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Compound-Specific Radiocarbon Analysis by Elemental Analyzer−Accelerator Mass Spectrometry: Precision and Limitations

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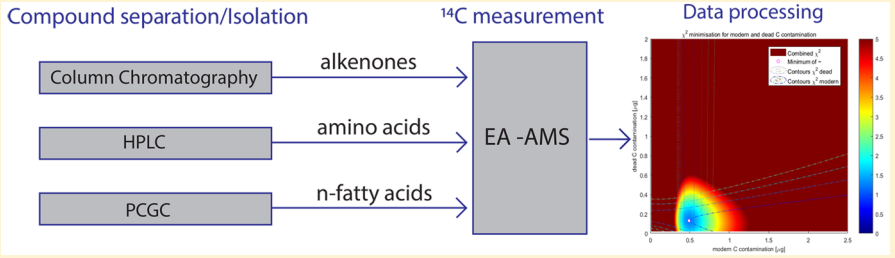
Supporting Information



ABSTRACT: We examine instrumental and methodological capabilities for microscale (10−50 μg of C) radiocarbon analysis of individual compounds in the context of paleoclimate and paleoceanography applications, for which relatively high-precision measurements are required. An extensive suite of data for ^{14}C-free and modern reference materials processed using different methods and acquired using an elemental-analyzer−accelerator-mass-spectrometry (EA-AMS) instrumental setup at ETH Zurich was compiled to assess the reproducibility of specific isolation procedures. In order to determine the precision, accuracy, and reproducibility of measurements on processed compounds, we explore the results of both reference materials and three classes of compounds (fatty acids, alkenones, and amino acids) extracted from sediment samples. We utilize a MATLAB code developed to systematically evaluate constant-contamination-model parameters, which in turn can be applied to measurements of unknown process samples. This approach is computationally reliable and can be used for any blank assessment of small-size radiocarbon samples. Our results show that a conservative lower estimate of the sample sizes required to produce relatively high-precision ^{14}C data (i.e., with acceptable errors of <5% on final ^{14}C ages) and high reproducibility in old samples (i.e., F^{14}C ≈ 0.1) using current isolation methods are 50 and 30 μg of C for alkenones and fatty acids, respectively. Moreover, when the F^{14}C is >0.5, a precision of 2% can be achieved for alkenone and fatty acid samples containing ≥15 and 10 μg of C, respectively.

The low natural abundance of ^{14}C atoms (10^{-12}, one part per trillion in modern samples) renders its detection and measurement highly challenging. The development of accelerator mass spectrometry (AMS) created a revolution in radiocarbon analysis, decreasing required sample sizes from grams of C to submilligrams of C ranges.[1] In recent years, continued reductions in sample-size limits have enabled different archives to be targeted for a variety of applications using radiocarbon (^{14}C) analysis and motivated several AMS laboratories worldwide to develop new techniques that push measurement boundaries and expand or open new analytical windows.[2−6]

Compound-specific radiocarbon analysis (CSRA) is a powerful tool that facilitates deeper insights into the carbon cycle through tracing of specific organic components, as well as radiocarbon dating of individual components within complex organic mixtures.[7,8] Over the past 20 years, CSRA has grown rapidly, and is now applied in a variety of subdisciplines, including different areas of earth science (e.g., paleoceanography, paleoclimatology, and terrestrial and marine biogeochemistry),[9−11] environmental science,[12] and archeology.[13] However, it remains challenging to produce reliable results with small sample sizes (e.g., < 50 μg of C), especially for organic compounds that require complex, multiple-step sample processing associated with isolation and purification prior to measurement, as well as for CSRA applications that require relatively high-precision data. In such cases, the challenges in the measurement of individual compounds are not only related to instrumental limitations but also to the specific isolation and purification methods involved. The latter requires minimization and careful assessment of the processing of chemistry blanks.

Until recently, most samples for CSRA were prepared via sealed-tube combustion and processing on a vacuum line, with subsequent analysis either as CO_2 in ampule cracker systems or

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as graphite targets.[14] The vacuum-line technique is labor intensive and requires significant consumables. In addition, accurate mass quantification using a vacuum line is hard to achieve. Direct coupling of an elemental analyzer (EA) to the gas-ion source of an AMS reduces both time and money investment and, in addition, minimizes handling steps, the latter resulting in smaller blanks and better recovery.

Following the recent developments in gas-accepting ion sources that enable online measurement of small ^{14}C samples, several studies have demonstrated applications involving direct combustion to CO_2 of reference materials, carbonates, and collagen.[15,16] However, the coupled (EA-AMS) measurement approach has not been rigorously tested for small-scale analysis of individual compounds or compound classes, particularly for applications that require relatively high precision and accuracy. The few reports of compound-specific radiocarbon analysis of samples where accuracy precision was concerned have typically involved measurement of relatively large sample sizes (i.e., greater than 100 or 200 μg of C) in applications such as paleoclimatology[17] and archeology.[18] Haas et al., 2017[19] described CSRA of smaller sample sizes; however, that study focused on a single compound type, a comprehensive blank assessment was not undertaken, and ^{14}C data from samples >20 ka are prone to large age uncertainty. A primary goal of the present study is to constrain and minimize the extraneous carbon associated with specific isolation methods for different compound types in a step toward accurate CSRA. Given that even minor quantities of contamination differing markedly in ^{14}C content from the target analyte can significantly bias the resulting ^{14}C data, a key aim of our study is to assess the limitations of CSRA using EA-AMS in applications where high-precision (<5%) data are required, such as in examining intervals of abrupt climate change. Various approaches for quantification of the $F^{14}C$ and mass of extraneous carbon (C_{ex}) during CSRA have previously been applied.[20−23] However, systematic assessment of the reproducibility and robustness of each method for each compound type is necessary because the variability associated with specific methods and analytes influences the overall uncertainty of the final result.

There are three novel aspects to this contribution: (1) "Routine" online ^{14}C analysis of compounds using EA-AMS is demonstrated. This setup allows increased sample throughput, and at the same time is very time- and cost-efficient. (2) Instrumental and methodological considerations associated with small-scale (5−50 μg of C), molecular-level ^{14}C dating with respect to paleoclimate and paleoceanographic applications are explored. The precision, accuracy, and reproducibility of the measurements are assessed through analysis of reference materials and two classes of compounds extracted from sediment cores from two different locations independently dated to ≈20 ka. (3) We introduce a computational approach that allows for robust quantification of the total mass and $F^{14}C$ of extraneous carbon and associated errors.

MATERIALS AND METHODS

EA Capsules. The overall method involves the combustion of samples (isolated compounds) in an elemental analyzer (varioMICRO cube, Elementar), and the resulting CO_2 is separated from other gases before being fed, using helium as carrier gas, into a gas-interface system (GIS) coupled with a Mini Carbon Dating System (MICADAS).[24,25] The pure CO_2 sample trapped in a zeolite trap is transferred via a syringe pump to the gas-ion source of the AMS.[4,6]

Samples dissolved in solvent (typically either dichloromethane, (DCM), or water) were transferred to tin capsules designed for liquids (Elementar). Because the extraneous carbon introduced by the capsules depends on their mass and composition, the smallest commercially available capsules were used. Tin and silver capsules of similar sizes (2.88 × 6 mm^2, foil thickness 0.1 mm, 25 μL) were compared. Tin capsules were precleaned by rinsing with DCM (GC grade, Honeywell), whereas silver capsules were combusted at 550 °C for 6 h prior to use. The combustion of silver capsules should be done no more than 24−48 h prior to AMS measurement as longer-term storage of silver capsules significantly increases the contamination, most likely because of activation of the surface of the silver. Because the ^{14}C backgrounds of cleaned tin and combusted silver capsules were found to be similar,[26] tin capsules were considered preferable for CSRA as they can be stored several weeks without showing increased contamination. Furthermore, tin enhances the combustion process in the EA.

Sediment Samples and Reference Materials. *Sediment Core I.* Core I (NGHP-01-16A; 16° 35′ N, 82° 40′ E) was collected on the continental margin (1268 m water depth) near the mouth of the Godavari River where it discharges into the Bay of Bengal.[27] About 60 samples were taken from the top 8.5 m of the sediment core, spanning the entire Holocene. The samples were then processed for foraminifera, bulk-total-organic-carbon (TOC), and long-chain (C_{26-32}) fatty acid analysis.

Sediment Core II. Core II (SHAK06-5K; 37° 34′ N, 10° 09′ W, 2646 m) was retrieved at Shackleton Site (Iberian margin) and sliced onboard at 1 cm intervals. Samples were individually stored in the freezer at −20 °C until they were used for this investigation, at which point they were processed for foraminifera, TOC, and alkenone analysis.

Both cores were independently dated by ^{14}C measurements on planktonic foraminiferal carbonate at high resolution (1 cm intervals). All foraminifera and bulk-TOC samples were measured as large targets (>200 μg of C). A detailed study of radiocarbon and related data for these cores will be presented elsewhere.[28,29] Here, we concern ourselves exclusively with the potential and limitations of ^{14}C dating with different purification methods applied in paleoclimate studies.

Most of the reference materials used in this study are commercially available. Additionally, in-house reference material was prepared (Table S1). We use "short term" to refer to measurements stemming from a single AMS or isolation-preparation sequence or batch and "long term" to describe measurements spanning a range of times and sequences. Processing blanks went through all the steps, whereas untreated material did not go through any steps.

Purification Methods. For purification, we used high-performance liquid chromatography (HPLC) for amino acids,[30] preparative capillary gas chromatography (PCGC)[31] for *n*-fatty acids, and column chromatography following ref 32 for alkenones.

PCGC (n-Fatty Acids). The separation of *n*-fatty acids was performed with preparatory capillary-gas-column GC (PCGC) using an Agilent 7890 GC equipped with Agilent VF-Ims (30 m × 530 μm × 0.5 μm) and a Gerstel fraction collector. The GC oven was programmed as follows: 40 °C (for 1 min), 130 °C (at 30 °C/min), 320 °C (at 6 °C/min), and 320 °C (6 min). We used a Gerstel PTV inlet in solvent-vent mode programmed as follows: 40 °C (0.01 min), 320 °C (at 12 °C/sec), and 320 °C (3 min).

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For the blank assessment of radiocarbon analysis of fatty acids, two commercial *n*-alkanes of known $F^{14}C$ values (i.e., *n*-C_{28} alkane, 0.003 ± 0.001, and *n*-C_{32} alkane, 1.07 ± 0.003) were used. Processing blanks were injected following the same GC method and number of injections (40 injections) as used for compound isolation from sediment samples. Following PCGC isolation, the traps were rinsed with 1 mL of DCM/hexane (1:1) into the precombusted GC vials. Samples were passed through a 1 cm precombusted silica gel in a glass pipet to remove any column bleed. An aliquot of each isolated compound was analyzed by analytical GC with a flame-ionization detector (GC-FID) to assess purities and recovery efficiencies prior to AMS measurement.

HPLC (Amino Acids). For amino acids, only data from short-term evaluations of processing blanks are available. Eight commercially available standards (listed in Table S1) were prepared using HPLC and yielded C masses between 10 and 60 μg of C. L-Alanine, glycine, and L-methionine were used as ^{14}C-free standards. L-Valine, L-phenylalanine, L-leucine, L-isoleucine, and L-glutamic acid were the modern ^{14}C standards (Table S1).

Preparative procedures for amino acid purification are described in detail by Ishikawa et al.[30] In brief, a solution containing a mixture of different amino acids was injected and separated by HPLC (1260 series, Agilent Technologies). To isolate individual amino acids, the HPLC was equipped with a reversed-phase CAPCELL PAK C18 MG column (preparative scale, 20 × 250 mm, 5 μm particle size, Shiseido) or a mixed-mode ion-exchange reversed-phase Primesep A column (preparative scale, 10 × 250 mm, 5 μm particle size, SIELC Technologies). The mobile phases were distilled water with 0.1% (v/v) TFA (solvent A) and acetonitrile with 0.1% (v/v) TFA (solvent B). All eight amino acids were injected in the first column (CAPSELL). The compounds with retention times between 60 and 100 min (Val, Met, Ile, Leu, and Phe) separated in the first column, and the compounds with retention times between 20 and 30 min (Gly, Ala, and Glu) were reinjected and separated in the second column (Primesep). The linear-solvent-gradient programs for each column were as follows: For the CAPSELL column, the initial condition was 100% solvent A, which was followed by 85% solvent A and 15% solvent B for 34 min; then, the column was flushed for 11 min with 100% solvent B and re-equilibrated for 10 min with solvent A. For the Primesep column, the initial condition was 100% solvent A, which was followed by 90% solvent A and 10% solvent B for 34 min; then, the column was flushed for 10 min with 100% solvent B and re-equilibrated for 10 min with solvent A. Dried fractions were then redissolved in 0.5 mL of 0.1 mol/L HCl and filtered through a membrane filter (GHP Nanosep MF, 0.45 μm pore size, ODGHPC34, Pall Life 127 Sciences) to remove possible column bleed (e.g., octadecyl silica derived from the stationary phase from the CAPCELL column).

Column Chromatography (Alkenones). Samples were purified and isolated on the basis of the method described by Ohkouchi et al.[32] Two approaches for blank assessment for radiocarbon analysis of alkenones were compared:

I. In the first approach, the processing blanks (no sample added to the columns) were spiked with varying masses of oxalic acid II (OXAII) and phthalic anhydride (PHA) ^{14}C reference standards.

II. In the second approach, ^{14}C-free alkenones from 60 g of sediment core GeoB1711-4 (depth 1065−1075 cm, age >130 000 a) were extracted. Total lipid extracts (TLEs) were obtained with microwave-assisted extraction of freeze-dried bulk-sediment samples using DCM/MeOH (9:1, v/v) and subsequently saponified with 0.5 M KOH/MeOH. The neutral fraction, containing the alkenones, was liquid−liquid extracted with hexane. Further silica-gel column chromatography (6 mm i.d. × 4 mL) was performed when necessary to isolate alkenones from an unknown compound using hexane (fraction 1), hexane/DCM (38:32, v/v, fraction 2), and DCM (fraction 3), with the alkenones eluting in fraction 2. For testing the reproducibility of the isolation method only, alkenones from a TLE were sampled after the saponification step.

After purification, samples were concentrated to 100 μL and stored in the freezer (about −20 °C) in sealed, freshly precombusted GC vials. Samples were transferred into EA capsules using different solvents (e.g., DCM or H_2O depending on the solubility of the target compound) in four washing steps to ensure quantitative (>95%) transfer. After the removal of solvent from the samples by evaporation under a stream of N_2, the capsules were wrapped and stored in freshly combusted GC vials prior to analysis. For each set of samples, additional solvent and capsule blanks were prepared in order to control for potential contamination introduced either by the solvent or by variations in the amount of C associated with different capsule batches.

AMS Measurements. Measurements were performed using a gas-ion-source (GIS)-equipped AMS (MICADAS) system in the Laboratory for Ion Beam Physics as ETH Zurich.[24,25] Oxalic acid II NIST SRM 4990C standard (gas bottle) was measured for fractionation correction and standard normalization and to ensure stable measurement conditions.[33] Radiocarbon-free CO_2 reference gas (5% CO_2 in helium, Messer Schweiz AG) was measured to quantify the ^{14}C background of the MICADAS. A precision of better than 5‰ can be achieved for the oxalic acid II standards, whereas for the radiocarbon free CO_2, an $F^{14}C$ background of less than 0.002 (i.e., 50 000 a) is attainable.[34] The background of the EA was assessed with phthalic anhydride (PHA, Sigma, PN-320064-500g, LN-MKBH1376V), which was weighed into the same capsules used for the samples and thus included the respective contamination. The measurement order for the samples was from ^{14}C-depleted to modern. Prior to running the samples with $F^{14}C < 0.1$, a conditioning run (i.e., without combusting any material) was included in the measurement sequence in order to purge the system, condition the trap, and minimize any cross-talk between samples. The trap-cleaning time was set to 2 min.

Data Reduction. *Standard Normalization.* In the first step, the data processing was done using the in-house BATS software.[33] Measured $^{14}C/^{12}C$ ratios are reported as $F^{14}C$, as described in refs 35 and 36.

Correction for Constant Contamination. For each class of compounds, two sets of reference materials were used, one with a modern ^{14}C value ($F^{14}C \approx 1$) and one ^{14}C-free contents ($F^{14}C \approx 0$). The mass and $F^{14}C$ of the contamination introduced during sample preparation was quantified with materials of known ^{14}C contents that were as chemically

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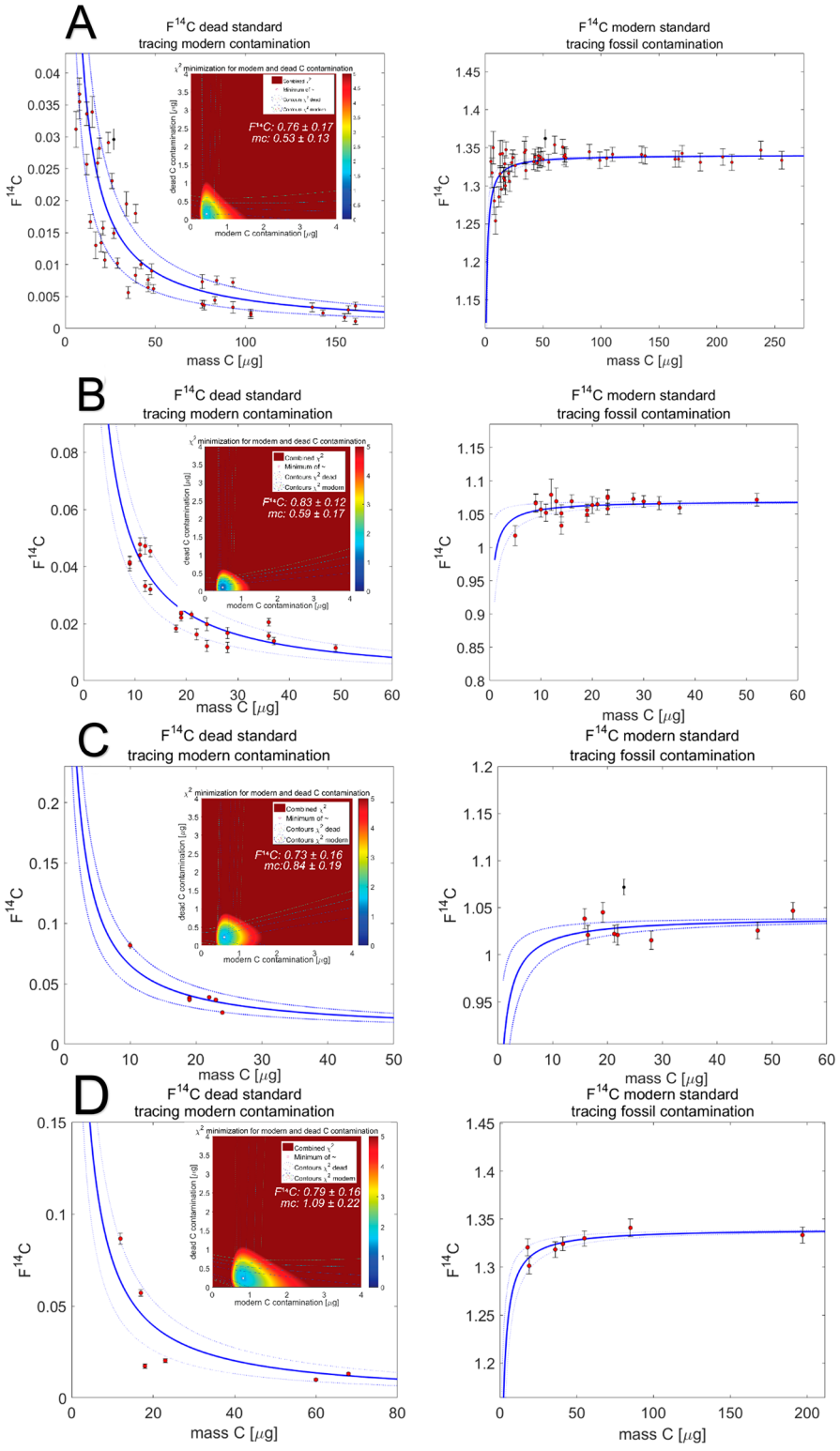

Figure 1. 3D-modeled constant contamination for (A) EA background using untreated material, (B) PCGC method for fatty acid isolation, (C) HPLC method for amino acid isolation, and (D) column chromatography for alkenone isolation. The red circles are measured standards. The black circles are the outliers. The solid blue curves represent the best fits, and the 1σ error ranges are shown with dotted lines. The 3D models show the best solution for the least χ^2 for each set of data (inset figures).

similar as possible to the target compounds. These materials will be denoted processing blanks hereafter.

To evaluate the mass and $F^{14}C$ of extraneous carbon for each method, we used a model of constant contamination based on the mass-balance correction represented by eq 1:

$$F^{14}C_s = \frac{F^{14}C_m \times m_m - F^{14}C_c \times m_c}{m_m - m_c} \quad (1)$$

where $F^{14}C_c$ is the $F^{14}C$ of the contaminant; the total mass of the sample measured by the AMS (m_m) is the sum of the mass

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of the sample (m_s) and the mass of the contaminant (m_c).[37] The $F^{14}C_m$ is the $F^{14}C$ measured by AMS.

The corresponding uncertainty of $F^{14}C_s$ (for the sample) is derived from error propagation:

$$\sigma^2_{F^{14}C_s} = \left[\sigma_{m_c}\left(\frac{F^{14}C_m \times m_m - F^{14}C_c \times m_c}{(m_m - m_c)^2}\right) - \left(\frac{F^{14}C_c}{m_m - m_c}\right)\right]^2 + \left[\sigma_{m_m}\left(\frac{F^{14}C_m}{m_m - m_c} - \frac{F^{14}C_m \times m_m - F^{14}C_c \times m_c}{(m_m - m_c)^2}\right)\right]^2 + \left[\sigma_{F^{14}C_m}\frac{m_m}{m_m - m_c}\right]^2 + \left[\sigma_{F^{14}C_c}\frac{-m_c}{m_m - m_c}\right]^2 \quad (2)$$

After applying a constant-contamination correction to the measured $F^{14}C$ of small samples, the variability of the contamination mass (i.e., σ of $m_{C_{ex}}$) and its radiocarbon concentration (σ of $F^{14}C_{C_{ex}}$) contribute significantly to the overall uncertainty of the sample. For a robust blank assessment using the model of constant contamination, it is critical to have a sufficient number of ^{14}C-free and modern standards, especially in the long term, in order to be able to better differentiate outliers and assess the reproducibility of different methods.

Evaluation of the Constant-Contamination-Model Parameters. We follow the data reduction procedure presented in ref 37. The masses of modern and dead contamination (C_{ex}) were determined using the least-squares method in MATLAB (R2017a, Mathworks; see the Supporting Information for the script). The model optimizes the fit by comparing the measured data with the modeled value for constant contamination. The χ^2 (sum of the squared residuals divided by the squared propagated uncertainties) is systematically evaluated for the continuous model-parameter space of modern and dead contamination in increments of 0.02 μg of C. This grid search yields a contaminant-mass pair model that minimizes the data misfit. The 95% limits of the χ^2 fit were used to determine the 2σ measurement uncertainties. The calculations could be run on a workstation within a minute.

RESULTS AND DISCUSSION

Corrections Associated with EA-AMS Analysis. Following Ruff et al.[26] and Welte et al.,[38] we combined between three and six empty tin capsules in order to obtain enough mass of blank C for EA and ^{14}C measurements (>1 μg of C), respectively. The C content directly measured for the tin liquid capsules was 0.28 ± 0.08 μg with an $F^{14}C$ value of 0.6 ± 0.2. These values are, within error, in good agreement with those from the study of Ruff et al.[26] (0.340 ± 0.13 μg, $F^{14}C$ = 0.6) The detection limit of EA and AMS is insufficient to independently determine the capsule-to-capsule variability in mass and the $F^{14}C$ of the contamination. However, the $F^{14}C$ values of untreated PHA and OXAII have been used to assess the instrumental ^{14}C-detection limit and to more precisely constrain contamination associated not only with EA capsules but also with potential cross contamination from the EA column, the zeolite trap, and the AMS syringe. The ^{14}C-free and modern reference materials were measured at different C masses ranging 5−200 μg (Table S2). Applying a correction according to the constant-contamination model, we calculated the contamination introduced by the EA capsules. The long-term contamination for about n = 100 measurements in several sequences gives m_c = 0.53 ± 013 with an $F^{14}C$ of 0.76 ± 0.17 (Figure 1A). The short-term (i.e., measured in one sequence) contamination for n = 10 gives m_c = 0.57 ± 011 with an $F^{14}C$ of 0.63 ± 0.17.

Corrections for Different Purification Methods. Using a model of constant contamination, we calculated the masses and $F^{14}C$ values of extraneous carbon and their associated errors for the different methods. The processing blanks used in this study are combinations of samples before and after the chemical-purification steps required for each isolation method.

PCGC Isolation Method (Fatty Acids). Short-term observations (n = 12) from PCGC yield a contamination of 0.62 ± 0.12 μg of C with an $F^{14}C$ value of 0.89 ± 0.16. Long-term results (n = 40) (Table S3) provide a mass of contamination 0.59 ± 0.12 μg of C with an $F^{14}C$ value of 0.83 ± 0.17 (Figure 1B). The close agreement in extraneous-carbon values (C_{ex}) between these short- and long-term measurements demonstrates the robustness of this method. The C_{ex} calculated in our study is lower than that reported in a prior study where the samples were prepared by sealed-tube combustion−vacuum line (1.3 ± 0.2 μg of C).[23] The minor contamination introduced by PCGC is also supported by a recent study.[18]

HPLC Isolation Method (Amino Acids). L-Alanine, glycine, and L-methionine were used as ^{14}C-free reference materials to assess external carbon associated with amino acid purification by HPLC. The reproducibility for L-methionine was very low, and a large offset was observed compared with those of the other two ^{14}C-free materials (Table S4). Most likely, this is caused by the different behaviors of the different amino acid compounds rather than by the isolation method, because other ^{14}C-free and modern amino acids showed good agreement with nominal values. L-Methionine is the only sulfur-containing amino acid, which may influence its chemical behavior relative to those of the other amino acids. The results obtained from the short-term-processing blanks measured by HPLC (n = 14) yielded a contamination mass of 0.84 ± 0.19 μg of C with an $F^{14}C$ value of 0.73 ± 0.16 (Figure 1C). However, the calculated C_{ex} value using L-methionine showed a larger mass of 1.25 ± 0.41 μg of C with an $F^{14}C$ value of 0.92 ± 0.16 with a less-defined model. Although methionine has a longer chromatographic retention time compared with those of the other ^{14}C-free amino acids, the modern contamination observed from L-methionine cannot be explained by column bleed, which is ^{14}C-free.

Column-Chromatography Isolation Method (Alkenones). The constant-contamination parameter determined for this method using spiked materials was 1.25 ± 0.16 μg of C with an $F^{14}C$ value of 0.83 ± 0.09 (Figure1D). These values are different from those found for alkenones from extracted sediment (m_c = 1.09 ± 0.22 μg of C, $F^{14}C$ = 0.79 ± 0.16).

Comparing the two approaches using the same isolation method but different materials indicates that the isolation method is sensitive to different reference materials. The handling of small samples that are not purified using either GC or HPLC may bias the results and needs further investigation for the source of impurity.

Graphical Indication of Prereduction. Visual inspection of the χ^2 in the model space (Figure S1A) reveals that there is no clear solution for χ^2. This can provide information about data quality and serve to identify underlying issues inherent from the preparation or analytical procedures. Figure S1A in the Supporting Information shows that the sensitivity of data misfit to the model parameters (two contaminant masses) is relatively high, and the misfit increases with minor

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modifications of contamination assumptions. In contrast, Figure S1B indicates that an increase in modern-contamination assumptions carries a lower misfit penalty, so the contamination determination is potentially less well-constrained. In addition, the notable inclination of χ^2 contours for the dead (i.e., ^{14}C-free) data alone hint that some sources of error are not independent.

Reproducibility and Uncertainties. The uncertainties on reported ^{14}C ages are based on a counting-statistics error and propagated with the uncertainties stemming from blank subtraction, fractionation correction, and standard normalization.[5] When correcting small samples for constant contamination, the uncertainties of $F^{14}C$ and the mass of the contamination must be considered as well. Poor reproducibility of processing blanks will increase these uncertainties. It is therefore crucial to investigate in detail the reproducibility of each purification method for each target compound by performing systematic tests and replicates. A thorough assessment of the uncertainties associated with the constant contamination for different sample-preparation methods is necessary both on a daily basis as well as over the long term to allow for proper data reduction. We address the latter using long-term data from samples with C masses ranging from 5 to 50 μg that were measured in different sequences over the course of one to two years (Figure 2). Our results show that some purification methods produce more variable processing blanks than others (Figure 2).

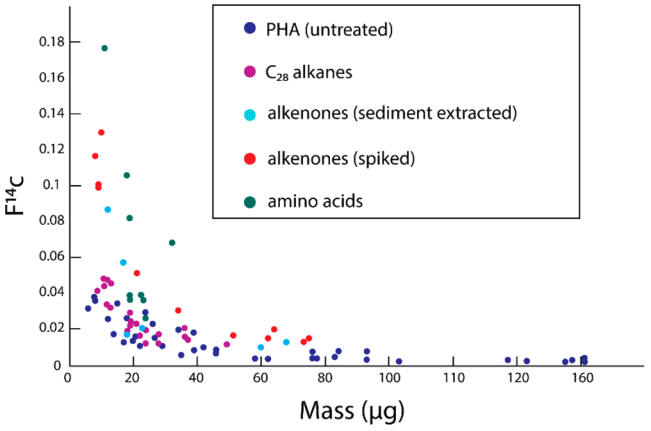

Figure 2. Comparison of the reproducibility of ^{14}C-free processing blanks for compounds isolated using different purification methods.

In the case of the C_{28} fatty acid, the measured data for the processing-blank samples indicate good reproducibility for samples with >10 μg of C, with the $F^{14}C$ values of the processing-blank samples returning a value better than 0.02 with a mass of >20 μg of C (Figure 2).

For the alkenones, both applied methods (i.e., spiked and extracted from the ^{14}C-free-sediment core sample) result in relatively poor reproducibility for samples with <20 μg of C. This low reproducibility of alkenones most likely reflects a combination of limitations of the isolation method as well as the specific chemical characteristics of the target compounds. For the processing blanks, an $F^{14}C$ of 0.02 could only be achieved for comparably large samples containing >50 μg of C.

In the case of amino acids, the reproducibility of the method depends on the selected compound. For example, although L-alanine and glycine show good reproducibility, the reproducibility is poorer for L-methionine, which is also associated with higher processing blanks.

Although EA-AMS allows the measurement of small samples with a precision of 1%,[4] the error on the final results after the constant-contamination correction may render the data unreliable. Here, we demonstrate the influence of contamination correction and discuss the limitations for different sample sizes and ages to reach a precision of <5% (Figure 2b). The size limit for constant contamination of 1.25 ± 0.16 and 0.83 ± 0.09 is 50 μg of C for samples with $F^{14}C$ of 0.1. Only small samples (<20 μg of C) with $F^{14}C$ > 0.6 give a precision better than 5% (Figure 3). For samples with $F^{14}C$ < 0.1 and

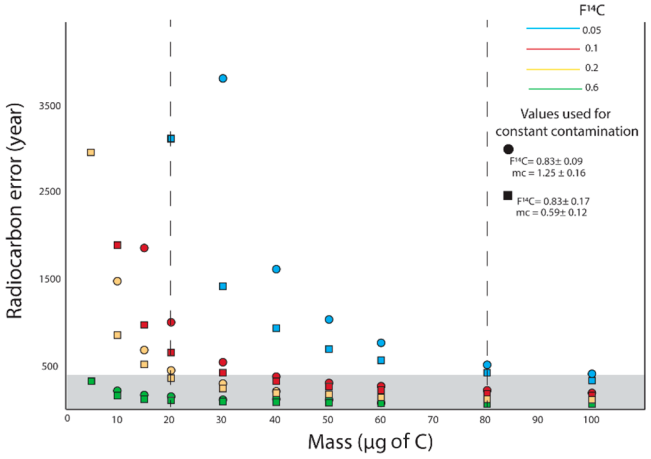

Figure 3. Limits of sample sizes and their associated errors applying the constant-contamination correction for different methods. The gray box highlights the area with error of less than 5%. Different colors indicate different $F^{14}C$ values. Different symbols show different constant-contamination values applied to the samples.

with masses smaller than 20 μg of C, the overall errors are more than 50%. The size limit for a constant contamination of 0.83 ± 0.17 and 0.59 ± 0.12 is 30 μg of C for samples with $F^{14}C$ of 0.2.

These findings imply that preparing samples for CSRA that are in the range of 5−20 μg of C is very challenging, particularly for $F^{14}C$ values <0.6. The size requirement for samples with $F^{14}C$ of 0.1 is 50 μg of C, and this value increases to 100 μg of C for samples with $F^{14}C$ < 0.1. However, it should be noted that higher values for constant contamination than those reported in this study (>2 μg of C) will put the size limit even higher (Figure 3)

CSRA of Sediment Cores Using EA-AMS: Two Case Studies. In two case studies, ^{14}C measurements of isolated compounds were compared with those of bulk TOC and foraminiferal carbonate from the same sediment depth interval in order to assess the limitations and achievable precision for small CSRA samples in marine sediments in paleoclimate applications.

^{14}C ages of *n*-fatty acids from core I from the Bay of Bengal are shown in Figure S2A. For samples larger than 20 μg of C, a precision of approximately 130 years was obtained if the samples were younger than 10 ka, whereas this precision diminished to 400 years for the range of 10−18 ka. In core II from the Iberian margin (Figure S2B), we obtained a precision of approximately 140 years for compound samples younger than 5 ka with more than 10 μg of C. The precision for samples older than 10 ka and with <40 μg of C deteriorated to

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>500 years, whereas the observed older ages of the target compounds (i.e., fatty acids and alkenones) in comparison with those of the foraminifera in the two case studies can be explained with preaged material incorporated in the sediment;[39−41] the offset of ages for samples in which the alkenones are younger than the foraminifera and bulk TOC is more difficult to explain (see the Supporting Information).

In summary, the results of the two case studies allow us to conclude that regardless of the purification method, the required precision and reproducibility necessary for ^{14}C dating is difficult to achieve for samples with <10 μg of C and older than 10 ka (Holocene). Our comparison of purification methods revealed that different minimal sample masses (μg of C) are required in order to gain acceptable precision for paleoclimate studies. This is not only caused by the amount of C_{ex} but also by the reproducibility of the method itself (Figure 2). Preparation of samples older than 20 ka and with <50 μg of C require special care as other factors besides the isolation method also have to be considered.

CONCLUSION

In this study, the results of both short- and long-term routine online CSRA using EA-AMS with different methods of compound isolation are compiled and compared. The presented data show that the processing-blank values obtained for the different methods and compounds are generally similar and in some cases are superior to those reported for samples prepared via offline (sealed-tube combustion and vacuum line) methods. This finding, together with its convenience and cost-effectiveness, renders EA-AMS well-suited for CSRA. We show that some purification methods produce larger variability in the magnitudes and $F^{14}C$ values of the processing blanks and samples, leading to poorer reproducibility and larger total uncertainties. Improvements in isolation methods (e.g., for alkenones) are necessary to yield more informative data. This is particularly the case for samples of compounds smaller than 15 μg of C where reproducibility tests of the methods are essential for acquisition of accurate data. The minimum sample mass allowing the required measurement precision depends both on the isolation method and on the target-compound type. For the isolation methods described in this study, our results suggest that the minimum C mass necessary to produce relatively high precision ^{14}C data (i.e., better than 5%) in low-concentration samples (i.e., with ^{14}C ages above 18 ka) is 60 μg of C, whereas this can be reduced to 15 μg of C for samples <10 ka. Of the isolation methods tested, PCGC was superior to HPLC and column-chromatography methods with regard to both reproducibility and extraneous-carbon contamination. Assessment of the uncertainties associated with the mass and $F^{14}C$ of the constant contamination is facilitated by the development of a minimization routine in MATLAB. Tests of the applicability and reproducibility of online ^{14}C measurements for CSRA should be expanded to other analytes, including more volatile compounds and more polar compounds.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b04491.

Tables S1−S6. Chemicals, samples, and data (PDF)

Figure S1. χ^2 minimization for modern and dead C contamination (PDF)

Figure S2. Comparison of ^{14}C measurements of different materials (PDF)

MATLAB example input file (XLSX)

Data-reduction and contamination-evaluation script for ^{14}C analyses (PDF)

Configuration file (PDF)

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Notes

The authors declare no competing financial interest.

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