A rapid method to collect methane from peatland streams for radiocarbon analysis

M. H. Garnett,[1]* P. Gulliver[1] and M. F. Billett[2]

[1] *NERC Radiocarbon Facility, East Kilbride, Scotland, UK*
[2] *School of Biological & Environmental Sciences, University of Stirling, Stirling, UK*

ABSTRACT

Peatland streams typically contain high methane concentrations and act as conduits for the release of this greenhouse gas to the atmosphere. Radiocarbon analysis provides a unique tracer that can be used to identify the methane source, and quantify the time elapsed between carbon fixation and return to the atmosphere as CH_4. Few studies – those that have focus largely on sites with bubble (ebullition) emissions – have investigated the ^{14}C age of methane in surface waters because of the difficulty in collecting sufficient CH_4 for analysis. Here, we describe new sampling methods for the collection of CH_4 samples from CH_4-oversaturated peatland streams for radiocarbon analysis. We report the results of a suite of tests, including using methane ^{14}C standards and replicated field measurements, to verify the methods. The methods are not restricted to ebullition sites, and can be applied to peatland streams with lower methane concentrations. We report the ^{14}C age of methane extracted from surface water samples (~4–13 l) at two contrasting locations in a temperate raised peat bog. Results indicate substantial spatial variation with ages ranging from ~400 (ditch in afforested peatland) to ~3000 years BP (bog perimeter stream). These contrasting ages suggest that methane in stream water can be derived from a wide range of peat depths. This new method provides a rapid (10–15 min per sample) and convenient approach, which should make $^{14}CH_4$ dating of surface water more accessible and lead to an increased understanding of carbon cycling within the soil–water–atmosphere system. © 2015 The Authors. *Ecohydrology* published by John Wiley & Sons Ltd.

KEY WORDS   radiocarbon; methane; peatland; stream; evasion

Received 30 July 2014; Revised 30 January 2015; Accepted 30 January 2015

INTRODUCTION

Inland waters, including streams, rivers and lakes are increasingly recognised for the important role that they play in the transport of carbon from terrestrial ecosystems to the atmosphere (Repo *et al.*, 2007; Dinsmore *et al.*, 2010; Aufdenkampe *et al.*, 2011). Indeed, it has been estimated that globally, 0.8 Pg C is lost annually from aquatic systems (excluding wetlands; Cole *et al.*, 2007). Although most of the carbon lost to the atmosphere is in the form of carbon dioxide (CO_2), many inland waters and in particular peatland streams contain large concentrations of methane (CH_4), another powerful greenhouse gas (e.g. Hope *et al.*, 2001; Billett and Harvey, 2013). While both gases are sparingly soluble in freshwater, CH_4 has a lower solubility and is rapidly lost to the atmosphere (Chanton, 2005). Methane release in streams is also known to occur from specific hot spots, e.g. natural sediment traps (Roulet *et al.*, 1997). To quantify the role of inland waters in greenhouse gas emissions, it is important to understand the hydrological and biogeochemical processes that link sources in the terrestrial system to aquatic fluxes (Vonk and Gustafsson, 2013).

Water-saturated horizons of a peatland provide both abundant substrate and the oxygen-free conditions required for CH_4 production via anaerobic decomposition (Clymo and Bryant, 2008). While conditions suitable for methanogenesis may span several metres depth of peat, it has been suggested that a zone of high methane concentration exists near the peat surface close to the water table (e.g. Laing *et al.*, 2010). Methane is also transferred to the atmosphere via a number of routes, which, in addition to export in drainage waters, includes diffusive emission from the peat surface, ebullition, transport through plants with aerenchymateous tissues (Chanton, 2005) and release from natural peatland pipes (Dinsmore *et al.*, 2011). New methodological developments have tended to focus on improved quantification of surface CH_4 fluxes (e.g. Baird *et al.*, 2010).

Radiocarbon (^{14}C) provides a unique natural tracer with the potential to greatly improve our understanding of carbon cycling through ecosystems (Levin and Hesshaimer, 2000), and to unravel the sources and pathways of greenhouse gases. For example, ^{14}C analysis

*Correspondence to: M. H. Garnett, NERC Radiocarbon Facility, East Kilbride, Scotland, UK.
E-mail: mark.garnett@glasgow.ac.uk

of CO_2 evaded from the surface of peatland streams has shown that UK peatlands emit CO_2 with a wide range of ^{14}C ages (Billett *et al.*, 2006, 2007), from modern to ~1450 years before present (BP, where 0 BP = AD 1950; Stuiver and Polach, 1977). This shows that evasion CO_2 in some peatlands is derived from carbon that was fixed within recent decades, whereas at other sites, the emitted carbon had been stored for many hundreds of years or derived from geogenic sources. The age of evaded CO_2 has been found to be much older than dissolved organic carbon (DOC) in some streams, indicating that CO_2 and DOC are derived from different sources (Billett *et al.*, 2007). There has been far less study of the radiocarbon content of CH_4 in inland waters primarily because the low (compared with CO_2 and DOC) concentration of CH_4 makes it more challenging to obtain sufficient material for analysis [typically ≥1 ml CH_4 is required for ^{14}C measurement by accelerator mass spectrometry (AMS)]. A notable exception to this is the dating of CH_4 from high-ebullition environments like Siberian thaw lakes, where ages of 35 260 – 42 900 years BP have been measured (e.g. Walter *et al.*, 2006).

A recent method has been developed to collect methane for ^{14}C analysis from peatland streams using floating chambers (Garnett *et al.*, 2013). This method collects evaded (rather than in-stream) CH_4 and is hampered by a requirement to leave the chamber for several days to allow sufficient CH_4 to accumulate. The existing method, while a step forward, is time-consuming and difficult to deploy at higher and changing flow conditions as floating chambers become less stable.

An alternative approach to collecting methane samples from water is to use headspace equilibration (Kling *et al.*, 1991; Hope *et al.*, 1995; Jahangir *et al.*, 2012). This is a common direct method used to collect *in situ* gas samples from within the water column and uses syringes or flasks as the equilibration container, and a headspace of ambient air or N_2. The ratio of headspace volume to water volume in the collection chamber can be varied according to the concentration of dissolved gas in the water body (Cole and Caraco, 1998; Reira *et al.*, 1999).

While studies have verified headspace equilibration for determining concentrations of dissolved gases in water (e.g. Hope *et al.*, 1995), it cannot be assumed that these methods are reliable for collection of samples for radiocarbon analysis because of potential issues such as contamination and isotopic fractionation. The latter is unlikely because ^{13}C isotopic fractionation of methane across the water–air interface has been shown to be small (Harting *et al.*, 1976 cited in Jancsó, 2002), and because radiocarbon results are normalised using the ^{13}C content (Stuiver and Polach, 1977), they should be insensitive to fractionation effects if they are mass-dependent. Here, we describe a new method based on headspace equilibration

to enable the collection of methane samples from potentially remote and inaccessible peatland streams and surface waters for radiocarbon analysis. Specifically, we report the following: (i) a simple and rapid field method for the estimation of CH_4 concentration in stream water, (ii) test data on the effectiveness of manual headspace equilibration of methane from stream water, (iii) a rapid field method for the collection of sufficient methane for radiocarbon analysis and (iv) test results performed to verify its accuracy and reliability.

METHODS

We used a Detecto Pak-Infrared gas analyser (DP-IR; Gas Measurement Instruments Ltd, UK) to determine the methane concentration when equilibrated with the head-space of two types of containers. First, we used an airtight glass container ('Kilner' jar, John Lewis, UK) of fixed 1 l volume as a field method to rapidly quantify the methane available in the stream water for collection. The jar was modified by drilling two holes (7 mm diameter) into the metal lid, into which were inserted ~5 cm length of Tygon tubing (R3603 diameter 8 mm, wall 1.6 mm, Fisher, UK). Quick-connect couplings, which automatically close on disconnection (from Colder Products Co., Tom Parker, Preston, UK), were inserted in the ends of the tubing, allowing connection to the DP-IR (via more Tygon tubing).

The methane concentration of a water sample was determined by partly filling the 1-l container with stream water, replacing the lid and shaking for a set amount of time, which we determined from a series of tests to ensure degassing was complete (discussed later in the text). The jar was cleaned between samples by flushing with atmospheric air; this left a small amount (~2 ppm) of atmospheric CH_4 in the headspace, which was insignificant compared with the amount of methane recovered from the water samples. The methane concentration in the head-space of the jar was measured by attaching the DP-IR using a short length of tubing (~1 m) in a closed loop. The volume of the water used for headspace equilibration was then determined in a measuring cylinder and the methane concentration of the water (ml l) estimated using

$$CH_4 = \left((1000 - V_w) \times \left(M_{ppm} / 1 \, x \, 10^6 \right) \right) / (V_w / 1000) \quad (1)$$

where V_w is the volume of stream water (ml) and M_{ppm} represents the methane concentration (ppm) in the chamber headspace. Although methane has a very low solubility in water (Chanton, 2005), a small amount is still likely to be present in the water after manual degassing. While this can be quantified using Henry's Law, our aim here was to determine the amount of CH_4 available to be collected for isotope analysis, rather than to accurately measure stream water dissolved gas concentrations.

We determined from the processing of multiple stream water samples that shaking the 1-l container for more than 2 min did not result in further increases in the methane concentration of the headspace. Therefore, we standardised the shaking time required for all samples using this method to 3 min, considering this was more than sufficient time for complete degassing and to accommodate slight variations in shaking by different operators. We verified the reliability of this approach by testing whether different water volumes (and therefore different headspace to water volume ratios) affected the amount of methane degassed by performing multiple measurements of different volumes of water collected from the same sampling locations.

To enable collection of sufficient methane in a single degassing operation for ^{14}C analysis, we used a collapsible 15-l airtight container ['Accordion Water Carrier' (AWC); Highlander, Livingston, UK], which was partly filled with ~4–9 l of sample water by submerging under the water surface over about 1 min. Great care was taken to fill the container as gently as possible to minimise degassing before it was sealed and avoid disturbance of the stream substrate. For this reason, we initially chose not to use a pump. However, subsequently, we found that with care (and using an appropriate flow rate of ~300 ml min^{-1}), a pump is preferable and allows water samples to be collected in shallower streams where it is more difficult to submerge an AWC. The container had initially been flushed with atmospheric air prior to sampling, and approximately 4–6 l of air remained in order to create the headspace. We accepted that this would introduce a small amount of contamination from atmospheric CH_4, which was corrected using the following equation:

$$Ci_{(corr)} = \left(Ci_{(meas)} - F_{(atmos)} \times Ci_{(atmos)}\right) / \left(1 - F_{(atmos)}\right) \quad (2)$$

where Ci represents the isotopic content (^{14}C % modern or $\delta^{13}C$ ‰) of methane in the atmospheric-corrected sample ($_{corr}$), measured sample ($_{meas}$) and atmosphere ($_{atmos}$; assumed to be 130 % modern, $\delta^{13}C = -47$; Lassey *et al.*, 2007). $F_{(atmos)}$ is the fractional contribution of atmospheric CH_4 in the recovered methane sample (determined by measuring the methane concentration in the headspace of the equilibrated sample).

After shaking for 3 min, the methane concentration in the headspace was measured by the DP-IR (the lid to the AWC had couplings inserted as described earlier for the 1-l container). The headspace gas was then transferred to a 10-l foil gas sampling bag (SKC Ltd, UK) by simply attaching the bag and manually deflating the container. The entire operation to collect one sample typically took 10–15 min (a certain amount of care is required when handling these gas bags to avoid damage, although we have found them to be reliable).

We performed a series of tests to verify the 'AWC' method. First, we manually equilibrated samples by shaking for different lengths of time in order to establish the optimum time required for equilibration (as earlier performed for the 1-l container). Second, we verified the reliability of the method for collecting samples for stable and radiocarbon analysis by analysing duplicate field samples from two locations on two separate occasions. Third, we investigated the potential impact of incomplete degassing on the carbon isotopic composition of the methane by analysing samples collected at different equilibration times; the complete headspace in duplicate water samples was recovered for analysis sequentially from the same water sample after it had been (i) shaken for an initial 15 s, (ii) shaken for a further 30 s (total shaking time 45 s) and (iii) shaken for a further 45 s (total shaking time 90 s). We anticipated that most methane would be removed within the first 15 s, and therefore, the headspace for samples collected after 15 s was recreated using high-purity N_2 gas to exclude the contaminating influence of atmospheric CH_4, which would be greater in small samples.

We also tested the AWC and laboratory processing methods using standards of known radiocarbon content. Firstly, we added ~8 ml of '^{14}C-dead' methane (BOC, UK) to an AWC containing 6 l of deionised water and a headspace of atmospheric air, and performed the same methane extraction and laboratory processing methods as used in the field for the stream water samples. We also generated a modern CH_4 standard using leaves from deciduous trees collected in 2012, which were decomposed under anaerobic conditions in a sealed glass vessel. Headspace gas was removed using a syringe and injected into a foil gas bag containing oxygen, and then after determining the CH_4 concentration using the DP-IR, processed using the same laboratory procedures as used for the samples.

Laboratory analyses

On return to the NERC Radiocarbon Facility, the CO_2 component of the field samples (and also the two ^{14}C standard gases) was removed by pumping (~500 ml min) through soda lime; an infrared gas analyser (EGM-4, PPsystems, Hitchin, UK) verified the complete removal of CO_2. The remaining gas was then passed through platinum-alumina beads (Johnson Matthey Chemicals, UK) heated to 950 °C to oxidise the methane (Garnett *et al.*, 2012). Samples that had been equilibrated into a N_2 headspace received high-purity oxygen prior to combustion; this was unnecessary for samples equilibrated into a headspace containing atmospheric air. The resulting methane-derived CO_2 was dried using a 'slush' trap (−78 °C; dry ice and methylated spirits) and the methane-derived CO_2 recovered using liquid nitrogen (−196 °C). The methane-derived CO_2 was split into aliquots. One was used for determination of $\delta^{13}C$ ($^{13}C/^{12}C$ ratio in ‰ expressed relative to the Vienna Pee

Dee Belemnite (PDB) international standard) by isotopic ratio mass spectrometry (Thermo Fisher Delta V) and the second graphitised by Fe:Zn reduction (Slota *et al.*, 1987) and the ^{14}C concentration measured by AMS at the Scottish Universities Environmental Research Centre (East Kilbride, UK). Following convention, ^{14}C results were corrected for mass-dependent fractionation by normalising to a $\delta^{13}C$ of $-25\%_0$ (using measured values of individual samples), and expressed as conventional radiocarbon ages (Stuiver and Polach, 1977).

Sampling test locations

Samples were collected from two contrasting sites at Langlands Moss, an ombrotrophic raised bog in central south-west Scotland, UK (see Table I for details). We collected samples from a small stream draining the south-east perimeter of the peatland ('Stream' site) and from a man-made ditch within an area of afforested peatland ('Plantation' site). The dominant plant species on Langlands Moss are *Calluna vulgaris*, *Erica tetralix* and *Eriophorum vaginatum* (Langdon and Barber, 2005); the Plantation site is covered with mixed coniferous trees.

RESULTS

During three field tests of the method on different days, we found a strong linear correlation ($p < 0.001$) between volume of methane in the 1-l container headspace and volume of water (Figure 1). This suggested that variations in the headspace to sample water ratio made no significant difference to the efficiency that methane was equilibrated using our sampling protocol. For example, methane concentration in water was the same whether equilibration involved a ~300 ml or ~600 ml volume of water. This finding was consistent despite the considerably different concentration of methane in water at the sites: Stream site = 0.2–0.3 ml CH_4 (l H_2O)$^{-1}$; Plantation = 1.6 ml CH_4 (l H_2O)$^{-1}$.

Figure 2 shows the change in methane concentration in the headspace of the AWC when water samples were manually equilibrated by shaking. Even in the most methane-rich water samples, the majority (>80%) of methane was released within the first 30 s of shaking. Slight increases in concentration in the headspace occurred

up to about 90 s of shaking, but shaking for more than 120 s did not appear to result in further degassing (headspace CH_4 concentrations were constant). Based on these results, we adopted a standardised sampling protocol of 180-s manual shaking for AWC samples (as well as the 1-l container), considering that this would result in water samples that were consistently equilibrated in full, while additionally factoring in potential operator differences.
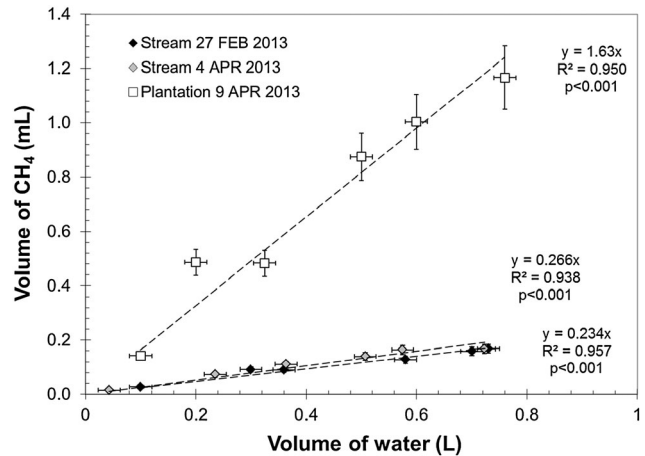

Figure 1. Volume of methane equilibrated from different amounts of water into the headspace of a 1-l container determined using the Detecto Pak-Infrared. Slope of the regression lines indicates the methane concentration (ml l^{-1}).
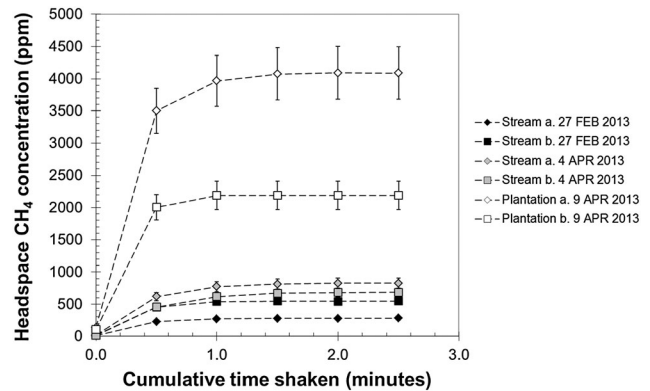

Figure 2. Methane concentration in the headspace of an Accordion Water Carrier after degassing water samples for different lengths of time at two sites. Error bars represent accuracy (±10%) of individual measurements made by the Detecto Pak-Infrared. Results show that methane concentration in the headspace no longer increased after shaking for 2 min.

Table I. Details of sampling locations.

Site	Lat/long	Sampling date	Water temperature (°C)	Water pH
Stream	55°44′5.5″N 04°10′25.8″W	27 Feb 2013	2.8	6.4
	55°44′5.5″N 04°10′25.8″W	4 April 2013	2.9	6.5
Plantation	55°44′10.68″N 04°10′39.24″W	9 April 2013	2.0	5.7

Using the AWC method with the 3-min shaking period, we collected duplicate samples from the two sites on three separate occasions for carbon isotope analysis. After equilibrating, methane concentrations in the headspace of the AWC ranged considerably between samples, partly reflecting the methane concentration in the water (but influenced also by the total volume of the headspace, which varied slightly and was not accurately known; Table II). All headspace CH_4 concentrations were >200 ppm, and therefore, the atmospheric CH_4 component (ambient concentrations ~2 ppm) was small (<1%), meaning that correction for atmospheric contamination made little difference to the results (corrected results differed from uncorrected results by <1σ). For two of the three sets of samples (Stream: 27 Feb 2013 and Plantation: 9 April 2013), there was extremely good agreement between duplicates, with atmosphere-corrected results differing by less than the 1σ measurement uncertainty of the ^{14}C analyses. There was greater difference between duplicate samples from the Stream site collected on 4 April 2013, with results agreeing within 3σ measurement uncertainty. The situation was similar for $\delta^{13}C$ results of duplicate samples, with two of the sets of duplicates agreeing closely (<1σ) and a greater difference (1‰) between the two Plantation samples. There was a considerable difference in the radiocarbon age of the methane between the two sites, with the samples from the Stream site (3047–3265 years BP) being much older than those from the Plantation site (371–433 years BP). At the Stream site, the average age of methane did not differ between sampling dates (Table II). The estimated methane concentration of the water determined using the AWC method was very similar to that determined with degassing samples using the 1 l container (Table II, Figure 1).

Table III presents data to investigate whether incomplete degassing had an effect on carbon isotope ratios. Samples were collected from the Stream site on 27 February 2013 to allow comparison with AWC samples collected using the standardised sampling protocol collected on the same day (Table II). For samples that were equilibrated by shaking for a total of 90 s, approximately 50–60% of the total methane was released in the first 15 s (Table III). However, at 3070 and 3291 years BP (after air-correction), these samples of the initially extracted CH_4 had a radiocarbon age that was virtually identical (within 1–3σ) to that collected using the standardised method. We collected insufficient CH_4 after 15 s of shaking for ^{14}C measurement. However, we were able to measure $\delta^{13}C$ of methane on samples at all three stages of degassing (after 0–15, 15–45 and 45–90 s), with results showing a trend consistent with lighter (i.e. ^{13}C-depleted) CH_4 being released first, and heavier (i.e. ^{13}C-enriched by ~1–3‰) CH_4 being removed later (Table III).

Radiocarbon results of the two standard materials were identical to expected values, indicating the absence of significant contamination and supporting the reliability of

Table II. Volumes of water and methane, and carbon isotope concentrations (before and after correction for a small atmospheric CH_4 component) of headspace equilibrated methane for three sets of duplicate water samples collected from two locations.

Site	Sampling date	Headspace CH_4 (ppm)	Water volume (l)	CH_4 volume (ml)	CH_4 concentration [ml CH_4 (l H_2O)$^{-1}$]	Publication code (SUERC-)	^{14}C age (years BP ± 1σ)	$\delta^{13}C_{VPDB}$ ‰ (±0.1)	Air-corrected ^{14}C age (years BP ± 1σ)	Air-corrected $\delta^{13}C_{VPDB}$ ‰ (±1σ)
Stream a.	27 Feb 2013	265	5.3	1.45	0.27	45738	3074±35	−64.0	3144±45	−64.2±0.2
Stream b.	27 Feb 2013	417	13.2	2.78	0.21	45739	3068±37	−64.0	3112±41	−64.1±0.2
Stream a.	4 April 2013	827	7.0	1.64	0.23	45973	3242±37	−64.5	3265±38	−64.6±0.1
Stream b.	4 April 2013	685	8.5	1.94	0.23	45974	3021±35	−64.4	3047±37	−64.4±0.1
Plantation a.	9 April 2013	4088	6.4	14.18	2.22	45975	369±35	−67.2	371±35	−67.2±0.1
Plantation b.	9 April 2013	2192	4.3	8.18	1.90	45976	430±35	−66.2	433±35	−66.2±0.1

Table III. Results of samples used to investigate carbon isotope fractionation during the equilibration of methane in stream water samples.

Site – degassing time (s)	Sampling date	Headspace CH$_4$ (ppm)	CH$_4$ volume (ml)	Publication code (SUERC-)	^{14}C age (years BP ± 1σ)	$δ^{13}C_{VPDB}$ ‰ (±0.1)	Air-corrected ^{14}C age (years BP ± 1σ)	Air-corrected $δ^{13}C_{VPDB}$ ‰ (±0.1)
Stream a. – 15	27 Feb 2013	198	1.13	45740	2978 ± 37	−64.2	3070 ± 53	−64.4 ± 0.3
Stream a. – 45	27 Feb 2013	159	0.61	—	—	−63.0[a]	—	−63.0 ± 0.1
Stream a. – 90	27 Feb 2013	47	0.02	—	—	n/a	—	n/a[b]
Isotope mass balance total								−63.9 ± 0.3
Stream b. – 15	27 Feb 2013	472	1.15	45741	3251 ± 35	−65.4	3291 ± 39	−65.5 ± 0.2
Stream b. – 45	27 Feb 2013	301	1.02	—	—	−64.1[a]	—	−64.1 ± 0.1
Stream b. – 90	27 Feb 2013	114	0.07	—	—	−62.8[a]	—	−62.8 ± 0.1
Isotope mass balance total								−64.8 ± 0.2

The volume of CH$_4$ recovered and its carbon isotope composition are presented for two water samples after being shaken for 0–15, 15–45 and 45–90 s. Carbon isotope results air-corrected (discussed in the text).
[a] Samples were equilibrated into a nitrogen headspace and therefore did not require correction for atmospheric CH$_4$.
[b] Estimated $δ^{13}$C value of −62.8‰ used in the isotope mass balance calculation, based on equivalent result from Stream b.

the sampling and laboratory methods; the '^{14}C-dead' CH$_4$ had a radiocarbon concentration of $0.35 ± 0.01$ %modern (SUERC-45742), which is only slightly above the laboratory background for methane samples (despite having an atmospheric CH$_4$ component), and the modern methane standard had a ^{14}C content of $103.83 ± 0.47$ % modern (SUERC-52758), i.e. within $1σ$ of the atmospheric ^{14}CO$_2$ in the year of sample collection.

DISCUSSION

Sampling methods and reliability tests

Our overall aim was to develop a reliable sampling method to enable the radiocarbon analysis of methane from surface waters. The key methodological advance was to recover sufficient CH$_4$ in the field for routine radiocarbon analysis by AMS using minimal equipment, thus providing a robust and rapid method suitable for inaccessible locations. For example, using this method, a single operator is likely to be capable of recovering 8–10 samples of headspace gas collected in sample bags, which might otherwise require the transport of at least 50–100 l of water if the gas had to be extracted in a laboratory.

The sampling methods we have developed are based on the same principles as existing headspace equilibrium methods used to determine dissolved gas concentrations in aquatic systems (e.g. Hope *et al.*, 1995; Jahangir *et al.*, 2012), but adapted to satisfy the previous criteria. Firstly, as has been demonstrated in the current study, the AWC sampling method can provide sufficient CH$_4$ (>1 ml) for AMS ^{14}C analysis, and we recovered between 1.5 and 14.2 ml CH$_4$ in the peatland surface waters that were sampled. The method is dependent on the methane concentration of the water, but literature values suggest that the technique could be applied to a wide variety of wetlands (e.g. Hope *et al.*, 2001; Repo *et al.*, 2007; Billett and Moore, 2008; Dinsmore *et al.*, 2011). Furthermore, the method can be scaled up, e.g. by sequentially combining the recovered headspace in multiple AWC samples (which was the case for the Stream b. sample collected on 27 February 2013; Table II). There will, however, be a limit when CH$_4$ concentrations in water are too low that this method might become impractical (e.g. <0.05 ml CH$_4$ (L H$_2$O)$^{-1}$). Although the minimum sampling requirement is a degassing container (AWC) and sample gas bag, a DP-IR and measuring cylinder are useful for verifying that the recovered sample will be sufficient for ^{14}C analysis. Similarly, the 1-l degassing container provides a simple and rapid (<5 min) field method to quantify the CH$_4$ available by headspace equilibration, and therefore to inform how much water is required to provide enough CH$_4$ for ^{14}C analysis.

The merits of the sampling methods described earlier are only of value if they provide reliable samples for carbon

isotope analysis. The cost of ^{14}C determinations limits the number of samples that can be analysed, so it is important to understand the reproducibility of the method. The ^{14}C results for duplicate samples were similar, and in most cases agreed within 1σ measurement uncertainty, suggesting that additional replication (with increased cost) may be unnecessary. As yet, we know little about how the ^{14}C content of CH_4 varies spatially in peatland streams and drainage systems, but slight differences in sampling locations of duplicate samples, or disturbance of sediments during sampling (despite care to avoid this), may explain the small difference in the ^{14}C results for one set of duplicate samples. We know already that both peatland stream water CH_4 concentration and the $\delta^{13}C$ of CH_4 show strong spatial and temporal variability in natural peat pipes, peatland streams and wetlands (Billett and Moore, 2008; Billett *et al.*, 2012; Dinsmore *et al.*, 2013), suggesting that peatland systems are heterogeneous and may show significant differences in CH_4 age.

We deliberately simulated incomplete degassing of samples to investigate the implications for carbon isotope results. We found that after only 15 s of manual shaking, more than half of the total CH_4 in the water was extracted. Moreover, there was only a small difference in $\delta^{13}C$ of less than 3‰ between samples collected after the first 15 s of shaking and the small amount of CH_4 released between 45 and 90 s (Table III); as expected, these results indicate that isotopically light methane (e.g. $^{12}CH_4$) is preferentially released into the headspace first, resulting in isotopic fractionation. Clearly, for studies of the $\delta^{13}C$ of CH_4, it will be important that fractionation issues are minimised; this appears to be achievable by analysing the CH_4 equilibrated after shaking for longer duration. It is notable that when the different components of the fractionated samples were combined using isotope mass balance, $\delta^{13}C$ values were very similar to the results obtained for samples collected using the standard method (Table III). Other headspace techniques have found 1-min or 2-min manual shaking of water samples to be sufficient when equilibrating samples for determining the concentration of dissolved gases (e.g. Repo *et al.*, 2007; Dinsmore *et al.*, 2011), although these are generally for volumetrically much smaller samples (e.g. Dinsmore *et al.*, 2013).

The main focus of this study concerned the ^{14}C analysis of methane, but if there had been differential isotopic fractionation of ^{12}C and ^{13}C isotopes, there will also have been fractionation of the ^{14}C. However, these fractionations are mass-dependent, and because all conventional radiocarbon ages are normalised to a $\delta^{13}C$ of $-25‰$, ^{14}C results are not impacted by isotopic fractionation (for the same reason, radiocarbon results are not affected by methane oxidation; Kessler and Reeburgh, 2005). Hence, we found considerable agreement in the ^{14}C age between replicates, even when incompletely equilibrated (Tables II and III). Consequently,

our results show that ^{14}C analysis of CH_4 using this method is robust and does not need the development of strict protocols that are required for other headspace methods concerned with accurately determining the concentration of dissolved gases in water (e.g. Jahangir *et al.*, 2012).

For most of our samples, we used atmospheric O_2 naturally present in air in the headspace of the AWC for combustion of CH_4 to CO_2, accepting that this would introduce a small amount of atmospheric CH_4 contamination. Our results show that this atmospheric component had an insignificant effect on the age of our samples, shifting them by less than the ^{14}C measurement uncertainty (atmospheric CH_4 represented ≤1% of the samples; Tables II and III). However, to avoid this problem, the headspace can be formed using CH_4-free gas, e.g. N_2 (as we did for small samples; Table III). For future application of the technique, the benefits of using a CH_4-free headspace should be considered, although given the current atmospheric CH_4 levels, it is only likely to be of significant benefit for samples that contain low concentrations of CH_4.

Implications of the carbon isotope results

The radiocarbon ages that we measured for CH_4 at the Stream site (3047–3265 years BP) were significantly older than evaded CO_2 previously measured for both this site (~300–520 years BP; Garnett *et al.*, 2013), and elsewhere in the UK (modern to 1450 years BP; Billett *et al.*, 2006, 2007). This observation supports earlier assertions that either the sources of CO_2 and CH_4 in peatland streams differ or they are derived from a different mixture of sources (Garnett *et al.*, 2013). The presence of younger CH_4 in water at the Plantation site (371–433 years BP) showed that CH_4 of a range of ages does enter the drainage system, indicating that the sources of methane vary spatially. Although we have few results, it may also be significant that at our Plantation site the age of the methane in the drainage water was very similar to the age of evaded CO_2 previously determined for the Stream site (Plantation CH_4 = ~371–433 years BP; Stream site evaded CO_2 = ~300–520 years BP; Garnett *et al.*, 2013). However, we did not analyse CO_2 at the Plantation site (the focus of the study being CH_4), and so have no information on how the age of CO_2 varies spatially at this location.

Investigations of peat pore waters have found that generally methane age increases with depth up to a reported maximum of ~4000 years BP in deep layers of ombrotrophic bogs (Clymo and Bryant, 2008; Garnett *et al.*, 2011). At Langlands Moss, CH_4 in peat pore waters was ~2800 years BP at 2 m and ~4000 years BP at 4 m depth (Garnett *et al.*, 2011). A recent ^{14}C measurement of methane from close to the base of the peat deposit (6 m) at Langlands Moss provided an age of 4291 ± 64 years BP (SUERC-42724; sample collected using the same method and location described in Garnett *et al.*, 2011). Given that

depths above 2 m all provided ages for methane in peat pore water of <2500 years BP; this suggests that the methane in the adjacent stream draining the peatland (~3000 years BP) was likely substantially derived from depths below 2 m. This contrasts with emissions from the peat surface, which were aged ~200–1400 years BP at Langlands Moss (Garnett *et al.*, 2012), and have been reported to be even younger at other sites (e.g. Wahlen *et al.*, 1989; Quay *et al.*, 1991; Chanton *et al.*, 1995). These results underline the considerable differences in the sources of methane emitted (i) directly to the atmosphere and (ii) indirectly to the aquatic system via sub-surface hydrological pathways, and further highlight the importance of flowing surface waters in fully understanding greenhouse gas emissions from peatlands.

Although there are few radiocarbon measurements of methane from peatland streams, we have previously dated methane evaded from the stream site at Langlands Moss using a floating chamber (Garnett *et al.*, 2013). Methane trapped using this method had an age between ~1600 and 2000 years BP, substantially younger than measured using the headspace method in the present study. Samples collected using both methods were processed using the same laboratory procedures, and both were corrected for an atmospheric CH_4 component, and therefore, we have no reason to suspect that the difference in results is due to a methodological issue. A potential explanation may be that an AWC sample is derived from methane dissolved in stream water, whereas a floating chamber would also trap any methane released from underlying sediments released by ebullition. Although bubble emissions were not directly observed, the possibility of capturing bubble emissions may have been high given that chambers were installed for several days, and even a small amount of ebullition could significantly contribute to the floating chamber samples because of the high methane concentration of bubbles (Strack *et al.*, 2005). Alternatively, although care was taken to avoid it, one technique may have been more prone to induce ebullition by disturbance of underwater sediments.

Previously, radiocarbon results for methane in surface waters have only been reported for a few studies, largely confined to boreal and arctic environments where locations with large ebullition fluxes have favoured collection of suitable-sized samples (e.g. Walter *et al.*, 2006, 2008). However, bubble emissions are often not evident in peatland streams even though they contain high quantities of methane gas that are transferred to the atmosphere via less-concentrated diffusive emissions, making sampling for radiocarbon analysis more challenging and necessitating the development of new methods like the one described here. There is an urgent need to improve our understanding of the response of carbon-rich environments to perturbation and climate change (e.g. permafrost thaw). Vonk and Gustafsson (2013) have called for a greater understanding of the links between carbon and hydrological cycles at a landscape scale, and ^{14}C approaches using the new method described here will help this to be achieved.

REFERENCES

Aufdenkampe AK, Mayorga E, Raymond PA, Melack JM, Doney SC, Alin SR, Aalto RE, Yoo K. 2011. Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. *Frontiers in Ecology and the Environment* **9**: 53–60.

Baird AJ, Stamp I, Heppell CM, Green SM. 2010. CH_4 fluxes from peatlands: a new measurement method. *Ecohydrology* **3**: 360–367.

Billett MF, Dinsmore KJ, Smart RP, Garnett MH, Holden J, Chapman P, Baird AJ, Grayson R, Stott AW. 2012. Variable source and age of different forms of carbon released from natural peatland pipes. *Journal of Geophysical Research* **117**: G02003. DOI: 10.1029/2011JG001807

Billett MF, Garnett MH, Hardie SML. 2006. A direct method to measure $^{14}CO_2$ lost by evasion from surface waters. *Radiocarbon* **48**: 61–68.

Billett MF, Garnett MH, Harvey F. 2007. UK peatland streams release old carbon dioxide to the atmosphere and young dissolved organic carbon to rivers. *Geophysical Research Letters* **34**: L23401. DOI: 10.1029/2007GL031797

Billett MF, Harvey FH. 2013. Measurements of CO_2 and CH_4 evasion from UK peatland headwater streams. *Biogeochemistry* **114**: 165–181.

Billett MF, Moore T. 2008. Supersaturation and evasion of CO_2 and CH_4 in surface waters at Mer Bleue peatland, Canada. *Hydrological Processes* **22**: 2044–2054.

Chanton JP. 2005. The effect of gas transport on the isotope signature of methane in wetlands. *Organic Geochemistry* **36**: 753–768.

Chanton JP, Bauer JE, Glaser PA, Siegel DI, Kelley CA, Tyler SC, Romanowicz EH, Lazrus A. 1995. Radiocarbon evidence for the substrates supporting methane formation within northern Minnesota peatlands. *Geochimica et Cosmochimica Acta* **59**: 3663–3668.

Clymo RS, Bryant CL. 2008. Diffusion and mass flow of dissolved carbon dioxide, methane, and dissolved organic carbon in a 7-m deep raised peat bog. *Geochimica et Cosmochimica Acta* **72**: 2048–2066.

Cole JI, Caraco NF. 1998. Atmospheric exchange of carbon dioxide in a low-wind oligotrophic lake measured by the addition of SF_6. *Limnology and Oceanography* **43**: 647–656.

Cole JJ, Prairie YT, Caraco NF, McDowell WH, Tranvik LJ, Striegl RG, Duarte CM, Kortelainen P, Downing JA, Middelburg JJ, Melack J. 2007. Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. *Ecosystems* **10**: 171–184.

Dinsmore KJ, Billett MF, Dyson K. 2013. Temperature and precipitation drive temporal variability in aquatic carbon and GHG concentrations and fluxes in a peatland catchment. *Global Change Biology* **19**: 2133–2148.

Dinsmore KJ, Billett MF, Skiba UM, Rees RM, Helfter C. 2010. Role of the aquatic pathway in the carbon and greenhouse gas budgets of a peatland catchment. *Global Change Biology* **16**: 2750–2762.

Dinsmore KJ, Smart RP, Billett MF, Holden J, Baird AJ, Chapman PJ. 2011. Greenhouse gas losses from peatland pipes: a major pathway for loss to the atmosphere? *Journal of Geophysical Research-Biogeosciences* **116**: G03041. DOI: 10.1029/2011JG001646

Garnett MH, Hardie SML, Murray C. 2011. Radiocarbon and stable carbon analysis of dissolved methane and carbon dioxide from the profile of a raised peat bog. *Radiocarbon* **53**: 71–83.

Garnett MH, Hardie SML, Murray C. 2012. Radiocarbon analysis of methane emitted from the surface of a raised peat bog. *Soil Biology and Biochemistry* **50**: 158–163.

Garnett MH, Hardie SML, Murray C, Billett MF. 2013. Radiocarbon dating of methane and carbon dioxide evaded from a temperate peatland stream. *Biogeochemistry* **114**: 213–223.

Harting P, Schütze H, Christoph G. 1976. Der thermodynamische Kohlenstoffisotopieeffekt im System CH_4-H_2O. *Isotopenpraxis* **12**: 232–234.

Hope D, Dawson JJC, Cresser MS, Billett MF. 1995. A method for measuring free-CO_2 in upland stream water using headspace analysis. *Journal of Hydrology* **166**: 1–14.

Hope D, Palmer S, Billett MF, Dawson JJC. 2001. Carbon dioxide and methane evasion from a temperate peatland stream. *Limnology and Oceanography* **46**: 847–857.

Jahangir MMR, Johnston P, Khalil MI, Grant J, Somers C, Richards KG. 2012. Evaluation of headspace equilibration methods for quantifying greenhouse gases in groundwater. *Journal of Environmental Management* **111**: 208–212.

Jancsó G. 2002. Interpretation of isotope effects on the solubility of gases. *Nukleonika* **47**: S53–S57.

Kessler J, Reeburgh W. 2005. Preparation of natural methane samples for stable isotope and radiocarbon analysis. *Limnology and Oceanography-Methods* **3**: 408–418.

Kling GWG, Kipphut W, Miller MC. 1991. Arctic streams and lakes as conduits to the atmosphere: Implications for tundra carbon budgets. *Science* **251**: 298–301.

Laing C, Shreeve T, Pearce DME. 2010. The fine scale variability of dissolved methane in surface peat cores. *Soil Biology and Biochemistry* **42**: 1320–1328.

Langdon PG, Barber KE. 2005. The climate of Scotland over the last 5000 years inferred from multiproxy peatland records: inter-site correlations and regional variability. *Journal of Quaternary Science* **20**: 549–566.

Lassey K, Lowe DJ, Smith A. 2007. The atmospheric cycling of radiomethane and the "fossil fraction" of the methane source. *Atmospheric Chemistry and Physics* **7**: 2141–2149.

Levin I, Hesshaimer V. 2000. Radiocarbon - A unique tracer of global carbon cycle dynamics. *Radiocarbon* **42**: 69–80.

Quay PD, King SL, Stutsman J, Wilbur D, Steele L, Fung I, Gammon R, Brown TA, Farwell G, Grootes P, Schmidt F. 1991. Carbon isotopic composition of atmospheric CH_4: fossil and biomass burning source strengths. *Global Biogeochemical Cycles* **5**: 25–47.

Reira J, Schindler J, Kratz T. 1999. Seasonal dynamics of carbon dioxide and methane in two clear-water lakes and two bog lakes in northern Wisconsin, USA. *Canadian Journal of Fisheries and Aquatic Sciences* **56**: 265–274.

Repo ME, Huttunen JT, Naumov AV, Chichulin AV, Lapshina ED, Bleuten W, Martikainen PJ. 2007. Release of CO_2 and CH_4 from small wetland lakes in western Siberia. *Tellus Series B-Chemical and Physical Meteorology* **59**: 788–796.

Roulet N, Crill P, Comer N, Dove A, Bourbonniere R. 1997. CO_2 and CH_4 flux between a boreal beaver pond and the atmosphere. *Journal of Geophysical Research* **102**: 29313–29319.

Slota P, Jull AJT, Linick T, Toolin LJ. 1987. Preparation of small samples for ^{14}C accelerator targets by catalytic reduction of CO. *Radiocarbon* **29**: 303–306.

Strack M, Kellner E, Waddington JM. 2005. Dynamics of biogenic gas bubbles in peat and their effects on peatland biogeochemistry. *Global Biogeochemical Cycles* **19**: GB1003.

Stuiver M, Polach HA. 1977. Reporting of ^{14}C data. *Radiocarbon* **19**: 355–363.

Vonk JE, Gustafsson O. 2013. Permafrost-carbon complexities. *Nature Geoscience* **6**: 675–676.

Wahlen M, Tanaka N, Henry R, Deck B, Zeglen J, Vogel JS, Southon J, Shemesh A, Fairbanks R, Broecker W. 1989. Carbon-14 in methane sources and in atmospheric methane: the contribution from fossil carbon. *Science* **245**: 286–290.

Walter K, Zimov S, Chanton J, Verbyla D, Chapin F III. 2006. Methane bubbling from Siberian thaw lakes as a positive feedback to climate warming. *Nature* **443**: 71–75.

Walter KM, Chanton JP, Chapin FS, III, Schuur EAG, Zimov SA. 2008. Methane production and bubble emissions from arctic lakes: isotopic implications for source pathways and ages. *Journal of Geophysical Research-Biogeosciences* **113**: G00A08. DOI: 10.1029/2007JG000569