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1   **A passive sampling method for radiocarbon**

2   **analysis of soil respiration using molecular sieve**

3

4   **Abstract**

5

6   Radiocarbon analysis of soil CO_2 can provide information on the age, source and

7   turnover rate of soil organic C. We developed a new method for passively trapping

8   respired CO_2 on molecular sieve, allowing it to be returned to the laboratory and

9   recovered for C isotope analysis. We tested the method on a soil at a grassland site,

10   and using a synthetic soil created to provide a contrasting isotopic signature. As

11   with other passive sampling techniques, a small amount of fractionation of the ^{13}C

12   isotope occurs during sampling, which we have quantified, otherwise the results

13   show that the molecular sieve traps a sufficiently large and representative sample

14   of CO_2 for C isotope analysis. Since ^{14}C results are routinely corrected for mass

15   dependent fractionation, our results show that passive sampling of soil respiration

16   using molecular sieve offers a reliable method to collect soil-respired CO_2 for ^{14}C

17   analysis.

18

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

20

21   **1. Introduction**

22

23   The largest flux of carbon (C) from terrestrial ecosystems is soil respiration

24   (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 (SOM) comes mainly from the decomposition

30   of the typically small pool of C derived from recent plant activity, or from the

31   larger and older soil organic C reservoirs. This must be addressed if we are to

32   predict the effect of global warming on soil organic C stocks and the potential for a

33   positive feedback to climate change. Measurements of the ^{14}C content of soil-

34   respired CO_2 can help us towards 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 SOM has been used to estimate soil C

39   cycling rates using models of C turnover (e.g. Harkness et al., 1986; Harrison et al.,

40   2000; Gaudinski et al., 2000). However, this modelling approach has a number of

41   drawbacks, for example, models assume a uniformly mixed soil and steady-state;

42   assumptions that are unlikely to be true for all soils. An alternative approach for

43   investigating soil organic C turnover is through radiocarbon analysis of soil

44   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 (MSCs) 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 MSC as previously described by Hardie et al. (2005), which was based

90   on a design by Bol and Harkness (1995). This cartridge has been successfully used

91   with a sampling system that incorporated a pump (e.g. Wookey et al., 2002; Billett

92   et al., 2006, 2007). In addition, this sieve cartridge design (utilising the same Type

93   13X molecular sieve) has already been successfully tested (Hardie et al., 2005) for

94   isotopic fractionation and contamination (e.g. memory effects, where small

95   quantities of a sample may remain on the sieve after discharge and therefore

96   contaminate the next sample).

97   To test the use of MSCs for passive sampling we established several

98   experiments, designed to answer the following two questions:

99　　　　　　　　1.　*Is CO_2 passively captured on the MSC at a rate which is*

100　　　*always proportional to the environmental CO_2 concentration?* If CO_2 is

101　　　not captured at a constant rate when the environmental CO_2 concentration

102　　　is constant, then the CO_2 being recovered may not be representative of the

103　　　total sampling period. For example, if the sieve starts to saturate with

104　　　CO_2, trapping rates may decline over time even with no change in

105　　　conditions and therefore the recovered sample may not be representative

106　　　of the total sampling period. Based on Fick's Law, the rate of CO_2 capture

107　　　in a diffusion sampler should be proportional to the CO_2 concentration of

108　　　the environment (see e.g. Bertoni et al., 2004). If this is so, then it should

109　　　be possible to estimate the environment's CO_2 concentration simply from

110　　　the rate of CO_2 trapping (i.e. CO_2 recovered/sampling time), providing

111　　　additional potentially useful information.

112　　　　　　　　2.　*Does the recovered CO_2 have an isotopic (^{13}C and ^{14}C)*

113　　　*composition the same as the environment?* The MSCs have insignificant

114　　　fractionation or memory effects when used for ^{13}C and ^{14}C with the pump-

115　　　based system (Hardie et al., 2005). If fractionation occurs during passive

116　　　sampling, this will only affect ^{13}C results. The MSCs would still be

117　　　suitable for passive collection of ^{14}C samples since ^{14}C results are

118　　　corrected for mass-dependent fractionation (Stuiver and Polach, 1977). If

119　　　fractionation does occur when sampling passively then this may be a

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

121　　　correctable.

122

123　**2. Materials and methods**

124

125   *2.1. Site and soil information*

126

127   We tested passive sampling of soil respiration using MSCs on two different

128   soils at contrasting times of year (see Table 1 for soil characteristics). Firstly, we

129   sampled from a grassland with a non calcareous surface-water gley soil during the

130   summer (2007) when respiration rates were expected to be at their maximum. The

131   grassland was located in a suburban area to the south of Glasgow, UK (55°46'N,

132   4°18'W) and most abundant plant species were: *Lolium perenne*, *Holcus lanatus*,

133   *Cynosurus cristatus*, *Anthoxanthum odoratum*, *Ranunculus repens*, *Veronica*

134   *chamaedrys* and *Trifolium repens*. The site has been a grassland lawn for several

135   decades at least, and it is extremely unlikely that it has ever contained any C4

136   plants. From previous results (e.g. Hahn et al., 2006), we expected that the CO_2

137   respired from the grassland would have a similar ^{14}C content to the contemporary

138   atmosphere. Therefore, for a contrast and thus to provide a more robust test of the

139   method, we created a synthetic soil for the second study in order to generate CO_2

140   that was much more ^{14}C-depleted than the contemporary atmosphere. This

141   synthetic soil was a mixture of approximately equal masses of compost (composed

142   predominantly of garden peat, but pre-mixed with lime $CaCO_3$; Homebase, UK)

143   and sand, and was placed in a large (30 x 40 x 25 cm deep) open-top container at

144   the same grassland field site. Sampling of the soil respiration from the synthetic

145   soil was performed from December 2007 to January 2008 (winter), when

146   respiration rates were expected to be at their annual minima. In addition, we

147   performed a further experiment to assess the CO_2 capacity of the MSCs when used

148   passively by exposing two further sieves at the grassland site for an extended

149   duration during the summer.

150

151   *2.2. Sieve design and sampling procedure*

152

153   The design of the molecular sieve cartridge (Fig. 1) has previously been

154   described by Hardie et al. (2005). It was constructed from quartz glass tube with a

155   central chamber (dimensions 11 mm ID, 70 mm length) filled with ~ 3-4 g of 13X

156   zeolite molecular sieve (1/16" pellets, BDH, UK). The tubing at either end of the

157   cartridge was slightly narrower than the central chamber (4 mm ID, 100 mm length

158   and 8 mm ID, 100 mm length) which, together with quartz wool, held the

159   molecular sieve in place. At either end of the MSC a short length of PVC tubing

160   (Tygon, Fisher Scientific, UK) was attached and into this an auto-shutoff Quick

161   Coupling[TM] (Colder Products Company, USA) was inserted; the couplings allow

162   minimal contamination from the atmosphere when attaching to other equipment

163   (e.g. the respiration chamber). Although the couplings automatically close when

164   detached, WeLoc[©] clips (Scandinavia Direct, UK) were also placed over the PVC

165   tubing to provide an additional seal. Prior to sampling all molecular sieve

166   cartridges were charged by heating (500°C) for 1.5 hours while attached to a

167   vacuum rig (see Hardie et al. (2005) for details) and subsequently filled with high

168   purity N_2.

169   A single respiration chamber (dimensions 10.4 cm diameter, 14.0 cm

170   length, volume 1190 ml) was constructed from PVC pipe which was open at the

171   base (for contact with the soil) and closed at the top with a rubber seal (Fig. 1).

172   Three couplings were inserted into the side of the chamber to which MSCs could

173 be attached. Therefore at any one time, three MSCs could be used to collect

174 passively CO_2 from the headspace of the chamber. Inside the chamber a

175 hydrophobic filter (Accurel PP V8/2 HF, Membrana GmbH, Germany) was

176 attached to the couplings which allowed gas exchange between the inside of the

177 chamber and the MSCs, but prevented liquid water from entering the MSCs.

178   The chamber was inserted to a depth of ~ 4 cm in both the grassland and

179 synthetic soils (vegetation had previously been removed from the grassland soil

180 one month earlier, and no vegetation was present in the synthetic soil). Prior to

181 attaching the MSCs, the atmospheric CO_2 inside the chamber had been removed

182 using a soda-lime based scrubbing system (Hardie et al., 2005) and left several

183 days for CO_2 to accumulate.

184   An experimental design based on that of Bertoni et al. (2004) was adopted

185 (Fig. 2). The design utilises three sieves to sample simultaneously from the same

186 chamber and was chosen as it allows a test of the sampling method even if there

187 are variations in chamber CO_2 concentration or isotopic signature over time (which

188 is likely to be the case). If changes in the chamber CO_2 occur, this should be

189 reflected in short period samples. However, the combined results from adjacent

190 short period samples should still be identical to the results for longer term samples

191 that were trapping CO_2 over the same period. For example, the volume of CO_2

192 recovered from samples S1 + S2 should be the same as from the single M1 sample

193 etc (Fig. 2). The principle applies similarly for the isotope results, although the

194 values are averaged (weighted by recovered CO_2 volume) rather than summed.

195 This sampling design therefore tests whether the MSCs trap representative samples

196 at different lengths of exposure.

197   To begin sampling, each cartridge was attached to the respiration chamber

198   with the coupling and then the clip nearest the chamber removed, allowing

199   chamber air into the MSC via the hydrophobic filter. The sieve cartridge was

200   protected by covering with a short length of pipe insulation. At the end of the

201   sampling period (which ranged from 2 to 56 d) the clip was simply replaced on the

202   MSC and the cartridge uncoupled from the chamber.

203   To test whether the sieves were collecting samples which were

204   representative of the headspace CO_2, in addition to the molecular sieve samples,

205   we collected CO_2 from the chamber using evacuated flasks (volume 65 ml). These

206   flask samples were collected at the start, middle and end of the experiments, and

207   were sampled by simply attaching the flask to the chamber using couplings and

208   leaving to equilibrate for 1 hour.

209

210   *2.3. Gas collection and isotope analysis*

211

212   On return to the laboratory, the CO_2 trapped on the molecular sieves was

213   recovered by heating ($500^{o}C$) while attached to a vacuum rig. The gas evolved

214   from the sieve was dried in a slush trap (mixture of dry ice and industrial

215   methylated spirits; $-78^{o}C$) and the CO_2 recovered using liquid N_2 (see Hardie et al.

216   (2005) for further details). The volume of the recovered CO_2 was measured and

217   divided into sub-samples for ^{13}C and ^{14}C measurement. Measurement of $\delta^{13}C$

218   ($^{13}C/^{12}C$ ratio in ‰ units relative to the standard Vienna Pee Dee Belemnite;

219   VPDB) was performed on a dual inlet isotope ratio mass spectrometer (VG

220   Optima, Micromass, UK). The ^{14}C sub-sample of CO_2 was reduced to graphite

221   using Fe/Zn reduction (Slota et al., 1987) and analysed by AMS at the Scottish

222 Universities Environmental Research Centre (SUERC), East Kilbride, UK

223 (Freeman et al., 2007). Following Stuiver and Polach (1977), ^{14}C results were

224 normalised to a $\delta^{13}C$ of -25‰ and expressed as %modern and conventional

225 radiocarbon ages (BP; i.e. relative to AD 1950). Following convention,

226 measurement uncertainties for isotope concentrations are expressed as standard

227 deviations. The 65 ml flasks were also returned to the laboratory, the CO_2

228 cryogenically recovered on a vacuum rig as described above, and the $\delta^{13}C$

229 measured. ^{14}C content was measured for one flask sample (F2 in the grassland

230 experiment).

231

232 **3. Results**

233

234 *3.1. Grassland soil*

235

236 　　　The sums of CO_2 recovered from the short-, medium- and long-period

237 MSCs were each very similar (Table 2). There was a highly significant correlation

238 ($P<0.001$) between CO_2 recovered from the sieve cartridges and sampling time,

239 which was linear even when the incubation was extended to 28 days, by which

240 time the MSCs had trapped >100 ml of CO_2 (Fig. 3). This suggests that the sieves

241 were collecting representative samples of the respired CO_2 independent of the

242 duration of the incubation.

243 　　　The $\delta^{13}C$ values were unaffected by multiple versus single MSC samplings

244 (Table 2). The results from all values when averaged for the full 8 days of the main

245 experiment were all identical to within 2 σ; this is despite the fact that the $\delta^{13}C$ of

246 the recovered CO_2 ranged from -30.4 to -29.5‰ over the course of the experiment,

247   as indicated by the results for the short period samples. The $\delta^{13}C$ values from the

248   MSCs are, however, more depleted than samples collected from the same chamber

249   using evacuated flasks.

250   There was a slight difference in the ^{14}C content of the two medium period

251   samples (although only significant at 1 σ), perhaps indicating a slight change in the

252   mean age of the respiration during the course of the experiment (Table 3).

253   Importantly, the average of the two medium period samples was not significantly

254   different (<1 σ) to the result for the long period sample. In addition, all samples

255   collected using the passive molecular sieve method had ^{14}C contents that did not

256   differ (<1 σ) from the evacuated flask sample when measurement uncertainty was

257   considered (Table 3).

258

259   *3.2. Synthetic soil*

260

261   The $\delta^{13}C$ value of the CO_2 recovered from the molecular sieve varied

262   during the experiment from -11.8 to -14.0‰ (Table 4). A similar range of variation

263   in chamber CO_2 $\delta^{13}C$ occurred for the flask samples (range -8.7 to -10.9‰; Table

264   4). Importantly, however, when the results from the MSCs for the short sampling

265   periods were combined the results were never significantly different (<2 σ) to the

266   longer period samples collected at the same time (Table 4). For example, the

267   average $\delta^{13}C$ value of the first two short period samples (S1 and S2) was -12.4‰,

268   whereas the first medium period sample (M1) had a $\delta^{13}C$ value of -12.5‰.

269   Similarly, combining the two medium period samples (M1 and M2) resulted in a

270    $\delta^{13}C$ value (-13.1‰) that was nearly identical to the long period sample (L; -

271   13.0‰).

272   However, unlike in the results from the grassland, combining the volumes

273   of CO_2 recovered from short period samples did not in all cases result in the

274   expected values based on the longer period results. For example, the total volume

275   of CO_2 recovered in S1 and S2 was 14.0 ml, whereas the equivalent longer period

276   sample (M1) had a CO_2 volume of 21.2 ml. A similar situation was apparent in

277   samples from the second half of the experiment where the total volume recovered

278   from samples S3 and S4 (11.7 ml) was substantially lower than the M2 sample

279   (15.3 ml).

280   Inspection revealed that two of the MSCs used to collect samples S1 and S4

281   from the synthetic soil were slightly different compared to the other MSCs. These

282   two cartridges were made from glass tubing with a narrower inner diameter than

283   the other MSCs (2 mm diameter compared to 4 mm for the other MSCs) at the end

284   that was connected to the respiration chamber. Since the two samples collected

285   using these cartridges recovered less CO_2 than expected, we performed an

286   additional experiment to test whether the inner diameter of this tube affected the

287   rate of CO_2 trapping. Soil CO_2 was passively collected from the grassland site

288   using three pairs of identical MSCs except for the inner diameter of the tube that

289   connected the MSC to the respiration chamber. The results show that the inner

290   diameter of the tube between the respiration chamber and molecular sieve strongly

291   affected CO_2 trapping rate (Fig. 4).

292   From the results of the experiment comparing MSCs with sampling tubes

293   of different inner diameter we were able to derive a factor (equation 1) to correct

294   the results for the synthetic soil experiment which had been collected using non-

295   standard MSCs (S1 and S4; Table 4).

296

297   Correction factor =

298   CO_2 trap rate (4 mm tubing) / CO_2 trap rate (2 mm tubing)   (equation 1)

299

300   Thus the CO_2 volumes recovered using non-standard MSCs (2 mm ID at sampling

301   end) were corrected by multiplying by the correction factor (1.880). This

302   correction resulted in a closer agreement between the sum of the recovered

303   volumes of CO_2 for short period samples and the corresponding medium period

304   sample. The correction made little difference to the weighted average $\delta^{13}C$ results –

305   these all remained within measurement error (<2 σ).

306   The volume of CO_2 recovered was highly correlated ($P<0.001$) with

307   sampling time for the synthetic soil (Fig. 3) even before applying the correction

308   factor. However, the R^2 value was less than had been calculated for the field test

309   results, and the value only slightly improved after correcting for differences in the

310   tube size of MSCs. The poorer correlation may simply result from greater variation

311   in the CO_2 concentration of the chamber during the experiment with synthetic soil

312   (as observed in flask samples), which was performed over a much greater period of

313   time compared to the field test.

314   The respired CO_2 collected using MSCs from the synthetic soil had a

315   considerably lower ^{14}C content compared to the contemporary atmosphere

316   (expressed as conventional radiocarbon ages, the results range from 6965 to 8542

317   years BP). The ^{14}C content of the chamber CO_2 varied considerably over the course

318   of the experiment (Table 5), with the result for the first half of the experiment (M1)

319   being considerably ^{14}C-depleted compared to the second half (M2). Despite this,

320   the combined result from the two medium period samples (37.67 %modern) was

321   within the measurement error of the long period sample (37.76±0.64 %modern).

322

4. Discussion

323

324

325 Models utilizing the radiocarbon content of SOM have frequently been

326 used to provide valuable information on soil organic C cycling (e.g. Harkness et

327 al., 1986; Harrison et al., 2000; Gaudinski et al., 2000). However, these models

328 generally rely on several assumptions which are unlikely to be true for all soils.

329 Radiocarbon analysis of soil respiration avoids the need for these assumptions and

330 provides a direct measurement of the mean age of soil-respired CO_2, therefore

331 providing more detailed information on the rate of C cycling in soil, and allowing

332 prediction of 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   ($\sim$ 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 $\sim$ 40,000 ppm, only 2 days were required to trap $\sim$ 7 ml CO_2, whereas

352   for the synthetic soil experiment, where concentrations were $\sim$ 10,000 ppm, similar

353   volumes required $\sim$ 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 $\pm$ 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 $3.8 \pm 0.4‰$ (grassland soil) and $3.2 \pm 0.8‰$ (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 $\sim$ 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 $\sim$ 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   It should be noted that this passive sampling technique for trapping soil

439   respired CO_2 may not be appropriate for all situations. For example, if soil CO_2

440   concentrations are extremely low, then chambers may need to be in place for

441   considerable lengths of time (e.g. months) in order to provide sufficient CO_2 for

442   analysis, which may not be practical. As with all chambers, some characteristics of

443   a soil may be affected by the installation of the chamber itself (e.g. perturbations to

444   soil CO_2 gradients), issues that may be more important with this passive sampling

445   method due to the extended time that a chamber may need to be in place.

446   Our aim was to test the sampling method, and in that context the

447   interpretation of the isotope results from the experiments is of lesser importance.

448   However, it is interesting to note that in the field experiment, the ^{14}C results were

449   close but slightly enriched, relative to the contemporary atmospheric ^{14}C value

450   (Levin et al., 2008) indicating, as expected, that most soil respiration from this

451   grassland soil was fixed within the last few years. In the synthetic soil experiment,

452   the low ^{14}C concentrations (equivalent to greater than 6000 years old) most likely

453   imply that CO_2 was derived not only from the organic component (peat) of the

454   compost, but also from the small amount of carbonate it contained. Further support

455   for a contribution from the carbonate is provided by the $\delta^{13}C$ of the CO_2 which was

456   very enriched in ^{13}C compared to the bulk soil (Table 1), suggesting a contribution

457   from a ^{13}C-enriched source such as carbonate. An atmospheric contribution ($\delta^{13}C$ =

458   -8.5‰; Hemming et al., 2005) would also increase the $\delta^{13}C$ of chamber CO_2, but

459   would need to be a major component of the chamber CO_2 to explain the $\delta^{13}C$

460   values, which would be inconsistent with the depleted ^{14}C of the chamber CO_2.

461   The increase in ^{14}C content between the first and second medium period samples

462   could potentially be explained by an increase in the atmospheric CO_2 component

463   (to ~ 11% of the CO_2 sampled). However, this explanation is unlikely since we

464   estimated a maximum atmospheric component of 6% based on the chamber CO_2

465   concentration (see above), and because the decreased ^{13}C content observed in the

466   second half of the experiment suggests a more ^{13}C-depleted source than

467   atmospheric CO_2.

468   In summary, from the results of this first test of the use of a molecular sieve

469   method to sample soil-respired CO_2 passively, we conclude that:

470   1.   The MSCs passively trapped CO_2 consistently over time and

471   collected representative samples.

472   2.   Used passively, the MSCs collected representative samples

473   up to at least 100 ml CO_2, therefore implying that the method could be used

474   for a large range of conditions – e.g. for a range of sampling timescales or

475   in situations where the chamber CO_2 concentration (which is a major

476   control on CO_2 trapping rate) is unknown.

477   3.   While we would recommend the MSC design we employed

478   for use over a wide range of conditions, modifications to the dimensions of

479   the cartridge could be used to alter trap rates to suit particular sampling

480   needs. For example, increasing the inner diameter of the sampling tube or

481   reducing the path from the chamber to the zeolite would both increase the

482   CO_2 trap rate, which may be advantageous if chamber CO_2 concentrations

483   are particularly low.

484   4.   As with other passive sampling techniques, isotopic

485   fractionation (~ 3-4‰) occurred during trapping when using the MSCs

486   passively, and future investigations aim to reduce the uncertainty in the

487   required adjustment factor.

488   5.   Passive collection of CO_2 using the MSCs provides an easy

489   and inexpensive method to reliably collect samples of soil-respired CO_2 for

490   [14]C analysis. As fossil fuel-derived CO_2 is "[14]C dead" we also suggest that

491   this technique could be used to measure leakage from industrial carbon

492   capture and storage ventures.

493   6.   Further tests of passive sampling using MSCs under a wider

494   range of conditions are being performed; preliminary results from sampling

495   atmospheric CO_2 confirm the relationship between trap rate and CO_2

496   concentration, and suggest a similar ^{13}C fractionation ($\sim 4‰$) during

497   trapping.

498

499   **Acknowledgements**

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508   **References**

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581

582   **Tables**

583

584   Table 1

585   Characteristics of soils used in the experiments.

586

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

587

588

Table 2

590 Volume (ml) and $\delta^{13}C$ (in brackets; ‰) of respired CO_2 collected by passive

591 trapping on molecular sieve from a grassland soil. Samples were collected for

592 different exposure times: short (S; 2 d), medium (M; 4 d) and long (L; 8 d).

593 Sampling was concurrent so that sieve cartridges experienced the same

594 conditions, and therefore, where results from short period samples have been

595 combined they should be identical to the results for the corresponding longer

596 period sample (see Fig. 2 and text for further explanation). $\delta^{13}C$ results for

597 evacuated flask samples (F) collected at the start, middle and end of the

598 experiment are shown; mean CO_2 concentration was ~ 40,000 ppm. $\delta^{13}C$ values

599 ±0.1‰ (1 σ).

600

601

	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)

602

603

604

605   Table 3

606   Radiocarbon concentration (%modern) of respired CO_2 from a grassland soil.

607   Samples taken by passive trapping on molecular sieve were collected for

608   different exposure times: medium (M; 4 d) and long (L; 8 d). The evacuated

609   flask sample (F2) was collected over a period of 1 hour during the middle of

610   the experiment. Radiocarbon publication codes given in brackets.

611

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

612

613

614   Table 4

615   Volume (ml) and $\delta^{13}C$ (in brackets; ‰) of respired CO_2 collected by passive

616   trapping on molecular sieve from a synthetic soil. Samples were collected for

617   different exposure times: short (S; 14 d), medium (M; 28 d) and long (L; 56 d).

618   Sampling was concurrent so that sieve cartridges experienced the same

619   conditions, and therefore, where results from short period samples have been

620   combined they should be identical to the results for the corresponding longer

621   period sample (see Fig. 2 and text for further explanation). $\delta^{13}C$ results for

622   evacuated flask samples (F) collected at the start, middle and end of the

623   experiment are shown; CO_2 concentration was 15800 ppm (Day 1), 7700 ppm

624   (Day 28) and 7000 ppm (Day 56). Superscript (^{N}) indicates if sample was

625   collected using a narrow tube MSC; results for S^C are corrected for tube size

626   (see text). $\delta^{13}C$ values ±0.1‰ (1 σ).

627

	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)

628

629

630   Table 5

631   Radiocarbon concentration (%modern) of respired CO_2 collected by passive

632   trapping on molecular sieve from a synthetic soil. Samples were collected for

633   different exposure times: medium (M; 28 d) and long (L; 56 d). Radiocarbon

634   publication codes given in brackets.

635

	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)	

636

637

Figure captions

639

640   1.   Schematic diagram showing a molecular sieve cartridge attached to the

641   respiration chamber. The cartridge was composed of quartz glass

642   containing 13X molecular sieve held in place by quartz wool. Quick

643   couplings allowed the cartridge to be easily connected or disconnected from

644   the chamber. A hydrophobic filter (Accurel PP V8/2 HF) was fitted inside

645   the chamber which restricted moisture entering the sieve cartridge, but

646   allowed gas exchange. Three cartridges were attached simultaneously to the

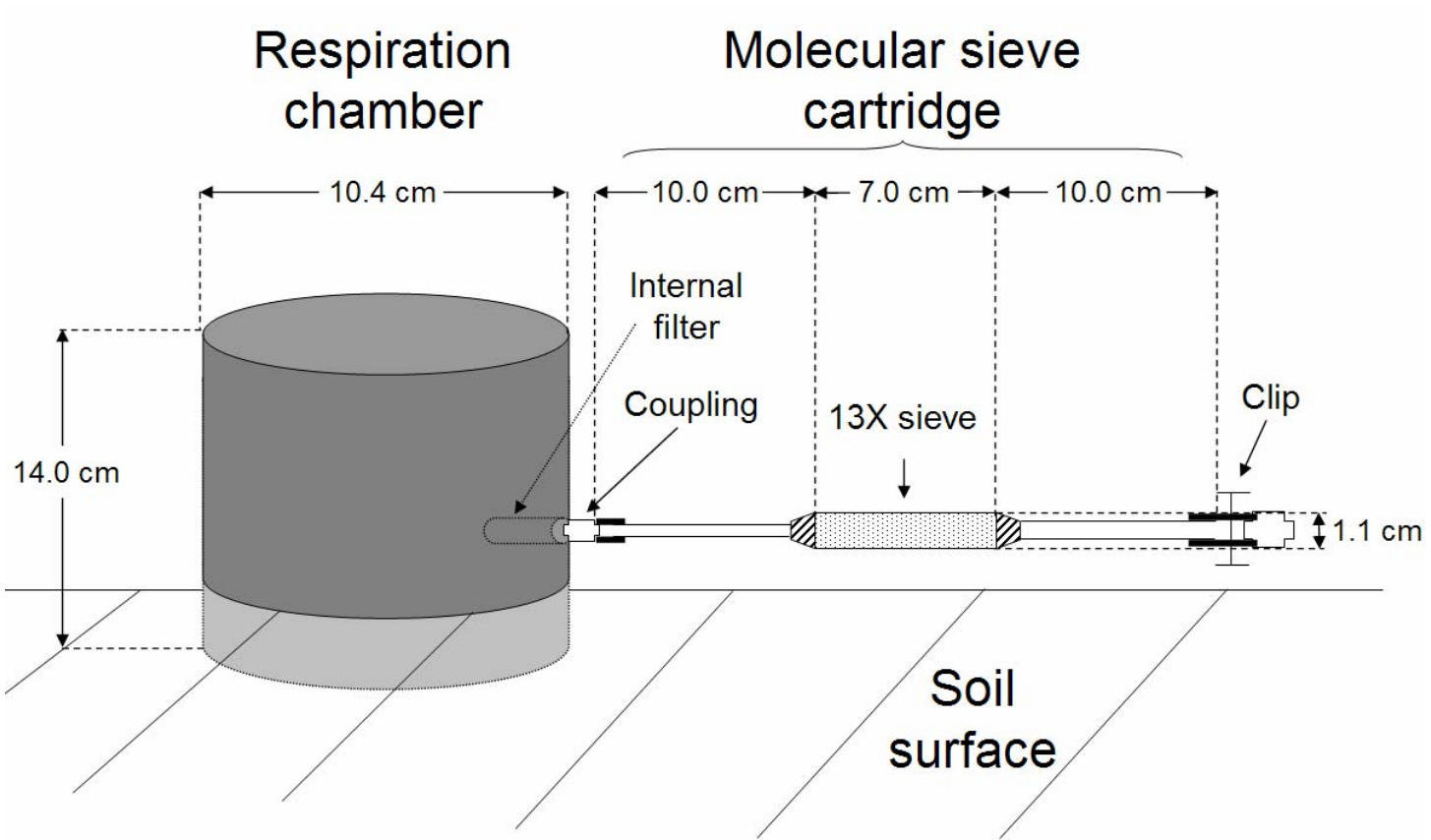647   chamber during the tests.

648

649   2.   Diagram illustrating the sampling design. At any one time, three sieve

650   cartridges were sampling; one from each of the short (S), medium (M) and

651   long (L) sampling periods. This design tests whether the sieve cartridges

652   collect a representative sample of CO_2 for a range of sampling times, even

653   if the concentration and isotopic characteristics of the chamber CO_2 vary;

654   the combined results from shorter period samples should equal the values

655   for the corresponding longer period samples. For example, for volume of

656   CO_2 recovered, S1+S2 should equal M1. Similarly, the average $\delta^{13}C$ value

657   for S1 and S2 should be identical to the result for M1. Samples collected at

658   three time points using evacuated flasks are also shown (F1, F2 and F3).

659   Total duration of the experiments was 8 and 56 d for the grassland and

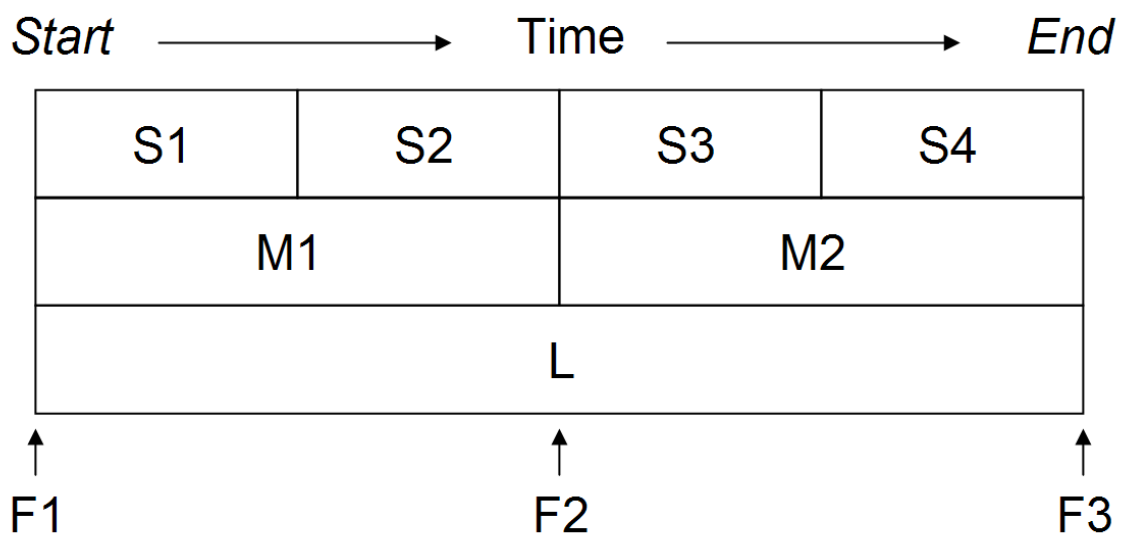660   synthetic soils, respectively.

661

662   3. Volume of CO_2 recovered from each molecular sieve plotted against the

663   length of sampling time. Results for both the field test on the grassland soil

664   and the synthetic soil are shown. Both correlations were highly significant

665   ($P<0.001$). Results for the synthetic soil experiment are not corrected for

666   tube size.

667

668   4. Rate of passive CO_2 trapping for MSCs connected to the same respiration

669   chamber with sampling tubes of different inner diameters. The chamber had

670   a CO_2 concentration of ~ 50,000 ppm and the sampling time was 2 days.

671   The correlation is highly significant ($P<0.001$).

672

Figure

Figure with axes: y-axis "CO$_2$ recovered (ml)" (0 to 120), x-axis "Exposure time (days)" (0 to 60).

Legend: □ Grassland soil, ▲ Synthetic soil

$y = 3.969x - 1.367$
$R^2 = 0.999$

$y = 0.723x - 3.278$
$R^2 = 0.952$

$y = 1.269x + 0.404$

$R^2 = 0.998$