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}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}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}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}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}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 CO_2 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 CO_2 adsorbent, which has the advantage that the sample CO_2 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 CO_2 evaded from a stream surface; once sufficient CO_2 has accumulated, chamber air is pumped through a molecular sieve trap to collect the CO_2, 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 CO_2 (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 ^{14}C age of aquatic CO_2 at a specific moment in time. This would allow results to be compared with other measurements (e.g. $DO^{14}C$, $PO^{14}C$), 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 ^{14}C dating of aquatic CO_2, 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 CO_2-free air was injected into the AWC using the 1 L syringe to create a headspace into which aquatic CO_2 was equilibrated. As with other, established headspace methods, equilibration was achieved by shaking.

Following equilibration, the CO_2 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 CO_2 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 CO_2 back-filling into the MSC. At least ~ 3 ml CO_2 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:

$$V = (3 \times 10^6) / C \qquad (1)$$

Where V is the volume of headspace gas (ml) at a CO_2 concentration of C (ppm) required to provide the minimum 3 ml of CO_2 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 CO_2 (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 CO_2-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 (CO_2 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 CO_2-free air, shaken for 3 minutes (to equilibrate), and transferred into a MSC using syringe-injection.

2.3 Field tests of sampling methods

Field tests initially focussed 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 CO_2-free air in between each sample collection). This test was performed in duplicate, with the CO_2 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 CO_2 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 CO_2 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 ^{14}C measurements of CO_2 and CH_4 (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 CO_2 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 CO_2 cryogenically recovered (Garnett and Murray, 2013). For the water sample, total DIC was recovered as CO_2 following acidification using ortho-phosphoric acid and cryogenic collection (Bryant *et al.*, 2013). 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 δ^{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 δ^{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 ($<1\ \sigma$) to the international consensus value (116.35 %modern; Gulliksen and Scott, 1995).

3.2 Field tests of sampling method

The δ^{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 δ^{13}C values (-24.6 ‰). For the second water sample where headspace gases were collected after different periods of shaking, all $\delta^{13}C$ values overlapped at <1 σ. The weighted mean $\delta^{13}C$ 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 CO_2 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 CO_2 concentration (Figure 2). The amount of CO_2 recovered from a 1 L headspace sample increased as the water:headspace ratio increased. For example, a mean of 3.2 ml of CO_2 was recovered in samples equilibrated at a ratio of 2:1, and a mean of 4.5 ml CO_2 recovered from a ratio of 5:1 (Table 3). $\delta^{13}C$ decreased when the volume of recovered CO_2 increased ($P < 0.05$) or the volume of water equilibrated increased ($P < 0.05$; and therefore $\delta^{13}C$ also decreased when the water:headspace ratio increased since the headspace volume was 1 L for all samples). The radiocarbon age of the dissolved CO_2 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 CO_2 recovered or volume of water equilibrated (and therefore no correlation between ^{14}C age and water:headspace ratio). However, $\delta^{13}C$ and %modern were significantly correlated at $P < 0.05$. All pairs of MSC and bag samples collected using the same water:headspace ratio had ^{14}C 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 CO_2 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 $DI^{14}C$ analysis. Whilst recent improvements have significantly reduced the volume of water required for $DI^{14}C$ 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 $\delta^{13}C$ 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 ^{14}C 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 ^{14}C 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 ^{14}C when using the syringe-injection method (tested using ^{14}C-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 σ 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 σ 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 σ 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}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}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 $^{14}CO_2$ 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 ~2 mg ^{14}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 ~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).

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Table 1. Laboratory tests of the sampling methods. CO_2 derived from ^{14}C-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. CO_2 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.

Sample	**Total gas used (ml)**	**Recovered CO_2 (ml)**	**%CO_2**	**$\delta^{13}C_{VPDB}$ ± 0.1 ‰**	**Publication code (SUERC-)**	**^{14}C content (%modern ± 1σ)**
Anthracite CO_2	-	6.3	-	-23.1	51692	0.43 ± 0.01
Mix gas (reference)	~6000	-	-	-25.2	-	-
Mix gas – bag 1	1000	7.6	0.76	-25.2	-	-
Mix gas – bag 2	1000	7.7	0.77	-25.2	-	-
Mix gas – MSC 1	1000	7.7	0.77	-25.4	-	-
Mix gas – MSC 2	1000	7.7	0.77	-25.4	-	-
TIRI barley mash CO_2	750	6.8	0.91	-26.4	60184	116.15 ± 0.54

Table 2. Field test to investigate the shaking time required for isotopic equilibration of AWC CO_2 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.

Sample	**Water volume (ml)**	**Shaking interval (sec)**	**CO_2 recovered (ml)**	**$\delta^{13}C_{VPDB}$ ± 0.1 ‰**
A	3550	0-30	6.3	-23.3
		30-90	5.6	-24.6
		90-180	5.0	-24.6
			Weighted mean	*-24.1*
B	2190	0-180	5.7	-23.8
C	2020	0-30	5.1	-23.8
		30-90	4.3	-23.9
		90-180	3.3	-23.8
			Weighted mean	*-23.8*

Table 3. Field test to investigate the effect of water:headspace ratio on the isotopic composition of CO_2 collected by equilibration of water samples in the AWC. 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.

Target water: headspace ratio	**Replicate no.**	**Water volume (ml)**	**CO_2 recovered (ml)**	**Sample storage method**	**$\delta^{13}C_{VPDB}$ ± 0.1 ‰**	**Publication code (SUERC-)-**	**Conventional ^{14}C age (years BP ± 1 σ)**	**^{14}C content (%modern ± 0.1 σ)**
2:1	1	1900	3.3	Bag	-22.6	-	-	-
2:1	2	1950	3.2	Bag	-22.4	51947	571 ± 35	93.14 ± 0.41
2:1	3	2000	3.2	MSC	-22.6	51952	630 ± 37	92.46 ± 0.43
3:1	1	2950	3.6	Bag	-22.3	-	-	-
3:1	2	2900	3.7	Bag	-22.5	51948	566 ± 35	93.19 ± 0.41
3:1	3	2960	3.4	MSC	-22.9	51956	544 ± 37	93.45 ± 0.43
4:1	1	3870	4.2	Bag	-22.8	-	-	-
4:1	2	4400	4.5	Bag	-23.2	51949	533 ± 35	93.58 ± 0.41
4:1	3	3950	4.1	MSC	-23.4	51955	478 ± 37	94.23 ± 0.44
5:1	1	5050	4.4	Bag	-23.0	-	-	-
5:1	2	4900	4.7	Bag	-23.2	51950	536 ± 37	93.55 ± 0.43
5:1	3	4730	4.3	MSC	-23.4	51951	561 ± 37	93.25 ± 0.43
ΣDIC (gas stripped)	-	485	9.3	Bag	-22.4	63602	906 ± 37	89.34 ± 0.41

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 CO_2-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 CO_2 is trapped. Exhaust gases (no longer containing sample CO_2) exit to atmosphere after passing through a cartridge containing soda lime, which prevents back filling with atmospheric CO_2.

Figure 2. Variation in mean headspace CO_2 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 ^{o}C and pH 6.35.

Fig. 1

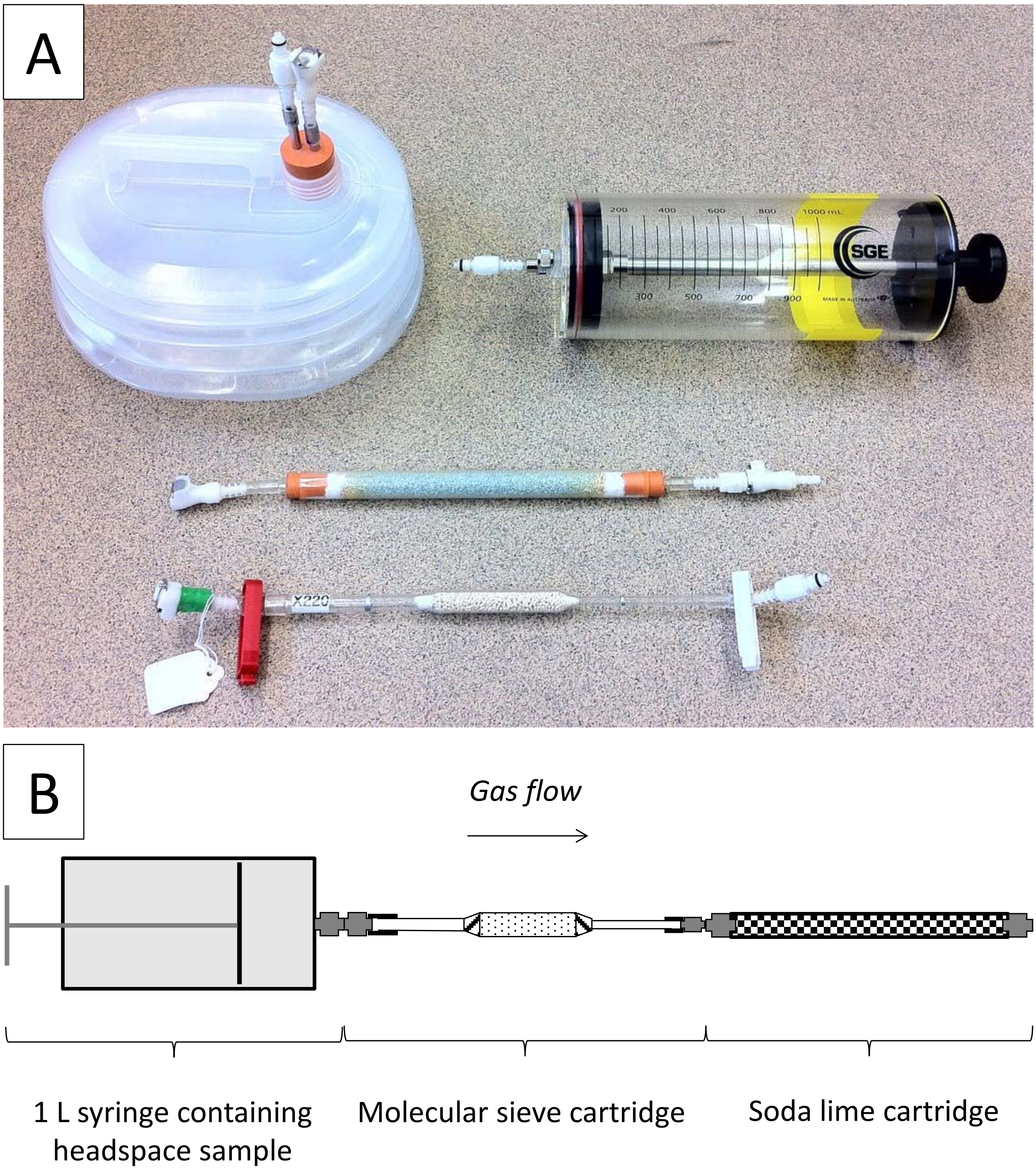
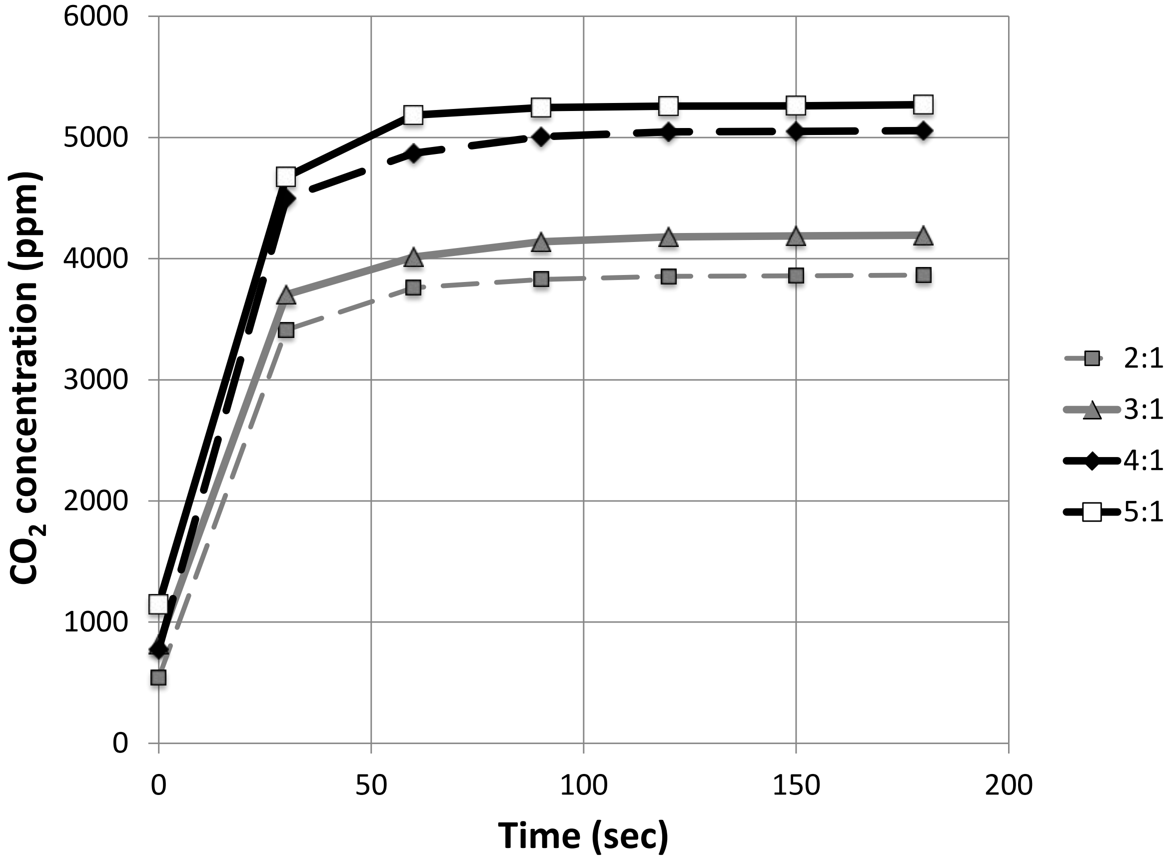

A
B
Gas flow
1 L syringe containing headspace sample
Molecular sieve cartridge
Soda lime cartridge

Fig.2


6000
5000
4000
3000
2000
1000
0
CO$_2$ concentration (ppm)
0
50
100
150
200
Time (sec)
2:1
3:1
4:1
5:1