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A new field approach for the collection of samples for aquatic $^{14}$CO$_2$ analysis using headspace equilibration and molecular sieve traps: The Super Headspace Method

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Abstract

Carbon dioxide evasion from inland waters such as lakes, rivers and streams, represents a significant component of the global carbon cycle, yet in many parts of the world relatively little is known about its source. Radiocarbon dating of aquatic CO$_2$ has the potential to provide new insights into C cycling in the terrestrial-aquatic-atmosphere continuum, and while a range of methods are available for the collection of samples for $^{14}$C analysis, they all have limitations or disadvantages (e.g. slow collection rates, potential non-equilibrium). These issues are further compounded in remote field sites. Here, we describe a new method for the field collection of CO$_2$ samples from low pH waters (pH<7) for radiocarbon analysis, which involves a scaled-up version of the widely-used headspace equilibration technique coupled with syringe-injection of samples into molecular sieve traps for convenient and stable storage. We present the results of laboratory and field tests to verify this “Super Headspace Method”, and discuss its advantages compared to existing techniques, particularly for sampling in remote locations. This includes its high portability, speed of use, and absence of any special sample preservation requirements.

1. Introduction

The importance of lakes, rivers and streams as globally significant sources of greenhouse gases is being increasingly recognised (Borges et al., 2015). CO$_2$ evasion from these inland waters has been estimated at ~2.1 Pg C yr$^{-1}$ (Raymond et al., 2013), which is broadly the same magnitude as the total terrestrial land carbon sink. Moreover, there is still considerable uncertainty concerning the sources of aquatic CO$_2$ (Raymond et al., 2013), which must be addressed if we are to more accurately predict the response of the global carbon cycle to change. This is particularly relevant to the Arctic regions where there is evidence that “old” carbon is being released into aquatic systems in response to warming (e.g. Vonk et al., 2015).
The isotopic composition of aquatic carbon can be used to identify the sources of different forms of carbon (e.g. particulate and dissolved organic carbon, dissolved inorganic carbon and methane), and the processes responsible for their transformations (Benner et al., 2004; Raymond and Bauer, 2001; Billett et al., 2012). Radiocarbon \(^{14}\text{C}\) analysis/dating provides unique information to enable the partitioning of different carbon sources (e.g. organic matter versus carbonate), its age and rate that it is cycled across the terrestrial-aquatic-atmospheric system (Billett et al., 2007).

Isotopic methods for the analysis of aquatic organic carbon are well established. However, analysis of aquatic CO\(_2\) has until recently received far less attention, particularly in regard to \(^{14}\text{C}\). This is a serious omission given the magnitude of the CO\(_2\) evasion flux (Raymond et al., 2013) and because an accurate quantification of the contemporary carbon balance of catchments containing old carbon (e.g. peatlands) requires an understanding of the age of the CO\(_2\) (Billett et al., 2015), which is best determined through measurement of the \(^{14}\text{C}\) content of aquatic CO\(_2\).

The “gas stripping” method is frequently used to determine the isotopic composition of dissolved inorganic carbon (DIC) and aquatic CO\(_2\) (the latter via temperature and pH dependent equilibria (Stumm and Morgan, 1981; Butler, 1982) and experimentally-derived isotopic fractionation factors (Zhang et al., 1995)). Originally developed for the analysis of sea waters (Kroopnick et al., 1970), the procedure involves the recovery of the total DIC following acidification of water samples with phosphoric acid to a point where all of the DIC is converted to CO\(_2\); the CO\(_2\) is then recovered for isotope analysis (including \(^{14}\text{C}\) measurement; e.g. McNichol et al., 1994; Dean et al., 2014). This, or similar, methods are also used for measurement of stable carbon isotopes in freshwaters (e.g. Gillikin and Bouillon, 2007). However, these methods have significant disadvantages for the preparation of radiocarbon samples because of the greater sample size requirements; stable carbon isotope analysis of DIC requires as little as 0.5 ml or less of water (e.g. Gillikin and Bouillon, 2007; Klein Gebbinck et al., 2014) whereas \(^{14}\text{C}\) analysis may require hundreds or thousands of millilitres even for measurements undertaken using accelerator mass spectrometry (AMS; the actual volume of water needed being dependent on the DIC concentration). There are also concerns about the reliability of the “gas stripping” method for determining the isotopic composition of aquatic CO\(_2\) since it has been suggested that some of the assumptions regarding equilibrium conditions and the calculations used may not hold for all (i.e. biogeochemically variable) aquatic systems (Billett and Garnett, 2010). In addition, when using water samples for isotope analysis consideration must be given to sample preservation,
especially given recent reports of the high lability of different forms of aquatic carbon (McCallister and Giorgio, 2012, Moody et al., 2013; Drake et al., 2015).

New methods for the radiocarbon analysis of aquatic CO2 have recently been developed. For example, Gao et al. (2014) reported a rapid method using headspace extraction of acidified water samples. Other methods exploit the convenience of zeolite molecular sieves as a CO2 adsorbent, which has the advantage that the sample CO2 can be quickly isolated and stabilised in the field, and stored in a concentrated, stationary phase inside gas-tight cartridges; this provides advantages of high portability of samples and avoids concerns relating to the preservation and transport of water samples. These molecular sieve traps have been used in conjunction with floating chambers in order to collect CO2 evaded from a stream surface; once sufficient CO2 has accumulated, chamber air is pumped through a molecular sieve trap to collect the CO2, allowing it to be returned to the laboratory, recovered and analysed (Billett et al., 2006). A passive (in situ) sampling method has also been developed whereby a gas permeable hydrophobic filter allows aquatic CO2 (but not water) to enter a cartridge containing the molecular sieve, where it is again trapped and held until returned to the laboratory for processing (Garnett et al., 2012). These methods have proven to be extremely valuable in a number of studies (e.g. Billett et al., 2007; Billett et al., 2012; Vihermaa et al., 2014), however, neither are particularly rapid, and both result in the collection of time-integrated samples, rather than the collection of a sample from a single instance in time (a floating chamber sample may take several hours to collect, whereas a passive sample may take 3-6 weeks). In aquatic systems prone to high temporal variability associated with rapid rainfall-runoff response, it is important for source/age attribution to be able to sample individual parts of the hydrograph. Hence while time-integrated sampling offers many advantages and is appropriate to many situations, we identified a need for a new and faster sampling approach that would allow the determination of the 14C age of aquatic CO2 at a specific moment in time. This would allow results to be compared with other measurements (e.g. DO14C, PO14C), tied to the same sampling chronology and which would, for example, have considerable benefits for capturing relatively short-term hydrological events (e.g. storm events). In addition, we required a sampling method that could be used in remote locations and therefore had low power requirements, was easily transportable (i.e. minimal equipment) and had no special storage requirements.

Here, we present a new sampling technique for 14C dating of aquatic CO2, which meets the requirements for remote field working. The technique combines established “headspace equilibration” techniques widely used as a method to directly measure aquatic
dissolved CO$_2$ (e.g. Kling et al., 1991; Dinsmore et al., 2013), scaled up to provide the larger sample size requirements of $^{14}$C analysis, with syringe injection of headspace gases into molecular sieve CO$_2$ traps. We present the results of tests used to verify the “Super Headspace Method”, which is rapid and highly portable, and has already proven to be reliable during field sampling campaigns in remote Arctic ecosystems.

2. Methods

2.1 Description of the new sampling method

We used a collapsible water carrier (5 L and 10 L Accordion Water Carrier – AWC; Highlander, Livingston, UK; Figure 1) for an equilibration chamber. The AWC was modified by replacing the screw cap lid with a 2-holed rubber bung which had stainless steel pipe inserted into each hole (~5 cm lengths of 6 mm o/d). Sampling ports were provided using quick connect couplings (Colder Products Co, USA) attached to the pipes via ~5 cm lengths of tubing (Tygon E3603, Saint-Gobain, France). Although the couplings had shut-off valves that automatically closed on disconnection, plastic clips were also placed across the Tygon tubing to provide an additional second seal (WeLoc, Scandinavia Direct, UK). Tests using an infrared gas analyser (IRGA; PPsystems EGM-4, Hitchin, UK) verified that the AWC was gas tight (when filled with 1 L of nitrogen, less than 0.0025 ml of CO$_2$ accumulated after 15 minutes, i.e. <0.1 % of a typical sample).

Immediately before use, the AWC was “collapsed” to remove atmospheric air and then flushed with CO$_2$-free air and evacuated using a 1 L “jumbo” syringe (Thames Restek UK Ltd); the gas port of the syringe had a quick coupling attached via a ~3 cm length of Tygon tubing providing a leak-tight connection to the AWC. CO$_2$-free air was generated on-site using the 1 L syringe by passing atmospheric air twice through a cartridge (1.8 cm o/d x 45 cm length) containing soda lime (i.e. when the syringe was filled and also when injected into the AWC; earlier tests using an IRGA had confirmed the complete removal of atmospheric CO$_2$ during this operation, and this can be repeated, as necessary, in the field using the IRGA to confirm a CO$_2$-free source). Stream water was then gently pumped into the AWC via Tygon tubing using a 12V aquarium pump at a flow rate of ~300 cc/minute (pump rate will vary according to water temperature and hydraulic head). The volume of water sampled was initially estimated by monitoring the pumping time, and later (after sample collection) more accurately measured using a 1 L measuring cylinder (alternatively a
balance/weighing scales (accurate to at least ± 0.01 kg) can be used to infer sample volume from the weight). A known volume of CO₂-free air was injected into the AWC using the 1 L syringe to create a headspace into which aquatic CO₂ was equilibrated. As with other, established headspace methods, equilibration was achieved by shaking.

Following equilibration, the CO₂ concentration of the headspace inside the AWC was measured using an IRGA. Measured volumes of headspace gas were then removed from the AWC using the jumbo syringe and injected into a cartridge containing Type 13X zeolite molecular sieve (Figure 1; see Hardie et al., 2005 for details about the molecular sieve cartridge). As the gas slowly (<500 ml/minute) passed through the cartridge, sample CO₂ was trapped by the molecular sieve. After passing through the molecular sieve cartridge (MSC), the remaining headspace gases were allowed to vent to atmosphere via a cartridge containing soda lime, which prevented atmospheric CO₂ back-filling into the MSC. At least ~ 3 ml CO₂ is recommended for collection of samples for radiocarbon analysis using these MSCs as established from tests performed on standard gases of known radiocarbon concentration (Garnett and Murray, 2013). Therefore, if necessary, several litres of equilibrated headspace gas were syringe-injected into the same MSC, as calculated using:

\[ V = \frac{3 \times 10^6}{C} \]  

(1)

Where \( V \) is the volume of headspace gas (ml) at a CO₂ concentration of \( C \) (ppm) required to provide the minimum 3 ml of CO₂ for a sample.

2.2 Laboratory tests of sampling methods

Laboratory tests investigated whether contamination and isotopic fractionation of samples occurred. First, to test for the introduction of contaminants during syringe-injection of a MSC, \(^{14}\)C-dead CO₂ (derived from the combustion of geologically-old anthracite) was sampled by syringe-injecting into a MSC and then recovered for \(^{14}\)C analysis using routine methods (Garnett and Murray, 2013). To test for isotopic fractionation during transfer using the 1 L syringe and also during injection into a MSC, a CO₂-enriched air reference gas ("Mix") with a known \( \delta^{13} \)C signature was aliquoted using syringe-injection into 2 foil gas bags (SKC Ltd, UK) and 2 MSCs. To test the complete headspace equilibration and MSC syringe-injection procedure, a gas standard of known \(^{14}\)C concentration (CO₂ derived from TIRI barley mash; Gulliksen and Scott, 1995) was introduced to an AWC containing 3 L of
deionised water and a 1 L headspace formed from CO2-free air, shaken for 3 minutes (to
equilbrate), and transferred into a MSC using syringe-injection.

2.3 Field tests of sampling methods

Field tests initially focused on establishing the optimum protocol for headspace
equilibration of stream water in an AWC. Firstly, the time required for isotopic equilibration
was assessed by collecting headspace gases after different periods of time (total shaking
times of 30, 90, and 180 seconds; the same water sample was used for each sample, with a
new headspace recreated using CO2-free air in between each sample collection). This test was
performed in duplicate, with the CO2 concentration being measured at 30 second intervals
and all headspace samples collected using syringe-injection into MSCs. In addition, a single
water sample was shaken for 180 seconds and the headspace CO2 recovered by syringe-
injection into a molecular sieve cartridge.

A second field test aimed to determine the optimum water:headspace ratio for
equilibrating samples. Water:headspace ratios of 2:1, 3:1, 4:1 and 5:1 were tested, by varying
the volume of stream water inside an AWC and maintaining the headspace volume at 1 L.
Each ratio was investigated in triplicate, with headspace CO2 concentrations measured at 30
second intervals throughout a total equilibration time of 3 minutes. For each water:headspace
ratio, the total headspace gas (~1 L) from one sample was collected by syringe-injection into
an MSC. The headspace gases (~1 L) from the 2 additional equilibrations at the same
water:headspace ratio were syringe-injected into foil gas bags.

All field tests were performed at a small (approximately 40 cm wide by 30 cm deep)
peatland stream adjacent to Langlands Moss, a domed ombrotrophic peat bog in central
south-west Scotland, UK (55°44′5.5″N 04°10′25.8″W). The site has been used previously for
aquatic 14C measurements of CO2 and CH4 (Garnett et al., 2015). Peat depth in the centre of
Langlands Moss is at least 8 m (Langdon and Barber, 2005). Water temperature and pH were
measured and a sample of stream water collected in a sealed foil bag for total DIC
measurement using gas-stripping.

2.4 Recovery of sample CO2 and carbon isotope analysis

Molecular sieve and gas bag samples from Langlands Moss were returned to the
NERC Radiocarbon Facility (located 2 km from the field site) and the CO2 cryogenically
recovered (Garnett and Murray, 2013). For the water sample, total DIC was recovered as CO2
following acidification using ortho-phosphoric acid and cryogenic collection (Bryant et al.,
The volume of CO$_2$ recovered from all samples was measured using a pressure transducer in a calibrated volume, and divided into aliquots for $^{13}$C and $^{14}$C analysis. The $^{13}$C/$^{12}$C ratio (expressed in ‰ relative to the Vienna Pee Dee Belemnite (PDB) international standard) was determined using isotope ratio mass spectrometry (Thermo Fisher Delta V). Only standard gases and samples from the field experiment investigating equilibration with different water:headspace ratios were selected for $^{14}$C analysis. These samples were measured by AMS at the Scottish Universities Environmental Research Centre (East Kilbride, UK) using an aliquot of sample CO$_2$ converted to graphite (Slota et al., 1987). Following convention, $^{14}$C results were corrected for mass-dependent fractionation by normalising to a $\delta^{13}$C of -25 ‰ and expressed as conventional radiocarbon ages (years BP, where 0 BP = AD1950) and %modern (Stuiver and Polach, 1977).

3. Results

3.1 Laboratory tests of sampling method

The $^{14}$C-dead anthracite-derived CO$_2$ that had been collected using syringe-injection into a MSC had a $^{14}$C content of 0.43 ± 0.01 %modern (43,699 ± 169 years BP; Table 1) and was comparable to the routine background for the molecular sieve method (Garnett and Murray, 2013). Samples of the “Mix” reference gas recovered from foil gas bags had identical $\delta^{13}$C values to the reference (all -25.2 ‰; Table 1). “Mix” gas samples recovered from MSCs following syringe-injection were slightly $^{13}$C-depleted (-25.4 ‰), but overlapped with the reference when considering measurement precision (Table 1). The TIRI barley mash-derived CO$_2$ standard gas processed through all stages of the sampling method (from AWC equilibration through to MSC syringe-injection and CO$_2$ recovery) had a $^{14}$C concentration of 116.15 ± 0.54 %modern (Table 1) and was therefore identical (<1 σ) to the international consensus value (116.35 %modern; Gulliksen and Scott, 1995).

3.2 Field tests of sampling method

The $\delta^{13}$C value of headspace CO$_2$ when the same water sample was equilibrated multiple times showed little variation with the exception of one sample (A 0-30 sec; Table 2) collected after just 30 seconds of shaking; this was slightly $^{13}$C-enriched compared to the other samples. Headspace gases collected subsequently from this water sample (after 90 and 180 seconds total shaking time) had identical $\delta^{13}$C values (-24.6 ‰). For the second water
sample where headspace gases were collected after different periods of shaking, all δ¹³C values overlapped at <1 σ. The weighted mean δ¹³C values of the two samples subjected to multiple headspace collections differed by only 0.3 ‰, and were in close agreement with the sample collected using a single sampling period of 180 seconds (sample B).

Measurement of the headspace during equilibration showed that CO₂ concentrations had consistently reached nearly 90 % of their final value after just 30 seconds of shaking, and that additional shaking beyond 120 seconds made little difference to the headspace CO₂ concentration (Figure 2). The amount of CO₂ recovered from a 1 L headspace sample increased as the water:headspace ratio increased. For example, a mean of 3.2 ml of CO₂ was recovered in samples equilibrated at a ratio of 2:1, and a mean of 4.5 ml CO₂ recovered from a ratio of 5:1 (Table 3). δ¹³C decreased when the volume of recovered CO₂ increased (P < 0.05) or the volume of water equilibrated increased (P < 0.05; and therefore δ¹³C also decreased when the water:headspace ratio increased since the headspace volume was 1 L for all samples). The radiocarbon age of the dissolved CO₂ collected ranged from 478 to 630 years BP (Table 3), a range of just 152 years, and therefore all samples overlapped with each other at just over the 2 σ measurement uncertainty. There was no significant correlation between radiocarbon concentration and either the volume of CO₂ recovered or volume of water equilibrated (and therefore no correlation between ¹⁴C age and water:headspace ratio). However, δ¹³C and %modern were significantly correlated at P < 0.05. All pairs of MSC and bag samples collected using the same water:headspace ratio had ¹⁴C contents that overlapped at < 1 σ measurement uncertainty.

4. Discussion

We had identified a clear need for a sampling method to enable the rapid collection of aquatic CO₂ samples for radiocarbon analysis, especially in remote locations. Existing approaches for the collection of these samples had a number of disadvantages, in particular, the relatively time consuming nature of passive and floating chamber molecular sieve sampling methods (Billett et al., 2006; Garnett et al., 2012), or logistical challenges associated with the collection and storage of water samples sufficiently large for DI¹⁴C analysis. Whilst recent improvements have significantly reduced the volume of water required for DI¹⁴C analysis (Gao et al., 2014), preservation of water samples was also a concern for remote fieldwork, especially given recent reports on the lability of some forms of aquatic carbon (Moody et al., 2013; Drake et al., 2015). Various methods have been used to
stabilise water samples, including freezing, acidifying or poisoning (with e.g. with HgCl), but these methods are either impractical for use in remote locations or create samples that are hazardous to process and dispose (Gulliver et al., 2010). In addition, the logistics of transporting large water volumes from remote locations to analytical laboratories remain, regardless.

This new sampling method employs headspace equilibration (Kling et al., 1991; Hope et al., 1995; Jahangir et al., 2012), which is widely used to provide samples for the determination of dissolved greenhouse gas concentrations and δ^{13}C of CO_2 in inland waters. In our case, we envisaged the method being used to collect aquatic CO_2 from relatively acidic streams associated with organic-rich soils and peatlands. In such waters, where pH is typically <7, greater than 50 % of the total DIC will exist as dissolved CO_2 (Polsenaere and Abril, 2012). Therefore, in common with established headspace equilibration methods, we considered that it would be unnecessary to acidify our water samples in order to recover sufficient sample as CO_2. We believe that our new method could also be reliable for sampling waters with higher pH or lower CO_2 concentrations. For example, acidification of water samples could be undertaken in the field to increase headspace CO_2 volumes, although this would bring additional complications (especially for sampling in remote locations) and would require additional tests for reliability.

In our method the established headspace equilibration technique (Kling et al., 1991; Hope et al., 1995; Jahangir et al., 2012) is scaled up to provide the necessary larger volume samples using a sampling method originally devised for the collection of methane from water for ^14C analysis (Garnett et al., 2015). Headspace gases are injected using a large syringe into the same molecular sieve traps that have previously been proven to be very reliable for the collection of ^14C samples of aquatic CO_2 either collected passively (Garnett et al., 2012) or from a floating chamber (Billett et al., 2006). The traps have also been shown to be reliable for the separation of CO_2 from methane (Garnett et al., 2011). Thus, some aspects of the new method are taken from existing techniques that have already undergone rigorous testing. Nevertheless, we considered it important to perform a series of laboratory and field based tests in order to verify the new method.

Background ^14C when using the syringe-injection method (tested using ^14C-dead anthracite) was almost identical to the routine background for similar-sized samples processed using the molecular sieve traps (Garnett and Murray, 2013). This indicates that the syringe-injection method does not introduce significant contamination from sources with
detectable $^{14}$C concentrations (the most likely being atmospheric CO$_2$). The absence of significant isotopic fractionation when sampling CO$_2$ using syringe injection was confirmed using the “Mix” reference gas; although the reference gas recovered from the molecular sieve samples was slightly depleted in $^{13}$C relative to the reference and bag samples; values from all samples overlapped when the 2 $\sigma$ measurement uncertainty was considered (Table 1). The absence of fractionation had been expected, since IRGA measurements of the CO$_2$ concentration of the exhaust gases of a cartridge during syringe injection had indicated complete trapping of CO$_2$ in similar-sized samples. However, it should be noted that the sieve cartridges do have a finite capacity (estimated at ~ 8-10 ml CO$_2$), above which syringe injection of CO$_2$ samples will result in incomplete trapping and possible isotopic fractionation (this would not affect the $^{14}$C results, since these are by convention corrected for isotopic fractionation by normalisation to a $\delta^{13}$C of -25 ‰; Stuiver and Polach, 1977). The final laboratory test was performed on the complete method, from equilibration, to syringe injection and CO$_2$ recovery. We chose to use CO$_2$ derived from the TIRI barley mash radiocarbon standard, which has an enriched $^{14}$C signature relative to the contemporary atmosphere, and is therefore very sensitive to detecting any contaminants with a $^{14}$C-dead signature (e.g. derived from the various plastic materials used), but would also indicate significant contamination from atmospheric CO$_2$. The $^{14}$C content of this standard was $< 1$ $\sigma$ of the consensus value, and therefore consistent with the absence of any significant contamination.

The field testing of the method addressed several key issues: 1) The shaking time required to fully equilibrate a sample, and 2) The optimal water:headspace ratio for the equilibration of samples.

Measurement of the CO$_2$ concentration in the headspace of the equilibration vessel during field sampling indicated that the CO$_2$ rapidly approached its maximum concentration, and that values barely increased after manual shaking for longer than 90 seconds (Figure 2). There was also very little difference in the $\delta^{13}$C values of the headspace CO$_2$, even when the entire equilibrated headspace had been removed and the headspace resampled from the same water sample on several occasions (Table 2). The latter can probably be explained by the fact that each headspace equilibration only removed a small ($< 10$-15 %) proportion of the total carbon available (estimated based on the total DIC concentration of the gas-stripped sample of 19.2 ml CO$_2$/L). Despite these field results suggesting that the samples were fully equilibrated within 2 minutes, we chose to standardise our sampling method with a shaking time of 3 minutes to allow for manual differences in shaking technique between operators.
This was also consistent with our headspace equilibration method for the collection of methane samples for $^{14}$C analysis (Garnett et al., 2015), thus permitting the collection of samples of both CO$_2$ and CH$_4$ for $^{14}$C analysis using the same equilibrated water sample.

Higher water:headspace ratios resulted in a higher CO$_2$ concentration (Figure 2) with slightly decreased $\delta^{13}$C in the headspace CO$_2$; at the extreme ratios of 2:1 and 5:1, $\delta^{13}$C ranged from -22.4 to -22.6 ‰ and -23.0 to -23.4 ‰, respectively (Table 3). This suggested that the $\delta^{13}$C value of the headspace CO$_2$ might be slightly affected by the water:headspace ratio, and therefore question the reliability of the $\delta^{13}$C values. However, we cannot exclude the possibility that the $\delta^{13}$C of the stream water CO$_2$ changed during the course of the sampling, even if there were no visual changes in flow regime. Samples were collected over several hours in the order presented in Table 3, and therefore if the $\delta^{13}$C of the stream water CO$_2$ became slightly more depleted over time, then this would explain the up to ~ 1 ‰ shift in the $\delta^{13}$C of the samples collected from different water:headspace ratios. We also note that the single sample of TIRI barley mash CO$_2$ that was recovered after being equilibrated with deionised water returned a $\delta^{13}$C value (Table 1) that was just 0.5 ‰ from the expected value (-26.9 ‰), which supports the reliability of the method for determining $\delta^{13}$CO$_2$. However, the primary aim of the new sampling method is the provision of reliable $^{14}$C measurements, and further investigation is required if the method is to be used specifically for the precise determination of aquatic $\delta^{13}$CO$_2$.

The $^{14}$C age of all eight headspace-equilibrated CO$_2$ samples, whether collected using syringe injection into molecular sieve cartridges or foil gas bags, were remarkably similar and marginally failed to all agree within 2 $\sigma$ measurement uncertainty (Table 3). There was no correlation between $^{14}$C age and sample CO$_2$ volume, water volume, or water:headspace ratio, suggesting that the method is resilient and any of the water:headspace ratios would be equally suitable for sample collection (however, in practice we have subsequently adopted a 3:1 water:headspace ratio). All paired MSC and gas bag samples for each water:headspace ratio gave $^{14}$C ages that overlapped at < 2 $\sigma$, indicating that there was no significant difference between gas storage methods. However, we consider molecular sieve cartridges to be more convenient and reliable for long term storage compared to gas bags, as the latter are cumbersome to handle, difficult to transport and can develop leaks.

The single sample of total DIC that was determined using the “gas stripping” method had a radiocarbon age that was significantly older than any of the samples collected using headspace equilibration (Table 3). Whilst this could be interpreted as highlighting a potential
problem with the new “Super Headspace Method”, such as atmospheric contamination, we consider that extremely unlikely given the tests that we have performed on the method; namely 1) there was no correlation between \(^{14}\text{C}\) age and volume of CO\(_{2}\) recovered which would be expected if there had been significant atmospheric contamination, 2) the CO\(_{2}\) from headspace-equilibrated samples in this study was similar, or older, in age compared to previous measurements at the same site (this study: 478-630 years BP, previous study: 303-521 years BP; Garnett et al., 2013), 3) tests with known \(^{14}\text{C}\) content standards were in agreement with consensus values, and 4) frequent monitoring with an IRGA confirmed the absence of atmospheric CO\(_{2}\) ingress in the “Super Headspace” sampling equipment. Hence we are confident that the new method produces accurate aquatic \(^{14}\text{CO}_{2}\) values.

Instead, we consider that the discrepancy between results for headspace-equilibrated and gas stripped samples more likely reflects an issue with the latter. Errors could arise due to either: 1) a small contribution of geological carbonate contributing to gas-stripped samples, and not the headspace-equilibrated samples (for practical reasons the water samples were not filtered, but a small particle of as little as \(\sim\)2 mg \(^{14}\text{C}\)-dead CaCO\(_{3}\) in the 485 ml water sample would be sufficient to account for the discrepancy between headspace-equilibrated and gas stripped results; although the stream flows from a peatland, the underlying geological formation (Clackmannan Group) does contain limestone; British Geological Society; http://mapapps.bgs.ac.uk/geologyofbritain/home.html), 2) disequilibrium between aquatic CO\(_{2}\) and DIC due to unfavourable mixing in the stream (suggested by Billett and Garnett (2010) who also reported older ages for gas stripped DIC samples compared to CO\(_{2}\) collected using a floating chamber), and 3) a sample preservation issue (the DIC sample was stored for \(\sim\)4 months in a refrigerator without any other means of sample preservation, prior to processing, and therefore transfer of carbon between different inorganic pools within the sample may have occurred. Although we did not measure methane during this study, it has previously been found to be abundant and much older than CO\(_{2}\) at this site (up to 3291 years BP; Garnett et al., 2015), and so methane oxidation during sample storage could explain a significant proportion of the offset between headspace-equilibrated and gas stripped samples). In this respect, it should be noted that by avoiding the need for the acidification of water samples, and instead relying on rapid equilibration of samples by shaking and the isolation of aquatic CO\(_{2}\) from the water sample in the field and stable storage on molecular sieve, the new method completely avoids all of the confounding issues associated with the gas stripping method.
5. Conclusions

The new method for the collection of samples of aquatic CO$_2$ for $^{14}$C dating has features that we believe provide considerable advantages over existing methods, particularly for fieldwork in remote locations. The benefits include high portability, speed (< 15 minutes collection time per sample) and minimal power requirements. Besides the IRGA, most items are low cost (~£400 for the 1 L syringe and <£100 for all other items). The recovered samples need no special storage or preservation as sample CO$_2$ can be stored on the molecular sieves for many months before processing. The $^{14}$C content of headspace equilibrated CO$_2$ using this method is not sensitive to different water:headspace ratios, though we have adopted a standardised 3:1 ratio with 3 minutes of manual shaking. The method has proved to be reliable and robust following extensive use in remote locations including northern Canada and Sweden. We suggest that the availability of a rapid, transportable, field based method for measuring aquatic $^{14}$CO$_2$ has considerable application in ecosystems where the terrestrial C cycle is being affected by climate warming like the Arctic (e.g. Vonk et al., 2015), or where peatland management is leading to significant destabilisation of soil C pools (e.g. Evans et al., 2014).

6. Acknowledgements

We thank staff at the NERC Radiocarbon Facility (NRCF010001) and the SUERC AMS Laboratory, the UK Natural Environment Research Council for funding the analyses, and South Lanarkshire District Council for site access.

7. References


Billett MF, Garnett, MH, and Hardie, SML. 2006. A direct method to measure 


Langdon PG, and 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.


Table 1. Laboratory tests of the sampling methods. CO₂ derived from ¹⁴C-dead anthracite was injected into a MSC to test for contamination during syringe-injection sampling. A standard gas (“Mix”) was aliquoted using a 1 L syringe into foil gas bags and MSCs to test for isotopic fractionation. CO₂ derived from an international radiocarbon standard (“TIRI barley mash”) was equilibrated with 3 L of deionised water in an AWC and syringe-injected into a molecular sieve trap, testing all stages of the new sampling method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total gas used (ml)</th>
<th>Recovered CO₂ (ml)</th>
<th>%CO₂</th>
<th>δ¹³CVPDB ± 0.1 ‰</th>
<th>Publication code (SUERC-)</th>
<th>¹⁴C content (%modern ± 1σ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthracite CO₂</td>
<td>-</td>
<td>6.3</td>
<td>-</td>
<td>-23.1</td>
<td>51692</td>
<td>0.43 ± 0.01</td>
</tr>
<tr>
<td>Mix gas (reference)</td>
<td>~6000</td>
<td>-</td>
<td>-</td>
<td>-25.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mix gas – bag 1</td>
<td>1000</td>
<td>7.6</td>
<td>0.76</td>
<td>-25.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mix gas – bag 2</td>
<td>1000</td>
<td>7.7</td>
<td>0.77</td>
<td>-25.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mix gas – MSC 1</td>
<td>1000</td>
<td>7.7</td>
<td>0.77</td>
<td>-25.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mix gas – MSC 2</td>
<td>1000</td>
<td>7.7</td>
<td>0.77</td>
<td>-25.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TIRI barley mash CO₂</td>
<td>750</td>
<td>6.8</td>
<td>0.91</td>
<td>-26.4</td>
<td>60184</td>
<td>116.15 ± 0.54</td>
</tr>
</tbody>
</table>
Table 2. Field test to investigate the shaking time required for isotopic equilibration of AWC CO₂ samples. Samples collected 8th May, 2014 from a small stream draining Langlands Moss, Scotland, UK. Samples A and C represent water samples where headspace gas was removed for analysis after different periods of equilibration (with the headspace being recreated after each sample collection). Sample B represents a single sample collected after equilibrating stream water for 180 seconds. Stream temperature was 9.5 °C and pH 5.75.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water volume (ml)</th>
<th>Shaking interval (sec)</th>
<th>CO₂ recovered (ml)</th>
<th>δ¹³CVPDB ± 0.1‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3550</td>
<td>0-30</td>
<td>6.3</td>
<td>-23.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30-90</td>
<td>5.6</td>
<td>-24.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90-180</td>
<td>5.0</td>
<td>-24.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Weighted mean</strong></td>
<td></td>
<td><strong>-24.1</strong></td>
</tr>
<tr>
<td>B</td>
<td>2190</td>
<td>0-180</td>
<td>5.7</td>
<td>-23.8</td>
</tr>
<tr>
<td>C</td>
<td>2020</td>
<td>0-30</td>
<td>5.1</td>
<td>-23.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30-90</td>
<td>4.3</td>
<td>-23.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90-180</td>
<td>3.3</td>
<td>-23.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Weighted mean</strong></td>
<td></td>
<td><strong>-23.8</strong></td>
</tr>
</tbody>
</table>
Table 3. Field test to investigate the effect of water:headspace ratio on the isotopic composition of CO₂ collected by equilibration of water samples in the AWC. Samples collected on 10<sup>th</sup> February, 2014 from a small stream draining Langlands Moss, Scotland, UK. Stream temperature was 4.7 °C and pH 6.35.

<table>
<thead>
<tr>
<th>Target water: headspace ratio</th>
<th>Replicate no.</th>
<th>Water volume (ml)</th>
<th>CO₂ recovered (ml)</th>
<th>Sample storage method</th>
<th>δ&lt;sup&gt;13&lt;/sup&gt;C&lt;sub&gt;VPDB ± 0.1 ‰&lt;/sub&gt;</th>
<th>Publication code (SUERC-)</th>
<th>Conventional ¹⁴C age (years BP ± 1 σ)</th>
<th>¹⁴C content (% modern ± 0.1 σ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:1</td>
<td>1</td>
<td>1900</td>
<td>3.3</td>
<td>Bag</td>
<td>-22.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2:1</td>
<td>2</td>
<td>1950</td>
<td>3.2</td>
<td>Bag</td>
<td>-22.4</td>
<td>51947</td>
<td>571 ± 35</td>
<td>93.14 ± 0.41</td>
</tr>
<tr>
<td>2:1</td>
<td>3</td>
<td>2000</td>
<td>3.2</td>
<td>MSC</td>
<td>-22.6</td>
<td>51952</td>
<td>630 ± 37</td>
<td>92.46 ± 0.43</td>
</tr>
<tr>
<td>3:1</td>
<td>1</td>
<td>2950</td>
<td>3.6</td>
<td>Bag</td>
<td>-22.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3:1</td>
<td>2</td>
<td>2900</td>
<td>3.7</td>
<td>Bag</td>
<td>-22.5</td>
<td>51948</td>
<td>566 ± 35</td>
<td>93.19 ± 0.41</td>
</tr>
<tr>
<td>3:1</td>
<td>3</td>
<td>2960</td>
<td>3.4</td>
<td>MSC</td>
<td>-22.9</td>
<td>51956</td>
<td>544 ± 37</td>
<td>93.45 ± 0.43</td>
</tr>
<tr>
<td>4:1</td>
<td>1</td>
<td>3870</td>
<td>4.2</td>
<td>Bag</td>
<td>-22.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4:1</td>
<td>2</td>
<td>4400</td>
<td>4.5</td>
<td>Bag</td>
<td>-23.2</td>
<td>51949</td>
<td>533 ± 35</td>
<td>93.58 ± 0.41</td>
</tr>
<tr>
<td>4:1</td>
<td>3</td>
<td>3950</td>
<td>4.1</td>
<td>MSC</td>
<td>-23.4</td>
<td>51955</td>
<td>478 ± 37</td>
<td>94.23 ± 0.44</td>
</tr>
<tr>
<td>5:1</td>
<td>1</td>
<td>5050</td>
<td>4.4</td>
<td>Bag</td>
<td>-23.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5:1</td>
<td>2</td>
<td>4900</td>
<td>4.7</td>
<td>Bag</td>
<td>-23.2</td>
<td>51950</td>
<td>536 ± 37</td>
<td>93.55 ± 0.43</td>
</tr>
<tr>
<td>5:1</td>
<td>3</td>
<td>4730</td>
<td>4.3</td>
<td>MSC</td>
<td>-23.4</td>
<td>51951</td>
<td>561 ± 37</td>
<td>93.25 ± 0.43</td>
</tr>
<tr>
<td>ΣDIC (gas stripped)</td>
<td>-</td>
<td>485</td>
<td>9.3</td>
<td>Bag</td>
<td>-22.4</td>
<td>63602</td>
<td>906 ± 37</td>
<td>89.34 ± 0.41</td>
</tr>
</tbody>
</table>
**FIGURE CAPTIONS**

**Figure 1.** A. Photograph of the sampling equipment used (accordion water carrier equilibration vessel, 1 L syringe and cartridges containing soda lime and molecular sieve). B. Schematic showing the syringe injection of a CO₂-rich headspace sample into a molecular sieve cartridge. Headspace gases are injected using the 1 L syringe into a cartridge containing molecular sieve where the CO₂ is trapped. Exhaust gases (no longer containing sample CO₂) exit to atmosphere after passing through a cartridge containing soda lime, which prevents back filling with atmospheric CO₂.

**Figure 2.** Variation in mean headspace CO₂ concentrations at different water:headspace ratios with increasing equilibration (shaking) time. Each data point represents the mean of three individual samples. Samples collected on 10th February, 2014 from a small stream draining Langlands Moss, Scotland, UK. Stream temperature was 4.7 °C and pH 6.35.
Fig. 1

A

Gas flow

B

1 L syringe containing headspace sample  Molecular sieve cartridge  Soda lime cartridge