

COLLAGEN QUALITY INDICATORS FOR RADIOCARBON DATING OF BONES: NEW DATA ON BRONZE AGE CYPRUS

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ABSTRACT. Radiocarbon dating of bones can be very useful in archaeological contexts, especially when dealing with funerary deposits lacking material culture, e.g. pottery vessels. ¹⁴C measurements of bone samples are usually performed on the extracted collagen residue. The content and the quality of collagen can vary significantly, mainly depending on bone preservation and diagenesis. Generally speaking, environmental conditions such as low pH level of soils, high temperatures, and percolating groundwaters, typical of arid and tropical zones, can affect the preservation of collagen; at the same time, bones recovered in such environments are more likely to be contaminated with carbon from the surrounding environment. Possible contamination of samples can also occur in temperate zones. While low collagen content is a condition we cannot overcome, we can use several chemical and elemental indicators in order to assess collagen quality. Among these, the C/N atomic ratio is considered a good parameter for detecting low-quality collagen and possibly contaminated samples. In a combustion and graphitization setup like that installed at INFN-LABEC, Florence, measurement can be easily performed using an elemental analyzer when combusting the sample prior to graphitization, thus requiring no extra effort (or extra amount of sample) during the preparation procedure. Bone samples recently ¹⁴C dated at INFN-LABEC have confirmed that the measurement of C/N atomic ratios can give some indications of the collagen quality. The bone material was collected from 3 necropolises of the Bronze Age period in Cyprus (Erimi-Laonin tou Porakou, Lophou-Kolaouzou, and Erimi-Kafkalla&Pitharka, along the Kouris Valley), an area characterized by environmental conditions that do not favor bone preservation. Samples were treated to extract collagen and measured by accelerator mass spectrometry (AMS). ¹⁴C results have been compared with the archaeological evidence, showing some relationship between measured C/N atomic ratios and collagen quality. In particular, when grouping the measured samples according to their C/N ratio, the agreement between ¹⁴C dates and archaeological evidence is good or inconsistent when the C/N ratio clearly falls inside or outside the “recommended” range, respectively, with a still reasonable agreement also when it is slightly above the upper limit of that range.

INTRODUCTION

The interest of archaeologists in radiocarbon dating of bones mainly rests in the possibility of dating a material (bone) that is usually strongly connected to the event to be dated. This is particularly true when dealing with necropolises, especially those consisting of many tombs, with no stratigraphical relation at all, or very poor or undefined grave goods.

Bone is a composite material, characterized by a complex hierarchical structure made up of a mineral phase, essentially hydroxyapatite, including calcium phosphate and calcium carbonate, and an organic matrix predominantly composed of collagen, a fibrous protein that ensures strength and flexibility for the bone (for an overview of bone structure, see Weiner and Traub 1992). Despite various attempts to obtain accurate ¹⁴C determinations from the inorganic component of bone (e.g. Haas and Banewics 1980; Saliège et al. 1995), collagen is still the preferable fraction to be collected and ¹⁴C dated. However, the content and the quality of collagen can vary significantly, mainly depending on bone preservation and diagenesis (DeNiro and Weiner 1988). In particular, the more deteriorated the collagen, the more relevant is its loss, and the larger the probability for the residual collagen to be contaminated (Hedges and van Klinken 1992). In addition, ¹⁴C dating of low-collagen bone samples can be time consuming, with the risk of destroying archaeologically significant material without obtaining any result.

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For these reasons, the preservation state of collagen is of crucial importance when ^{14}C dating bones, and many studies have been performed over the last decades concerning both the mechanisms involved in bone diagenesis (see Collins et al. 2002 for a review) and the environmental parameters affecting the deterioration of collagen (see e.g. Nielsen-Marsh et al. 2007). In fact, it is generally accepted that environmental conditions such as the low pH level of soils, high temperatures, and percolating groundwaters, typical of arid and tropical zones, can impair conservation of collagen. In light of these considerations, the key issues involved in bone sample preparation for ^{14}C dating concern 2 main questions:

1. Is collagen available in sufficient quantity?
2. Is the residual collagen of good quality, or likely to be contaminated?

As mentioned above, collagen loss is strictly dependent upon bone diagenesis: all the diagenetic processes can in fact deteriorate the organic fraction to the point that not enough organic matter to be ^{14}C dated has remained in the bone sample. Many attempts to define different states of bone preservation through analytical techniques have been made. These include porosity, Fourier transform infrared spectroscopy (FTIR; see Gianfrate et al. 2007), amino acid analysis, protein (percent nitrogen) content, X-ray diffraction, histological studies, and mercury intrusion porosimetry (see Brock et al. 2010 for a review of the most recent prescreening techniques for identification of bone samples suitable for ^{14}C dating).

With respect to the second question above, extensive research has been done in order to assess a range of criteria capable of estimating the quality of the extracted collagen. An overview of bone collagen quality indicators for ^{14}C measurements can be found in van Klinken (1999). Among these, the yield of collagen after extraction and the carbon/nitrogen atomic ratio, measured on the collagen residue, are considered good parameters for detecting low-quality collagen and possibly contaminated samples. In particular, C/N measurements can be easily performed using an elemental analyzer when combusting the sample prior to graphitization, thus requiring no extra effort (or extra amount of sample) during the preparation procedure.

In this paper, we discuss bone samples, recently dated at INFN-LABEC in Florence, which have confirmed the importance of measuring collagen quality indicators in order to be confident in the reliability and accuracy of the obtained ^{14}C dates of bones. Samples were collected from 3 necropolises of the Bronze Age period on the southern coast Cyprus, an area characterized by environmental conditions that do not favor bone preservation, in the framework of a project conducted with the Department of Antiquities of Cyprus.

BONE DATING AT INFN-LABEC

At INFN-LABEC, ^{14}C dating of bone samples is performed on the extracted collagen residue, after demineralization of the sample and cleaning of the extracted material. Bones are first mechanically cleaned with a scalpel and afterward ground in a mortar, then the chemical pretreatment is performed by following a modified procedure derived from the Longin (1971) method:

- Demineralization in 0.5M HCl at room temperature for 24 hr;
- Purification in 0.1M NaOH at room temperature for 2 hr;
- Further bath in 1M HCl at room temperature for 2 hr to remove any CO_2 possibly absorbed from atmosphere during the second step;
- Gelatinization of the collagen-based acidified solution at 80 °C for 12 hr.

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The concentration of the acidic HCl solution for the first step is usually chosen according to the state of preservation of the bone: if the sample is suspected to have been strongly affected by diagenesis, the concentration is kept as low as possible, for a gentle attack. In this case, when necessary, the duration of this pretreatment step can be maintained for more than 24 hr, until complete demineralization is achieved. Moreover, the HCl solution is frequently changed to improve the efficiency of the reaction.

Only samples whose collagen extraction yield is 1 wt% (collagen weight with respect to the processed bone) are combusted and converted to graphite using the procedure standard at LABEC (Fedi et al. 2007), and measured by accelerator mass spectrometry (AMS). Combustion is performed by a CN elemental analyzer, Thermo Flash EA 1112, thus measuring the collagen quality indicator C/N ratio at the same time. When possible, 2 C pellets from the same purified sample are prepared. Once verified that the results are consistent, the best estimate of the ¹⁴C concentration of each sample is then given by the weighted average of its value in the 2 measured pellets.

All the data presented in this paper were obtained by correcting the measured ¹⁴C/¹²C isotopic ratios for isotopic fractionation (¹³C/¹²C isotopic ratios are also measured in the accelerator) and for background; NIST oxalic Acid II (SRM 4990C) was used as primary standard. Calibration of the measured ¹⁴C ages was performed by OxCal v 4 (Bronk Ramsey 2009), using the IntCal09 calibration curve (Reimer et al. 2009). Different ranges of good C/N atomic ratios are indicated as recommended in the literature (e.g. DeNiro 1985; van Klinken 1999). We typically consider the interval 2.9–3.6 as reference.

THE CASE STUDY: BRONZE AGE NECROPOLES IN SOUTH CYPRUS

The Bronze Age (~2400–1050 BC) is one of the most formative periods in Cypriot prehistory, especially when considering all the cultural and social changes that turned the Cypriot society from a village-based culture, during the second half of the 3rd millennium BC, into a multicultural, town-centered polity, towards the end of the 2nd millennium BC (Knapp 2008). The periodization of the Bronze Age in Cyprus has been developed over the last decades by a number of different scholars, and it is essentially based on the results of archaeological excavations and pottery classifications, properly synchronized with the Near Eastern and Egyptian chronologies. Nevertheless, it is still under continuous revision. It is important to note that, considering the lack of textual sources that can describe the developments of the Cypriot society, an absolute chronology of Cyprus is necessarily dependent on ¹⁴C dates (Manning 2007).

In this paper, we focus the discussion on bone samples collected from 3 Bronze Age necropolises located on the southern coast of Cyprus (see Figure 1): Erimi-Laonin tou Porakou–southern Cemetery Area E (Scirè Calabrisotto et al. 2012); Lophou-Kolaouzou (Violaris et al., forthcoming); and Erimi-Kafkalla&Pitharka. From an archaeological point of view, the 3 cemeteries show many features in common so that they have been selected as a starting point for mapping and matching necropolises in the south coast region of Cyprus:

- They present a period of utilization spanning since the Early Cypriot until the beginning of the Late Cypriot period (~2400–1450 BC);
- They pertain to the same territory, namely the Kourion region (Limassol District);
- They are characterized by a peculiar topographic arrangement, with tombs occurring on natural limestone sloping terraces;
- They consist of tombs displaying a similar funerary architecture, mainly corresponding to single rock-cut chambers or pit graves.

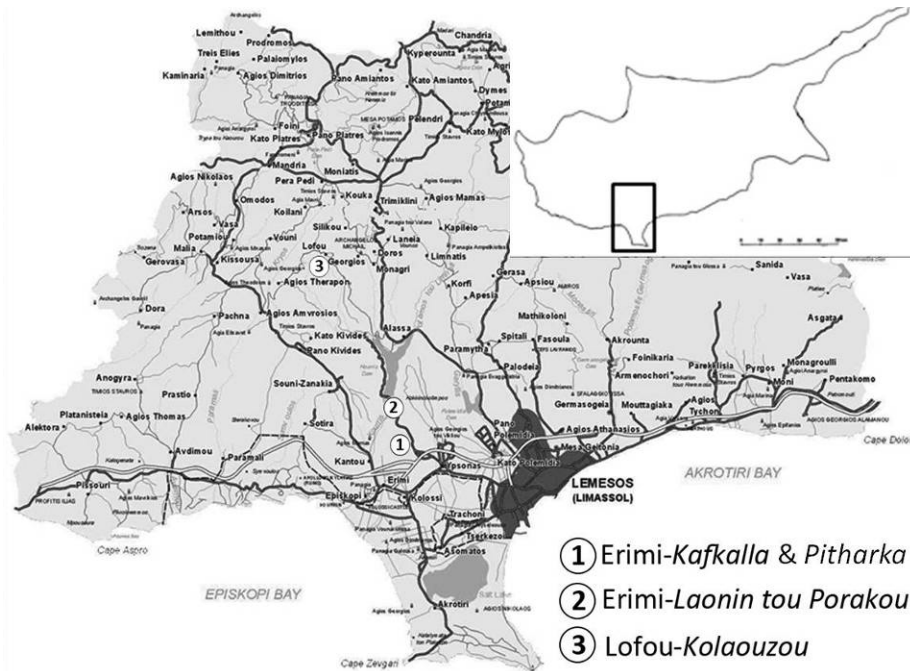


Figure 1 Map of the 3 sites along the Kouris Valley in southern Cyprus studied in this paper

The environmental conditions of the 3 sites do not favor bone preservation. In fact, the 3 areas are characterized by moderate to high calcareous soils contributing to an alkaline environment, and it has been recognized that an alkaline pH can affect the survival of collagen, causing swelling of the protein and acceleration of hydrolysis. In addition, the soils are rich in groundwaters flowing in highly retentive rocks such as chalk interbedded with marls, and even though not all the mechanisms have been explained yet, the hydrography and moisture of the soil appear to also contribute to bone diagenesis. Finally, collagen deterioration is also caused by high temperatures and Cyprus is indeed characterized by hot temperatures, which can even exceed 40 °C during summer.

Only long bones, such as femurs or humeri, were chosen for this study, as is typically done in ¹⁴C dating (see e.g. Tripp et al. 2010). Unfortunately, as might be expected on the basis of the Cyprus environmental conditions, we actually found that many collected bones were in a very bad state of preservation (not always evident at first sight): about half of the collected samples were not measured since their collagen yield after the pretreatment was insufficient (i.e. <1 wt%). The collagen yield of the measured samples was instead in the range 2–5%.

DISCUSSION OF C/N AND RADIOCARBON RESULTS

Erimi-Laonin tou Porakou

The Bronze Age settlement of Erimi-Laonin tou Porakou is located in southern Cyprus, in the Limassol District. The chronology of the site, as inferred from the interpretation of the stratigraphic deposits and analysis of the ceramic assemblage of the 3 areas excavated up to now (Workshop Complex, Domestic Quarter, and Southern Cemetery), points to a period of occupation characterized by 2 main phases, for which 2 possible ranges can be suggested: Early Cypriote III–Middle

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Cypriote I/II (EC III–MC I/II, ~2100–1750 BC) for the earlier Phase B, and Middle Cypriote III–Late Cypriote I (MC III–LC I, ~1750–1450 BC) for the more recent Phase A (Bombardieri 2011).

As far as ¹⁴C dating is concerned, the dated bone samples were collected from 3 tombs, identified as tombs 228, 230 and 248, all ascribed to phase A on an archaeological basis (~1750–1450 BC). These were multiple burials from which a single sample per individual (identified after anthropological observations [Bombardieri et al. 2011]) was initially taken for dating. In 1 case (Tomb 230, individual 1), both a femur and a humerus were sampled from the same individual. The results are summarized in Table 1 together with the C/N atomic ratios measured during sample combustion, as explained above. For each sample, the first part of the label identifies the tomb where it was found, the second, after the underscore sign, the individual to whom the bone belongs.

Table 1 Results of C/N atomic ratios, measured conventional ¹⁴C ages, and calibrated ages of bone samples from Erimi-Laonin tou Porakou. Calibrated ages are compared to ages as expected on the basis of the archaeological evidence (Merrillees 1992). Experimental uncertainties on ¹⁴C ages are quoted at 1σ level. T228_sub, T230_1_o, T230_2 samples were collected from humeri, the other samples from femurs.

Sample	C/N (±0.2)	¹⁴ C age (yr BP)	Calibrated age (BC) (95% confidence level)	Expected age (BC)
T228_1	3.4	3145 ± 30	1500–1380	~1750–1450
T228_sub	5.7	n.a.	n.a.	~1750–1450
T228_3	4.0	2140 ± 50	360 BC–AD 45	~1750–1450
T230_1_fa	3.4	3500 ± 65	1890–1690	~1750–1450
T230_1_o	3.5	3450 ± 55		
T230_2	3.4	3240 ± 40	1610–1430	~1750–1450
T248_1	3.7	3620 ± 40	2050–1885	~2000/1950–1450
T248_2	3.8	3570 ± 55	2040–1750	~2000/1950–1450

^aT230_1 (weighted average).

Before going into the details of the calibrated ages, the measured C/N ratios must be discussed. Two samples, T228_sub and T228_3, both from Tomb 228, are characterized by C/N ratios well outside the DeNiro (1985) range (2.9–3.6). After combustion, T228_sub was not measured, since the amount of collected CO₂ was too low. On the other hand, T228_3, even though outside the “recommended” C/N ratio, was measured, providing a ¹⁴C date of the period from the 4th century BC to the 1st century BC, i.e. the Hellenistic Age. However, the offering goods deposits of Tomb 228 do not present any material pertaining to this period: the inconsistency of the measured age, which is much younger with respect to the archaeological evidence, thus reinforces the reliability of the C/N ratio criterion as a risk indicator for ¹⁴C bone dating.

All the other samples are characterized by C/N ratios that either fall within or only slightly outside the DeNiro range, within the experimental uncertainty. Samples from Tomb 228 (T228_1) and from Tomb 230 are indeed roughly consistent with the expected age (compare the Calibrated Age column and the Expected Age column in Table 1). As far as Tomb 248 is concerned, the archaeological evidence suggests that it was used in a very large timespan (embracing both Phase B and Phase A, see the Expected Age column) for multiple burials. The dated samples T248_1 and T248_2, consistent with the earlier Phase B, can support the actual use of the tomb in the older times at least.

Lophou-Kolaouzou

The cemetery of Lophou-Kolaouzou is situated northwest to the Kouris Dam and presently consists of 16 rock-cut tombs, most of which were either interfered with by clandestine digging or by recent bulldozing operations (Violaris et al., forthcoming). The general chronology of the site, as pointed out by the preliminary overall analysis of the funerary architecture types and the material assemblages, suggests a sequence of occupation ranging from the beginning of the Early Cypriot to the end of the Middle Cypriot period (EC I–MC III, ~2400–1650 BC).

The results of ^{14}C dating performed on bone samples from tombs 8, 15, 20 (multiple burials), and 21 (single burial) are shown in Table 2. Sample labels are defined as for the case of Erimi-Laonin tou Porakou.

Table 2 Results of C/N atomic ratios, measured conventional ^{14}C ages, and calibrated ages of bone samples from Lophou-Kolaouzou. Calibrated ages are compared to ages as expected on the basis of the archaeological evidence (Merrillees 1992). Experimental uncertainties on ^{14}C ages are quoted at the 1σ level. All samples were collected from long bones as femurs.

Sample	C/N (± 0.2)	^{14}C age (yr BP)	Calibrated age (BC) (95% confidence level)	Expected age (BC)
LT8_1	5.5	2580 \pm 60	845–520	~1750–1450
LT8_2	5.9	3015 \pm 75	1430–1045	~1750–1450
LT15_1	3.9	3685 \pm 40	2150–1950	~2150–1850
LT15_2	3.7	3685 \pm 40	2150–1955	~2150–1850
LT20_2	3.8	3710 \pm 45	2210–1955	~2150–1850
LT21	4.2	3445 \pm 45	1885–1635	n.a.

As discussed above, samples showing a C/N atomic ratio outside the recommended range of 2.9–3.6 can be considered as “problematic” samples, likely to be contaminated. More specifically, the measured values of C/N atomic ratios for samples LT8_1, LT8_2, and LT21 fall well outside the recommended range, thus indicating low-quality collagen samples. Considering the C/N ratios, these samples appear unsuitable for ^{14}C dating, and this is indeed confirmed if we compare their measured ages with the expected ages. Actually, LT8_1 and LT8_2, though collected from the same Tomb 8, are not even consistent with each other: they date to the periods 845–520 and 1430–1045 BC, respectively, in disagreement with the expected age that has been estimated on the basis of the analysis of the funerary goods (~1750–1450 BC). As for sample LT21 (from Tomb 21), unfortunately, a possible dating on the basis of archaeological criteria is not feasible at the moment, because diagnostic materials from this tomb have not been identified yet. We can only comment that the measured ^{14}C age alone cannot be considered a reliable indication, as suggested by the “anomalous” C/N ratio.

The other samples LT15_1, LT15_2, collected from Tomb 15, and LT20_2, collected from Tomb 20, are characterized by C/N atomic ratios that, although not within the recommended C/N range, are near its upper limit. They have been ^{14}C dated to a period broadly ranging from the last 2 centuries of the 3rd millennium BC to the 1st century of the 2nd millennium BC, hence consistent with the chronological range proposed on an archaeological basis (Early Cypriot II/III–Middle Cypriot I, ~2150–1850 BC). Thus, in these cases, even though the C/N ratios may point out to the presence of a small contamination, this is not large enough to affect the ^{14}C data. This result can suggest that the limits of the good C/N range should be taken with some critical sensibility, considering the experimental uncertainty on the measured atomic ratios.

Erimi-Kafkalla&Pitharka

The wide cemetery area of Erimi-Kafkalla&Pitharka is located about 3 km north of the modern village of Erimi, north and south of a present freeway. The Department of Antiquities of Cyprus has excavated over 150 tombs for the last 5 yr. Unfortunately, a large number of the excavated tombs were found looted.

As far as chronology is concerned, exhaustive analyses of the offering good deposits of the 2 necropoles have not been performed yet. At present, it can be argued that the cemetery area of Kafkalla points to a main period of use during the Early Cypriote I to Middle Cypriote III (~2400–1650 BC), while the necropolis of Pitharka provides evidence for the subsequent Late Cypriote and Iron Age periods (~1650–850 BC). In addition, Hellenistic and Roman attestations have also been found within the tombs of the 2 necropoles.

The results of ^{14}C dating performed on bone material from Erimi-Kafkalla&Pitharka are shown in Table 3. Samples KT90_1 and KT90_4, belonging to 2 different individuals, were collected from Tomb 90, located in the necropolis of Pitharka; actually, even though the architecture of this tomb was Bronze Age, only Roman offerings were found during the excavation (Y Violaris, personal communication). Samples KT80_1 and KT78_1 were taken from Tomb 80 and Tomb 78, in the cemetery of Kafkalla. Concerning sample KT78_1, only the C/N atomic ratio is given because the amount of the collected graphite was too small to allow for an AMS measurement.

As seen in Table 3, acceptable C/N values were measured for all the dated samples. The calibrated ages are not in agreement with the Bronze Age (compare the Calibrated Ages column and the Expected Ages column in Table 3): samples are dated to the Hellenistic and Roman periods instead. It is worth noting that long-term reuse of Bronze Age funerary areas at Kafkalla and Pitharka is not an isolated occurrence in the region. Actually, evidence of Hellenistic-Roman burials within EBA-MBA tombs has been recently documented at Ypsonas-Vounaros, i.e. the eastern tombs cluster of Erimi-Laonin tou Porakou settlement (Christofi et al., forthcoming). Therefore, besides having found grave goods of the Roman period in Tomb 90, the ^{14}C data themselves suggest the hypothesis of a possible reuse of the older tomb in later periods. In the case of Tomb 80, which is near Tomb 90, assuming the reliability of the ^{14}C dating owing to the measured C/N ratio, the date also indicates a later reuse, although this is not corroborated, as for Tomb 90, by relevant goods pointing to any definite period (the burial had been looted in the past).

Table 3 Results of C/N atomic ratios, measured conventional ^{14}C ages, and calibrated ages of bone samples from Erimi-Kafkalla and Pitharka. Calibrated ages are compared to ages as expected on the basis of archaeological evidence (Merrillees 1992). Experimental uncertainties on ^{14}C ages are quoted at the 1σ level. All samples were collected from long bones as femurs.

Sample	C/N (± 0.2)	^{14}C age (yr BP)	Calibrated age (95% confidence level)	Expected age (BC)
KT90_1	2.9	1650 \pm 60	AD 260–540	~1650–950
KT90_4	3.2	1870 \pm 60	AD 0–260	~1650–950
KT80_1	3.0	2000 \pm 100	220 BC–AD 240	~2400–1650
KT78_1	2.6	n.a.	n.a.	~2400–1650

CONCLUSIONS

^{14}C dating of bones offers archaeologists the opportunity to date an event that is usually strictly associated to the context of the archaeological find. However, different problems can affect the accuracy

of ^{14}C dates obtained from bone material. Strong diagenesis processes of the bones, principally due to the duration and the environmental conditions of deposition, might cause a severe loss of the datable organic fraction, mainly collagen, or, at the least, might make the residual collagen more exposed to contamination.

In this article, we have discussed some examples of dated bones collected from archaeological sites in Cyprus whose environmental conditions can be responsible for a strong diagenesis. The dated bones can be divided into 3 different groups. One group (samples T228_sub and T228_3 from Erimi-Laonin tou Porakou; LT8_1, LT8_2, and LT21 from Lophou-Kolaouzou) is characterized by high C/N atomic ratios, definitely falling outside the “recommended” range. If we compare their ^{14}C dates with the archaeological evidence, we find a disagreement, suggesting that such high C/N ratios indeed indicate a severe contamination. On the contrary, another group (samples T228_1, T230_1_o, T230_1_f, T230_2 from Erimi-Laonin tou Porakou; and KT90_1, KT90_4, KT80_1 from Erimi-Kafkalla&Pitharka) is characterized by “good” C/N ratios. In this case, the measured ^{14}C data are consistent with the archaeological information, at least when the latter is conclusive. Finally, the other samples (T248_1 and T248_2 from Erimi-Laonin tou Porakou; and LT15_1, LT15_2 and LT20_2 from Lophou-Kolaouzou) have C/N ratios that are close to the recommended range, being consistent with the upper limit within the experimental uncertainty. In these cases, the comparison of the measured ^{14}C data with the archaeological evidence suggests that such data can be considered reliable. Of course, we cannot exclude the presence of a contamination altering the C/N ratios, which is small enough, however, to not significantly affect the ^{14}C concentration. Even though these data represent a small data set, they seem to indicate that measured C/N ratios that are close to the limits of the recommended range should not be *a priori* rejected but discussed with a critical sensibility. More indications might likely be given by comparing ^{14}C data obtained after different pretreatment methods, as we plan to do in the future.

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