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REGULAR ARTICLE

Comparing the closed static versus the closed dynamic chamber flux methodology: Implications for soil respiration studies

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Abstract Soil respiration is the largest C-flux component in the terrestrial carbon (C) cycle, yet in many biomes this flux and its environmental responses are still poorly understood. Several methodological techniques exist to measure this flux, but mostly there remain comparability uncertainties. For example, the closed static chamber (CSC) and the closed dynamic chamber (CDC) systems are widely used, but still require a rigorous comparison. A major issue with the CSC approach is the generally long manual gas sampling periods causing a potential underestimation of the calculated fluxes due to an asymptotic increase in headspace CO_2 concentrations. However, shortening the sampling periods of the static chamber approach might provide comparable results to the closed dynamic chamber system. We compared these two different chamber systems using replicated CSC cover boxes and a Li-Cor 8100 CDC system under field conditions, and performed tests on both, mineral and peat soil. Whereas the automated CDC system calculated fluxes during the first two minutes, the CSC approach considered either all seven manual sampling points taken over 75 min, or only the first three sampling points over 15 min. Although flux variation was fairly large, there were considerable and statistically significant differences between the calculated fluxes considering the two chamber systems, yet this depended on soil type and the number of CSC sampling time points. The cover-box approach underestimated the chamber-based fluxes by 30% for combined samples, 21% for mineral and 39% for peat soils when calculated over 75 min but was comparable over the first 15 min. The chamber flux comparison demonstrates that the CSC approach can provide CO_2 flux measurements comparable to the CDC system when sampling at an appropriate initial frequency, preventing flux underestimation due to a build up of CO_2 headspace concentrations.

Keywords Soil respiration · Static chamber · Closed dynamic chamber · Chamber comparison · Gas sampling · CO_2 headspace concentration

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Introduction

Soils form the largest terrestrial carbon pool, approximately 3500 Gt of C (Tarnocai et al. 2009), and contribute the second largest terrestrial efflux of CO_2

to the atmosphere (Raich and Schlesinger, 1992), accounting for approximately 80% of total ecosystem respiration (Goulden et al. 1996; Longdoz et al. 2000). Peatlands in particular store large amounts of this SOC supporting their importance in the global C cycle (Rhodegioro et al. 2009). In recent years soil CO_2 efflux has been the subject of intense investigation because of its potential, and controversial, role in amplifying global warming (e.g. Cox et al. 2000; Giardina and Ryan, 2000). A better understanding on the terrestrial C cycle and its environmental responses is pivotal to more accurate model predictions of terrestrial C sink source relations and atmosphere-plant-soil C cycle feedbacks (Raich et al. 2002). However, current global models do not adequately represent peatland soil C stocks and fluxes and as such predicted potential climate feedbacks are questionable (Heinemeyer et al. 2010). This is largely a result of insufficient or inadequate field data and consequent process level understanding of soil C dynamics. Monitoring soil CO_2 efflux therefore requires adequate inter-comparisons of methodologies and any methodological bias to be overcome.

To date, many methods are being used to measure C fluxes, and their advantages and shortcomings have been discussed (see Liang et al. 2004), in particular emphasising chamber-based comparisons (i.e. Norman et al., 1997; Pumpanen et al. 2004). For example, pressure artefacts (e.g. Davidson et al. 2002) or collar insertion issues (e.g. Heinemeyer et al. 2011) are crucial factors to be considered. Two major approaches are the closed static chamber (CSC) and the closed dynamic chamber (CDC) system. Whereas the former is mostly operated manually with subsequent analysis at a laboratory gas chromatograph (GC) and as such is cheap, the latter is an *in situ* automated system based on a closed loop gas flow connected to an Infrared Gas Analyser (IRGA) and thus more costly. A major issue for any chamber system is the CO_2 headspace increase during the sampling period. This is of particular importance for the CSC with its normally rather long (> 30 min) incubation periods, which tend to result in considerable flux underestimation (e.g. Norman et al. 1997; Rochette et al. 1997) as the chamber CO_2 build up considerably alters the diffusion gradient (e.g. Gao and Yates, 1998). Although the CSC cover-box approach has been compared with the CDC flux approach (see Norman et al. 1997), so far no attempt has been made to assess whether or not the reported underestimation of the CSC approach could be reduced by altering the gas sampling design. The CSC cover-box method is widely used in peatlands or high latitude systems where rates of biological activity can be very low (Bubier et al. 2005; Nykanen et al. 2003; Roehm and Roulet 2003; Waddington and Roulet 2000), reflecting long incubation times needed to capture the small C-fluxes, CO_2 and particularly methane (CH_4). Importantly, both the fluxes have been, and still are, frequently measured as part of the same hourly (or even longer) manual sampling period (e.g. Ward et al. 2007; 2009), applying a mostly linear relationship to the concentration increase over time from which the flux is derived. Whilst this addresses the low CH_4 fluxes it leads to very high accumulation of CO_2 concentrations in the chamber headspace, potentially causing flux reductions as the soil to atmosphere gradient is changed (Davidson et al. 2002). Sometimes measurements include dark plant respiration or net ecosystem exchange (NEE) in the light. Although the appropriateness of the linear relationship assumption has been tested previously for both CO_2 and CH_4 (see Davidson et al. 2002; Forbich et al. 2010), the latter study also relates to the issue of potential flux reductions due to the headspace increase in cover-box systems, but only for CH_4. However, it should be pointed out that gas flux studies commonly justify a reliable CO_2 flux based on high (i.e. > 0.9) R^2 (coefficient of determination) regression values (see Savage et al. 2008), an indicator often used to support fluxes obtained even from long cover box incubation periods. However, this assumption might be a methodological artefact (i.e. limited time points over a long period are likely to result in a smooth linear regression, whereas a higher sample frequency might show a different curve shape, i.e. an asymptotic increase with an initially steeper increase in headspace concentrations) and thus could be misleading.

So far no direct comparison between the two different flux approaches has been made, i.e. specifically comparing fluxes when considering reducing the CSC headspace CO_2 increase through more frequent initial sampling, and there remains the potential for a general underestimation of CO_2 fluxes due to long incubation periods in past and present CSC cover-box flux approaches. Moreover, using the CSC system, the measured C flux and its environmental response might not reflect natural *in situ* flux conditions and as such would not be suitable for underpinning accurate model development and vali-

dation. Here we describe such a comparison of the two soil CO_2 efflux methodologies, the manual CSC cover-box system with subsequent GC analysis versus an automated CDC IRGA system.

Material and methods

The field site and experimental layout

During 26 May 2005 we performed our flux chamber methods comparison in the experimental garden of the Biology Department at the University of York, UK. The ground was bare soil on which were placed eight 20 cm diameter PVC soil collars (Plumb Centre, Wolseley UK, Ripon, UK) glued to a plastic base plate. The eight soil collars (with 2 m distances) were randomly allocated a soil treatment (four replicates each), i.e. either filled with the loamy soil from the garden (see Heinemeyer et al. 2003) or with a peat based soil substrate (John Innes No 1 compost). This setup allowed measurements to be taken from the two different soil types ($n=4$) using either a dynamic chamber-based or a standard cover-box approach; to avoid any sample artefacts (i.e. increased CO_2 headspace during cover-box sampling) the CDC system was monitored first and immediately after each measurement a cover boxes of the CSC was put over the same soil collar. The weather during measurements at mid day was sunny and stable with low wind (< 1 m s^{-1}) and air temperatures ranging between 18 and 20°C.

The flux monitoring systems

The CSC system consisted of a PVC collar lid (20 cm diameter, 35 cm height) and included a 25.5 mm Suba Seal (Scientific Laboratory Supplies, UK) gas sampling port at the top allowing repeated needle insertion. The entire box was covered in a reflective material (aluminium foil) to avoid overheating. The CSC cover-box was attached to the soil collar using an air-tight rubberized band during sampling. The cover-box was put over the soil collar after it was measured by the CDC and left in place for 75 min. Headspace gases were sampled 3, 5, 15, 30, 45, 60 and 75 mins after closure, using a luer lock syringe fitted with a 0.5 mm needle, and transferred into evacuated 3 ml exetainers (Labco Ltd, UK) for storage before laboratory analysis. One additional sample needle was inserted to allow pressure equilibration during sampling. All gas samples were analysed for CO_2 by GC within 2 weeks of collection, on a GC (Perkin Elmer Autosystem XL, UK) with flame ionization detector containing a methanizer. The injector temperature was 150°C, the detector temperature was 350°C, and a 2m Poropak Q 50–80 mesh column in the oven was operated at 40°C. Results were calibrated against certified gas standards comprising 500 μmol mol^{-1} CO_2 (Air Products, UK). Gas fluxes were reported as mg C m^{-2} h^{-1} after adjusting for chamber sampling temperature, headspace volume and soil area in accordance with methods detailed in Holland et al. (1999).

The CDC system was a custom-built multiplexed (University of York, Biology Department, Electronic Workshop) automated soil CO_2 flux system (Li-Cor 8100, Li-Cor, Lincoln, Nebraska, USA; using the improved Li-Cor vent; see http://www.licor.com/env/PDF/8100poster.pdf) for monitoring CO_2 (1 s readings). This system allowed automated hourly measurements from up to 16 long-term chambers (Li-Cor 8100–101) in turn. Consequently, the CO_2 flux rates (μmol CO_2 m^{-2} s^{-1}) can be calculated from the increase in CO_2 concentration over time (here a chamber closure period of 5 min), considering the systems's total air volume and the soil surface area. We performed the calculations in the existing software (i.e. Li-Cor Viewer 1.3.0), defining the volumes (4,503 cm^3), area (283.5 cm^2) and time periods for linear flux calculations (over the first two minutes) which excluded an initial 20 s mixing period. For a comparison with the CSC flux data we converted into mg C m^{-2} hr^{-1}.

Statistical analysis

Statistical analyses were carried out using SPSS (Version 15, SPSS Science, UK). We checked data for normality and homogeneity of variances using the Kolmogorov-Smirnov and Levene's tests, respectively. We then applied a paired t-test (i.e. measured over the same soil collar) to the flux data to determine statistical differences in mean proportional fluxes, considering the two systems and the different soil types (separately or combined). However, to overcome the large flux variations between replicates, we also tested the normalised proportional fluxes (i.e. CDC Li-Cor chamber fluxes=1.0) for each soil collar pair per soil

type (i.e. CSC/CDC flux) and acknowledge this violates the assumption of equal variances.

Results and discussion

Comparing the different flux methods

The weather conditions represented near perfect conditions for the flux comparison (i.e. average air temperatures just below 20°C and low wind speeds, see Materials and Methods), limiting overheating inside the CSC cover-boxes or any pressure effects (Davidson et al. 2002; Massmann et al. 1997) due to wind. However, in our system, the soil collars had a sealed bottom, so any lateral leakage (e.g. due to CO_2 headspace build-up or pressure pumping) was prevented. The CSC CO_2 headspace concentration increase showed a good linear increase over the entire closure times in all replicates with R^2 values of 0.9 or higher (Fig. 1a). This might lead to confirming those fluxes as 'accurate' fluxes based on the high coefficient of determination (i.e. R^2). However, for all collars the calculated slope was considerably higher (by about 5 μmol mol^{-1} per minute) when only the first three sample points were considered for flux calculations (Fig. 1b). Importantly, the R^2 values did not indicate either of the periods as more accurate, and were similar between both calculation periods (Fig. 1; for clarity the regression line is only shown for one replicate). The actual asymptotic CO_2 increase over time was more evident in the CDC concentrations at a one second IRGA measurement interval (Fig. 2); for all CDC flux calculations the individual R^2 values were>0.99 (P<0.001). The mean (± SE) soil CO_2 efflux from all collars measured by the CDC system was 58±12 mg C m^{-2} h^{-1}, an average soil decomposition flux in spring in Northern England.

Crucially, the CSC cover-box fluxes compared well to the CDC Li-Cor fluxes, but only if the initial CSC CO_2 increase (i.e. higher slope) was considered (i.e. excluding the last four gas sample measurements) and there were no significant differences, either for all soil samples (Fig. 3a; P=0.877) or the individual soil types, peat (Fig. 1b; P=0.771) or mineral (Fig. 1c; P=0.632) soil. However, the overall calculated mean CSC flux (including all seven gas samples) was 30%, 39% and 21% lower than the corresponding CDC fluxes, for all (P=0.029), peat (P=0.104) and mineral (P=0.053) soil collars, respectively. As a normalized

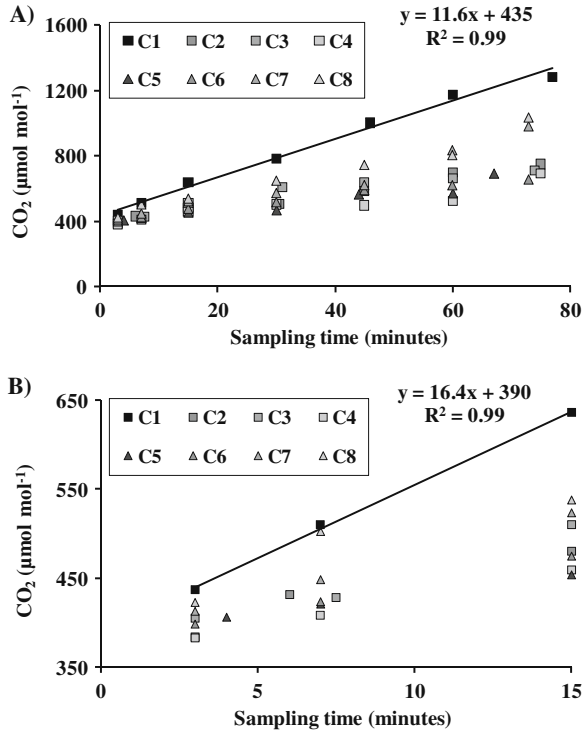

Fig. 1 Closed static chamber (**CSC**) headspace CO_2 concentrations during sampling of the eight individual CSC cover-boxes (**C1–C4**=peat soil; squares versus **C5–C8**=mineral soil; triangles). Whereas figure **a)** displays the CO_2 increase over the entire 75 min, **b)** shows the initial 15 min period. For both periods an example of a linear regression with the equation and associated R^2 (P<0.001) is provided for C1. Note slight time differences were due to the sampling procedure

proportion (comparing corresponding flux pairs per soil collars, see methods) the significant differences were P=0.030, P=0.014 and P=0.091, respectively. Although the statistical significances were only minor, due to the low (n=4) sample number (not dissimilar to most field studies), the overall flux differences were considerable and show the importance of considering carefully the gas sampling design in order to obtain a 'true' flux measurement.

Measurement implications

Previous studies have clearly shown the issue of flux underestimation on the CSC systems (Norman et al. 1997) due to CO_2 chamber headspace increase (Rochette et al), resulting in a non-linear concentration increase (Healy et al. 1996; Gao and Yates 1998). Our study confirms these previous findings but crucially offers a simple solution to this issue, supporting the

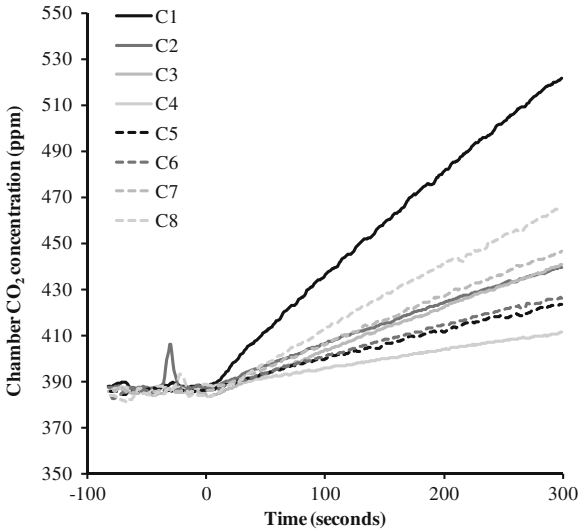

Fig. 2 Closed dynamic chamber (**CDC**) headspace CO_2 concentrations during the automated monitoring of the eight individual CDC Li-Cor chambers (**C1–C4**=peat soil; solid lines versus **C5–C8**=mineral soil; dashed lines). CO_2 concentrations were recorded during chamber closure (80 s) and over 5 min after chamber closure. Flux periods only considered two minutes (20–140 s) after chamber closure (equal to time=0 s)

continued use of CSC systems for obtaining accurate flux measurements compared to CDC systems. Generally, flux calculations for the two different systems provided very comparable data when the initial steeper increase in CO_2 was considered (i.e. during the first 15 min). On the contrary, when not considering the flux reduction over time, calculated CSC fluxes were much lower than those of the CDC fluxes. Although statistically only marginally significant for the combined and the mineral soil type, the overall magnitude of the flux difference of around 30% is considerable. Such an underestimation might not be important when qualitatively comparing 'like for like' treatments (e.g. Ward et al. 2009) but has implications when such data are used by other projects, reviews, and, particularly in meta-analyses and model development or validation. However, ideally even shorter sampling times should be considered (e.g. 10 min) in order to constrain CO_2 head space increase (e.g. around 50 ppm) and reduce the measurement error even further, but we acknowledge this is often impossible to achieve in the field due to man-power and time constraints.

Nevertheless, the observed flux underestimation might be even larger when the observed flux reduction due to collar insertion in most CSC cover-box studies (and the subsequent loss in root-derived fluxes, partic-

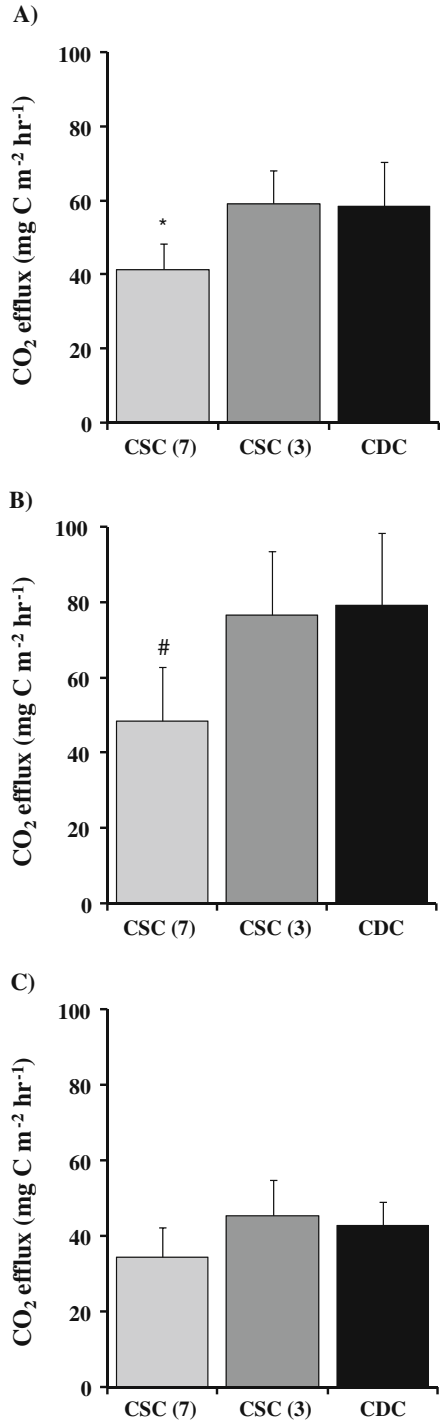

Fig. 3 Mean ± SE CO_2 efflux from the closed static chambers (**CSC**) versus the closed dynamic chambers (**CDC**), considering either using all (**7**) or only the first (**3**) gas samples for the CSC flux calculation: **a**) fluxes for the mean of all peat and mineral soil collars (n=8); **b**) fluxes for the peat collars only (n=4); **c**) fluxes for the mineral collars only (n=4). Indicated are significant differences based on a paired t-test between CSC (7) and CDC fluxes (*=$P <$ 0.05; for normalised fluxes (see text) this is denoted by #)

ularly in peatland studies with shallow root systems and average insertion depth of around 10 cm) is also considered (see Heinemeyer et al. 2011). It would be much better to use markers and revisit sites with collars placed into position only during active measurement periods. However, a seal between the CSC cover box and the soil surface needs to be maintained as otherwise lateral leakage might affect measured fluxes; sand or other sealants might then be needed to assure a seal (Heinemeyer et al. 2011). Such leakage issues depend on the porosity of the soil and its moisture content; any porosity ('leakage') related underestimation in the CSC method decreases during high soil moisture contents compared to dry conditions, particularly in peat soil, which would lead to false interpretations on the moisture effects on soil respiration. Conversely, any apparent increase in soil CO_2 efflux during high soil moisture would be the result of smaller leakage from the chamber rather than a biotic effect. A further complication of collar insertion related soil moisture changes is prevalent in peatlands, where collar insertion is known to prevent lateral drainage (Heinemeyer et al. 2011) with subsequent moisture effects on respiration from roots and decomposition. Consequently, CSC cover-box studies can provide reliable estimates of soil CO_2 efflux but only if adequate sampling is considered, avoiding flux limiting chamber headspace CO_2 concentration increases, avoiding cutting of roots and altering soil moisture due to collar insertion.

Conclusions

While new automated and dynamic systems are rapidly evolving we anticipate that CSC systems are still likely to be widely utilised as a means to determine soil CO_2 fluxes; for example, in developing countries where resources are limited, or in remote ecosystems with no easy access to electricity. Our methodological comparison revealed that although there are potential shortfalls in the CSC cover-box approach (i.e. asymptotic headspace increase) and consequent flux underestimations of around 30%, it can provide a cheap (e.g. one cover-box~£50) and reliable alternative to the relatively costly (e.g. one Li-Cor chamber~£5,000) CDC approach. This requires considering more frequent gas sampling during the initial closure time to prevent changing the soil-atmosphere diffusion gradient; for CO_2 fluxes in particular the increase in the cover-box headspace should be less than 100 μmol mol^{-1}, ideally even lower. Furthermore, if both, CO_2 and CH_4 fluxes are to be measured from one CSC then the gas sampling needs to account for the initially faster CO_2 headspace concentration increase. This study clearly shows that the R^2 value of the linear regression alone is not adequate enough to justify reliable cover-box fluxes. However, this could also apply to other trace gases such as CH_4, and short measurements are now possible with the available high precision instrumentation (e.g. Los Gatos Research, Inc. or Picarro, Inc.). Previous CSC fluxes based on the linear relationship over long time periods with high CO_2 headspace build-up, although possibly comparable in relative terms within a measurement or treatment campaign, will have to be considered carefully and might have to be corrected to enable a calculation of a 'true' initial flux for subsequent comparison studies or model development projects. Although, to derive any such correction term is difficult as it depends on the soil conditions (e.g. porosity) and specific circumstances (e.g. headspace concentration increase). In conclusion, flux rates tend to be underestimated with the CSC cover-box method but this error can be reduced by taking more frequent initial samples over shorter time periods.

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