**Vegetation matters: Correcting chamber carbon flux measurements using plant volumes**

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**Abstract**

Chamber carbon flux measurements are routinely used to assess ecosystem carbon sink/source dynamics. Often these point measurements enclose considerable vegetation biomass, with fluxes upscaled in space and time for each vegetation type. Here we assess the importance of including the volume of peatland dwarf shrub vegetation in chamber flux calculations and outline a simple but effective method of assessing plant volumes. We show that inclusion of plant volumes significantly affects fluxes and that this effect becomes greater as the proportion of chamber volume occupied by plants increases. Moreover, we demonstrate that, with an initial destructive laboratory assessment for each plant species and a little practice at volume estimation, plant volumes can be accurately assessed non-destructively in the field.

**Keywords**

Net ecosystem exchange; vegetation volume; chamber flux measurements; *Calluna vulgaris*; peatlands.

Globally, atmospheric carbon dioxide (CO2) levels are rising; this exacerbates climate change and may lead to further CO2 release (IPCC, 2014). Identifying ecosystems acting as either carbon (C) sinks (net CO2 uptake) or C sources (net CO2 release) requires accurate measurement of the net ecosystem exchange (NEE) CO2 flux, which is the net balance between CO2 uptake by photosynthesis and CO2 losses from respiration by plants and soil (Chapin et al., 2006; Livingston and Hutchinson, 1995).

Peatlands represent a vast C store and, where intact and fully functioning, act as substantial C sinks (Bain et al., 2011). NEE based C accumulation measurements on northern hemisphere peatlands encompass values from -2 g C m-2 y-1 to -136 g C m-2 y-1 (Helfter et al., 2015; Nilsson et al., 2008; Roulet et al., 2007). Although the range of these NEE fluxes is large, the values represent a substantial C sink strength and demonstrate the potential of peatlands to mitigate rising atmospheric CO2 concentrations, and hence regulate climate (Billett et al., 2010). This potential does however need balancing against the quantity of CH4, a much more potent greenhouse gas than CO2, released by peatlands (Bain et al., 2011).

Whilst NEE can be measured across a large area of peatland using eddy covariance flux towers, peatlands can be very heterogeneous environments, with each vegetation community exhibiting a different C balance (Poyatos et al., 2014). As climate change can alter the composition of vegetation communities (Bragazza et al., 2013), for accurate assessment and upscaling, NEE fluxes should therefore be measured for each community (Fox et al., 2008). This is typically achieved using ground-based chambers placed over the vegetation. As the chamber volume is used in calculating the NEE flux (Holland et al., 1999), large plant volumes reduce the effective chamber volume and may result in inaccurate fluxes. However, plant volumes are rarely, if ever, measured or included in NEE flux calculations.

In this study, plant volumes were estimated in the field and then validated in the laboratory. The aim was to verify whether including plant volumes in NEE flux calculations was necessary and, if so, whether volume estimates made in the field could be used as a proxy for volume measurements, without the need for destructive sampling. As NEE measured in the dark is ecosystem respiration (Reco), Reco measurements were used with NEE measurements (those made in the light) to verify whether including the plant volumes was necessary. Whilst this study only considered NEE and Reco fluxes from peatlands, the methods and findings detailed hereafter are applicable to any ecosystem and vegetation assemblage suitable for chamber measurements, and indeed chamber measurements of fluxes of any gas.

NEE fluxes were measured on three heather (*Calluna vulgaris*) dominated peatlands (Sites 1-3) in northwest England using a circular custom built clear Perspex chamber (Biology Mechanical Workshop, University of York, UK) with an internal diameter of 29.5 cm, a height of 60 cm and a volume of 39.6 L, which was connected to an infrared gas analyser (IRGA; Model 8100, Li-Cor, Lincoln, NE, USA). Each site had 24 permanent plots and fluxes were measured over three consecutive days (one day on each site) in July, October and December 2012. A PAR (photosynthetically active radiation) sensor (QS5 – PAR Quantum Sensor, Delta-T Devices, Cambridge, UK) was positioned free from shadows within a demarcated circle on each plot and the chamber was carefully placed over the sensor and all plants rooted within this area. Wet *Sphagnum* moss was tucked around the base to seal the chamber to the atmosphere, thus avoiding issues related to entrenching soil collars (as demonstrated in Heinemeyer et al., 2011).

The CO2 concentration within the chamber was measured every second (s) for 45-90 s (the shorter times were used under warmer conditions) for periods of varying light conditions. Firstly, measurements were made in more than 90% of the total PAR (light reduction by the Perspex chamber was ~10%; A. Heinemeyer, unpublished data). Without removing the chamber, a shading mesh was placed over it (providing on average 30% of total PAR) and CO2 concentrations recorded for another 45-90 s period. In July and October, a second shading mesh was placed over the first (resulting in 10% of total PAR on average) and another flux period was recorded. For the final flux period, ecosystem respiration was measured by placing a custom made cover (Environment Department, University of York, UK) over the chamber, blocking out all light. The *Sphagnum* moss seal was removed from plots after measurements. The internal chamber temperature increase was less than 3ºC during the light period and did not affect flux rates (i.e. the slope of CO2 increase during the measurement period did not change).

During the October NEE flux measurements, the volume of *Calluna* plants was estimated *in situ* as a percentage of the chamber volume to the nearest 5%. The same two observers estimated volume at all sites by conferring. In March 2013, all plants within the NEE measurement circles were cut at the stem bases, bagged and sealed. Stems were cut so that the *Calluna* from each NEE circle fitted as compactly as possible into its bag. Using tongs, each bag was slowly submerged in a 20 L bucket, which was filled to the brim with water and inside a larger container. The bags were mainly sealed but one corner was left open to allow air to escape as it was forced up by the water pressure. The water displaced by the sample was measured and the bucket refilled. An empty bag was also measured five times in the same manner but using a 1 L beaker. The average of this was subtracted from each sample to give the *Calluna* volume.

LiCor Viewer software (version 1.3) was used to derive the CO2 fluxes from the most linear 30-60 s portion (Li-Cor Biosciences, 2007) of each NEE measurement period under each light condition. The measured *Calluna* volume was subtracted from the chamber volume and all CO2 fluxes were calculated using both this reduced (effective) and the original (uncorrected) chamber volume. All fluxes were expressed in µmol CO2 m-2 s-1 where negative values represent NEE dominated by gross primary productivity (i.e. CO2 uptake via photosynthesis) and positive values represent NEE dominated by ecosystem respiration (i.e. CO2 release). The fluxes were calculated using a linear regression fit (with additional temperature, pressure, chamber volume and soil area adjustments performed by the software). The reduction in the effective chamber volume caused a proportional decrease in the corresponding plant volume corrected flux calculation based on:

Fcorr = Fraw \* Veff/Vraw

Where F is the uncorrected (raw) or the corrected (corr) flux and V is the uncorrected (raw) or the plant volume adjusted effective (eff) chamber volume.

A paired Student’s t-test (using the function “t.test” in the R “stats” package; R Core Team, 2016) was used to determine whether subtracting the plant volume from the chamber volume significantly affected the NEE fluxes. A linear regression model test (employing the function “lm” in the R “stats” package; R Core Team, 2016) was used to determine the relationship between the estimated percentage volume and the measured volume of *Calluna* within the chamber. Separate linear regressions were also used on the same data split by site.

Across the different light conditions, NEE fluxes ranged from -14.48 µmol CO2 m-2 s-1 to 12.67 µmol CO2 m-2 s-1 with a mean of 1.53 µmol CO2 m-2 s-1. The difference between uncorrected fluxes and NEE fluxes corrected for *Calluna* volume ranged from -0.59 µmol CO2 m-2 s-1 to 0.76 µmol CO2 m-2 s-1 with a mean of 0.04 µmol CO2 m-2 s-1. Whilst this appears a relatively small change, the difference between the corrected and uncorrected fluxes was highly significant (t791 = 10.0, p < 0.0001), highlighting the importance of incorporating plant volumes into NEE flux calculations. The larger the NEE fluxes (i.e. the further from zero the fluxes were) and the larger the *Calluna* plants were, the greater the absolute change in NEE fluxes (Figure 1). Therefore, the inclusion of plant volume becomes increasingly important as the proportion of the chamber occupied by vegetation increases, where fluxes from large mature vegetation are compared to fluxes from small or immature vegetation (e.g. as in Quin et al., 2015), and where NEE fluxes are upscaled, as any errors or inaccuracies in flux calculations would also be scaled (Holland et al., 1999).

Although there is a significant difference between the corrected and uncorrected fluxes, the mean of this difference was only 0.04 µmol CO2 m-2 s-1, just 2.6% of the mean of the uncorrected NEE fluxes (1.53 µmol CO2 m-2 s-1). This correction could therefore be construed to be unimportant, particularly given that other measurement errors associated with chamber techniques are likely to be included in these flux calculations. In this study, the IRGA CO2 measurements have an accuracy of 1.5% of the reading with a precision error of well below <1 ppm at 370 ppm for 1 second signal averaging (Li-Cor Biosciences, 2007), which is lower than the average difference between corrected and uncorrected fluxes and lower than the average (±SE) CO2 concentration change for the regression fit, which was lowest for the first shade period with 4.3 ± 0.4 ppm. Additionally the span drift of the IRGA is 0.03%/°C (Li-Cor Biosciences, 2007) and the internal temperature of the chamber was monitored – on no occasion did the temperature increase by more than 3°C during the full light flux period. Moreover, the chamber was vented to equalise the internal pressure, meaning that errors caused by differing wind speeds and vapour pressure should be negligible. Whilst none of these measurement errors exceeds the 2.6% mean difference between the corrected and uncorrected fluxes, some could be substantial errors nonetheless. However, it is crucial to note that these measurement errors represent random uncertainties: not including plant volume represents a well-defined bias where the flux will always be larger if plant volume is ignored (Figure 2; for those few points where there was no flux bias, i.e. the corrected and uncorrected fluxes were the same, this was because these fluxes were incredibly low). Additionally, whilst the average difference between the corrected and uncorrected fluxes is 2.6%, Figure 2 illustrates that ignoring plant volume can result in the fluxes calculated being over 11% more than they should be. On sites where plants occupy greater proportions of chamber volumes, this overestimation will be even more pronounced.

The estimated percentage of chamber volume occupied by *Calluna* ranged from 10% to 65% with a mean of 33%. The percentage of the chamber volume which was actually occupied by *Calluna* ranged from 0.93% (370 cm3) to 6.54% (2590 cm3) with a mean of 2.73% (1081 cm3), approximately 12 times lower than the estimated percentage volume. However, whilst this was an order of magnitude different, the proportionate difference was relatively consistent, with a significant linear relationship between the estimated and measured volumes across all three sites (R2 = 0.60, p < 0.0001; Figure 3). Similarly, there was significant linear relationship at each individual site but the strength of the relationship was weakest at the first site (Site 1; R2 = 0.33, p < 0.0001), stronger at the second (Site 2; R2 = 0.63, p < 0.0001) and strongest at the last (Site 3; R2 = 0.73, p < 0.0001). As there was an attempt to remove any individual bias between observers by allowing them to confer and settle on a consensus value, most likely this strengthening relationship between estimated and measured volume at each site is because the observers became more consistent at estimating volumes with practice. Moreover, there was no verification of the *Calluna* volume estimates between site visits (i.e. all *Calluna* was cut and measured after all volume estimates were made), meaning that this trend was probably due purely to practice and not due to observers altering estimates because they knew previous ones were incorrect. The three sites were measured on three consecutive days, showing a strengthening of the relationship between the estimated and measured volumes over those three days. Therefore, with practice, non-destructive field estimations of *Calluna* plant volumes are likely to be sufficiently accurate to improve NEE flux calculations. However, as the proportionate difference between estimated and observed volumes was considerable and is likely to be different between groups of observers and for different plant species, additional destructive estimates are necessary to establish a robust correction factor.

Here the NEE fluxes only took *Calluna* plant volume into account, despite other plant species being present in most chambers. Across the sites, *Eriophorum angustifolium*, *E. vaginatum*, *Sphagnum* species, berry species (e.g. *Vaccinium myrtillus*), *Hypnum jutlandicum*, other mosses, liverworts, and lichens (*Cladonia* spp.) were present in varying proportions. Whilst these other plants took up substantially less space than the *Calluna* plants, both because they are generally less bulky and because *Calluna* was the dominant species included in the chambers, investigation into the inclusion of these other plant volumes, including mosses, is warranted as inclusion of their volumes could still improve accuracy. As incorporation of *Calluna* volume into flux calculations significantly affected NEE fluxes, the results of this study are applicable to any other ecosystem for which woody dwarf shrubs or larger plants are present during NEE measurements, particularly when comparing chamber fluxes to those from eddy covariance systems and when upscaling fluxes.

**a)**

**b)**

**Figure 1**: The relationship between the difference in uncorrected and corrected NEE fluxes and **a)** the original (uncorrected) NEE fluxes and **b)** the measured volume of *Calluna vulgaris* as a percentage of the volume occupying the 39.6 L NEE measurement chamber for Sites 1, 2 and 3. Note that there are no negative values in b) as the absolute value of the NEE fluxes is used.

**Figure 2:** Relationship between *Calluna* volume and the percentage of the corrected NEE flux that would be calculated if ignoring the *Calluna* volume (i.e. using the uncorrected flux).

**Figure 3**: Relationship between the measured and estimated volume of *Calluna vulgaris* occupying the NEE measurement chamber at Sites 1, 2 and 3. The line shown is the linear regression for all three sites combined.

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**References**

Bain, C., Bonn, A., Stoneman, R., Chapman, S., Coupar, A.M., Evans, M., Gearey, B., Howat, M., Joosten, H., Keenleyside, C., Labadz, J.C., Lindsay, R., Littlewood, N.A., Lunt, P., Miller, C.J., Moxey, A., Orr, H., Reed, M., Smith, P., Swales, V., Thompson, D.B.A., Thompson, P.S., Van de Noort, R., Wilson, J.D., Worrall, F., 2011. IUCN UK Commission of Inquiry on Peatlands. IUCN UK Peatland Programme, Edinburgh.

Billett, M.F., Charman, D.J., Clark, J.M., Evans, C.D., Evans, M.G., Ostle, N.J., Worrall, F., Burden, A., Dinsmore, K.J., Jones, T., McNamara, N.P., Parry, L., Rowson, J.G., Rose, R., 2010. Carbon balance of UK peatlands: current state of knowledge and future research challenges. Climate Research 45, 13–29. doi:10.3354/cr00903

Bragazza, L., Parisod, J., Buttler, A., Bardgett, R.D., 2013. Biogeochemical plant-soil microbe feedback in response to climate warming in peatlands. Nature Climate Change 3, 273–277. doi:10.1038/nclimate1781

Chapin, F.S., Woodwell, G.M., Randerson, J.T., Rastetter, E.B., Lovett, G.M., Baldocchi, D.D., Clark, D.A., Harmon, M.E., Schimel, D.S., Valentini, R., Wirth, C., Aber, J.D., Cole, J.J., Goulden, M.L., Harden, J.W., Heimann, M., Howarth, R.W., Matson, P.A., McGuire, A.D., Melillo, J.M., Mooney, H.A., Neff, J.C., Houghton, R.A., Pace, M.L., Ryan, M.G., Running, S.W., Sala, O.E., Schlesinger, W.H., Schulze, E.-D., 2006. Reconciling Carbon-cycle Concepts, Terminology, and Methods. Ecosystems 9, 1041–1050. doi:10.1007/s10021-005-0105-7

Fox, A.M., Huntley, B., Lloyd, C.R., Williams, M., Baxter, R., 2008. Net ecosystem exchange over heterogeneous Arctic tundra: Scaling between chamber and eddy covariance measurements. Global Biogeochemical Cycles 22, GB2027. doi:10.1029/2007GB003027

Heinemeyer, A., Di Bene, C., Lloyd, A.R., Tortorella, D., Baxter, R., Huntley, B., Gelsomino, A., Ineson, P., 2011. Soil respiration: implications of the plant-soil continuum and respiration chamber collar-insertion depth on measurement and modelling of soil CO2 efflux rates in three ecosystems. European Journal of Soil Science 62, 82–94. doi:10.1111/j.1365-2389.2010.01331.x

Helfter, C., Campbell, C., Dinsmore, K.J., Drewer, J., Coyle, M., Anderson, M., Skiba, U., Nemitz, E., Billett, M.F., Sutton, M.A., 2015. Drivers of long-term variability in CO2 net ecosystem exchange in a temperate peatland. Biogeosciences 12, 1799–1811. doi:10.5194/bg-12-1799-2015

Holland, E.A., Robertson, G.P., Greenberg, J., Groffman, P.M., Boone, R.D., Gosz, J.R., 1999. Soil CO2, N2O, and CH4 exchange, in: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (Eds.), Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, Oxford, pp. 185–201.

IPCC, 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)] (Synthesis report). IPCC, Geneva, Switzerland.

Li-Cor Biosciences, 2007. LI-8100 automated soil CO2 flux system & LI-8150 multiplexer instruction manual.

Livingston, G.P., Hutchinson, G.L., 1995. Enclosure-based measurement of trace gas exchange: applications and sources of error, in: Matson, P.A., Harriss, R.C. (Eds.), Biogenic Trace Gases: Measuring Emissions from Soil and Water. Marston Lindsey Ross International Ltd., Oxford.

Nilsson, M., Sagerfors, J., Buffam, I., Laudon, H., Eriksson, T., Grelle, A., Klemedtsson, L., Weslien, P., Lindroth, A., 2008. Contemporary carbon accumulation in a boreal oligotrophic minerogenic mire – a significant sink after accounting for all C-fluxes. Global Change Biology 14, 2317–2332. doi:10.1111/j.1365-2486.2008.01654.x

Poyatos, R., Heinemeyer, A., Ineson, P., Evans, J.G., Ward, H.C., Huntley, B., Baxter, R., 2014. Environmental and Vegetation Drivers of Seasonal CO2 Fluxes in a Sub-arctic Forest–Mire Ecotone. Ecosystems 17, 377–393. doi:10.1007/s10021-013-9728-2

Quin, S.L.O., Artz, R.R.E., Coupar, A.M., Woodin, S.J., 2015. Calluna vulgaris-dominated upland heathland sequesters more CO₂ annually than grass-dominated upland heathland. The Science of the Total Environment 505, 740–747. doi:10.1016/j.scitotenv.2014.10.037

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Roulet, N.T., Lafleur, P.M., Richard, P.J.H., Moore, T.R., Humphreys, E.R., Bubier, J., 2007. Contemporary carbon balance and late Holocene carbon accumulation in a northern peatland. Global Change Biology 13, 397–411. doi:10.1111/j.1365-2486.2006.01292.x