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* Revision Notes

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Dears Sirs

RE. MS. NO. SBB3571 Garnett et al.

We have revised our manuscript to take into account all the comments made by the reviewers. Below we provide a detailed breakdown of the changes which have been made. We hope the manuscript is now suitable for publication in *Soil Biology and Biochemistry*.

Yours sincerely

Dr Mark H Garnett (corresponding author)

Revision notes MS. NO. SBB3571 GARNETT ET AL.

Reviewers comments in italics
Line and Table numbers refer to those in original document

Reviewer #1

General comments

"...I suggest the author to elaborate on the interpretation of the results in order to produce a full research paper rather than a technical paper. Particularly, as a synthetic soil is included with "expected" contrasting soil characteristics but these have not been reported. The synthetic soil would make it possible to discuss the isotopic results in relation to two C sources for CO2"
We are presenting this work as a technique that investigators may wish to consider applying to their research on SOM dynamics and land-atmosphere exchange of CO_2. The intention was to publicize this method promptly following validation. The technique can be applied in many different ways and in contrasting contexts. We tested the method in two contrasting soils to provide a robust test of the new method but the soils were not selected because we wanted to better understand how C cycles through these particular soils. Turning this manuscript into a full research paper would require a lot of additional ^{14}C measurements which are not justifiable based on the only parochial importance of soils chosen. In addition, we feel that a fuller analysis of carbon cycling in these soils would potentially deflect attention away from the primary purpose of the work; to present the passive-sampling method.

Specific points

L. 16: to use the word simple seems not appropriate.
We have removed the word "simple".

L. 35-63: this section is to long for a technical paper on CO2 gas sampling. I suggest the section being shortened (and L. 48-63 deleted). An introduction to CO2 sampling would be more helpful. The focus on technically testing a new method stands in contrast to the long introduction to the application of radiocarbon analysis.
As requested by the reviewer, we have reduced this section of the manuscript, including completely removing L. 48-55. We have retained L. 56-63 as we considered it important to introduce existing techniques which can be used to collect soil respired CO_2 (although they are usually relatively impractical compared to passive sampling with molecular sieves).

L. 44: The sentence: "Radiocarbon analysis of soil respiration avoids the need for these assumptions and provides a direct measurement of the mean age of soil-respired CO2 needs references or deleted here but discussed later.
We have now moved this sentence and incorporated it into the Discussion.

L 96-97: I agree and that is the reason why this study should be published
We thank the reviewer for this comment.

L 110: Not a constant rate but a rate proportion to the soil CO2 production - as soil CO2 production will fluctuate over time (at least over 24 h at most sites).
The rate of CO_2 capture by the MSC should be proportional to the CO_2 concentration of the environment (in this case, the CO_2 concentration of the chamber). This is different to the rate of soil CO_2 production, because there is also the likelihood of transport of the CO_2 in and out of the chamber. Unlike soil CO_2 production rate, soil CO_2 concentration may vary much less over time. On L 124 we already stated that we were testing whether the rate of CO_2 capture is proportional to the CO_2 concentration of the environment, which we believe is the same as the

reviewers comment. However, we have modified L 110 in response to the reviewers comment to make it clearer that the constant rate of trapping must only apply when conditions are constant. We have also added some text to explain the main reason why trap rates may not be constant even under fixed conditions (due to sieve saturation with CO_2), which also relates to comments in the Discussion.

L 135-141: There is a lack of background information: minimum information should include: soil C content and pH and 13C and 14C values of the bulk solid phase. This is the only way to validate the method of collecting CO2 gas as a measure of age and fraction of soil C being mineralized. Grass species should be noted as well as land use and recent land use changes (any C4-plants?).
We agree with the reviewer that more background information on the soil used in the grassland experiment should be provided. We have therefore created a new table to present this information which as requested includes: soil pH, %carbon and $\delta^{13}C$. We also provide a list of the most abundant plant species that are present on the grassland, as well as information on past and present land use. It is extremely unlikely that the site has ever been occupied by C4 plants and this is now stated in the manuscript.

We have not provided a ^{14}C value for the bulk soil. This is because ^{14}C analysis of the bulk soil from the grassland experiment would not benefit the main aims of the manuscript. Our aim was to provide a test of a method to sample soil respired CO_2 which we believe is best achieved by using the sampling approach adopted in our study and by comparing results with samples collected using evacuated flasks (an accepted method). Therefore the actual isotopic characteristics and source of the CO_2 are largely irrelevant in terms of testing the method. If the aim of the study was to investigate the fraction of soil C being mineralized, and potential long-term response of C storage to global change then we would certainly agree that the bulk soil should be analysed for ^{14}C. However, that was not the aim of the present study, and we would consider that an investigation of the source of soil C being mineralized would require a large number of additional ^{14}C analyses (including ^{14}C analysis of several soil fractions). Again given the only parochial importance of the soils chosen, and our concerns over detracting from the main aim of the study (to validate the method), we do not feel that this is justifiable.

L. 144: T here is an important lack of information when a synthetic soil is produced and the "contrasting" soil conditions are not reported. At least soil C, pH, 13C and C14 of garden peat, the lime and the mixture. This is the only way to provide any insight in contrasting results being reported. A mixture ratio has not been reported.
As in the above item, we agree that more background information on the synthetic soil should be provided. We therefore include soil pH, %carbon and $\delta^{13}C$ in a new Table. We now comment that approximately equal masses of compost and sand were used to create the artificial soil, as requested.

We cannot provide separate values for the lime fraction of the synthetic soil because the compost came with the lime already added, and as above, we do not feel that it is necessary to provide a ^{14}C value for the bulk soil or fractions. Again, the main aim of the study was to test the new method of collecting representative samples of CO_2 for ^{14}C analysis, and we believe that this was best achieved by comparing the results of the passive sampling with those from the evacuated flasks. As stated in the manuscript, interpretation of the isotope results in the context of the source of the CO_2 was of a much lower importance. We were using a synthetic soil and can think of no reason why understanding how C cycles through this soil in the long term would be of value to the scientific community. However, we have rewritten and increased part of the Discussion dealing with the interpretation of the results in the context of the CO_2 sources, and also relate the chamber $\delta^{13}C$ results with that of the soil $\delta^{13}C$.

L. 166: Three couplings have been applied per chamber, where do we see the results of replicates?
We think that the reviewer has slightly misunderstood the sampling design. Only one chamber was used, and it had three couplings inserted so that three passive MSCs could be attached at the same time. The three passive MSCs represented the short, medium and long period samples which were required to be exposed to the same CO_2 (therefore the same chamber headspace). We have tried to make this sampling design clearer in the text by stressing that only a single chamber was used for the experiments, by making modifications in the text.

L. 172: "Inserted to a depth of 4 cm" - have you tried or considered to insert chambers after removal of 10, 20 and 30 cm of soil to provide any insight into depth-dependent release of CO2?
We had realized that this passive sampling technique could be used to collect CO_2 from different soil depths as described by the referee, however, we have not yet undertaken any sampling. We thank the reviewer for his valuable suggestion, but consider that such samples are outside the scope of the present manuscript which is primarily concerned with testing the method.

L. 173: Vegetation has been removed - ok, but that means non-steady state conditions for a period. Any evaluation of removal? Time since removal needs to be stated.
Vegetation was removed one month before the passive molecular sieve sampling began, and this fact has now been included in the manuscript as requested by the reviewer. However, we do not consider that an evaluation of the effects of vegetation removal and possible non-steady state conditions is necessary in the context of the present manuscript, as we are primarily concerned with testing the method of trapping CO_2 from a chamber headspace. In that respect, the source of the CO_2 is of secondary importance, and it would be unlikely that we could say a great deal about the effects of vegetation removal from the results of a single chamber. However, the sampling design utilized was specifically chosen so that changes in chamber CO_2 throughout the experiment would not affect the test of the method. Indeed, variation in chamber characteristics, caused by the non-steady state conditions during the experiment (e.g. CO_2 concentration or isotope characteristics), provide a more rigorous test of the sampling method (as in the synthetic soil experiment).

L. 176: Left for several days - that means some oxygen depletion - any effect? If CO2 is being removed how will total pressure be affected? Will there be any marked shift from diffusion and advective transport.
We do not consider that leaving the chamber several days would have had any significant effect in terms of oxygen depletion because the base of the chamber was completely open to soil allowing gas to exchange with the soil atmosphere. Therefore, the chamber was not a closed system. Therefore, we consider that the chamber headspace would have equilibrated with the soil atmosphere, thus preventing any possible oxygen depletion. The headspace CO_2 concentration reached ~4% which reflects the CO_2 concentration in the soil air at the insertion depth. Assuming a respiratory quotient of 1, the oxygen concentration in the headspace would only have been depleted by 4%. Similarly, the CO_2 removal during the experiment would not have affected the total pressure (except temporally immediately after sampling with the evacuated flask) because the chamber was open to the soil atmosphere. Since the soil atmosphere is a far greater volume than the small chamber that we used, any pressure difference caused by removing CO_2 (we collected a total of 87ml of CO_2 across the three sets of sieves that were sampling from the chamber) would quickly have disappeared through equilibration between the chamber and soil.

Reviewer #2:

General comments

"My main concern for this paper is that both tests were conducted under quite high CO2 conditions: 4% for the grassland site and 0.7-1.7% for the artificial soils. These conditions are much higher than the normal soil CO2 concentration, which may alternate the microbial respiration, and may even, be poison to them. The high CO2 can also result in acidic condition and cause some CaCO3 in soil to dissolve. I guess the high CO2 condition is inevitable for this method because the camber is closed for days. Then the question need to be answer is - can the CO2 collected by this method still reflect the real soil respiration? They should have conducted a parallel sampling using the well-tested method of molecular sieve with pumping system to evaluate this potential problem."

We do not agree with the reviewer that the soil CO_2 concentrations in our experiments were much higher than normal soil CO_2 concentrations. For example, Santruckova and Simek (1997) state that soil CO_2 concentrations of 1-5 vol.% CO_2 are typical and that values of greater than 10% CO_2 have also been reported. We agree with the reviewer that high CO_2 concentrations in soil can effect the soil microbial community and decrease soil pH (again, see Santruckova and Simek (1997)). However, in our method, we are simply allowing the CO_2 concentration in the chamber to equilibrate with the soil atmosphere – chamber CO_2 will not be any higher than the soil CO_2 because the base of the chamber is open to the soil. Therefore, the chamber CO_2 will still be representative of the soil CO_2.

Reference: Santruckova, H., Simek, M., 1997. Effect of soil CO_2 concentration on microbial biomass. Biology and Fertility of Soils 25, 269-273.

The other concern I have is the method will still collect some component from the atmosphere because there will be some leaking no matter how "closed" the chamber is, especially if the collection time is for months. The 14C recovered from the synthetic soil did show much depleted value than the contemporary atmosphere. However it cannot rule out the atmospheric component if they don't know the 14C value of the stating material in the synthetic soil (that is, the starting material could be even more 14C depleted). They should measure the 14C of the synthetic soil, then incubate the synthetic soil in a closed jar, and measure the 14C in this incubated CO2. In this way, they would be able to compare these 14C values to the 14C in the recovered CO2 collected by their method to evaluate any possible leaking. Other researchers have been using 13C to correct for this atmospheric component. But, since the 13C collected by this method is fractionated, how can the authors correct for this component?

The reviewer makes a very good point in that we cannot rule out an air component in the chamber in either the grassland or synthetic soil experiments. However, it is not necessary to undertake ^{14}C analysis of the soil and the CO_2 evolved in a closed jar at the end of the experiment. We can estimate the **maximum** air component in the chambers based on a simple calculation using CO_2 concentrations. The maximum contribution of the atmosphere to the chamber can be calculated as the CO_2 concentration of the atmosphere divided by the CO_2 concentration of the chamber – as it is not possible that atmospheric CO_2 would be concentrating somehow in the chamber. For the grassland experiment, this gives a maximum atmospheric component of (380/40000) which is less than 1% of the sample, while for the synthetic soil experiment the range is (380/15800) to (380/7000) i.e. 2.4 to 5.4%. Such a small atmospheric component to the grassland experiment chamber makes an insignificant difference to the ^{14}C results which are almost identical to the atmosphere anyway. If there was a 5% atmospheric contribution to the chamber of the synthetic soil, removing this using mass balance would shift the average ^{14}C value of the CO_2 from 38 %modern to 34.5 %modern, which would not at all affect our interpretation of the results.

Although we are confident that the possibility of an air component in our samples would not be of much significance, we agree with the reviewer that this is an important point that needs to be discussed in the manuscript. We have therefore made substantial changes to the Discussion where we now discuss the possible air component. We now include the above calculations to demonstrate that the maximum air component in our samples is small, and insignificant in the context of the current study. Contrary to the reviewer, we consider that the $\delta^{13}C$ values determined using the passive MSC method are still valuable. These values can be corrected for fractionation, but will be subject to some uncertainty because of the variation in the amount of ^{13}C fractionation caused by the passive sampling (between ~3-4‰). Therefore there is a 1‰ uncertainty when calculating the $\delta^{13}C$ value of the CO_2 in the chamber. However, in C3 ecosystems the difference between atmospheric CO_2 and soil respired CO_2 $\delta^{13}C$ values is approximately 15-20‰ (-9‰ versus -24 to -29‰). Therefore, even if the headspace contains only 50% soil respired CO_2 we have a ~10% uncertainty when isotopically quantifying the contribution of atmospheric CO_2 to the headspace. This uncertainty declines as the contribution of soil respired CO_2 to headspace CO_2 increases. In summary, because of the variable fractionation there is an increased uncertainty in the estimate of the proportion of air in a chamber, based on ^{13}C measurements. But the ^{13}C data are still useful especially because in many ecosystems, the ^{14}C content of soil respiration can be similar, but slightly enriched, compared with the atmosphere, (as was the case in the grassland soil sampled in this study) and therefore the small uncertainty in the contribution of the atmospheric component will make little difference to calculation of the ^{14}C content of the respired CO_2.

All that being said, the referee raises valid points and if the technique is being used in situations in which atmospheric contamination to the headspace is substantial then fractionation tests should be undertaken prior to ^{14}C analysis. We now make this clear on page 18 of the revised manuscript.

The authors should also test if the passive trapping works when CO2 concentration is less than 0.2% (a more normal situation). Will the linear relationship of CO2 trapped with trapping time still hold when the CO2 concentration is much lower? How is the fractionation going to be at this lower concentration condition?
We have tested the passive MSC sampling method at atmospheric CO_2 concentrations (0.038%). The results are indeed consistent with the results in this manuscript; e.g. the relationship between CO_2 trap rate and CO_2 concentration holds even at lower concentrations, and a ^{13}C fractionation of ~4‰ also occurs at this lower CO_2 concentration. We have modified the summary section in the Discussion to comment on these preliminary results, however, we are not yet in a position to publish these results, and in any case, think it would be more appropriate to present them in an atmospheric science journal to highlight the potential utility of the technique at atmospheric flask sampling stations.

However this method is not appropriate for 13C measurement, and it yet to be calibrated if the authors intend to use it for measuring soil CO2 flux as well.
We agree that at the current time, this method requires some further tests before it can be perfected for collection of ^{13}C samples because ^{13}C results need to be corrected for an as yet uncertain fractionation factor (although this is only at most a 1‰ uncertainty, clearly better methods for collecting $^{13}CO_2$ samples currently exist). It was with this in mind that we focused the manuscript (e.g. the title) on sampling of CO_2 for radiocarbon measurement (by convention radiocarbon measurements are normalised to a $\delta^{13}C$ of -25‰ and are therefore not affected by this fractionation issue). Samples collected using absorption in hydroxide also suffer ^{13}C fractionation, but this has not prevented the method being used for collection of ^{13}C samples (see Davidson, 1995; reference in manuscript). If as we expect, we are able to reduce uncertainty in the ^{13}C fractionation factor in the future, this will improve the technique for collection of ^{13}C samples. Similarly, all our evidence to date suggests that we will be able to provide a very good calibration between CO_2 trap rates and the CO_2 concentration of the

chamber atmosphere, however, there are insufficient results from the current study to present a calibration factor.

Most importantly, the authors need to answer the questions with regarding to if the high CO2 condition occurred during sample collection (in the closed chamber for days) will alternate the normal soil respiration; and secondly, how can they correct for any atmospheric component in their sample that may come from leaking.
See responses above.

Specific comments

Ln 154: What is the size of the tube? (Inner diameter and length). Later on (in Table 3 and Figure 2) the authors reported the amount of CO2 trapped is strongly corrected with tube ID. How about the length of the tube from chamber to the molecular sieve? How does this diffusive path length affect the trapping? Most importantly, what is the recommended size of the trap tube, both ID and length? Should you use a bigger tube at a lower CO2 concentration condition?
As requested we have now provided the dimensions (inner diameter and length) of the tube in the text at this point. We now also refer to Fig. 1 at this point as this has a schematic diagram of the cartridge design. The rate of CO_2 trapping will be affected by changes in the dimensions of the molecular sieve cartridge, and in the original manuscript we suggested that this would be a possible way to tailor CO_2 trap rates for particular needs. To address the reviewers comments, we have elaborated on this and in the summary section at the end of the Discussion we have added text to state possible ways to alter the CO_2 trap rates. We include in this the suggestion by the reviewer that the path from the chamber to the molecular sieve can be used to alter the CO_2 trap rate. We now also state in the text that we recommend the use of our design of cartridge for a wide range of conditions, but this is mainly because it is the only design that we have tested and was suitable for our particular sampling needs.

Ln 162: How long had the molecular sieve trap been heated at 500°C under vacuum?
The molecular sieves were heated for 1.5 hours while under vacuum to charge them. We have amended the manuscript to include this information as requested by the reviewer.

Ln 164: What is the size of the chamber?
The chamber had dimensions of 10.4 cm diameter by 14.0 cm length, and a volume of 1190 ml. These details have now been provided in the manuscript as requested.

Ln 214: Size of the flasks? Does the drawing of the flask sample affect the CO2 concentration in the chamber?
The volume of the evacuated flask was 65 ml and this has been added to the text (Methods). Drawing of chamber air into the evacuated flask would have caused a slight pressure decrease in the chamber headspace, which would have been compensated by a small amount of soil gas entering the chamber (the chamber was open to the soil). Since the volume of the evacuated flask compared to the chamber volume (1190 ml) and chamber headspace (850 ml) was very small (<10%), and because the gas being drawn into the chamber due to the slight pressure difference was open to and equilibrated with the chamber headspace, we do not consider that the drawing of the flask sample would have substantially affected the CO_2 concentration in the chamber and the time for equilibration would have been short relative to the passive-sample collection period. This should be clearer in the manuscript since both the chamber volume and flask volumes are now provided. In addition, as stated in the manuscript, flask samples were allowed to equilibrate with the chamber for 1 hour during sampling, to ensure the sample being removed was representative (both in terms of CO_2 concentration and isotopic characteristics).

Ln 282: How was the correction factor derived? Your synthetic site had much lower CO2 concentration than the grassland site, and Fig 4 was conducted under a 5% CO2 condition. You would expect the correlation of CO2 trapping rate with tube diameter be different at a much lower CO2 condition, based on Fick's Law.
As requested, we now provide full details of how the correction factor was derived at this point in the text. We disagree with the reviewer that the correlation of CO_2 trapping rate with inner diameter of the sampling tube would be different at much lower CO_2 concentrations based on Fick's law. Using Fick's law we have calculated theoretical trap rates for sampling tubes of different inner diameter under atmospheres of different CO_2 concentrations. We have found that the ratio (i.e. the correction factor in the manuscript) of the CO_2 trap rate between a 4 mm and 2 mm inner diameter tube is a constant. Therefore, our correction factor derived from a CO_2 concentration of 5%, should be reliable for correcting the CO_2 volumes from the synthetic soil experiment. However, we accept that we had failed in the original manuscript to provide sufficient detail on how the correction factor was derived (now we hope rectified), and therefore it was appropriate for the reviewer to raise this query.

Ln 339: What is the precision of your CO2 volume measurement?
We estimate that the precision of our CO_2 volume measurement is +/- 0.1 ml, and this information has been provided in the manuscript as requested.

Ln 363: Take out "concentration" after d13C.
As requested, this word has been removed.

Ln 373: The trap 13C values were lighter by 3-4? (about 3.1? for the synthetic soil site).
We agree with the reviewer that it would be more accurate to describe the ^{13}C fractionation during passive trapping as 3-4‰ rather than ~4‰. We have therefore modified the manuscript at this point so that where we previously described the fractionation as ~ 4‰, it is now described as 3-4‰. Further, we have also modified this part of the Discussion to comment that there did appear to be a slight difference in the amount of fractionation that occurred in the different experiments (as commented by the reviewer). We retain our statement that further research into this area is required.

Ln 378: The discrepancy appears to be constant at one site, but different between the two sites. It is 3.7? and 3.1? for the grassland and the synthetic soil respectively.
We have made substantial changes to the manuscript to deal with this comment. We now give the calculated mean fractionation caused during passive trapping with the molecular sieve cartridges, and have added several additional sentences to discuss why there may have been differences in the calculated fractionation factor between the two experiments.

Table 1 and 3: Please be consistent with the significant numbers you reported on the volume (ml) of CO2 trapped.
We thank the reviewer for pointing out this discrepancy. The CO_2 volumes reported in Table 1 have now been amended so that the results are presented to one decimal place, consistent with Table 3.

Fig 3: For the grassland plot, the two longest samples are 28 days, however the longest period sample from grassland site shown in Table 1 is only 8 days. Why didn't you include the 28-day data in Table 1?
The 28-day experiment was primarily used to assess the CO_2 capacity of the molecular sieve cartridges and was performed entirely separately and at a different time to the other experiments (but using the same equipment). The results in Table 1 are for an 8-day experiment on the grassland which had an experimental design specifically requiring that the sieves from the short, medium and long periods all sampled from the same chamber at the same time. Since all sieves (when results were grouped according to sampling period) sampled the same period of time, it did not matter if conditions had changed during the 8-day

experiment. However, the 28-day experiment was performed at a different time, under potentially different conditions, therefore it is not correct to compare the results from the 28-day experiment with the 8-day experiment in Table 1 (differences may reflect changing conditions rather than a problem with the sampling). In reality, we found that the CO_2 trapping rate in the 28-day sieves was very similar to the trapping rates in the 8-day experiment, suggesting that conditions were probably similar, but more importantly, that the sieves had not begun to become saturated with CO_2. Hence, we consider it valuable to provide the results from the 28-day experiment in Fig 3, but we do not think it appropriate to also provide the two CO_2 volumes for the 28-day samples in a table.

Fig 4: Ln 272 indicates the narrow trap used is 1 mm diameter. Why didn't you do a 1 mm point in this plot so that you can be sure the linear relationship extends to 1 mm?
We thank the reviewer for drawing our attention to this. There was an error in the original manuscript at Ln 272 because the narrow trap actually had a 2 mm diameter (not a 1 mm diameter as in the original manuscript). This is why the lowest point in Fig 4 is at 2 mm diameter. We have now corrected the manuscript at Ln 272 to provide the correct tube diameters.

A passive sampling method for radiocarbon analysis of soil respiration using molecular sieve

Regular paper

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A passive sampling method for radiocarbon analysis of soil respiration using molecular sieve

Abstract

Radiocarbon analysis of soil CO_2 can provide information on the age, its source and the rate of soil C turnover. We developed a new method for passively trapping respired CO_2 on molecular sieve, allowing it to be returned to the laboratory and recovered for C isotope analysis. We tested the method on a soil at a grassland site, and using a synthetic soil that we created to provide a contrasting isotopic signature. As with other passive sampling techniques, a small amount of fractionation of the ^{13}C isotope occurs during sampling, which we have quantified, otherwise the results show that the molecular sieve traps a sufficiently large and representative sample of CO_2 for C isotope analysis. Since ^{14}C results are routinely corrected for mass dependent fractionation, our results show that passive sampling of soil respiration using molecular sieve offers a reliable method to collect soil-respired CO_2 for ^{14}C analysis.

Keywords: Soil respiration, CO_2, Radiocarbon, Molecular sieve.

1. Introduction

The largest flux of carbon (C) from terrestrial ecosystems is soil respiration (Raich and Tufekcioglu, 2000), but the processes involved in this flux, and how

25 they will respond to global change, remain poorly understood (Subke et al., 2006).
26 There are several reasons for this including practical issues associated with
27 measuring processes occurring below ground. One of the major challenges facing
28 soil biogeochemists is determining whether the CO_2 derived from the
29 decomposition of soil organic matter comes mainly from the decomposition of the
30 typically small pool of C derived from recent plant activity, or from the larger and
31 older soil C reservoirs. This must be addressed if we are to predict the effect of
32 global warming on soil C stocks and the potential for a positive feedback to climate
33 change. Measurements of the ^{14}C content of soil-respired CO_2 can help us towards
34 these ends.

35 Soil contains organic material at various stages of decomposition and
36 microbial resynthesis, and we know from direct radiocarbon analysis that it can
37 range in age from a few years up to several thousand (e.g. Bol et al., 1999). Indeed,
38 natural abundance radiocarbon analysis of soil organic matter has been used to
39 estimate soil C cycling rates using models of C turnover (e.g. Harkness et al., 1986;
40 Harrison et al., 2000; Gaudinski et al., 2000). However, this modelling approach
41 has a number of drawbacks, for example, models assume a uniformly mixed soil
42 and steady-state; assumptions that are unlikely to be true for all soils. An
43 alternative approach for investigating soil C turnover is through radiocarbon
44 analysis of soil respiration.

45 Radiocarbon analysis of soil respiration has become feasible through the
46 use of accelerator mass spectrometry (AMS). Recent developments in the use of
47 molecular sieves to collect respired CO_2 for ^{14}C analysis (e.g. Gaudinski et al.,
48 2000; Hardie et al., 2005) have further increased the feasibility of such studies.
49 More 'traditional' methods of CO_2 collection are impractical due to the large

50 volumes of gas required (e.g. for sampling bags, evacuated flasks), or are
51 potentially hazardous (e.g. trapping in liquid nitrogen or hydroxide) in field
52 situations. The collection of respired CO_2 by the pump-based molecular sieve
53 sampling methods described by Gaudinski et al. (2000) and Hardie et al. (2005) are
54 ideal for situations where respiration rates are relatively high, sampling times
55 relatively short (<1 d), and study sites are readily accessible. However, in some
56 ecosystems (e.g. high altitude or high latitude), access to remote sampling sites
57 may be extremely restricted for a considerable portion of the year, especially
58 during winter, even though soil respiration can continue and represent an important
59 proportion of the annual total (Elberling, 2007).
60 With a view to collecting samples of CO_2 derived from soil respiration
61 during winter in a remote Arctic location we developed and tested the use of
62 molecular sieve cartridges for the collection of CO_2 without the need for a
63 pumping system. The technique uses 'passive sampling' whereby instead of
64 pumping a gas through a molecular sieve, the gas enters by diffusion (passive
65 samplers are also known as 'diffusive' samplers; Bertoni et al., 2004). Due to the
66 properties of the molecular sieve, CO_2 is adsorbed from the air preferentially over
67 any other gas except water vapour. On return to the laboratory, the CO_2 can be
68 released from the sieve by heating. Passive sampling is simple and inexpensive and
69 does not require an energy source during sampling; cartridges only require
70 installation, followed by recovery after the required sampling time. Thus, they are
71 extremely suitable for sampling in locations where access is only periodic, or in
72 situations where sampling involving pumps might cause unacceptable disturbance
73 (e.g. beneath a snow-pack).

74 Passive sampling has previously been used for $^{14}CO_2$ measurement by
75 Cooper et al. (1998), however they employed hydroxides as the adsorbent, were
76 measuring ^{14}C concentrations much higher than natural abundance, and required
77 larger volumes of sample than would be practical for soil respiration experiments
78 (^{14}C measurement was by liquid scintillation counting). Similar to us, Godbout et
79 al. (2006) utilised molecular sieve (zeolite 5A) in passive samplers, but in contrast,
80 they collected samples of N_2O and CH_4 (and not for ^{14}C analysis). Hydroxides have
81 been utilised for the collection of soil CO_2 for stable isotope measurement, but as
82 described by Davidson (1995), considerable care must be taken as even fresh
83 hydroxide may contain a significant quantity of CO_2 (leading to sample
84 contamination). Furthermore, contrary to past assumptions, trapping is unlikely to
85 be quantitative, leading to isotopic fractionation (Davidson, 1995). To our
86 knowledge, no one has applied molecular sieve in passive samplers for the
87 measurement of natural abundance radiocarbon in CO_2.
88 In our approach, a considerable advantage is that we employ the same
89 design of molecular sieve cartridge (MSC) as previously described by Hardie et al.
90 (2005), which was based on a design by Bol and Harkness (1995). This cartridge
91 has been successfully used with a sampling system that incorporated a pump (e.g.
92 Wookey et al. 2002; Billett et al., 2006, 2007). In addition, this sieve cartridge
93 design (utilising the same Type 13X molecular sieve) has already been
94 successfully tested (Hardie et al., 2005) for isotopic fractionation and
95 contamination (e.g. memory effects, where small quantities of a sample may
96 remain on the sieve after discharge and therefore contaminate the next sample).

To test the use of molecular sieve cartridges (MSCs) for passive sampling we established several experiments, designed to answer the following two questions:

1. *Is CO_2 passively captured on the MSC at a rate which is always proportional to the environmental CO_2 concentration?* If CO_2 is not captured at a constant rate when the environmental CO_2 concentration is constant, then the CO_2 being recovered may not be representative of the total sampling period. For example, if the sieve starts to saturate with CO_2, trapping rates may decline over time even with no change in conditions and therefore the recovered sample may not be representative of the total sampling period. Based on Fick's Law, the rate of CO_2 capture in a diffusion sampler should be proportional to the CO_2 concentration of the environment (e.g. Bertoni et al., 2004). If this is so, then it should be possible to estimate the environment's CO_2 concentration simply from the rate of CO_2 trapping (i.e. CO_2 recovered/sampling time), providing additional potentially useful information.

2. *Does the recovered CO_2 have an isotopic (^{13}C and ^{14}C) composition the same as the environment?* The MSCs have insignificant fractionation or memory effects when used for ^{13}C and ^{14}C with the pump-based system (Hardie et al., 2005). If fractionation occurs during passive sampling, this will only affect ^{13}C results. The MSCs would still be suitable for passive collection of ^{14}C samples since ^{14}C results are corrected for mass-dependent fractionation (Stuiver and Polach, 1977). If fractionation does occur when sampling passively then this may be a

constant, or quantifiable, and therefore the ^{13}C results should be correctable.

2. Materials and methods

2.1. Site and soil information

We tested passive sampling of soil respiration using MSCs on two different soils at contrasting times of year (see Table 1 for soil characteristics). Firstly, we sampled from a grassland with a non calcareous surface-water gley soil during the summer (2007) when respiration rates were expected to be at their maximum. The grassland was located in a suburban area to the south of Glasgow, UK (55°46'N, 4°18'W) and most abundant plant species were: *Lolium perenne*, *Holcus lanatus*, *Cynosurus cristatus*, *Anthoxanthum odoratum*, *Ranunculus repens*, *Veronica chamaedrys* and *Trifolium repens*. The site has been a grassland lawn for at least several decades, and it is extremely unlikely that it has ever contained any C4 plants. From previous results (e.g. Hahn et al., 2006), we expected that the CO_2 respired from the grassland would have a similar ^{14}C content to the contemporary atmosphere. Therefore, for a contrast and thus to provide a more robust test of the method, we created a synthetic soil for the second study in order to generate CO_2 that was much more ^{14}C depleted than the contemporary atmosphere. This synthetic soil was a mixture of approximately equal masses of compost (composed predominantly of garden peat, but pre-mixed with lime $CaCO_3$ (Homebase, UK) and sand, and was placed in a large (30 x 40 x 25 cm deep) open-top container at the same grassland field site. Sampling of the soil respiration from the synthetic

146 soil was performed from December 2007 to January 2008 (winter), when
147 respiration rates were expected to be at their annual minima. In addition, we
148 performed a further experiment to assess the CO_2 capacity of the MSCs when used
149 passively by exposing two further sieves at the grassland site for an extended
150 duration during the summer.
151
152 *2.2. Sieve design and sampling procedure*
153
154 The design of the molecular sieve cartridge (Fig. 1) has previously been
155 described by Hardie et al. (2005). It was constructed from quartz glass tube with a
156 central chamber (dimensions 11 mm ID, 70 mm length) filled with ~ 3-4 g of 13X
157 zeolite molecular sieve (1/16" pellets, BDH, UK). The tubing at either end of the
158 cartridge was slightly narrower than the central chamber (4 mm ID, 100 mm length
159 and 8 mm ID, 100 mm length) which, together with quartz wool, held the
160 molecular sieve in place. At either end of the MSC a short length of PVC tubing
161 (Tygon, Fisher Scientific, UK) was attached and into this an auto-shutoff Quick
162 Coupling™ (Colder Products Company, USA) was inserted; the couplings allow
163 minimal contamination from the atmosphere when attaching to other equipment
164 (e.g. the respiration chamber). Although the couplings automatically close when
165 detached, WeLoc© clips (Scandinavia Direct, UK) were also placed over the PVC
166 tubing to provide an additional seal. Prior to sampling all molecular sieve
167 cartridges were charged by heating (500^{o}C) for 1.5 hours while attached to a
168 vacuum rig (see Hardie et al. (2005) for details) and subsequently filled with high
169 purity N_2.

A single respiration chamber (dimensions 10.4 cm diameter, 14.0 cm length, volume 1190 ml) was constructed from PVC pipe which was open at the base (for contact with the soil) and closed at the top with a rubber seal (Fig. 1). Three couplings were inserted into the side of the chamber to which MSCs could be attached. Therefore at any one time, three MSCs could be used to collect passively CO_2 from the headspace of the chamber. Inside the chamber a hydrophobic filter (Accurel PP V8/2 HF, Membrana GmbH, Germany) was attached to the couplings which allowed gas exchange between the inside of the chamber and the MSCs, but prevented liquid water from entering the MSC.

The chamber was inserted to a depth of ~ 4 cm in both the grassland and synthetic soils (vegetation had previously been removed from the grassland soil one month earlier, and no vegetation was present in the synthetic soil). Prior to attaching the MSCs, the atmospheric CO_2 inside the chamber had been removed using a soda-lime based scrubbing system (Hardie et al., 2005) and left several days for CO_2 to accumulate.

An experimental design based on that of Bertoni et al. (2004) was adopted (Fig. 2). The design utilises three sieves to sample simultaneously from the same chamber and was chosen as it allows a test of the sampling method even if there are variations in chamber CO_2 concentration or isotopic signature over time (which is likely to be the case). If changes in the chamber CO_2 occur, this should be reflected in short period samples. However, the combined results from adjacent short period samples should still be identical to the results for longer term samples that were trapping CO_2 over the same period. For example, the volume of CO_2 recovered from samples S1 + S2 should be the same as from the single M1 sample etc (Fig. 2). The principle applies similarly for the isotope results, although the

values are averaged (weighted by recovered CO_2 volume) rather than summed. This sampling design therefore tests whether the MSCs trap representative samples at different lengths of exposure.

To begin sampling, each cartridge was attached to the respiration chamber with the coupling and then the clip nearest the chamber removed, allowing chamber air into the MSC via the hydrophobic filter. The sieve cartridge was protected by covering with a short length of pipe insulation. At the end of the sampling period (which ranged from 2 to 56 d) the clip was simply replaced on the MSC and the cartridge uncoupled from the chamber.

To test whether the sieves were collecting samples which were representative of the headspace CO_2, in addition to the molecular sieve samples, we collected CO_2 from the chamber using evacuated flasks (volume 65 ml). These flask samples were collected at the start, middle and end of the experiments, and were sampled by simply attaching the flask to the chamber using couplings and leaving to equilibrate for 1 hour.

2.3. Gas collection and isotope analysis

On return to the laboratory, the CO_2 trapped on the molecular sieves was recovered by heating (500^{o}C) while attached to a vacuum rig. The gas evolved from the sieve was dried in a slush trap (mixture of dry ice and industrial methylated spirits; -78^{o}C) and the CO_2 recovered using liquid N_2 (see Hardie et al. (2005) for further details). The volume of the recovered CO_2 was measured and divided into sub-samples for ^{13}C and ^{14}C measurement. Measurement of $\delta^{13}C$ ($^{13}C/^{12}C$ ratio in ‰ units relative to the standard Vienna Pee Dee Belemnite;

220 VPDB) was performed on a dual inlet isotope ratio mass spectrometer (VG
221 Optima, Micromass, UK). The ^{14}C sub-sample of CO_2 was reduced to graphite
222 using Fe/Zn reduction (Slota et al., 1987) and analysed by AMS at the Scottish
223 Universities Environmental Research Centre (SUERC), East Kilbride, UK
224 (Freeman et al., 2007). Following Stuiver and Polach (1977), ^{14}C results were
225 normalised to a $\delta^{13}C$ of -25‰ and expressed as %modern and conventional
226 radiocarbon ages (BP; i.e. relative to AD 1950). Following convention,
227 measurement uncertainty for isotope concentrations are expressed as standard
228 deviations (i.e. ±1 σ = 68% probability, and ±2 σ = 95% probability). The 65 ml
229 flasks were also returned to the laboratory, the CO_2 cryogenically recovered on a
230 vacuum rig as described above, and the $\delta^{13}C$ measured. ^{14}C content was measured
231 for one flask sample (F2 in the grassland experiment).
232
233 **3. Results**
234
235 *3.1. Grassland soil*
236
237 The sum of CO_2 recovered from the short-, medium- and long-period MSCs
238 were each very similar (Table 2). There was a highly significant correlation
239 (P<0.001) between CO_2 recovered from the sieve cartridges and sampling time,
240 which was linear even when the incubation was extended to 28 days by which time
241 the MSCs had trapped >100 ml of CO_2 (Fig. 3). This suggests that the sieves were
242 collecting representative samples of the respired CO_2 independent of the duration
243 of the incubation.

244 The $\delta^{13}C$ values were unaffected by multiple versus single MSC samplings
245 (Table 2). The results from all values when averaged for the full 8 days of the main
246 experiment were all identical to within 2 σ; this is despite the fact that the $\delta^{13}C$
247 concentration appears to have changed by ~ 1‰ over the length of the experiment,
248 as indicated by the results for the short period samples. The $\delta^{13}C$ values from the
249 MSCs are, however, more depleted than samples collected from the same
250 chambers using evacuated flasks.
251 There was a slight difference in the ^{14}C content of the two medium period
252 samples (although only significant at 1 σ), perhaps indicating a slight change in the
253 mean age of the respiration during the course of the experiment (Table 3).
254 Importantly, the average of the two medium period samples was not significantly
255 different (<1 σ) to the result for the long period sample. In addition, all samples
256 collected using the passive molecular sieve method had ^{14}C contents that did not
257 differ (<1 σ) from the evacuated flask sample when measurement uncertainty was
258 considered (Table 3).
259
260 *3.2. Synthetic soil*
261
262 The $\delta^{13}C$ value of the CO_2 recovered from the molecular sieve varied
263 during the experiment from -11.8 to -14.0‰ (Table 4). A similar range of variation
264 in chamber CO_2 $\delta^{13}C$ occurred for the flask samples (range -8.7 to -10.9‰; Table
265 4). Importantly, however, when the results from the MSCs for the short sampling
266 periods were combined the results were never significantly different (<2 σ) to the
267 longer period samples collected at the same time (Table 4). For example, the
268 average $\delta^{13}C$ value of the first two short period samples (S1 and S2) was -12.4‰,

269 whereas the first medium period sample (M1) had a $\delta^{13}C$ value of -12.5‰.
270 Similarly, combining the two medium period samples (M1 and M2) resulted in a
271 $\delta^{13}C$ value (-13.1‰) that was nearly identical to the long period sample (L; -
272 13.0‰).

273 However, unlike in the results from the grassland, combining the volumes
274 of CO_2 recovered from short period samples did not in all cases result in the
275 expected values based on the longer period results. For example, the total volume
276 of CO_2 recovered in S1 and S2 was 14.0 ml, whereas the equivalent longer period
277 sample (M1) had a CO_2 volume of 21.2 ml. A similar situation was apparent in
278 samples from the second half of the experiment where the total volume recovered
279 from samples S3 and S4 (11.7 ml) was substantially lower than the M2 sample
280 (15.3 ml).

281 Inspection revealed that two of the MSCs used to collect samples S1 and S4
282 from the synthetic soil were slightly different compared to the other MSCs. These
283 two cartridges were made from glass tubing with a narrower inner diameter than
284 the other MSCs (2 mm diameter compared to 4 mm for the other MSCs) at the end
285 that was connected to the respiration chamber. Since the two samples collected
286 using these cartridges recovered less CO_2 than expected, we performed an
287 additional experiment to test whether the inner diameter of this tube affected the
288 rate of CO_2 trapping. Soil CO_2 was passively collected from the grassland site
289 using three pairs of identical MSCs except for the inner diameter of the tube that
290 connected the MSC to the respiration chamber. The results show that the inner
291 diameter of the tube between the respiration chamber and molecular sieve strongly
292 affected CO_2 trapping rate (Fig. 4).

293 From the results of the experiment comparing MSCs with sampling tubes
294 of different inner diameter we were able to derive a factor (equation 1) to correct
295 the results for the synthetic soil experiment which had been collected using non-
296 standard MSCs (S1 and S4; Table 4).
297
298 Correction factor =
299 CO_2 trap rate (4 mm tubing) / CO_2 trap rate (2 mm tubing) (equation 1)
300
301 Thus the CO_2 volumes recovered using non-standard MSCs (2 mm ID at sampling
302 end) were corrected by multiplying by the correction factor (1.880). This
303 correction resulted in a closer agreement between the sum of the recovered
304 volumes of CO_2 for short period samples and the corresponding medium period
305 sample. The correction made little difference to the weighted average $\delta^{13}C$ results –
306 these all remained within measurement error (<2 σ).
307 The volume of CO_2 recovered was highly correlated (P<0.001) with
308 sampling time for the synthetic soil (Fig. 3) even before applying the correction
309 factor. However, the R^2 value was less than had been calculated for the field test
310 results, and the value only slightly improved after correcting for differences in the
311 tube size of MSCs. The poorer correlation may simply result from greater variation
312 in the CO_2 concentration of the chamber during the experiment with synthetic soil
313 (as observed in flask samples), which was performed over a much greater period of
314 time compared to the field test.
315 The respired CO_2 collected using MSCs from the synthetic soil had a
316 considerably lower ^{14}C content compared to the contemporary atmosphere
317 (expressed as conventional radiocarbon ages, the results range from 6965 to 8542

318 years BP). The ^{14}C content of the chamber CO_2 varied considerably over the course
319 of the experiment (Table 5), with the result for the first half of the experiment (M1)
320 being considerably ^{14}C-depleted compared to the second half (M2). Despite this,
321 the combined result from the two medium period samples (37.67 %modern) was
322 within the measurement error of the long period sample (37.76±0.64 %modern).
323

324 **4. Discussion**

325 Models utilizing the radiocarbon content of SOM have frequently been
326 used to provide valuable information on soil C cycling (e.g. Harkness et al., 1986;
327 Harrison et al., 2000; Gaudinski et al., 2000). However, these models generally
328 rely on several assumptions which are unlikely to be true for all soils. Radiocarbon
329 analysis of soil respiration avoids the need for these assumptions and provides a
330 direct measurement of the mean age of soil respired CO_2, therefore providing more
331 detailed information on the rate of C cycling in soil, and allowing prediction of
332 how respiration will respond to changing environmental conditions.

333 The attractive features of passive (diffusion) sampling, such as ease of use
334 and inexpensiveness, have resulted in its adoption in a number of applications
335 ranging from monitoring air quality (e.g. Bertoni et al., 2004; Cooper et al., 1998)
336 to trapping of soil–generated gases (e.g. Davidson, 1995; Godbout et al., 2006). In
337 the present study, our aim was to develop a method to sample passively the CO_2
338 released by soil in the field, allowing it to be recovered later in the laboratory for
339 measurement of the stable and radiocarbon concentration. To be a reliable
340 technique, the sampling method would need to fulfill certain criteria, which formed
341 the basis of the tests described here.

342 Firstly, it is essential that the sampling technique allows the collection of a
343 representative sample of CO_2 throughout the sampling period. That we found a
344 very strong correlation between CO_2 recovered from the MSCs and the exposure
345 time (Fig. 3) shows that, within the limits of the present studies, CO_2 was
346 continuously trapped. There will clearly be maximum exposure times and volumes
347 of CO_2 that can be adsorbed on the sieve, however it would appear that those limits
348 (~ 120 ml CO_2 / 56 d exposure time) were not exceeded in the present studies.
349 Consistent with Fick's Law, the rate that CO_2 was trapped was highest when the
350 chamber CO_2 concentration was greatest; for the grassland experiment, where CO_2
351 levels were ~40,000 ppm, only 2 days were required to trap ~7 ml CO_2, whereas
352 for the synthetic soil experiment, where concentrations were only ~10,000 ppm,
353 similar volumes required ~14 days of sampling.
354 The correlations in Fig. 3 provide support for the conclusion that the MSCs
355 collect a representative sample over time; they are, however, potentially sensitive
356 to variations in chamber CO_2 concentration. A better test is therefore one in which
357 a series of samples were collected simultaneously for varying durations of
358 exposure so that they can be combined; if the combined results from shorter period
359 samples are identical to longer period samples collected at the same time, it shows
360 that representative samples were collected no matter what the exposure time.
361 The results from both the field and synthetic soil were consistent with
362 sampling rate not being affected by exposure time. In the grassland experiment, the
363 combined results from all shorter period samples all differed by < 0.5 ml from the
364 volume of corresponding longer period samples, which is close to the precision of
365 the volume measurement (approximately ±0.1 ml). The results for some of the
366 samples from the synthetic soil experiment did differ considerably from what was

367 expected, but the difference was greatly reduced when a correction was made for
368 two of the samples being collected using non-standard sieve cartridges. That the
369 difference in the inner diameter of the sampling tube made such a difference to the
370 trapping rate should not be surprising, since it is predicted by Fick's Law. Indeed,
371 altering the cross-sectional area of the sampling tube offers one method of
372 modifying the cartridge design to tailor trap rates and exposure times for particular
373 needs, although we have not explored this possibility systematically at this stage.
374 The sieve cartridges we utilized had already undergone tests which showed
375 that they do not suffer from sample carry over or contamination (Hardie et al.,
376 2005). When used with a pump-based sampling system the MSCs have also been
377 shown not to fractionate CO_2 isotopically, and although it was unsurprising that
378 passive trapping with the molecular sieve seemed to result in some isotopic
379 fractionation, we were not concerned about this in the context of ^{14}C measurements
380 as they are routinely corrected for mass-dependent fractionation (Stuiver and
381 Polach, 1977). Our results support the use of the technique for ^{14}C analysis: in both
382 the field and synthetic soil experiments, the combined results of the medium period
383 samples were within measurement error of the respective long period sample
384 collected over the same time period (Tables 3 and 5). Furthermore, in the grassland
385 experiment, the evacuated flask sample had a near identical ^{14}C content to all the
386 passive MSC-collected samples.
387 Although of secondary importance in the present study, the $\delta^{13}C$ of soil
388 respired CO_2 can provide valuable information on the C source and turnover of soil
389 organic matter. In addition, if an atmospheric component is suspected in a soil
390 respiration sample, the $\delta^{13}C$ value of the recovered CO_2 can be used to estimate the
391 proportion of air in the sample, thus allowing the ^{14}C results to be corrected for the

392 air contaminant (e.g. Gaudinski et al., 2000). Our tests show that, as for the volume
393 and ^{14}C content, different lengths of exposure time did not affect the $\delta^{13}C$ of the
394 recovered CO_2. However, we found significant differences in $\delta^{13}C$ between the
395 passive MSC samples and those collected using evacuated flasks.
396 Samples collected in both experiments using evacuated flasks returned $\delta^{13}C$
397 values that were enriched relative to passively collected MSC samples by on
398 average 3.8 ± 0.4‰ (grassland soil) and 3.2 ± 0.8‰ (synthetic soil). The results
399 probably indicate that some fractionation is occurring when the sieves are used
400 passively to collect CO_2. Again, this result is not surprising, since we know that
401 other passive sampling techniques (e.g. adsorption in hydroxide) fractionate during
402 trapping (Davidson, 1995). The ~3-4‰ fractionation is most likely associated with
403 diffusion through air (the value is close to that described by Davidson (1995)).
404 That the variation in the calculated fractionation was greater in the MSC samples
405 from the synthetic soil may be because the flasks collected samples representative
406 of a single moment in time, whereas the MSCs provided chamber CO_2 over several
407 weeks. Therefore if the $\delta^{13}C$ of the chamber CO_2 varied over the course of the
408 experiment (as shown by results from the flasks in the synthetic soil experiment),
409 then this could introduce error in the calculated amount of ^{13}C fractionation.
410 Further investigation into the discrepancy between the MSC and evacuated flask
411 results is being undertaken, which we hope will lead to a more reliable adjustment
412 factor.
413 Based on the results in the present study, the amount of ^{13}C fractionation
414 due to passive sampling with a MSC ranged between ~3 to 4‰. Therefore, using
415 this range of values to correct the $\delta^{13}C$ of chamber CO_2 samples collected by
416 passive MSC sampling increases the uncertainty in the proportion of air in a

417 chamber sample, in turn increasing the uncertainty in the air-corrected ^{14}C value of
418 soil respiration. However, in a C_3 ecosystem where the difference in the $\delta^{13}C$ ratios
419 of atmospheric and respired CO_2 are likely to be in the order of 15-20‰, the
420 current 1‰ uncertainty in the adjustment factor will only cause substantial errors
421 (>10%) in the calculation of the proportion of air present when the contribution of
422 respired CO_2 to the headspace is less than 50%. In such circumstances, issues with
423 analytical precision would, in any case, limit our ability to accurately estimate the
424 ^{14}C value of respired CO_2. Samples with high atmospheric contamination are often
425 discarded for these reasons. In the present study, the chamber CO_2 concentrations
426 were such that the maximum contribution of the atmospheric CO_2 (~380 ppm)
427 would only represent ~1% and 6% of the chamber CO_2 in the grassland and
428 synthetic soil experiments, respectively. In addition, the increased uncertainty in
429 the proportion of atmospheric contamination would only likely be significant if soil
430 respiration had a ^{14}C content very different from the contemporary atmosphere
431 (which is unlikely except in soils with extremely slow turnover rates or carbonate
432 contamination). However, to avoid all these issues, if a passive sampling
433 experiment is being carried out in a situation in which atmospheric contamination
434 of the samples is expected to be large, to allow for mass-balance corrections to be
435 made, we recommend that prior to the experiment, the degree of ^{13}C fractionation
436 be quantified using large closed chambers, isotopic standards and the same
437 molecular sieves as will be subsequently used for $^{14}CO_2$ sample collection.
438 Our aim was to test the sampling method, and in that context the
439 interpretation of the isotope results from the experiments is of lesser importance.
440 However, it is interesting to note that in the field experiment, the ^{14}C results were
441 close but slightly enriched, relative to the contemporary atmospheric ^{14}C value

(Levin et al., 2008) indicating, as expected, that most soil respiration from this grassland soil was fixed within the last few years. In the synthetic soil experiment, the low ^{14}C concentrations (equivalent to greater than 6000 years old) most likely imply that CO_2 was derived not only from the organic component (peat) of the compost, but also from the small amount of carbonate it contained. Further support for a contribution from the carbonate is provided by the $\delta^{13}C$ of the CO_2 which was very enriched in ^{13}C compared to the bulk soil (Table 1), suggesting a contribution from a ^{13}C-enriched source such as carbonate. An atmospheric contribution ($\delta^{13}C$ = -8.5‰; Hemming et al., 2005) would also increase the $\delta^{13}C$ of chamber CO_2, but would need to be a major component of the chamber CO_2 to explain the $\delta^{13}C$ values, which would be inconsistent with the depleted ^{14}C of the chamber CO_2.

In summary, from the results of this first test of the use of a molecular sieve method to sample soil-respired CO_2 passively, we conclude that:

1. The MSCs passively trapped CO_2 consistently over time and collected representative samples.
2. Used passively, the MSCs collected representative samples up to at least 100 ml CO_2, therefore implying that the method could be used for a large range of conditions – e.g. for a range of sampling timescales or in situations where the chamber CO_2 concentration (which is a major control on CO_2 trapping rate) is unknown.
3. While we would recommend the MSC design we employed for use over a wide range of conditions, modifications to the dimensions of the cartridge could be used to alter trap rates to suit particular sampling needs. For example, increasing the inner diameter of the sampling tube or reducing the path from the chamber to the zeolite would both increase the

CO_2 trap rate, which may be advantageous if chamber CO_2 concentrations are particularly low.

4. As with other passive sampling techniques, isotopic fractionation (~3-4‰) occurs during trapping when using the MSCs passively, and future investigations aim to reduce the uncertainty in the required adjustment factor.

5. Passive collection of CO_2 using the MSCs provides an easy and inexpensive method to reliably collect samples of soil-respired CO_2 for ^{14}C analysis. As fossil fuel-derived CO_2 is "^{14}C dead" we also suggest that this technique could be used to measure leakage from industrial carbon capture and storage ventures.

6. Further tests of passive sampling using MSCs under a wider range of conditions are being performed; preliminary results from sampling atmospheric CO_2 confirm the relationship between trap rate and CO_2 concentration, and suggest a similar ^{13}C fractionation (~4‰) during trapping.

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564

Tables

Table 1

Characteristics of soils used in the experiments.

Experiment	Soil type	pH	%carbon	$\delta^{13}C_{V\text{-}PDB}$‰
Grassland soil	Non calcareous surface-water gley	6.0	6.3	-28.6
Synthetic soil	Mixture of peat-based compost and sand	6.9	28.5	-25.9

571 Table 2

572 Volume (ml) and $\delta^{13}C$ (in brackets; ‰) of respired CO_2 collected by passive
573 trapping on molecular sieve from a grassland soil. Samples were collected for
574 different exposure times: short (S; 2 d), medium (M; 4 d) and long (L; 8 d).
575 Sampling was concurrent so that sieve cartridges experienced the same
576 conditions, and therefore, where results from short period samples have been
577 combined they should be identical to the results for the corresponding longer
578 period sample (see Fig. 2 and text for further explanation). $\delta^{13}C$ results for
579 evacuated flask samples (F) collected at the start, middle and end of the
580 experiment are shown; mean CO_2 concentration was ~40,000 ppm. $\delta^{13}C$ values
581 ±0.1‰ (1 σ).

582

583

	Days 1-2	**Days 3-4**	**Days 5-6**	**Days 7-8**	**Total**
S	7.4 (-30.4)	7.7 (-29.8)	7.7 (-29.5)	6.5 (-29.6)	29.2 (-29.8)
S (combined)	15.1 (-30.1)		14.1 (-29.6)		29.2 (-29.8)
M	14.6 (-30.4)		14.3 (-29.6)		28.9 (-30.0)
M (combined)	28.9 (-30.0)				28.9 (-30.0)
L	28.6 (-30.1)				28.6 (-30.1)
F	(-26.8)	(-25.7)		(-25.9)	(26.1)

584

585

586

587 Table 3

588 Radiocarbon concentration (%modern) of respired CO_2 from a grassland soil.
589 Samples taken by passive trapping on molecular sieve were collected for
590 different exposure times: medium (M; 4 d) and long (L; 8 d). The evacuated
591 flask sample (F2) was collected over a period of 1 hour during the middle of
592 the experiment. Radiocarbon publication codes given in brackets.

593

	Days 1-4	**Days 5-8**
M	106.12 ± 0.50 (SUERC-16183)	107.21 ± 0.51 (SUERC-16184)
M (combined)	106.66	
L	106.17 ± 0.50 (SUERC-16185)	
F2	106.43 ± 0.52 (SUERC-16182)	

594

595

Table 4

Volume (ml) and $\delta^{13}C$ (in brackets; ‰) of respired CO_2 collected by passive trapping on molecular sieve from a synthetic soil. Samples were collected for different exposure times: short (S; 14 d), medium (M; 28 d) and long (L; 56 d). Sampling was concurrent so that sieve cartridges experienced the same conditions, and therefore, where results from short period samples have been combined they should be identical to the results for the corresponding longer period sample (see Fig. 2 and text for further explanation). $\delta^{13}C$ results for evacuated flask samples (F) collected at the start, middle and end of the experiment are shown; CO_2 concentration ranged from 7000-15800 ppm. Superscript (^{N}) indicates if sample was collected using a narrow tube MSC; results for S^{C} are corrected for tube size (see text). $\delta^{13}C$ values ±0.1‰ (1 σ).

	Days 1-14	**Days 15-28**	**Days 29-42**	**Days 43-56**	**Total**
S	5.7^{N} (-11.8)	8.3 (-12.7)	8.2 (-14.0)	3.5^{N} (-13.4)	25.7 (-13.0)
S (combined)	14.0 (-12.4)		11.7 (-13.8)		25.7 (-13.0)
S^{C}	10.6 (-11.8)	8.3 (-12.7)	8.2 (-14.0)	6.5 (-13.4)	33.7 (-12.9)
S^{C} (combined)	18.9 (-12.2)		14.7 (-13.7)		33.7 (-12.9)
M	21.2 (-12.5)		15.3 (-14.0)		36.5 (-13.1)
M (combined)	36.5 (-13.1)				36.5 (-13.1)
L	36.3 (-13.0)				36.3 (-13.0)
F	(-8.7)	(-10.9)		(-10.1)	(-9.9)

611 Table 5

612 Radiocarbon concentration (%modern) of respired CO_2 collected by passive
613 trapping on molecular sieve from a synthetic soil. Samples were collected for
614 different exposure times: medium (M; 28 d) and long (L; 56 d). Radiocarbon
615 publication codes given in brackets.

616

	Days 1-28	**Days 29-56**
M	34.53 ± 0.67	42.02 ± 0.61
	(SUERC-18749)	(SUERC-18750)
M (combined)	37.67	
L	37.76 ± 0.64	
	(SUERC-18751)	

617

618

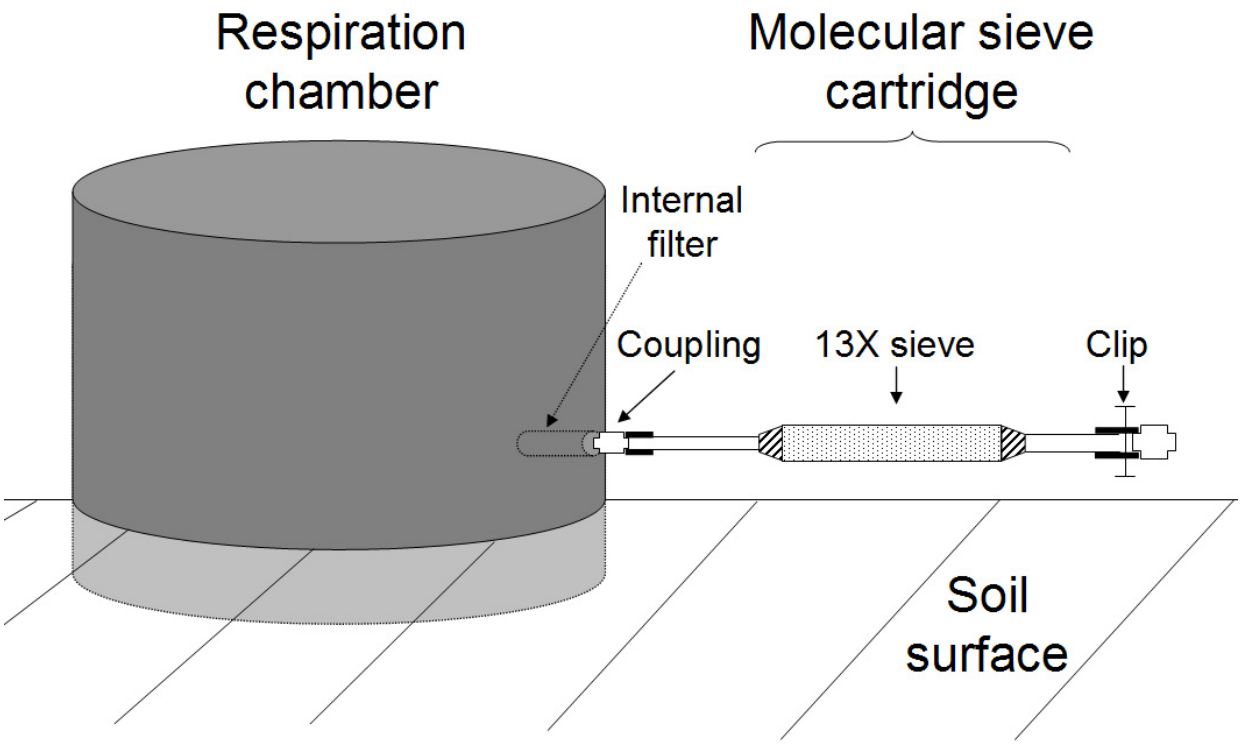
Figure captions

1. Schematic diagram showing a molecular sieve cartridge attached to the respiration chamber. The cartridge was composed of quartz glass containing 13X molecular sieve held in place by quartz wool. Quick couplings allowed the cartridge to be easily connected or disconnected from the chamber. A hydrophobic filter (Accurel PP V8/2 HF) was fitted inside the chamber which restricted moisture entering the sieve cartridge, but allowed gas exchange. Three cartridges were attached simultaneously to the chamber during the tests.

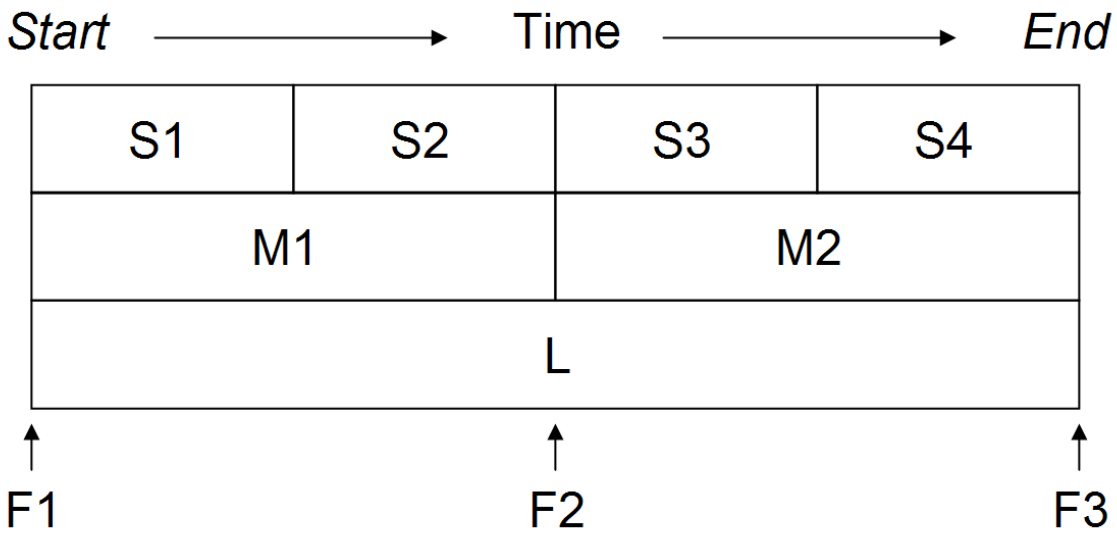
2. Diagram illustrating the sampling design. At any one time, three sieve cartridges were sampling; one from each of the short (S), medium (M) and long (L) sampling periods. This design tests whether the sieve cartridges collect a representative sample of CO_2 for a range of sampling times, even if the concentration and isotopic characteristics of the chamber CO_2 vary; the combined results from shorter period samples should equal the values for the corresponding longer period samples. For example, for volume of CO_2 recovered, S1+S2 should equal M1. Similarly, the average $\delta^{13}C$ value for S1 and S2 should be identical to the result for M1. Samples collected at three time points using evacuated flasks are also shown (F1, F2 and F3). Total duration of the experiments was 8 and 56 d for the grassland and synthetic soils, respectively.

643 3. Volume of CO_2 recovered from each molecular sieve plotted against the
644 length of sampling time. Results for both the field test on the grassland soil
645 and the synthetic soil are shown. Both correlations were highly significant
646 (P<0.001). Results for the synthetic soil experiment are not corrected for
647 tube size.
648
649 4. Rate of passive CO_2 trapping for MSCs connected to the same respiration
650 chamber with sampling tubes of different inner diameters. The chamber had
651 a CO_2 concentration of ~50,000 ppm and the sampling time was 2 days.
652 The correlation is highly significant (P<0.001).
653

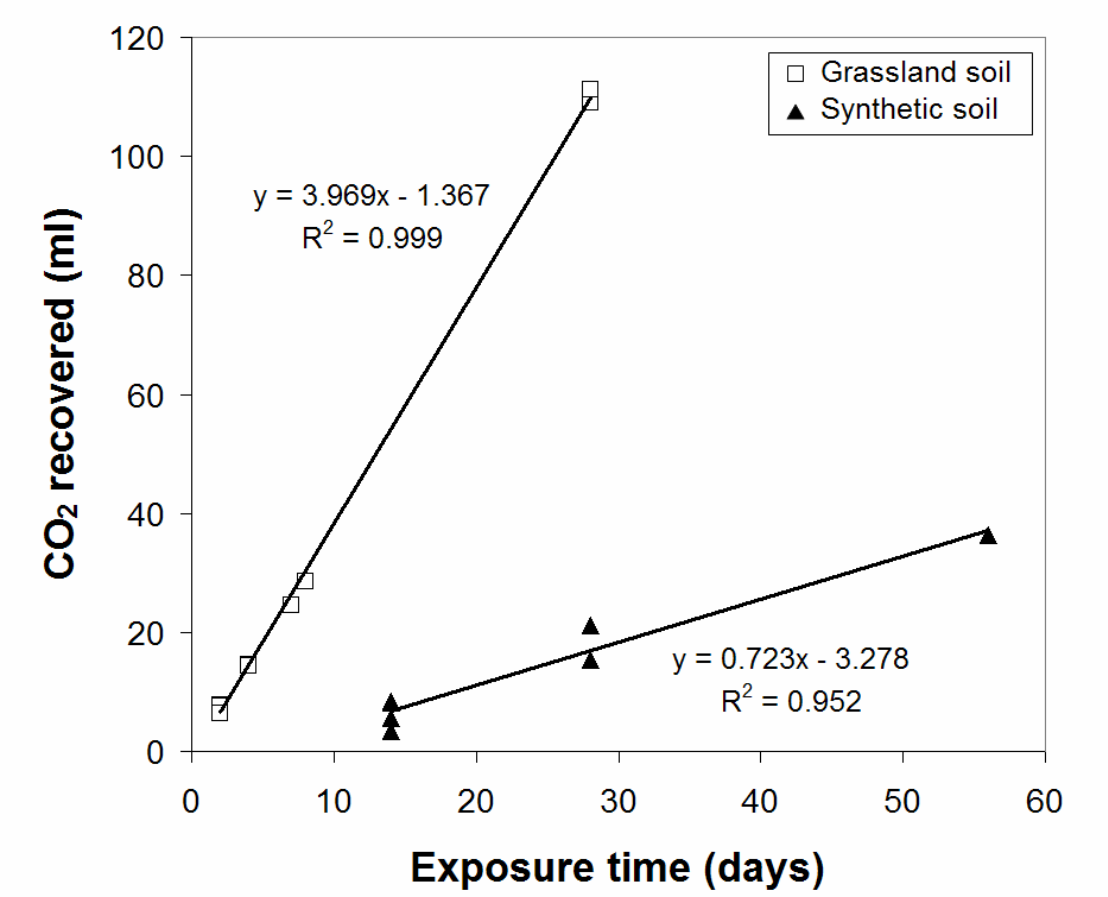
Figure


Respiration chamber
Molecular sieve cartridge
Internal filter
Coupling
13X sieve
Clip
Soil surface

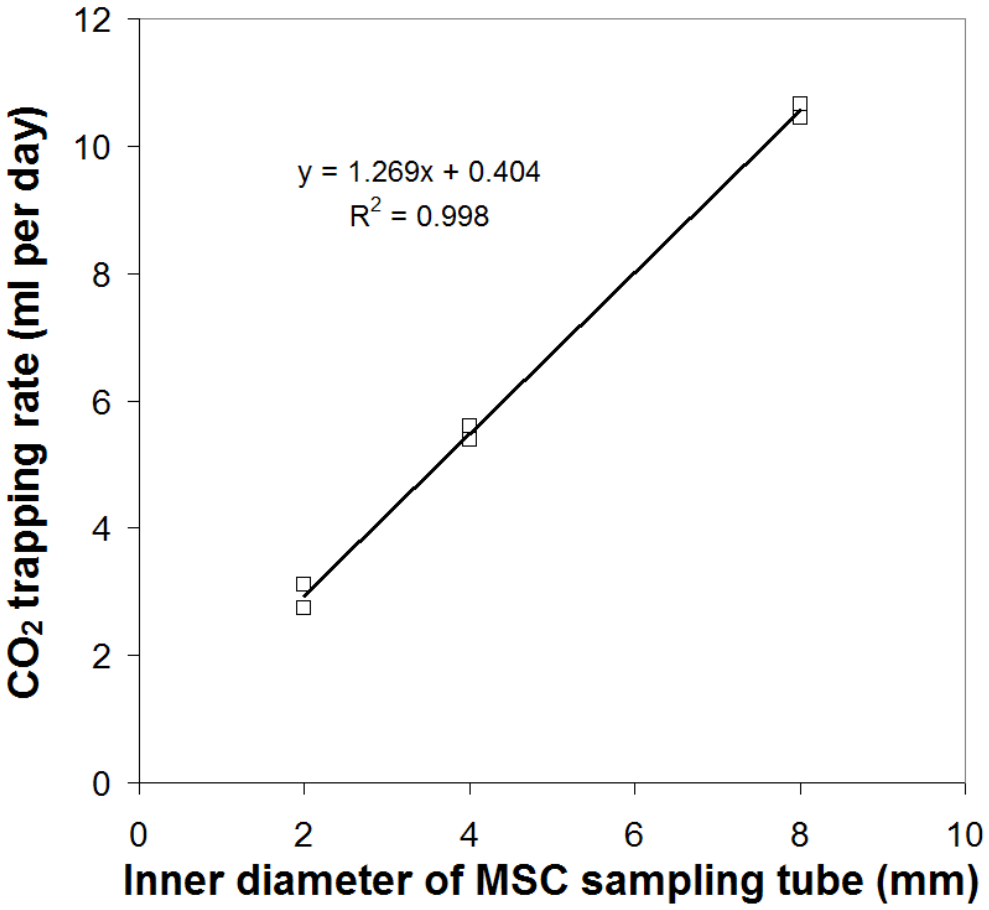
Figure



Figure


120
100
80
60
40
20
0
0
10
20
30
40
50
60
CO2 recovered (ml)
Exposure time (days)
Grassland soil
Synthetic soil
y = 3.969x - 1.367
R2 = 0.999
y = 0.723x - 3.278
R2 = 0.952

Figure


y = 1.269x + 0.404
R2 = 0.998
CO2 trapping rate (ml per day)
Inner diameter of MSC sampling tube (mm)
0
2
4
6
8
10
12