The model excludes the effect of taphonomy (model specification), which is presently justified, however this is a significant factor that needs addressing as the model becomes more refined. To what extent is this affecting the output?

It would indeed affect the output, although for our archaeological case studies the chosen scenarios are based on sound archaeological evidence and with detailed faunal assemblages available. We should add that this applies in generic terms to the interpretation of fatty acid data even when mixing models are not applied. Our modelling has the advantage of making quite explicit the assumptions on which it is based. A possible approach to demonstrate the impact of taphonomic effects is to perform a sensitivity test by comparing different scenarios (as we did in the simulated case studies). We have added the following to the text: "In cases where robust archaeological information is lacking and taphonomic effects may be unaccounted for different scenarios should be tested to verify the sensitivity of model estimates."

I am not sure I agree with the decision not to consider the input of freshwater fish in the model because their presence is rare in the faunal assemblage. Would the model not be biased by their exclusion?

See previous reply!

How does the programme deal with bias when using archaeozoological and archaeobotanical data, which may not necessarily reflect the true contributions, given, among others, taphonomic bias?

In the text: "One has to be aware of the fact that while imposing correct relationships can help efficiency, imposing wrong relationships can be entirely detrimental to the whole model (results can be easily biased heavily)."

The models presented in the article do not consider any quantitative data from faunal remains but we do consider likely sources to include in the model based on these data. If there were compelling reasons to think that particular plants or animals must have made a minimum relative contribution, considering taphonomic bias,



then this fact may be used to constrain the model as we suggest. Similarly, if there were good reasons to think that a particular source may be missing due to taphonomic or recovery biases then these could be included in the model. The latter is unlikely to be the case in the two studies we have chosen since they benefit from detailed faunal reports and were excavated recently using modern techniques.

Also, what is the logic behind the chosen uncertainty factor of 0.6ppm?

There is indeed need for a better quantification of this value. The typical error on measurement reproducibility is 0.3%. However, have used a more conservative value to account for additional uncertainties associated with the process of lipid absorption . We have added the following to the text: “We believe this to be a conservative uncertainty since it represents the double of the value (0.3%) commonly used for measurement reproducibility. “

What control measures are in place when adding prior information to the model? Can this not significantly bias the output?

As mentioned above the model user needs to ensure that the priors are only applied when supported by strong archaeological evidence and taphonomic effects are taken into account. There will certainly be instances where the disproportion in well-preserved archaeological remains allows for the use of the described priors (e.g. source A smaller/larger than source B).

Please find below a few minor typos in the text:

- \* Page 5 line 13: space needed before 'is positive...'
- \* Page 6 line 5: check for possible extra space
- \* Page 7 line 11: typo - 'collection' instead of 'collections'
- \* Page 11 line 7: check 'Wild ruminant adipose' or 'Domestic ruminant adipose'? 'Wild ruminant adipose' was not reported for scenario 2. Check also wording on Figure 2 Scenario 4.
- \* Page 15 line 23: '...thought to have been used exclusively...'

All typos have been corrected!