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An assessment of chamber ^{14}C methodologies for sampling aquatic CO_2 evasion

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ABSTRACT

The development of new methods to directly measure the radiocarbon age of dissolved and evaded aquatic carbon dioxide has enhanced our ability to understand carbon transport and cycling in the soil-water-atmosphere system. One of the methods involves collecting enough carbon dioxide for radiocarbon dating by allowing carbon dioxide to outgas from the water surface into an enclosed floating chamber, with the gas subsequently trapped onto a zeolite molecular sieve cartridge. There are, however, several different methodological approaches that can be used for the collection of floating chamber samples and it is currently unknown whether these different approaches influence the isotopic (stable carbon and radiocarbon) composition of the measured sample. Here, we evaluate four different floating chamber approaches and compare the stable and radiocarbon composition of the evaded carbon dioxide. Chamber conditions varied considerably with the different methodologies, with for example, maximum chamber CO_2 concentration ranging from ca. 400–6300 ppm during sampling. Despite the varying chamber conditions, our results indicate no significant differences in the ^{14}C age of evasion (range: 1276–1364 years BP) with any of the methodological approaches (in chambers where atmospheric carbon dioxide had been excluded). This confirms the methodologies are both robust and widely applicable.

INTRODUCTION

Although the measurement of the stable carbon ($\delta^{13}\text{C}$) and radiocarbon (^{14}C) composition of aquatic dissolved inorganic carbon (DIC), dissolved organic carbon (DOC) and particulate organic carbon (POC) is well established (e.g. McNichol *et al.*, 1994), methods to measure gaseous carbon (C) species (carbon dioxide (CO_2) and methane (CH_4)) are more challenging. For the collection of CO_2 for ^{14}C analysis, portable molecular sieve cartridges have been developed in the UK (e.g. Bol and Harkness, 1995; Hardie *et al.*, 2005) and elsewhere (e.g. Gaudinski *et al.*, 2000; Palonen, 2015; Wotte *et al.*, 2017). These cartridges have been used extensively to

improve understanding of the terrestrial carbon cycle in arctic, boreal and temperate regions (e.g. Gaudinski *et al.*, 2000, Billett *et al.*, 2007, Hartley *et al.*, 2012). Molecular sieve cartridges have been used to collect and date atmospheric CO₂ (Garnett and Hartley, 2010), soil-respired CO₂ (Briones *et al.*, 2010), and CO₂ lost from water surfaces by evasion or degassing (Billett *et al.*, 2006; 2007). For the aquatic environment this development has been a significant methodological step, since previous approaches relied upon the indirect measurement of the isotopic composition of CO₂ by “gas stripping” (sample acidification) of DIC (e.g. Palmer *et al.*, 2001) and the determination of δ¹³CO₂ or ¹⁴CO₂ by calculation.

The direct collection of evaded CO₂ in a chamber floating on the water surface has removed a significant amount of uncertainty associated with the previous indirect method (Billett and Garnett, 2010). However, the fact that chambers produce artefacts has been widely recognized in measurements of soil CO₂ fluxes (Davidson *et al.*, 2002). Since the conditions in the floating chamber differ from the ambient atmosphere, it has been argued that the isotopic composition of the CO₂ lost from the water surface will be altered (see Billett and Garnett, 2010). For example, the mass differences of individual carbon isotopes are known to affect rates of diffusion of CO₂ in air, with ¹²CO₂ diffusing at a rate of 1.044 and 1.088 times faster than ¹³CO₂ and ¹⁴CO₂, respectively (Craig, 1953; Cerling *et al.*, 1991; Egan *et al.*, 2014). Although such “mass-dependent” isotopic fractionation effects do alter the ¹⁴C concentration relative to the other isotopes, conventional ¹⁴C age results are corrected for this by normalising to a standardised δ¹³C of -25 ‰ (Stuiver and Polach, 1977).

However, it has been shown that chambers can produce isotopic fractionation effects that are not accounted for by routine ¹³C normalisation (Egan *et al.*, 2014). Using a modelling approach, Egan *et al.* (2014) found that for soil respired CO₂ the rates of ¹³C and ¹⁴C accumulation in a chamber were not always a constant multiple of each other. Hence, the assumption that ¹⁴C fractionates double that of ¹³C, may not be universally applicable. While this suggests that ¹³C-normalisation to correct ¹⁴C measurements may not always be reliable, the error was considered minor and far smaller than the routine measurement error associated with accelerator mass spectrometry (AMS).

A similar situation may occur during CO₂ evasion from water surfaces. Fluxes of seawater-atmosphere gas exchange can be described by the equation:

$$F_{gas} = -kS(pCO_2^w - pCO_2^a) \quad (1)$$

Where F_{gas} (mass area⁻¹ time⁻¹) represents the CO₂ flux, $-k$ is the gas exchange coefficient (length time⁻¹), S is the solubility (mass volume⁻¹ pressure⁻¹) of CO₂ and pCO_2 the partial pressure (pressure) of CO₂ in water (^w) and air (^a) (Zhang and Quay, 1997; Wanninkhof, 2014). Fluxes of different carbon isotopes (e.g. ¹³C or ¹⁴C) can also be expressed dependent on their individual isotope concentrations in the atmosphere and water (Zhang and Quay, 1997). For example, for ¹³C:

$$F_{gas}^{13} = -k\alpha_k\alpha_{aq-g}S[pCO_2^w (^{13}C/^{12}C)_{DIC}/\alpha_{DIC-g} - pCO_2^a (^{13}C/^{12}C)^a_g] \quad (2)$$

Where F_{gas}^{13} (mass area⁻¹ time⁻¹) represents the flux of ¹³CO₂, α_k represents the air-water kinetic fractionation factor, and α_{aq-g} and α_{DIC-g} represent the equilibrium fractionation factors between aqueous and gaseous CO₂, and DIC and gaseous CO₂, respectively (Zhang and Quay, 1997; Billett and Garnett, 2010).

From this model, it follows that the flux of individual carbon isotopes from the water surface is at least partly dependent on the concentration of individual isotopes in both the water and the overlying atmosphere, resulting in a fractionation effect that is not solely based on the mass of individual isotopes. This “mass-independent” isotopic fractionation would not be corrected for during routine ¹³C normalisation of ¹⁴C results. In this scenario, in a floating chamber used to trap CO₂ evasion, the rate of efflux of individual isotopes would be influenced by the isotopic composition of the chamber atmosphere itself, which will differ considerably from the free atmosphere as the evaded CO₂ builds up. This implies that the chamber could produce an artefact and that samples collected for isotope analysis using the floating chamber approach may not be representative of evasion into the real atmosphere. To overcome this potential problem ¹⁴CO₂ results have previously been mathematically corrected based on equation (2) to account for the fact that the chamber conditions differ from the free atmosphere (see Billett and Garnett, 2010 for details of this “gas flux correction”).

Here, we investigate whether the methodological approach to sample evaded CO₂ from surface waters using a floating chamber influences the measurement of carbon isotope concentration. We do this by performing multiple synchronous collections of evaded CO₂ from a small section of a peatland stream using a standard floating chamber method (Billett *et al.*, 2006). We deliberately manipulated chamber

atmosphere CO₂ concentrations in order to provide four different “test” scenarios. We hypothesise that there will be no significant differences in the ¹⁴C concentration of all samples, because any isotopic fractionation effects will be mass-dependent (or insignificant) and corrected for using routine ¹³C normalisation procedures (Stuiver and Polach, 1977).

MATERIALS AND METHODS

Over two days (26–27th June 2013) we carried out a series of replicated experiments using multiple floating chambers to test the above hypothesis. The work was undertaken in Black Burn (Auchencorth Moss, SE Scotland, 55°47'40"N; 03°14'54"W), a DOC- and CO₂-rich, coloured peatland stream, that is a well-established site for the study of aquatic C fluxes and their isotopic composition (e.g. Billett *et al.*, 2006; 2007; Dinsmore *et al.*, 2013; Leith *et al.*, 2014). The study reach was a linear stream section (bank height 1.5 m, stream width 2 m, stream length 5 m, water depth 20–25 cm) 10 m upstream from the Black Burn discharge control structure.

At the beginning and end of each sampling day a “spot” 100 ml sample of streamwater was collected and filtered on-site (0.45 µM GF/F syringe-driven filter, Whatman) for the determination of DOC and DIC using a Pollution and Process Monitoring LABTOC Analyser (detection range 0.1–4000 mg L⁻¹). Concentrations of CO₂ and CH₄ were measured using the headspace technique, which involved equilibration of 40 ml streamwater with 20 ml of atmospheric CO₂ (Kling *et al.*, 1991; Hope *et al.*, 2001). The headspace was analysed in the laboratory using a GC (HP5890 Series II) with detection limits of 7 ppmv (CO₂) and 84 ppbv (CH₄; Dinsmore *et al.*, 2013). With this data the streamwater dissolved CO₂ and CH₄ concentrations were calculated using Henry's Law (Hope *et al.*, 1995). Supporting measurements of the stream temperature and pH, and of the conductivity were performed using portable sensors (HI 9124, Hanna Instruments, precision ± 0.4°C and 249 0.01 pH units; HI9033, Hanna Instruments, ± 1 %).

To quantify any changes in underlying hydrochemical conditions a Campbell Scientific CR1000 logger system was used to make continuous measurements of stream temperature and conductivity (CS547A, Campbell Scientific) as well as streamwater dissolved CO₂ concentrations using a Vaisala CARBOCAP® (transmitter

series GMT221) non-dispersive infra-red absorption (NDIR) sensor (Johnson *et al.*, 2010). Continuous stage height was measured separately (Level Troll 400, In-situ) and converted to discharge using a ratings equation (Dinsmore *et al.*, 2013). Data from the continuous sensors were averaged and stored every 10 minutes.

We aimed to compare the isotopic composition of evasion CO₂ collected on molecular sieve cartridges in the field, using four different methods with chamber conditions deliberately modified (both isotope and CO₂ concentration) in order to test whether such differences affected the isotopic composition of CO₂ lost by evasion from the water surface:

- The “constant CO₂” method maintained CO₂ at ambient concentrations (ca. 400±50 ppmv) throughout the sampling period, both during the removal of atmospheric CO₂ and sample collection on the molecular sieve. When removing atmospheric CO₂ we monitored the volume of chamber headspace that was scrubbed and proceeded with the sample collection only after at least five chamber volumes had been scrubbed. Compared to the other three methods, this approach most closely mimics the degassing of CO₂ from the water surface to the ambient atmosphere.
- The “conventional scrub” method (Billett *et al.*, 2006) involved firstly removing all atmospheric CO₂ (by soda lime scrubbing) from the floating chamber, then allowing sufficient time (ca. 1 h) for the CO₂ to build up to suitable concentrations (ca. 1400 ppm) before collecting the CO₂ with a molecular sieve.
- The “conventional no scrub” method was identical to the “conventional scrub” method, apart from not removing all atmospheric CO₂ from the floating chamber at the beginning of the process. Thus, the isotopic CO₂ composition of this headspace differed considerably from the others at the start of the CO₂ build up period. Samples collected using this method will therefore be “contaminated” with a small amount of atmospheric CO₂, which we corrected (*corr*) using the following isotope mass balance equation:

$$I_{corr} = ((V_{end} \times I_{end}) - (V_{start} \times I_{start})) / (V_{end} - V_{start}) \quad (3)$$

Where I represents the isotopic concentration (pMC or $\delta^{13}\text{C}$), V is the CO_2 concentration (in parts per million; ppm), as measured by a portable infrared gas analyser (IRGA; PP Systems EGM4) when the chamber was first deployed and only contained air (*start*), and when the molecular sieve sample was recovered (*end*). We collected one sample of atmospheric CO_2 on a molecular sieve cartridge which we used to provide the carbon isotopic (pMC and $\delta^{13}\text{C}$) composition of the contemporary atmosphere. We varied the atmospheric CO_2 concentration at the start of the sampling across the four chambers by scrubbing with soda lime to simulate different levels of atmospheric CO_2 contamination (ranging from 227 to 400 ppm).

- The “equilibrium” method was like the conventional scrub method except that after scrubbing, the CO_2 was allowed to build up over time to a constant chamber concentration, where it reaches equilibrium with the water concentration. Although evasion rate in the first 10 minutes of buildup is linear, over time as the CO_2 concentration gradient decreases, the rate of buildup decreases until it reaches equilibrium. On both sampling days we allowed the duplicate floating chambers to equilibrate for 2.5 and 4.5 h. In addition, we left two chambers for an extended period to equilibrate overnight (17 h), before trapping the evaded CO_2 .

Evasion CO_2 was collected in multiple identical black plastic floating chambers (height 14 cm, width 25 cm, length 30 cm) with a volume of ca. 10.5 L. The chambers were deployed on the surface of the stream and (if required) securely attached to the stream bank with rope to prevent drifting during the duration of the experiment. Each chamber had an inlet and an outlet tube with automatic shut-off couplings on the upper surface to allow (1) measurement of temporal changes in CO_2 concentrations using an IRGA and (2) removal and trapping of CO_2 onto an in-line molecular sieve cartridge or soda lime trap. The latter was part of a portable, closed loop $^{14}\text{CO}_2$ sampling system (Hardie *et al.*, 2005; Billett *et al.*, 2006) that pumped (500 ml min^{-1}) and trapped the evaded CO_2 onto a zeolite molecular sieve (type 13X, BDH, UK) once it had accumulated a

sufficient (>3 ml) volume in the floating chamber. The portable system can be used to scrub atmospheric CO₂ from the floating headspace using a soda lime trap prior to the collection of a “pure” (i.e. uncontaminated with atmospheric CO₂) evasion sample (also at 500 ml min⁻¹).

After sampling, the molecular sieve cartridges were returned to the NERC Radiocarbon Facility, where the evaded and trapped CO₂ was recovered by heating (500°C) and cryogenic trapping (Garnett and Murray, 2013). The removed CO₂ was then split into aliquots. The first subsample (>0.2 ml) was analysed for ¹³C content using an Isotope Ratio Mass Spectrometer (Thermo-Fisher Delta V) and ¹³C concentrations expressed as δ¹³C (‰) relative to the Vienna Pee Dee Belemnite international standard (VPDB). A second subsample (>1 ml) was analysed for ¹⁴C after graphitisation associated with Fe-Zn reduction, using AMS at the Scottish Universities Environmental Research Centre AMS Facility, East Kilbride, UK. According to convention ¹⁴C results are expressed as radiocarbon ages (BP) and pMC (Stuiver and Polach, 1977), after being normalised to a δ¹³C of -25 ‰. This standardisation of the ¹⁴C results is used to correct for mass-dependant fractionation in all conventional radiocarbon age results.

In total we carried out 16 dual isotope (δ¹³CO₂ and ¹⁴CO₂) measurements of evasion CO₂ collected using the four different methods; “constant CO₂” (n=2), “conventional scrub” (n=4), “conventional no scrub” (n=4) and “equilibrium” (n=6).

We calculated δ¹³CO₂ and ¹⁴CO₂ values for the “conventional scrub” samples to simulate CO₂ fluxing into a free atmosphere (using the results from the atmospheric CO₂ sample: 400 ppmv; δ¹³C = -9 ‰; ¹⁴CO₂ = 102.6 pMC) using the “gas flux correction” (based on equation (2) and described in detail in Billett and Garnett (2010)). In this case, equilibrium conditions are not assumed and sample pH, DIC and temperature values are used in combination with our directly measured evasion isotopic CO₂ values to estimate the isotopic composition of DIC. This value is then used to predict what the evasion CO₂ isotopic composition would be if fluxed into a free atmosphere.

RESULTS

An important pre-condition for this method comparison was that stream flow conditions and underlying hydrochemistry were stable before and throughout the 27 h study period (Table 1 and Figure 1). There was no rainfall during the study period until 11:00

hrs on Day 2 when a small amount of light rain occurred. Streamwater chemistry reflected these stable hydrological conditions, with pH varying by a maximum of 0.13 pH units. Streamwater DOC concentrations were lower (by 1.47 mg L⁻¹) on Day 2, whereas concentrations of DIC, CO₂ and CH₄ were slightly higher. Continuous measurement of conductivity and temperature before, during and after the period showed a consistent diurnal pattern with both variables reaching minimum and maximum values around 5:30 hrs and 16:00 hrs, respectively. The maximum amplitude of the day/night variation was 5.3°C and 13 μS cm⁻¹. Likewise, CO₂ concentration showed a consistent diurnal pattern with highest values at 05:00 hrs and lowest at 10:30 hrs with a change in concentration during the study period of approximately 500 ppmv.

Table 2 shows that we successfully managed to manipulate the chamber CO₂ conditions of the four different collection methods to create a wide range of chamber CO₂ concentrations, and by inference, different water:air CO₂ concentration gradients. We maintained the “constant CO₂” chambers at 400±50 ppmv (for 100–110 minutes) to mimic fluxing into a free atmosphere. The maximum CO₂ concentrations achieved using the “conventional scrub” method ranged from 1428 to 1474 ppmv (mean 1458 ppmv), whereas the “conventional no scrub” method were sampled at slightly higher chamber concentrations (range 1920 to 2370, mean 2183 ppmv). The six “equilibrium” chambers vary in maximum CO₂ concentration at least partly because each pair of chambers were allowed to accumulate evasion CO₂ for different lengths of time. Hence, the shortest collection period (ca. 2.5 h) resulted in the lowest mean chamber CO₂ concentration (3486 ppmv), the intermediate period (ca. 4.5 h) in a greater mean concentration (5328 ppmv) and the longest (overnight) equilibrium period (17 h) in the highest mean concentration (6125 ppmv).

Dual isotope δ¹³CO₂ and ¹⁴CO₂ measurements are presented in Table 2 and Figure 2. On both sampling days, the δ¹³CO₂ values collected using the “conventional scrub” and “constant CO₂” methods were similar (within measurement uncertainty), suggesting no difference between these two collection methods. The difference in δ¹³CO₂ between the “conventional” and “equilibrium” samples was ~1.2 ‰ (26th June) and ~0.6 ‰ (27th June), suggesting there was a slight difference between these two methods, with the latter producing more ¹³C-enriched values.

Samples collected using the “conventional no scrub” method were (as expected) relatively ¹³C-enriched, since they had an atmospheric (δ¹³CO₂ = -9.0 ‰)

component. Replicate measurements of this method taken on both days were also the most variable (differing by 0.7 ‰) compared to the other three collection methods.

The ^{14}C enrichment values (Table 2 and Figure 2) of evasion CO_2 collected in chambers in which atmospheric CO_2 had been removed by scrubbing, varied between 84.38 and 85.32 pMC reflecting ^{14}C ages of 1364 to 1276 years BP. The ^{14}C age of all samples collected using the “constant CO_2 ”, “conventional scrub” and “equilibrium” methods was therefore not significantly different (within measurement uncertainty). The “conventional no scrub” chambers produced higher pMC values (85.48 to 86.71 pMC) with slightly younger ^{14}C ages (1260 to 1146 years BP). They were also the most variable set of replicates compared to the other three collection methods. Isotopic mass balance correction to account for the atmospheric component overcorrected the ^{14}C values producing lower pMC values (83.00 to 84.02; with equivalent radiocarbon ages between 149 to 351 years older; Table 3).

Finally, for the “conventional scrub” method only, we compared the actual measured (uncorrected) evasion $\delta^{13}\text{C}$ and ^{14}C values with values that had been produced using the “gas flux correction” based on the assumption that evasion CO_2 would have fluxed out into a free atmosphere (see Billett and Garnett, 2010). Table 4 shows that the corrected values are slightly less ^{14}C enriched and produced older conventional ages (by on average 91 years). The corrected $\delta^{13}\text{C}$ values were also slightly more depleted (mean -1.1 ‰), however, the uncorrected values are closer to the results from the other three collection methods and the differences are relatively small considering the measurement uncertainty.

DISCUSSION

By using several methodological approaches, we collected evasion in chambers with different (400 to 6300 ppmv) CO_2 concentrations. If chamber conditions influenced the isotopic composition of the evaded CO_2 , we would expect to obtain different results. In fact, for chambers where atmospheric CO_2 had been excluded from the sample, ^{14}C ages for evasion ranged narrowly from 1276 ± 35 to 1364 ± 37 years BP, with all results having measurement uncertainties that overlap at less than 2σ . This suggests that different collection methodologies did not result in different ^{14}C ages. Furthermore, it supports our hypothesis that any isotopic fractionation effects caused by the different chamber sampling approaches are either too small to be significant (as reported by

Egan *et al.*, 2014), or are corrected for by using the existing ^{13}C normalisation approach (Stuiver and Polach, 1977).

Our finding that evasion samples collected under different chamber conditions did not result in significantly different ^{14}C ages, suggests that the “gas flux correction” that we have previously employed (Billett and Garnett, 2010) is not necessary. This is further supported by the fact that when applied, the ^{14}C ages treated with the “gas flux correction” were more dissimilar to the samples collected using the other chamber methods. In the present study, the correction only resulted in an age shift of between 87 and 95 years, which is not much greater than the 2σ analytical uncertainty of the ^{14}C measurements. We acknowledge that this aspect of the study is not as conclusive as it might have been. Previously when samples were collected at this same location, the conditions influencing the “gas flux correction” (e.g. pH, temperature etc) led to a greater age shift (up to 313 years when pH was 5.87 and stream temperature 1.4 °C; site A10 in Billett and Garnett, 2010), which would have allowed for a more sensitive test.

The samples collected from chambers that had not had atmospheric CO_2 removed before sample collection had higher variability in $\delta^{13}\text{C}$ which likely reflected the different atmospheric CO_2 components since the variation reduced after isotopic mass balance correction (Table 3). This atmospheric correction overcorrects the evasion $\delta^{13}\text{CO}_2$ values by between -1.2 to -2.7 (mean -2.0) units resulting in more ^{13}C -depleted values (-22.9 to -23.7 ‰) compared to the “conventional scrub” method. Hence, an atmospheric correction based on the atmospheric component at the start of CO_2 build up in the chamber is unable to correctly calculate the evasion $\delta^{13}\text{CO}_2$ value.

Evasion collected using the “conventional no scrub” method was also ^{14}C -enriched relative to samples collected without an atmospheric CO_2 component. At the start of sampling the non-scrubbed chambers would have contained up to 4.2 ml of atmospheric CO_2 with a significantly higher ^{14}C concentration compared to the aquatic CO_2 . Like for $\delta^{13}\text{C}$, isotope mass balance correction for atmospheric CO_2 (equation (3)) overcorrected for this contamination because it calculated ^{14}C concentrations (ranging from 83.00 to 84.02 pMC) that were lower than the other chambers (ranging from 84.38 to 85.32 pMC). Reducing the atmospheric component in the correction of the “conventional no scrub” chambers (e.g. ranging from 84.73 to 85.05 pMC when halving the atmospheric CO_2 component in “conventional no scrub” chambers) makes

the results more comparable with the “conventional scrub” results, suggesting that an unquantified amount of invasion (as well as evasion) is occurring in the chambers during CO₂ build up and that equilibrium conditions have not been established. As a result of this uncertainty, the “conventional no scrub” methodology is the only one that we would not recommend based on our tests.

We found that the “constant CO₂” and “conventional scrub” chamber methodologies resulted in similar $\delta^{13}\text{C}$ values for evasion. In contrast, the results for “equilibrium” chambers were ¹³C-enriched by ca. 1 to 2 ‰ compared to the other chamber results when allowed to equilibrate for 4.5 h or more. Interestingly, experimentally derived equations for the prediction of isotopic fractionation between water and air under equilibrium conditions predict a similar (~1.2 ‰) ¹³C enrichment in gaseous CO₂ compared to aqueous (dissolved) CO₂ (Zhang *et al.*, 1995). Since this ¹³C enrichment appears to be absent in the non-equilibrium chambers, this suggests that CO₂ evasion has a similar isotopic composition to the aqueous pool of CO₂ in the streamwater. This implies that little isotopic fractionation occurs when CO₂ evades from the water into the floating chamber. The results also suggest that the $\delta^{13}\text{C}$ values from the “conventional” method more accurately reflect the true evasion value. We also note that the range of $\delta^{13}\text{C}$ values that we determined (-22.8 to -21.0 ‰, excluding the “conventional no scrub” samples) was similar to our previous floating chamber measurements of evasion on this stretch of the Black Burn (i.e. -21.6 to -21.0 ‰, Billett *et al.*, 2006; -21.5 to -18.3 ‰, Billett *et al.*, 2007; -21.0 to -19.5 ‰, Billett and Garnett, 2010).

The radiocarbon age of evasion CO₂ that we measured in chambers in June 2013 (1276 to 1364 years BP; excluding the “conventional no scrub” method) compared favourably with ages of 1443 and 1454 years BP for evasion CO₂ collected at the same site in August 2004 (Billett *et al.*, 2006). During both summer sampling periods (June and August) the stream was in low flow conditions characterised by circum-neutral pH and high DIC concentrations. Whether the change in age of evasion of ca. 100 years BP at this location over nine calendar years reflects a significant shift in the contribution of carbon sources producing the evasion is difficult to say from just two sets of measurements. We cannot imply from the close agreement of ¹⁴C results collected over the two days of this study that short-term variations in the age of evasion are unlikely, since we purposefully chose a period with stable conditions. Moreover, at a location 500 m downstream from our sampling point ¹⁴C ages of dissolved CO₂

ranged from 311 to 1077 years BP (Billett *et al.*, 2007); these samples were collected during different seasons and included periods of high discharge. Therefore, seasonal changes in the ^{14}C content of evasion at this site easily exceed the difference that we found between the summers of 2004 and 2013.

We have also measured the annual variability in the ^{14}C age of dissolved CO_2 at the same site using molecular sieves deployed below the water surface for periods of 4 to 12 weeks (Garnett *et al.*, 2012). Although this is a “passive” in stream method, which measures an average $^{14}\text{CO}_2$ age over a much longer time period (weeks) compared to short-term (hours) measurements using floating chambers, we found that the ^{14}C age varied between 707 and 1210 years BP with the oldest dissolved CO_2 occurring when discharge was lowest in late spring/early summer. These results show that the age of evasion and dissolved CO_2 of a specific stream can clearly be highly variable and differ to a much greater extent than the values we observed in our test of different methodological approaches to chamber sampling.

CONCLUSIONS

This study represents the most thorough test of the floating chamber method since it was first published (Billett *et al.*, 2006), and shows that statistically identical ^{14}C ages were obtained for CO_2 evasion collected using different methodological approaches. The results support our hypothesis that any fractionation effects during chamber sampling are either insignificant or are corrected for using routine ^{13}C normalisation procedures. The tests that we performed suggest that the “gas flux correction” that has been previously applied, is not necessary. In global aquatic systems where the degree of CO_2 oversaturation and evasion are highly variable and often unpredictable (e.g. Butman and Raymond, 2011), we argue that floating chambers provide a robust and relatively straight-forward method to source and age CO_2 released to the atmosphere.

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REFERENCES

- Billett, MF, Garnett, MH. (2010). Isotopic composition of carbon dioxide lost by evasion from surface water to the atmosphere: Methodological comparison of a direct and indirect approach. *Limnology and Oceanography: Methods*, 8, 45-53. doi:10.4319/lom.2010.8.45
- Billett, MF, Garnett, MH, Dinsmore, KJ. (2015). Should aquatic CO₂ evasion be included in contemporary carbon budgets for peatland ecosystems? *Ecosystems*, 18(3), 471-480. doi:10.1007/s10021-014-9838-5
- Billett, MF, Garnett, MH, Hardie, SML. (2006). A direct method to measure ¹⁴CO₂ lost by evasion from surface waters. *Radiocarbon*, 48(1), 61-68. doi:10.1017/S0033822200035396
- 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 CO₂ and CH₄ evasion from UK peatland headwater streams. *Biogeochemistry*, 114(1-3), 165-181. doi:10.1007/s10533-012-9798-9
- Bol, RA, Harkness, DD. (1995). The use of zeolite molecular sieves for trapping low concentrations of CO₂ from environmental atmospheres. *Radiocarbon*, 37(2), 643-647. doi:10.1017/S0033822200031155
- Briones, MJJ, Garnett, MH, Ineson, P. (2010). Soil biology and warming play a key role in the release of 'old C' from organic soils. *Soil Biology & Biochemistry*, 42, 960-967. doi:10.1016/j.soilbio.2010.02.013
- Butman, D, Raymond, PA. (2011). Significant efflux of carbon dioxide from streams and rivers in the United States. *Nature Geoscience*, 4, 839. doi:10.1038/ngeo1294
- Cerling, TE, Solomon, DK, Quade, J, Bowman, JR. (1991). On the isotopic composition of carbon in soil carbon-dioxide. *Geochimica Et Cosmochimica Acta*, 55(11), 3403-3405. doi: 10.1016/0016-7037(91)90498-T
- 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. doi:10.1007/s10021-006-9013-8
- Craig, H. (1953). The geochemistry of the stable carbon isotopes. *Geochimica Et Cosmochimica Acta*, 3, 53-92.

- Davidson, EA, Savage, K, Verchot, LV, Navarro, R. (2002). Minimizing artifacts and biases in chamber-based measurements of soil respiration. *Agricultural and Forest Meteorology*, 113(1-4), 21-37. doi:10.1016/s0168-1923(02)00100-4
- 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. doi:10.1111/gcb.12209
- Egan, J, Nickerson, N, Phillips, C, Risk, D. (2014). A numerical examination of $^{14}\text{CO}_2$ chamber methodologies for sampling at the soil surface. *Radiocarbon*, 56(3), 1175-1188. doi:10.2458/56.17771
- Garnett, MH, Dinsmore, KJ, Billett, MF. (2012). Annual variability in the radiocarbon age and source of dissolved CO_2 in a peatland stream. *Science of the Total Environment*, 427-428, 277-285. doi:10.1016/j.scitotenv.2012.03.087
- Garnett, MH, Hartley, IP. (2010). A passive sampling method for radiocarbon analysis of atmospheric CO_2 using molecular sieve. *Atmospheric Environment*, 44, 877-883. doi:10.1016/j.atmosenv.2009.12.005
- Garnett, MH, Murray, C. (2013). Processing of CO_2 samples collected using zeolite molecular sieve for ^{14}C analysis at the NERC Radiocarbon Facility (East Kilbride, UK). *Radiocarbon*, 55(2), 410-415. doi:10.1017/S0033822200057532
- Gaudinski, JB, Trumbore, SE, Davidson, EA, Zheng, S. (2000). Soil carbon cycling in a temperate forest: radiocarbon-based estimates of residence times, sequestration rates and partitioning of fluxes. *Biogeochemistry*, 51(1), 33-69. doi:10.1023/a:1006301010014
- Hardie, SML, Garnett, MH, Fallick, AE, Rowland, AP, Ostle, NJ. (2005). Carbon dioxide capture using a zeolite molecular sieve sampling system for isotopic studies (^{13}C and ^{14}C) of respiration. *Radiocarbon*, 47(3), 441-451. doi:10.1017/S0033822200035220
- Hartley, IP, Garnett, MH, Sommerkorn, M, Hopkins, DW, Fletcher, BJ, Sloan, VL, Phoenix, GK, Wookey, PA. (2012). A potential loss of carbon associated with greater plant growth in the European Arctic. *Nature Climate Change*, 2, 875-879. doi:10.1038/NCLIMATE1575
- Hope, D, Dawson, JJC, Cresser, MS, Billett, MF. (1995). A method for measuring free CO_2 in upland streamwater using headspace analysis. *Journal of Hydrology*, 166(1), 1-14. doi: 10.1016/0022-1694(94)02628-O

- Hope, D, Palmer, SM, Billett, MF, Dawson, JJC. (2001). Carbon dioxide and methane evasion from a temperate peatland stream. *Limnology and Oceanography*, 46(4), 847-857. doi:10.4319/lo.2001.46.4.0847
- Johnson, MS, Billett, MF, Dinsmore, KJ, Wallin, M, Dyson, K. (2010). Direct in situ measurement of dissolved carbon dioxide in freshwater aquatic systems - method and applications. *Ecohydrology*, 3, 68-78. doi:10.1002/eco.95
- 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. doi:10.1126/science.251.4991.298
- Leith, FI, Garnett, MH, Dinsmore, KJ, Billett, MF, Heal, KV. (2014). Source and age of dissolved and gaseous carbon in a peatland–riparian–stream continuum: a dual isotope (^{14}C and $\delta^{13}\text{C}$) analysis. *Biogeochemistry*, 119(1), 415-433. doi:10.1007/s10533-014-9977-y
- Mayorga, E, Aufdenkampe, AK, Masiello, CA, Krusche, AV, Hedges, JI, Quay, PD, Richey, JE, Brown, TA. (2005). Young organic matter as a source of carbon dioxide outgassing from Amazonian rivers. *Nature*, 436(7050), 538-541. doi:10.1038/nature03880
- McNichol, AP, Osborne, EA, Gagnon, AR, Fry, B, Jones, GA. (1994). TIC, TOC, DIC, DOC, PIC, POC — unique aspects in the preparation of oceanographic samples for ^{14}C -AMS. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, 92(1), 162-165. doi: 10.1016/0168-583X(94)95998-6
- Palmer, SM, Hope, D, Billett, MF, Dawson, JJC, Bryant, CL. (2001). Sources of organic and inorganic carbon in a headwater stream: Evidence from carbon isotope studies. *Biogeochemistry*, 52(3), 321-338. doi:10.1023/a:1006447706565
- Palonen, V. (2015). A portable molecular-sieve-based CO_2 sampling system for radiocarbon measurements. *Review of Scientific Instruments*, 86, 125101. doi:10.1063/1.4936291
- Stuiver, M, Polach, HA. (1977). Reporting of ^{14}C data. *Radiocarbon*, 19(3), 355-363. doi: 10.1017/S0033822200003672
- Wallin, MB, Grabs, T, Buffam, I, Laudon, H, Ågren, A, Öquist, MG, Bishop, K. (2013). Evasion of CO_2 from streams – The dominant component of the carbon export through the aquatic conduit in a boreal landscape. *Global Change Biology*, 19(3), 785-797. doi:10.1111/gcb.12083

Wanninkhof, R. (2014). Relationship between wind speed and gas exchange over the ocean revisited. *Limnology and Oceanography: Methods*, 12(6), 351-362. doi:10.4319/lom.2014.12.351

Wotte, A, Wordell-Dietrich, P, Wacker, L, Don, A, Rethemeyer, J. (2017). $^{14}\text{CO}_2$ processing using an improved and robust molecular sieve cartridge. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, 400, 65-73. doi:https://doi.org/10.1016/j.nimb.2017.04.019

Zhang, J, Quay, P. (1997). The total organic carbon export rate based on ^{13}C and ^{12}C of DIC budgets in the equatorial Pacific region. *Deep-Sea Research II*, 44(9-10), 2163-2190. doi: 10.1016/S0967-0645(97)00032-5

Zhang, J, Quay, PD, Wilbur, D. (1995). Carbon isotope fractionation during gas-water exchange and dissolution of CO_2 . *Geochimica Et Cosmochimica Acta*, 59, 107-114. doi: 10.1016/0016-7037(95)91550-D

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Table 1. Streamwater chemical characteristics during the two-day sampling period. DOC = dissolved organic carbon, DIC = dissolved inorganic carbon.

Sample date (time)	pH	Conductivity ($\mu\text{s cm}^{-1}$)	Temperature ($^{\circ}\text{C}$)	DOC (mg L^{-1})	DIC (mg L^{-1})	$\text{CO}_2\text{-C}$ (mg L^{-1})	$\text{CH}_4\text{-C}$ ($\mu\text{g L}^{-1}$)
26-Jun-13 (11:00 hrs)	6.95	142.7	10.3	9.64	16.50	3.75	15.5
26-Jun-13 (17:00 hrs)	7.08	140.6	13.9	9.69	16.67	3.44	15.4
27-Jun-13 (10:30 hrs)	7.06	147.7	10.1	8.34	17.58	4.12	17.9
27-Jun-13 (14:30 hrs)	7.08	145.0	11.5	8.05	17.15	3.67	16.7

Table 2. Isotopic composition of atmospheric CO₂ and evaded CO₂ collected using the four different sampling methods. n/a = not applicable. The “conventional no scrub” values have not been corrected for the atmospheric component.

Public- ation code (SUERC-)	Sampling method	Date	Atmospheric CO ₂ scrubbed?	Chamber CO ₂ (ppmv)	Sample collection time (h)	¹⁴ C enrichment (pMC ± 1σ)	Conventional ¹⁴ C age (years BP±1 σ)	δ ¹³ C _{VPDB} ± 0.3 (‰)
50489	Atmospheric CO ₂	26-Jun-13	n/a	n/a	1	102.60+/-0.47	n/a	-9.0
50490	Constant CO ₂	26-Jun-13	Y	400	2	84.75+/-0.37	1329+/-35	-22.3
50499	Constant CO ₂	27-Jun-13	Y	400	2	84.95+/-0.37	1310+/-35	-22.8
50491	Conventional scrub	26-Jun-13	Y	1428	1	85.15+/-0.39	1292+/-37	-22.4
50494	Conventional scrub	26-Jun-13	Y	1466	1	84.98+/-0.39	1307+/-37	-22.1
50500	Conventional scrub	27-Jun-13	Y	1465	1	85.32+/-0.37	1276+/-35	-22.7
50501	Conventional scrub	27-Jun-13	Y	1474	1	85.20+/-0.39	1286+/-37	-22.5
50497	Conventional no scrub 1	26-Jun-13	N	2114	1	86.71+/-0.38	1146+/-35	-20.7
50498	Conventional no scrub 2	26-Jun-13	N	1920	1	85.97+/-0.37	1214+/-35	-21.4
50508	Conventional no scrub 1	27-Jun-13	N	2326	1	86.46+/-0.40	1169+/-37	-21.3

50509	Conventional no scrub 2	27-Jun-13	N	2370	1	85.48+/-0.39	1260+/-37	-22.0
50504	Equilibrium (2.5 h)	27-Jun-13	Y	3470	2.5	85.02+/-0.37	1304+/-35	-22.1
50505	Equilibrium (2.5 h)	27-Jun-13	Y	3501	2.5	85.28+/-0.37	1279+/-35	-21.9
50496	Equilibrium (4.5 h)	26-Jun-13	Y	5242	4.5	84.38+/-0.39	1364+/-37	-21.0
50495	Equilibrium (4.5 h)	26-Jun-13	Y	5414	4.5	84.93+/-0.39	1312+/-37	-21.1
50506	Equilibrium (17 h)	27-Jun-13	Y	5920	17	85.02+/-0.37	1303+/-35	-21.6
50507	Equilibrium (17 h)	27-Jun-13	Y	6330	17	84.81+/-0.39	1324+/-37	-21.5

Table 3. Comparison of isotopic values of evasion CO₂ collected using the “conventional no scrub” method before and after correction for atmospheric CO₂ using equation (3).

Publication code (SUERC-)	Atmospheric CO ₂ in chamber		¹⁴ C enrichment (pMC)			Conventional ¹⁴ C age (years BP)			$\delta^{13}\text{C}_{\text{VPDB}}$ (‰)		
	at start (ppm)	Un-corrected	Corrected	Difference	Un-corrected	Corrected	Difference	Un-corrected	Corrected	Difference	
50497	400	86.71	83.00	-3.71	1146	1497	351	-20.7	-23.4	-2.7	
50498	227	85.97	84.02	-1.95	1214	1398	184	-21.4	-22.9	-1.5	
50508	385	86.46	83.26	-3.20	1169	1472	303	-21.3	-23.7	-2.4	
50509	200	85.48	83.90	-1.58	1260	1410	149	-22.0	-23.2	1.2	
<i>Mean</i>		86.16	83.55	-2.61	1197	1444	247	-21.4	-23.3	-1.4	

Table 4. Comparison of corrected and uncorrected isotopic values of evasion CO₂ collected using the “conventional scrub” method before and after applying the “gas flux correction”.

Sample ID	¹⁴ C enrichment (pMC)			Conventional ¹⁴ C age (years BP)			δ ¹³ C _{VPDB} (‰)		
	Uncorrected	Corrected	Difference	Uncorrected	Corrected	Difference	Uncorrected	Corrected	Difference
SUERC-50491	85.15	84.21	-0.94	1292	1381	89	-22.4	-23.5	-1.1
SUERC-50494	84.98	84.07	-0.91	1307	1394	87	-22.1	-23.2	-1.1
SUERC-50500	85.32	84.33	-0.99	1276	1369	93	-22.7	-23.9	-1.2
SUERC-50501	85.20	84.20	-1.00	1286	1381	95	-22.5	-23.7	-1.2
<i>Mean values</i>	<i>85.16</i>	<i>84.20</i>	<i>-0.96</i>	<i>1290</i>	<i>1381</i>	<i>91</i>	<i>-22.4</i>	<i>-23.6</i>	<i>-1.1</i>

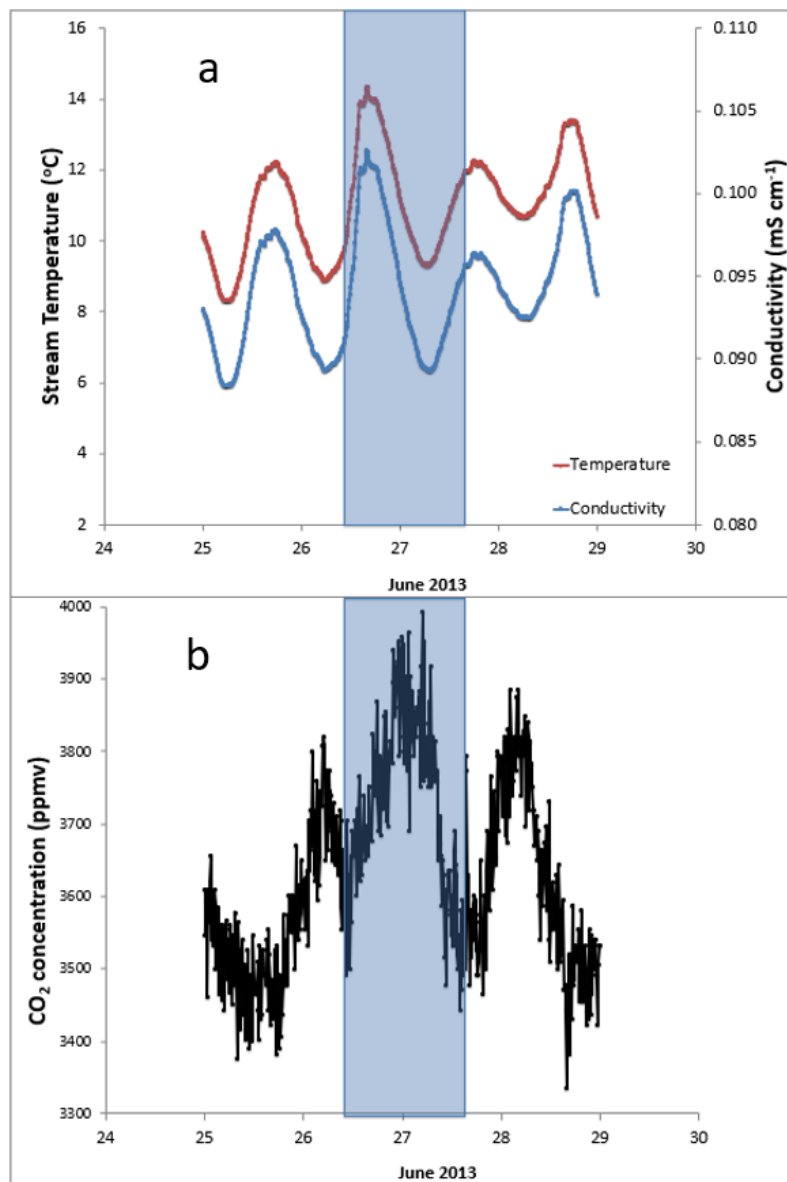


Figure 1. Continuous (10 minute interval) changes in stream temperature and conductivity (a) and aquatic CO₂ concentration (b) before, during and after the experimental period (26–27 June). The shaded area represents the 27 h study period.

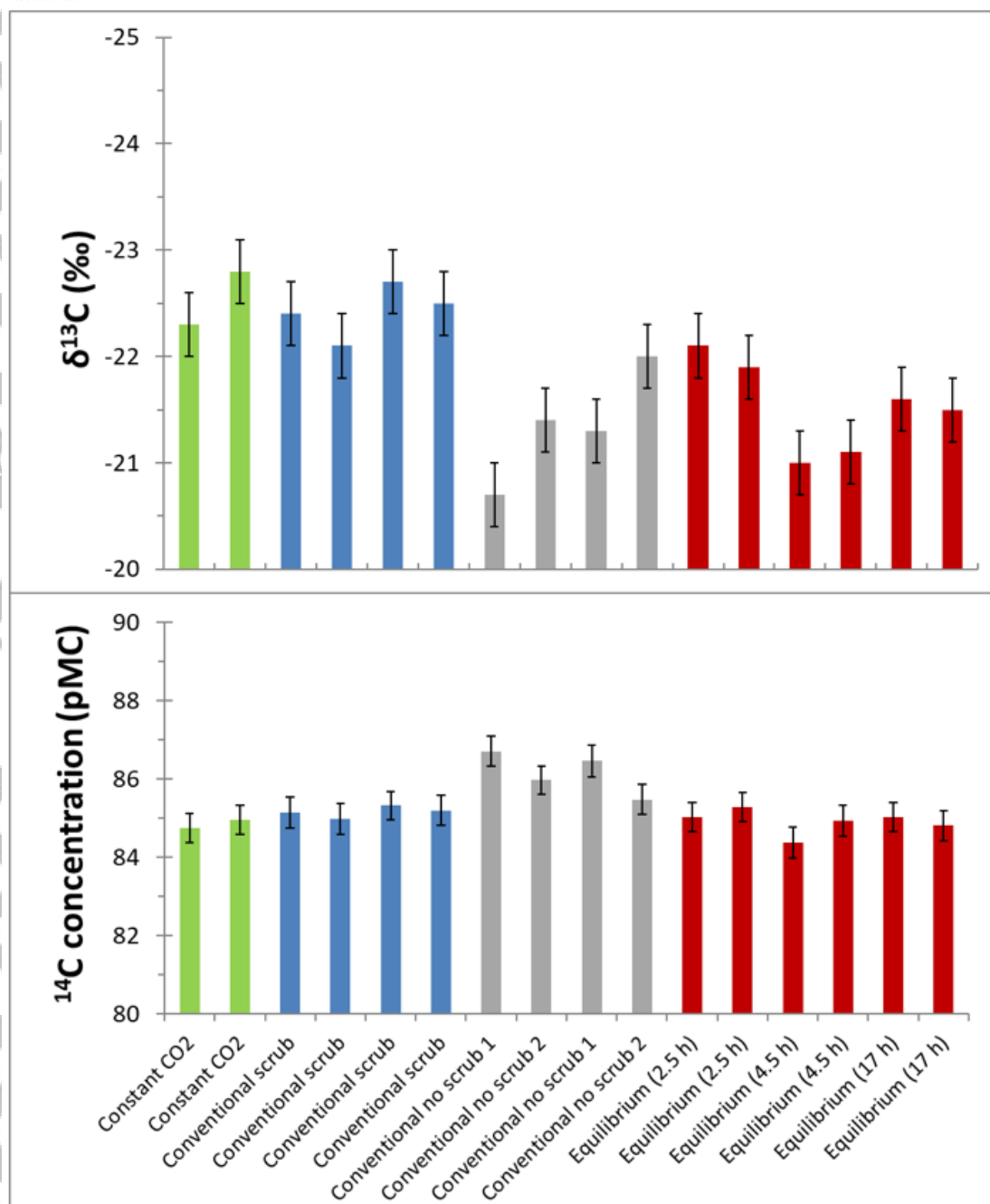


Figure 2. $\delta^{13}\text{C}$ and ^{14}C enrichment values of evasion CO₂ collected using four different methods reflecting different chamber CO₂ conditions. Error bars represent 1 σ .