Grass material as a modern process standard for ^{14}C analysis of *n*-alkanes

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ABSTRACT

One of the difficulties in reporting accurate radiocarbon results from compound-specific radiocarbon analysis (CSRA) is the lack of suitable process standard materials to correct for the amount and ^{14}C content of carbon added during extensive sample processing. We evaluated the use of *n*-alkanes extracted from modern grass material (1.224 ±0.006 fraction modern) as process standards for CSRA. The *n*-alkanes were isolated using preparative capillary gas chromatography (PCGC) from two independent chemical extraction methods applied to the grass. Since this was our first assessment of the ^{14}C content of the grass *n*-alkanes, we corrected for extraneous carbon derived from PCGC isolation using commercially available single compounds of modern and ^{14}C-free content. Results were consistent across the two extraction methods showing that the C_{29} *n*-alkane has a fraction modern value that is within 1σ of the bulk value of the grass while C_{31} *n*-alkane and less abundant *n*-alkanes have values within 2σ of the bulk value of the grass. C_{29} and C_{31} *n*-alkanes were the most abundant *n*-alkanes in the grass and, as such, the more feasible for collection of sufficient amounts of carbon for accelerator mass spectrometry (AMS) analysis. Our results suggest that choosing a grass *n*-alkane with an elution time closest to that of the unknowns may be advisable due to possibly greater effect from GC column bleed (^{14}C-free) at later elution times. We conclude that C_{29} and C_{31} *n*-alkanes in modern grass of known ^{14}C content can be used as in-house standards to correct for the addition of ^{14}C-free carbon during sample preparation for ^{14}C analysis of *n*-alkanes.

INTRODUCTION

Compound-specific radiocarbon analysis (CSRA) is a powerful tool to investigate carbon cycling and/or as a dating technique in paleoclimate reconstructions (Uchida et al. 2001; Rethemeyer et al. 2005; Ohkouchi and Eglinton 2008; Uchikawa et al. 2008; Kramer et al. 2010; Kusch et al. 2010; Douglas et al. 2014; McIntosh et al. 2015; Tao et al. 2015). The ^{14}C content of individual compounds can be used to estimate residence times, identify carbon sources of organic matter or establish chronologies if traditional dating materials (e.g. macrofossils, pollen, charcoal) are not available. However, the isolation of compounds from parent material (e.g. plant material, soil, lacustrine or marine sediments) involves chemical extractions and isolation procedures that result in carbon contamination. In addition, the target compounds are often present in low concentrations and thus it is inevitable that the extracted quantities of carbon are often as little as tens of micrograms (μg), which amplifies the effect from carbon contamination. In CSRA, apart from carbon contamination derived from routine procedures of combustion and graphitisation (corrected for by using internationally accepted ^{14}C standards), carbon contamination is also derived from the chemical extraction and compound isolation, often achieved by preparative capillary gas chromatography (PCGC). In order to report accurate values from CSRA, efforts must be made to correct for carbon contamination derived from these procedures, hereafter referred to as extraneous carbon (C_{ex}).

In order to correct for C_{ex}, the amount and the ^{14}C content of C_{ex} must be determined by either using process blanks or process standards (materials processed in the same manner as unknowns at matching sizes) of known ^{14}C content (Mollenhauer and Rethemeyer 2009; Ziolkowski and Druffel 2009; Santos et al. 2010). The use of process blanks, known as the "direct method" involves the processing of solvent only (no sample or standard). The difficulty with this approach is that the amount of carbon obtained is often too small (<10 μg C) for a reliable AMS measurement. The use of process standards, known as the "indirect method", aims to estimate the old (^{14}C-free) and the modern (modern ^{14}C content) component of C_{ex} by using standard materials of modern ^{14}C content and ^{14}C-free, respectively. This approach assumes that the process standard has been diluted with a constant amount of C_{ex}, which causes a deviation in its ^{14}C content from its consensus (or in-house determined) value.

Different methods have been used to include process blanks or standard materials of known ^{14}C age to assess C_{ex} in studies involving CSRA. In a coastal sediments study, a mixture of commercially available compounds that ranged from ^{14}C-free to modern ^{14}C content was added to sea sand and used as a process standard (Santos et al. 2010). In a study of ^{14}C analysis of phospholipid fatty acids (PLFA) extracted from mineral soil, two commercially available fatty acid methyl esters (FAMEs; nC18:0 and nC16:0) of modern ^{14}C content were individually isolated by PCGC to determine the amount of ^{14}C-free C_{ex} added during PCGC isolation (Kramer et al. 2010). In ^{14}C analysis of PLFA and *n*-alkanes extracted from ocean sediments, Druffel et al. (2010) used several approaches to determine modern and ^{14}C-free C_{ex} during PCGC isolation that included solvent only, a modern methyl stearate standard and a ^{14}C-free C_{22} *n*-alkane standard and the assessment of the combined procedures of chemical extractions and PCGC was achieved by using blanks (no sample added). In the isolation of black carbon (BC), Ziolkowski and Druffel (2009) used commercially available modern and ^{14}C-free vanillin to determine carbon addition during PCGC isolation and BC reference materials from the BC Ring Trial (modern grass char, ^{14}C-free hexane soot; Hammes et al. 2007) and blanks (no sample added), to evaluate the chemical and PCGC isolation steps combined. Coppola et al. (2013) used a similar approach to assess C_{ex} during isolation of BC using reference materials from the BC Ring Trial (modern grass char, wood char, and ^{14}C-free hexane soot) in addition to NIST Standard Reference Material urban dust aerosol (SRM 1649a), marine sediment (SRM 1941b) added to wood char, and US Geological Survey Green River Shale. Tao et al. (2015) used solvents-only through the entire sample preparation procedure and solvents spiked with compounds of ^{14}C-free and of modern ^{14}C content after PCGC isolation as process standards.

One of the challenges for CSRA is the lack of suitable process standard materials, i.e. materials of known ^{14}C content, containing the compounds of interest and which can be subjected to the same chemical extractions and isolation procedures used on unknowns. Here we present the potential of using single year-growth grass as a modern process standard for the extraction and PCGC isolation of *n*-alkanes for radiocarbon analysis. We started from the assumption that the ^{14}C content of the grass leaf waxes, such as the long chain *n*-alkanes ($>C_{21}$), will be equal to the ^{14}C content of the bulk grass, which is representative of the carbon fixed from atmospheric CO_2 during one growing season (i.e. preceding collection). Our results showed that the *n*-alkanes extracted from the grass are indeed of modern ^{14}C content similar to the bulk grass and thus can be suitable for the assessment of ^{14}C-free C_{ex} derived from sample preparation for CSRA. Grass material can be subjected to the same chemical extractions used on unknown samples (e.g. soils,

sediments, plant matter) and has a similar composition to that of the unknowns (e.g. terrestrial material) thus constituting a good option as a process standard material.

MATERIALS AND METHODS

Grass material

While any modern grass material could be used for the purpose described in this paper, we took advantage of an earlier collection of grass near our Facility from which a large stock of material is still available. Single year-growth grass was collected locally (55.76 °N, -4.18 °W) in East Kilbride (EK), UK near the NERC Radiocarbon Facility during the growing season of 1984 and stored in dry, cool and dark conditions. The ^{14}C content of the bulk grass, initially measured by Liquid Scintillation Counting (LSC) at the NERC Radiocarbon Laboratory (n=2), is 1.2301 ± 0.008 fraction modern, which agrees with atmospheric values reported for Central Europe and the Northern Hemisphere during 1984 (Levin et al 1985; Hua et al, 2013). For this study, we performed several ^{14}C measurements by AMS of a sub-sample of this grass. Approximately 500 grams of the grass was ground (using a new grinder to avoid cross-contamination) to pass a 500 µm mesh size, freeze-dried and stored in an air-tight clean container. Three subsamples (~9 mg) were combusted to CO_2 and converted to graphite (in replicates of 3) following established protocols (Slota et al. 1987). Graphites from bulk combusted grass were sent to the SUERC AMS in East Kilbride and to the KECK CCAMS Facility in the University of California, Irvine (UCI) for analysis, with each Facility measuring 1 or 2 graphites from each combustion. ^{14}C concentration in this study is reported as fraction modern ($F^{14}C$) according to international conventions (Stuiver and Polach 1977; Reimer et al. 2004). The average $F^{14}C$ value of all measurements by AMS (n=9) is 1.2224 ± 0.0051. For the purpose of this study, we used all ^{14}C measurements of the bulk grass available, including the two historical values obtained by LSC (Figure 1), to obtain the average bulk $F^{14}C$ value of the grass of 1.2238 ± 0.0058 (n=11).

Extraction of *n*-alkanes

Two independent extractions of *n*-alkanes from the grass material were carried out at Newcastle University and Rothamsted Research, hereafter “extraction 1” and “extraction 2”, respectively, using two different methods. Two extractions were performed in order to obtain an additional set of *n*-alkane fractions. Extraction 1 consisted of microwave assisted solvent extraction (MARS 5, CEM Microwave Technology Ltd. UK) of ~24 grams of grass material

using 15 ml dichloromethane (DCM):methanol (3:1). A blank (no sample, solvent only) was also processed in the same manner as the grass sample. Glassware was cleaned with Decon90 (Decon Laboratories Limited), rinsed with ultra-pure water, dried in furnace then rinsed with solvents before use. Pipettes and vials were heated for 1 hr at 450 °C. Approximately 1-2 grams of grass were extracted in a single microwave vessel and extracts from multiple vessels were combined. The microwave program ramped to 70 °C and was held for 5 minutes. Total extracts were centrifuged then the solvent decanted and dried down using a rotary evaporator and nitrogen stream. The solvent extract was re-dissolved and added to aluminium oxide (150 mesh) before being added to 5% activated silica gel 60 columns which were used to elute the hydrocarbon fraction using hexane (four column volumes). Extracts were subsequently dried using a rotary evaporator and nitrogen stream. The total hydrocarbon fraction and blank were analysed by gas chromatography/mass spectrometry (GC/MS) to check purity of the extracts. The GC column used was a 30-m length Hewlett-Packard (HP) 5 and temperature program used was 50 °C for 2 min, then 5 °C/min to 310 °C for 21 min. Extraction 2 consisted of the Soxhlet extraction of ~12 grams of grass. Glassware was cleaned by washing with critical detergent, rinsing in ultra-pure water then drying with acetone, before heating in a muffle furnace for 1 hr at 450 ºC. Grass sample was extracted for 24 hr using DCM:acetone (9:1 v/v) to obtain a total lipid extract (TLE). The solvent was removed using a rotary evaporator and nitrogen stream. The TLE was re-dissolved in DCM:isopropanol (2:1 v/v) and filtered over defatted cotton wool. Glass columns packed with dried activated silica gel 60 (120 ºC, >12 h) were pre-eluted with hexane. The TLE was re-suspended in hexane and applied to the column. The hydrocarbon fraction was eluted using hexane under positive pressure supplied by a stream of nitrogen. The solvent was evaporated under nitrogen at 40 ºC.

Isolation of compounds and preparation for ^{14}C analysis

All Pyrex glassware and GC vials were cleaned by using either Decon90 or soaking in 5M nitric acid overnight, rinsed with ultra-pure water and dried then heated for 1 hr at 450 °C. U-traps for collection of isolated compounds (see below) were rinsed with DCM 5 times, dried in fume hood overnight and heated for 1 hr at 450 °C. Quartz glassware was heated for 1 hr at 900 °C the day before use (aluminium foil and tweezers were heated for 1 hr at 450 °C). All clean glassware was kept in air tight containers along with desiccant (Silica gel, Fisher Scientific) and CO_2 adsorbent (BDH Laboratory Supplies) and was heated again if stored for several weeks.

Separation of compounds was performed with a HP 5890 Series II GC with a fused silica capillary column (Rxi-1ms Restek, 30-m length, 0.32-mm ID, 0.25 um thickness), equipped with a HP 7673 injector and HP 5972 mass selective detector (MSD). The GC temperature program for the separation of grass *n*-alkanes was 50 °C for 2 minutes then 10 °C/min to 320 °C and held for 5 minutes. The same temperature program but ramping to 250 °C was used for isolation of the standard material docosane (see below). The injection volume was 2µl splitless for all samples (injection volume limited by the use of a standard GC injector). Compounds were isolated using a Gerstel preparative fraction collector (PFC) interfaced to the HP GC/MSD in a set up similar to that used by Eglinton et al. (1996). Approximately 1% of the flow eluting from the GC column was diverted to the MSD and 99% was sent to the PFC. Transfer line and PFC oven were kept at the maximum GC temperature program in use. The PFC was equipped with 6 U-traps for collection of compounds and one trap for waste. Care was taken to collect the entire peak of the target compound to avoid isotopic fractionation (Eglinton et al. 1996; Zencak et al. 2007). The U-traps for collection were kept at -10 °C using a cooling system of 50%/50% mixture of glycol/water. To prevent cross contamination, all samples were first injected 10 times and collected into U-traps which were then replaced with clean traps to start the sequence of injections for trapping. The total number of injections for trapping varied from 200 to 325 (see below) and final data corrections accounted for this.

Trapped compounds were retrieved by rinsing the U-traps 4 times with 250 µl of DCM into a clean GC vial. An aliquot of 100 µl was taken for determination of purity and yield by GC/MSD. Compounds were then transferred to a clean quartz insert (45 mm long, 5 mm ID) and solvent was removed under a stream of ultra-high purity nitrogen. The quartz insert was handled with tweezers and kept inside a clean 4 ml GC vial during solvent removal, covered loosely with clean aluminium foil (perforated at the top) to keep the insert clean. Solvent was removed to dryness and ~100-150 mg of copper oxide (pre-cleaned for 1 hr at 900 °C) was added to the quartz insert. The insert was then placed inside a quartz tube (270 mm long, 9 mm ID on one end and 3 mm ID on the other end) and the quartz tube was flamed-sealed at the 9 mm ID end. Tubes were evacuated to $10x^{-5}$ Torr, flame-sealed and combusted for 6 hr at 900 °C followed by 8 hrs at 700 °C. These combustion temperatures were not chosen for any particular reason other than the convenience of combusting samples along with other samples in our Facility (using ramped cooling to optimize purity of combusted gas). All samples in this study, including those not prepared via PCGC were combusted using the

same type of quartz tubes and same combustion temperatures. After combustion, CO_2 was cryogenically purified and reduced to graphite using standard procedures (Slota et al. 1987). Graphite targets from isolated compounds were analyzed at the KECK CCAMS Facility at UCI normalized to OXII primary standard and fractionation corrected to -25‰ by using the AMS $\delta^{13}C$. Data corrections for combustion and graphitization procedures were done following the "non-matching" method (Santos et al. 2007) using internationally accepted ^{14}C standards and in-house ^{14}C-free materials. Data corrections for PCGC preparation (isolation and solvent removal) accounted for ^{14}C-free C_{ex}. Modern C_{ex} was assessed for solvent removal and applied to PCGC too (see explanation below). ^{14}C-free C_{ex} and modern C_{ex} were evaluated using commercially available compounds of known ^{14}C content (indirect method) as described below.

Correction for the amount and ^{14}C content of C_{ex}

Since this was our first assessment of the usefulness of grass *n*-alkanes as process standards, that is, whether their $F^{14}C$ values agree with the bulk $F^{14}C$ value of the grass, we corrected the $F^{14}C$ values of the grass *n*-alkanes for C_{ex} derived from PCGC isolation and solvent removal (after correcting for combustion and graphitisation). The chemical extraction procedure (prior to PCGC) was not evaluated (apart from processing a blank for GC/MS analysis, see Results and Discussion) since it is a relatively simple procedure that does not require derivatization and thus it is unlikely to introduce as much C_{ex} compared to PCGC isolation and solvent removal. It should be noted that evaluating the chemical extraction becomes relevant if there are co-eluting compounds in the reagents and solvents used in the extraction procedure and/or extensive chemical pre-treatments are used (Ziolkowski and Druffel 2009; Coppola et al. 2013). Our results showed that our extraction methods do not contribute co-eluting compounds (see Results and Discussion).

To assess C_{ex}, we followed the indirect method by using commercially available compounds of modern ^{14}C content and ^{14}C-free as standard materials to estimate the ^{14}C-free and modern components of C_{ex}, respectively. The bulk $F^{14}C$ values of these compounds were measured in duplicate by combusting an amount equivalent to ~0.8 mg C of the unprocessed material following the procedures described above. As a modern standard we used docosane (C_{22} *n*-alkane, Aldrich, 134457, Lot# MKBJ6726V), bulk $F^{14}C$ value = 1.059 ± 0.003 (n=2) and as ^{14}C-free standards we used adipic acid (Acros Organics, 102815000, lot# A0306460), bulk $F^{14}C$ value = 0.0015 ± 0.0001 (n=2) and vanillin (Sigma Aldrich,

W310700), bulk $F^{14}C$ value = 0.0022 ± 0.0001 (n=2). To determine the amount of modern and ^{14}C-free C_{ex} derived from PCGC and solvent removal, different amounts of the compounds were dissolved in 1 ml DCM (usual volume in unknown samples) and subjected to these procedures before preparation for ^{14}C analysis. The deviation in the $F^{14}C$ values of the standard materials measured after PCGC isolation and solvent removal from their bulk $F^{14}C$ values (measured on unprocessed standard materials) was used to estimate the amount of C_{ex} (of modern or ^{14}C-free content depending on the standards used; Table 1). The amount of C_{ex} was estimated by mass balance using the formulae by Santos et al. (2007) adding an extra term for "dead carbon correction" to include our ^{14}C-free C_{ex} derived from PCGC isolation and solvent removal. Our modern component of C_{ex} corresponded to the "modern carbon correction" term in the formulae by Santos et al. (2007). We estimated the amount of C_{ex} as the mass of extraneous carbon needed to correct the $F^{14}C$ values of the processed standard materials to within 1σ of their bulk $F^{14}C$ value. We express C_{ex} derived from PCGC isolation and solvent removal in µg C per minute, per 50 (1 µl) injections for consistency with published literature (Ziolkowski and Druffel 2009; Coppola et al. 2013) although C_{ex} values are unique to each laboratory and procedure. In the case of solvent removal, C_{ex} is expressed as µg C (Table 1).

Modern C_{ex} was only evaluated for solvent removal due to technical issues with the GC/MSD interfaced to the PCGC collector. We used the amount of modern C_{ex} estimated for solvent removal as the amount of modern C_{ex} for PCGC isolation. Nevertheless the modern component of C_{ex} derived from PCGC processing is generally less significant than the ^{14}C-free component (Druffel et al. 2010; Kramer et al. 2010; Coppola et al. 2013). In addition, the modern $F^{14}C$ value of our grass material makes the evaluation of ^{14}C-free C_{ex} relatively more relevant.

RESULTS AND DISCUSSION

The distribution and relative abundance of *n*-alkanes extracted from the grass are shown in Figure 2 and were similar across the two independent extractions. The most abundant *n*-alkanes were C_{29} and C_{31} and these compounds were targeted for PCGC isolation. In addition, a group of compounds from extraction 2, consisting of $C_{23\text{-}27+}C_{33}$ *n*-alkanes, was also PCGC-isolated for ^{14}C analysis (combined to obtain enough carbon for AMS analysis). The $F^{14}C$ values of the *n*-alkanes and the total *n*-alkane fraction (before PCGC isolation of individual *n*-alkanes) from each extraction are shown in Table 2 as "uncorrected" (corrected

only for combustion and graphitisation) and "corrected" for C_{ex} derived from PCGC isolation and solvent removal. The $F^{14}C$ values of C_{29} and C_{31} *n*-alkanes were in agreement across the two extractions and they were within 1σ and 2σ, respectively, of the $F^{14}C$ value of the bulk grass (Figure 3). The grouped $C_{23\text{-}27+}C_{33}$ had a $F^{14}C$ value that was within 2σ of the bulk grass. The $F^{14}C$ value of the total *n*-alkane fraction from extraction 1 agreed with that of the bulk grass while for extraction 2 it was within 3σ of the bulk grass. The blank processed through the chemical extraction 1 and analysed by GC/MS showed a clean extract and without compounds co-eluting with the *n*-alkanes. Although we did not evaluate the chemical extraction 2 with a blank, the difference in the $F^{14}C$ value of the total *n*-alkane fraction with respect to extraction 1 is likely due to a different overall composition of the total extract, e.g. varying trace amounts of compounds other than *n*-alkanes, (rather than co-eluting compounds, see below), which could be possible given differences in the protocols between the two extractions. Regardless, trace compounds other than the targeted *n*-alkanes are excluded during PCGC isolation and thus do not affect the ^{14}C content of the target compounds.

As explained earlier, the $F^{14}C$ values of the grass *n*-alkanes shown in Table 2 and Figure 3 were corrected for C_{ex} derived from PCGC and solvent removal (Table 1) for the purpose of our initial assessment of their ^{14}C content. We can also use the uncorrected $F^{14}C$ values of C_{29} and C_{31} *n*-alkanes isolated from the grass to estimate C_{ex} derived from the entire sample procedure (chemical extraction + PCGC + solvent removal), assuming that the grass *n*-alkanes have the same ^{14}C content of the bulk grass (our initial assumption) and thus any deviation represents C_{ex} (^{14}C-free) added during the entire sample procedure (assuming addition of modern C_{ex} during PCGC isolation is relatively insignificant; Druffel et al. 2010; Kramer et al. 2010; Coppola et al. 2013). Our estimates show that ~ 0.91 ± 0.46 to 1.3 ± 0.65 μg C per minute, per 50 (1μl) injections is derived from the entire sample preparation procedure versus 0.75 ± 0.38 derived from PCGC isolation and solvent removal only (Table 3). The difference between these two estimates would suggest some contribution from the chemical extraction of grass *n*-alkanes. However this contribution is likely small as the GC/MS analysis of the chemistry blank from extraction 1 revealed a clean chromatogram (dominated only by column bleed) showing that the extraction method 1 can produce clean extracts and free of co-eluting compounds. Although we did not evaluate extraction 2 in the same way, the similarity in the value of C_{ex} between the two extraction methods (Table 3) suggests that extraction 2 also produces *n*-alkanes free of co-eluting compounds. Since the extraction of *n*-alkanes does not require extensive processing or the use of derivatization (which adds carbon and requires an additional correction; Eglinton et al. 1996; Ziolkowski

and Druffel 2009), we should not expect the correction for C_{ex} due to the prior chemical extraction alone to be significant relative to the correction due to PCGC isolation and solvent removal. GC column bleed on the other hand, can contribute carbon (^{14}C-free) to target compounds during PCGC isolation and this could explain the small difference in the estimated C_{ex} values between PCGC and the entire procedure. Relatively greater GC column bleed occurs with later elution times and thus C_{31} *n*-alkane may be affected to a greater extent by column bleed relative to C_{29} (Figure 2) and both of these compounds may receive more column bleed relative to docosane (C_{22} *n*-alkane), which elutes the earliest. Given that docosane was used to estimate C_{ex} derived from PCGC and the grass *n*-alkanes were used to estimate C_{ex} from the entire procedure, the small differences in the estimated C_{ex} values (Table 3) could be due to the effect of different degrees of GC column bleed on each compound rather than the chemical extraction of grass *n*-alkanes.

We compared the effect of correcting for PCGC + solvent removal versus correcting for the entire sample procedure on the $F^{14}C$ values of the grass *n*-alkanes, namely C_{31} and the group $C_{23\text{-}27+}C_{33}$. To correct for the entire procedure, we used the C_{ex} values based on the C_{29} *n*-alkane (matching its value to the bulk grass) which are 0.93 ± 0.47 and 0.91 ± 0.46 µg C per minute, per 50 (1 µl) injections for extraction 1 and 2, respectively (Table 3). This correction brings the $F^{14}C$ value of C_{31} to within 1σ of the grass value for extraction 1 but it does not make much difference to the $F^{14}C$ value of C_{31} from extraction 2 (Figure 4). A similar effect is observed on the correction of the $F^{14}C$ value of the combined $C_{23\text{-}27+}C_{33}$. Again, this may be due to different amounts of ^{14}C-free C_{ex} added to each compound derived from GC column bleed at different elution times and therefore the use of a single C_{ex} value based on the C_{29} *n*-alkane does not fully correct the $F^{14}C$ values of compounds that elute relatively later. The estimated correction factors C_{ex} based on the $F^{14}C$ value of C_{31} *n*-alkane (matching to the $F^{14}C$ value to the bulk grass) are 1.3 ± 0.65 and 1.00 ± 0.50 µg per minute, per 50 (1 µl) injections for extraction 1 and 2, respectively, which are slightly higher than those estimated based on C_{29} (Table 3). We did not estimate the correction factor based on the grouped *n*-alkanes collected from extraction 2 as the combined collection time is naturally much longer than the collection time needed for single compounds and thus artificially reduces the value of C_{ex}, which is normalised to time. We collected this group of *n*-alkanes to have enough carbon for an AMS measurement and be able to compare their combined ^{14}C content to the ^{14}C content of the bulk grass despite their much lower abundance.

Further in support of the effect from GC column bleed, the difference in ^{14}C content among the grass *n*-alkanes does not seem to be related to sample size. Lower uncorrected ^{14}C content would be expected with smaller sample sizes due to greater effect from ^{14}C-free carbon on samples < 100 µg C (Santos et al. 2010). Although our data corrections accounted for this sample-size effect, we note that the PCGC isolated sample size of C_{31} *n*-alkane matched that of C_{29} or was bigger, yet had relatively lower ^{14}C content across the two extractions (Table 2). Thus greater GC column bleed (^{14}C-free) at a later elution time seems to explain the relatively lower ^{14}C content of C_{31} *n*-alkane and to some extent that of the grouped $C_{23\text{-}27+}C_{33}$ (Figure 3). Taking this into account, when using the grass material as a modern *n*-alkane process standard, it may be advisable to choose the grass *n*-alkane that has an elution time closest to the elution time of the unknown compound to be corrected for C_{ex}. Table 3 shows that the C_{ex} value estimated for a given compound is similar across the two extractions, which supports this approach.

CONCLUSIONS

C_{29} and C_{31} *n*-alkanes were the most abundant *n*-alkanes in our modern grass and have $F^{14}C$ values that are within 1σ and 2σ of the $F^{14}C$ value of the bulk grass (1.224 ± 0.006), respectively, thus constituting a good choice of compounds using the grass material as a process standard. Based on our results and our PCGC set up, 25 grams of ground and homogenised grass material was sufficient to obtain enough carbon from C_{29} and C_{31} *n*-alkanes for the tests and PCGC isolation presented here. The chemical extraction of the grass *n*-alkanes did not seem to contribute much extraneous carbon relative to PCGC isolation. The $F^{14}C$ values of the grass C_{29} and C_{31} *n*-alkanes were corrected for ^{14}C-free extraneous carbon derived from PCGC isolation, using commercially available docosane, which has a relatively earlier elution time. Our results suggest small differences may exist among the size of ^{14}C-free blank of the individual compounds, including the different grass *n*-alkanes, related to different elution times and associated with contribution from GC column bleed. Therefore, when using the grass material as an *n*-alkane standard, it may be advisable to choose the ^{14}C-free blank of the grass *n*-alkane that has an elution time closest to the elution time of unknowns. The use of the grass *n*-alkanes as process standards allows for the determination of the ^{14}C-free component of carbon addition during preparation of similar sample materials (e.g. terrestrial plant material) for ^{14}C analyses. Based on these results, other compounds of interest in CSRA (e.g. alkanoic acids, lignin phenols) could also be explored using modern grass as process standards.

ACKNOWLEDGMENTS

Thanks to Paul Donohoe and Berni Bowler for GC/MS analytical support at Newcastle University. This work was supported by the NERC Radiocarbon Facility NRCF010001.

Table 1. Materials and sample sizes used to assess extraneous carbon (C_{ex}) added during PCGC isolation and solvent removal (^{14}C-free only) and solvent removal (^{14}C-free and modern ^{14}C content).

Procedure	Material	Bulk $F^{14}C$[(a)]	Sample size (µg C)	Lab code (UCIAMS #)	$F^{14}C$[(b)]	Error (AMS)	C_{ex}[(c)]	
PCGC + Solvent removal								
	Docosane (Aldrich, 134457)	1.0593 ± 0.0034					µg per minute, per 50 (1 µl) injections	$F^{14}C$[(d)]
			37	149741	0.912	0.010	0.75 ± 0.38	0.0
			90	149740	1.030	0.004		
			102	154551	1.025	0.004		
Solvent removal[(e)]							C_{ex}[(c)]	
	Docosane (Aldrich, 134457)	1.0593 ± 0.0034					µg C	$F^{14}C$[(d)]
			161	149743	1.044	0.002	1.55 ± 0.78	0.0
			260	154548	1.058	0.002		
			540	154547	1.059	0.002		
			857	149742	1.058	0.002		
	Adipic acid (Acros Organics, 102815000)	0.0015 ± 0.0001						
			106	149745	0.0128	0.0002	0.9 ± 0.45	1.0
			643	144623	0.0016	0.0001		
			964	154556	0.0016	0.0001		
	Vanillin (Sigma Aldrich, W310700)	0.0022 ± 0.0001						
			123	164455	0.0091	0.0002	0.9 ± 0.45	1.0
			493	155326	0.0025	0.0001		
			995	155330	0.0023	0.0001		

[(a)] Average $F^{14}C$ value (n=2) of unprocessed material combusted in sample sizes of 0.6-0.9 mg C.
[(b)] Corrected for combustion and graphitisation procedures only.
[(c)] Estimated by mass balance using the formulae in Santos et al. (2007) based on the deviation in the $F^{14}C$ value of each processed sample (corrected for combustion and graphitisation) from the $F^{14}C$ value of unprocessed material (bulk $F^{14}C$). This is the mass of extraneous carbon needed to correct the $F^{14}C$ values of the processed samples to within 1σ of the bulk $F^{14}C$ value. Uncertainty is estimated as 50% of the carbon mass.
[(d)] ^{14}C-free or modern ^{14}C component of C_{ex} as evaluated.
[(e)] Unprocessed material dissolved in ~1ml of dichloromethane and solvent evaporated under a stream of ultra-high purity nitrogen.

Table 2. $F^{14}C$ values of *n*-alkanes extracted from the grass material before and after correction for extraneous carbon (C_{ex}) added during PCGC isolation and solvent removal (excludes chemistry prior to PCGC)

Fraction extracted from grass	Lab code (UCIAMS #)	Sample size (µg C)	$F^{14}C$ uncorrected[(a)]	Error (AMS)	$F^{14}C$ corrected	Error (propagated)
Extraction 1						
C_{29} *n*-alkane	139052	70	1.132	0.008	1.189	0.037
C_{31} *n*-alkane	139053	70	1.088	0.008	1.143	0.035
Total *n*-alkane[(b)]	139051	96	1.201	0.006	1.221	0.015
Extraction 2						
C_{29} *n*-alkane	133585	64	1.143	0.007	1.206	0.040
C_{31} *n*-alkane	133586	102	1.107	0.004	1.144	0.023
C_{23}-C_{27}, C_{33} *n*-alkanes	133588	48	1.052	0.009	1.131	0.052
Total *n*-alkane[(b)]	133591	79	1.131	0.006	1.154	0.017
Bulk grass $F^{14}C$ value: 1.224 ± 0.006						

[(a)] Corrected for combustion and graphitisation only.

[(b)] Aliquot of the total *n*-alkane extract before PCGC isolation of individual *n*-alkanes.

Table 3. Estimation of the ^{14}C-free component ($F^{14}C=0$) of extraneous carbon (C_{ex}) derived from PCGC and solvent removal procedures (using docosane) and derived from the entire sample preparation procedure (chemistry + PCGC + solvent removal, using the grass) for the isolation of *n*-alkanes.

Material	Bulk $F^{14}C$	C_{ex} evaluated[(b)]	n	Chemical extraction	PCGC	Number of injections	C_{ex}	
							µg per minute, per 50 (1 µl) injections	$F^{14}C$
Docosane[(a)]	1.0593 ± 0.0034	^{14}C-free	3	No	Yes	200	0.75 ± 0.38[(c)]	0.0
Grass Material	1.2238 ± 0.0058	^{14}C-free	1	Yes	Yes			
C_{29}, Extraction 1						325	0.93 ± 0.47[(d)]	0.0
C_{29}, Extraction 2						239	0.91 ± 0.46[(d)]	0.0
C_{31}, Extraction 1						325	1.30 ± 0.65[(d)]	0.0
C_{31}. Extraction 2						239	1.00 ± 0.50[(d)]	0.0

[(a)] PCGC-isolated as indicated in Table 1.

[(b)] The ^{14}C component of the carbon added (C_{ex}) during sample processing.

[(c)] Estimated by mass balance using the formulae in Santos et al. (2007) based on the deviation in the $F^{14}C$ values of PCGC-isolated fractions (as in Table 1, corrected only for combustion and graphitisation) from the bulk $F^{14}C$ value. This is the mass of ^{14}C-free extraneous carbon needed to correct the $F^{14}C$ values of the fractions to within 1σ of the bulk $F^{14}C$ value. Uncertainty is estimated as 50% of the carbon mass.

[(d)] Estimated by mass balance using the formulae in Santos et al. (2007) based on the deviation in the $F^{14}C$ value of the *n*-alkane fraction (as in Table 2, "uncorrected") from the bulk $F^{14}C$ value of the grass material. This is the mass of ^{14}C-free extraneous carbon needed to correct the $F^{14}C$ values of the fractions to within 1σ of the bulk grass $F^{14}C$ value. Uncertainty is estimated as 50% of the carbon mass.

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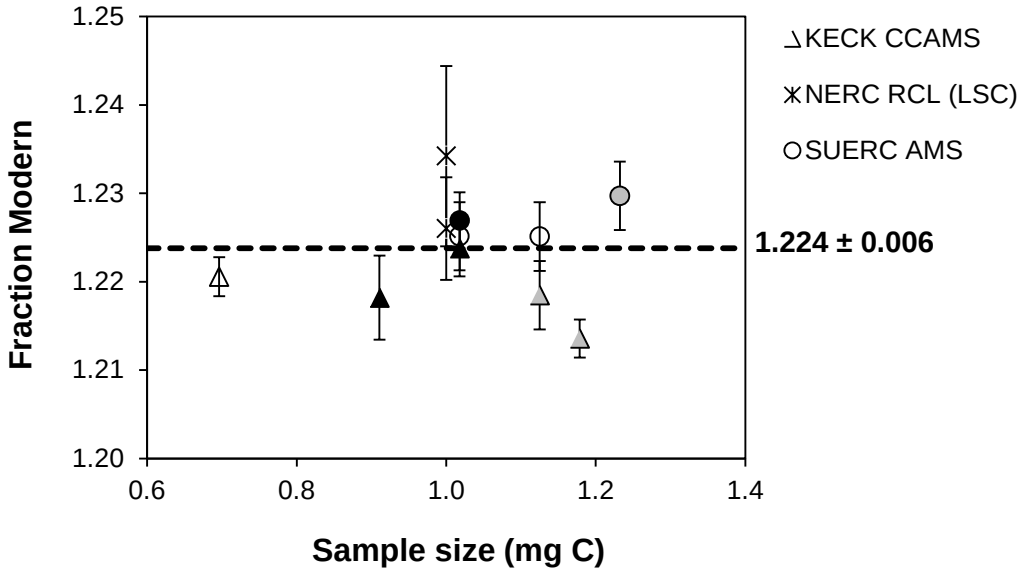

Figure 1. Fraction modern ($F^{14}C$) values (error bars denote AMS uncertainty) from separate measurements of the grass material (bulk combusted in amounts varying from 0.7 mg C to 1.0 mg C). These include triplicate measurements by accelerator mass spectrometry (AMS) of 3 independent combustions (each shown as black, grey and white symbols; n=9), measured at the KECK CCAMS and SUERC AMS facilities as indicated by triangles and circles, respectively. Also included are two historical measurements by liquid scintillation counting (LSC) measured at the NERC Radiocarbon Laboratory (NERC RCL; a sample size of 1 mg of carbon is used for plotting purposes). Dashed line shows average ± standard deviation (1.224 ± 0.006 fraction modern; n=11).

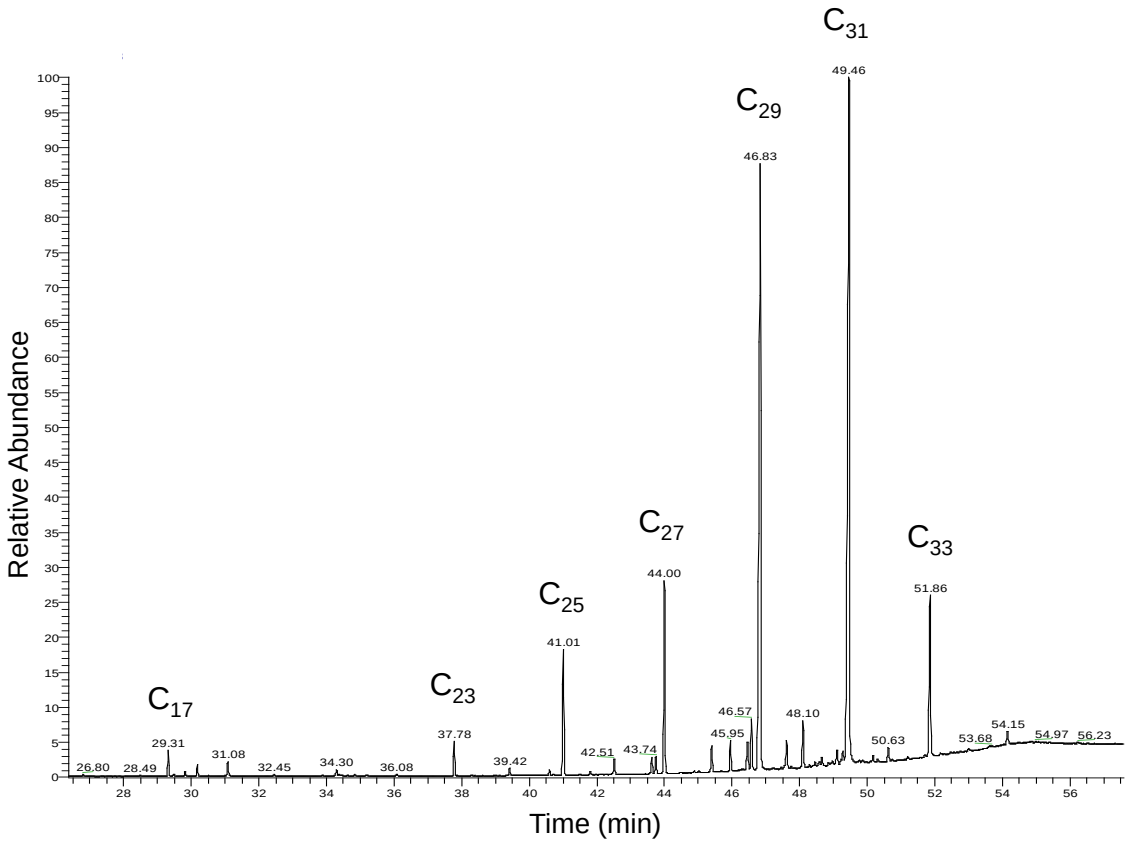

Figure 2. Relative abundance of *n*-alkanes extracted from the grass material.

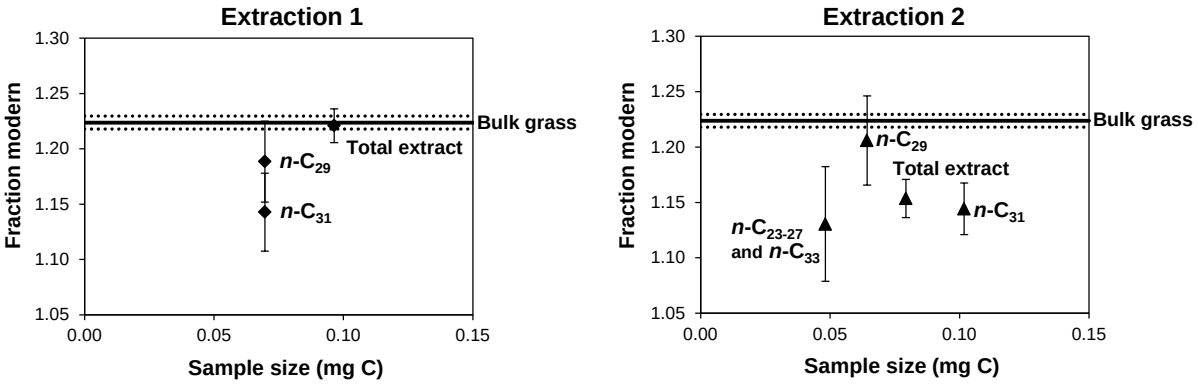

Figure 3. Fraction modern ($F^{14}C$) values of grass *n*-alkanes PCGC-isolated from two independent extraction methods. Also shown are the $F^{14}C$ values of the total *n*-alkane fraction ("Total extract"; before PCGC isolation of individual *n*-alkanes) from each method. The $F^{14}C$ value of the grass (bulk combusted; n=11) and standard deviation are shown as solid and dotted lines, respectively.

a)

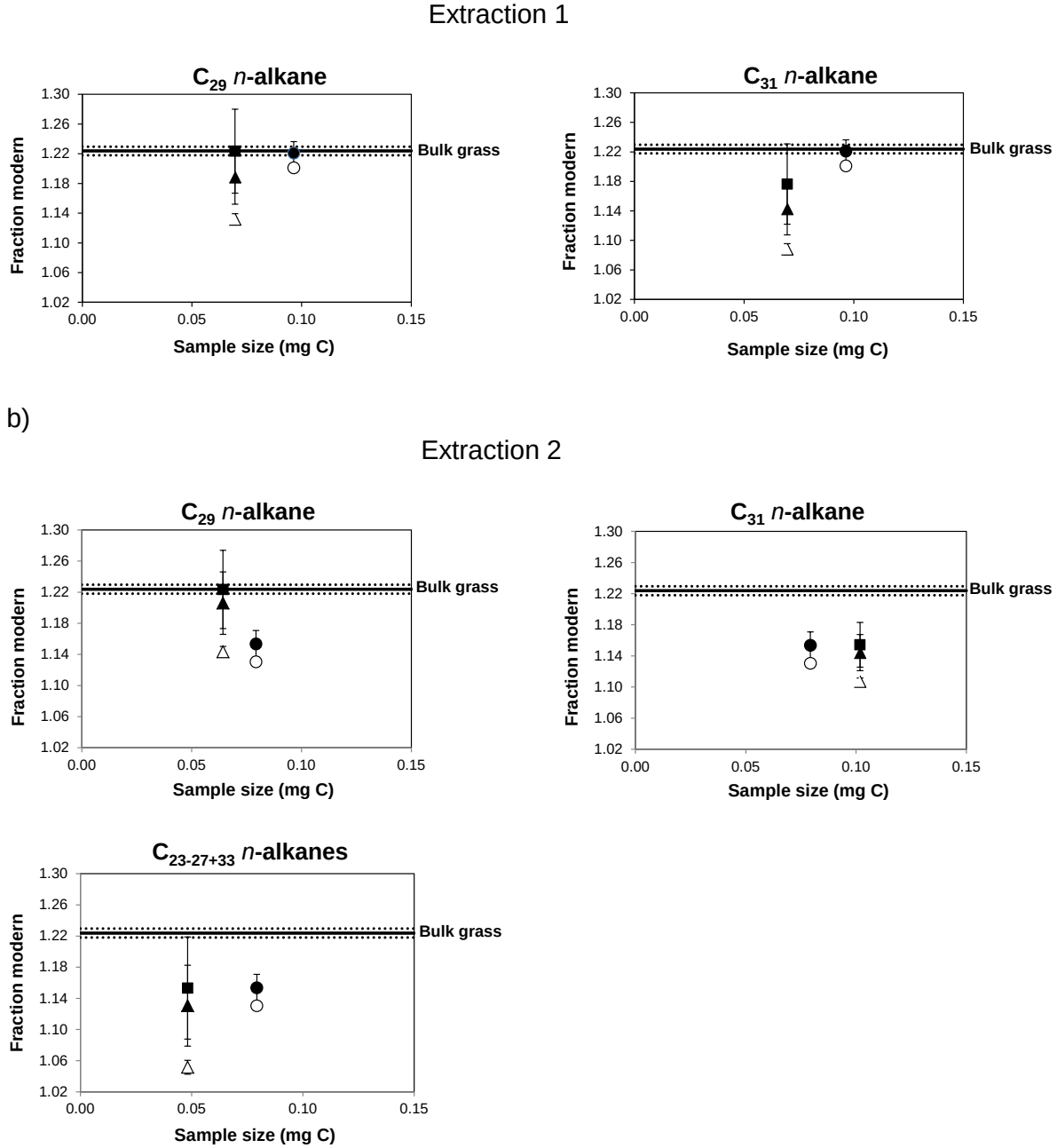

Figure 4. Fraction modern ($F^{14}C$) values of *n*-alkanes (triangles and squares) and total *n*-alkane extract (circles) from a) extraction 1 and b) extraction 2. $F^{14}C$ values before and after correction for extraneous carbon (C_{ex}) added during sample preparation are shown as open and closed symbols, respectively. Correction for C_{ex} added during PCGC isolation and solvent removal (excludes chemical extraction, Table 1) is shown in triangles and correction for C_{ex} added during the entire sample procedure (chemical extraction + PCGC + solvent removal, based on C_{29} *n*-alkane isolated from the grass, Table 3) is shown in squares. The total *n*-alkane extract was corrected for C_{ex} derived from solvent removal. The $F^{14}C$ value of the grass (bulk combusted; n=11) and standard deviation are shown as solid and dotted lines, respectively.