

Methods for determining peat humification and for quantifying peat bulk density, organic matter and carbon content for palaeostudies of climate and peatland carbon dynamics

F.M. Chambers¹, D.W. Beilman² and Z. Yu³

¹Centre for Environmental Change and Quaternary Research, University of Gloucestershire, UK

²Department of Geography, University of Hawai'i at Mānoa, Honolulu, Hawaii, USA

³Department of Earth and Environmental Sciences, Lehigh University, Bethlehem, Pennsylvania, USA

SUMMARY

Quantitative palaeostudies of climate change and carbon dynamics are reliant on precise and accurate laboratory measurements. Here we present laboratory protocols for the colorimetric determination of peat humification and for bulk density and organic matter content, including an overview of methodological considerations for the quantification of the carbon content of peat.

KEY WORDS: bogs, carbon cycle, colorimetry, laboratory protocol, mires, palaeoclimate.

1. INTRODUCTION

Actively growing peatlands (i.e. bogs and fens, also known as mires) accumulate organic mass, and thereby sequester carbon (C) as the excess of vegetation production over decay. C is taken in by mire plants through the process of photosynthesis from carbon dioxide (CO₂), largely from the atmosphere; but not exclusively, as there is some evidence to suggest fixation of 'old' CO₂ from soil respiration (see Kilian *et al.* 1995 and Pancost *et al.* 2000 for discussion). However, peatlands also release C as a direct result of decay processes. Most of the vegetation decay takes place aerobically in the surface horizons (the acrotelm), and 'peat humification' is often taken to be a measure of this initial plant breakdown and decomposition. However, anaerobic decay also continues, albeit at a much slower rate, at depth in cold, anaerobic horizons (the catotelm; Belyea & Clymo 2001), releasing methane (CH₄). The degree of peat humification influences both the recognisability of the macrofossil components that make up the peat matrix and the total C contained within peat layers; continuing decay also influences CH₄ production.

Peatland C is increasingly recognised as a persistent and dynamic component of the terrestrial biosphere and the global climate system. Its importance stems from (1) the large proportion of the global soil C pool (~1500 Gt; Amundson 2001) that occurs below ground in peatland ecosystems (530–694 Gt; Yu *et al.* 2010); (2) the climate- and land-cover sensitivity of emissions of CH₄ and exchange of CO₂ from about four million km² of northern peatlands (MacDonald *et al.* 2006) and

368,500 km² of tropical peatlands (this is a combined country-based estimate, within previously estimated total ranges of 333,900 to 418,100 km²; CARBOPEAT 2009); and (3) the link to aquatic systems and oceans *via* dissolved C (e.g. Prokushkin *et al.* 2007, Frey & McClelland 2009). A useable assessment of peatlands in the global C cycle requires accurate estimates of pools and fluxes—particularly as peatland ecosystems are included in Earth system models (Frolking *et al.* 2009)—and an understanding of their role in Holocene and glacial C cycles *via* reconstruction of past C accumulation and atmospheric exchange. For proxy palaeoclimate and palaeoenvironment data, determination of peat C is also relevant to the handling and interpretation of C data (e.g. ¹³C signatures of important biomarkers) as well as the corrections applied to humification data.

This article describes methods for determination of peat humification, bulk density and organic matter content of peat. It includes a laboratory protocol for the colorimetric peat humification method, used for proxy-climate studies, and for bulk density and organic matter content; and provides an overview of methodological considerations for the quantification of the C content of peat, particularly for use in palaeostudies.

2. DETERMINATION OF HUMIFICATION

Peat humification, as a proxy or indicator of the degree of breakdown or decomposition of peat, can be assessed in the field or in the laboratory using a range of methods, including measurement of

physical properties such as fibrosity; chemical properties (Klavins *et al.* 2009); and chemical extraction of soluble material (see Blackford & Chambers 1993). In the field, peat humification can be estimated visually from freshly extracted peat, using either a 10-point (von Post & Granlund 1926) or a 5-point (Troels-Smith 1955) scale. This gives a reasonable assessment for rapid description of peat stratigraphy. However, for palaeoclimatic reconstruction, more detailed high-resolution sampling and laboratory analysis is desirable.

A colorimetric method for laboratory determination of peat humification was proposed by Bahnson (1968), and applied to raised bog peat initially by Aaby & Tauber (1975) in Denmark and subsequently widely elsewhere (see De Jong *et al.* 2010 for a recent account). Colorimetric analysis is usually conducted on contiguous samples of 1 cm in raised mire peat, or 0.5 cm in some slow-growing blanket peats (e.g. Chambers 1984). Particular care is recommended for sedge-rich peats, notably *Eriophorum* peats, which are difficult to sample at close intervals, especially when employing a mini-monolith cutter (Cloutman 1987, Amesbury *et al.* 2010), but even when using a sharp knife. In addition, the stark difference between coarse *Eriophorum* fibres and more humified matrix can yield spurious differences between adjacent samples, potentially resulting in unreliable humification data. The rationale for determining peat humification, together with a discussion as to the validity of the resulting data, is given in De Jong *et al.* (2010).

2.1 Microwave system

A revised protocol, based on those by Bahnson (1968), Aaby & Tauber (1975) and Blackford & Chambers (1993), was developed for the Europe-wide ACCROTELM mire-based palaeoclimate project (Chambers 2006), and is shown in Table 1 (note that a colorimeter can be used instead of a spectrophotometer).

Blackford & Chambers (1993) recommended using percentage light transmission values rather than Absorbance (Optical Density) data, which have been used for calculating percentage humification, because ‘percentage humification’, derived by formula (cf. Bahnson 1968) from the Absorbance readings, is a dubious concept, given the uncertainties as to the compounds extracted (see Caseldine *et al.* 2000, Morgan *et al.* 2005). However, practitioners use either or both. Corrections can be made to take account of the proportion of mineral matter in the sample, using loss-on-ignition (LOI) data (Blackford & Chambers 1993, Roos-Barraclough *et al.* 2004, Payne &

Blackford 2008). Note, however, that the original formula quoted by Blackford & Chambers (1993) is for converting percentage humification data rather than percentage light-transmission values, and that the term described as ‘corrected humification value’ by Payne & Blackford (2008) is the corrected light transmission value.

Interpretation of the resulting data can be facilitated by smoothing using a 3-point running mean (moving average), to which further Gaussian smoothing can be applied (see Blackford & Chambers 1995), whilst any notable long-term trend in the data (such as might result from the continuing slow decay of peat in the catotelm) can be removed by detrending. There is, however, often a pronounced trend towards high percentage light transmission (i.e. low peat humification) in the acrotelm; near-surface data may not be directly comparable with those from within the catotelm and may need to be treated or interpreted differently.

2.2 Use of peat humification data

Peat humification data provide an indicator of local bog surface wetness and so have been interpreted (qualitatively) to indicate past changes in regional climate, especially pronounced shifts to climatic wetness, because bog surface wetness is believed to be driven primarily by precipitation reinforced by temperature (Charman *et al.* 2009). If these are the only proxy-climate data available from a peat profile, then it is recommended that radiocarbon dating is focussed on inferred ‘wet shifts’ at which the light-transmission data jump to previously unattained values. These pronounced ‘wet shifts’ often provide the best opportunity for radiocarbon wiggle-match dating (van Geel & Mook 1989) because solar forcing may play a role not only in cosmogenic isotope production (Beer & van Geel 2008), but also in climate changes that are reflected in changes in bog surface wetness (Mauquoy *et al.* 2004).

Owing to reservations expressed by some authors as to the derivation and meaning of peat humification data (e.g. Yeloff & Mauquoy 2006), it is recommended that the technique be used alongside others, such as analysis of plant macrofossils (Quadrat Leaf-count Macrofossil Analysis: QLCMA; Mauquoy *et al.* 2010) or of plant biomarkers in highly humified peats (De Vleeschouwer *et al.* 2010/11) and analysis of testate amoebae (Booth *et al.* 2010), whenever possible (e.g. Hughes *et al.* 2006). When a multiproxy approach is taken, and the resulting data normalised, there can be close correspondence between the proxy-climate indicators, but also periods in which there are differences (see Figure 1).

Table 1. Laboratory protocol for the determination of peat humification (after ACCROTELM humification protocol, available online at <http://www2.glos.ac.uk/accrotelm/humproto.html>).

1. Sample sufficient peat (e.g. for very fibrous and/or very wet peat, use 1 × 1 × 0.5 cm; for less fibrous peat, use less) and place each sample in a separate weighing boat. Cut up the peat if very stringy; otherwise just leave in boat. Dry samples in boats in an oven (~50°C) or under infra-red heat lamps.
2. Prepare 1 litre 8% NaOH solution. To do this, dissolve 80 g NaOH granules of AnalaR (or suprapur) grade in about 500 ml deionised water and make up to one litre.
3. Grind up each peat sample separately in an agate pestle and mortar* and return to its weighing boat.
*Note: clean the pestle and mortar between each grind using dry paper or clean cloth—do not wash.
4. Weigh out 0.2 g of each sample accurately on a top-loading balance (to 3 decimal places)—use a piece of weighing paper and tare weight—then shake into a 150 ml beaker (one beaker per sample). Process a batch of 12 or 16 samples at a time.
5. Turn on hotplate to preheat to 85–100°C.
6. Add 100 ml of 8% NaOH solution to each beaker using a 100 ml measuring cylinder.
7. Place beakers on hotplate and simmer at 95°C. Do not cover or boil fiercely.
8. Top up beakers occasionally with deionised water to prevent drying out and to ensure solution does not become too concentrated.
9. Turn off hotplate after one hour of slow boiling (simmering).
10. Pour contents of each beaker into a separate 200 ml labelled volumetric flask using a separate clean funnel for each sample, and wash all residue into flask with deionised water.
11. Top up flasks (when slightly cooled) to mark; stopper each flask, then shake well.
12. For each batch of samples, place the equivalent number of clean and dry filter funnels in a rack, with a 50 ml labelled volumetric flask stood beneath each funnel. Using Whatman No. 1 grade papers (size 15 cm), filter 50 ml of the contents of each 200 ml flask into the corresponding labelled 50 ml volumetric flask. Use filter paper in a corrugated folding mode to speed filtration. Ensure only 50 ml of filtrate is transferred.
13. Decant filtrate into labelled 100 ml volumetric flask.
14. Turn on spectrophotometer to stabilise. Set to 540 nm.
15. Top up 100 ml flask to mark with distilled water and stopper, then shake well.
16. When all flasks are ready, pipette a small volume from the first one into each of three* cuvettes and, using spectrophotometer (previously allowed to stabilise), measure and record Absorbance and % light transmission of first cuvette.
*Note: alternatively, re-use cuvette twice more for the same sample only.
17. Repeat for the second cuvette (or using the first cuvette a second time) and, if a different reading is obtained, repeat for third cuvette (or time). Usually, the readings will be identical or very close; if they are not, this may be due to insufficient shaking at Stage 15. The two (or three) readings obtained for each peat horizon are averaged.

N.B. DO THESE READINGS IN NUMERICAL FLASK ORDER!

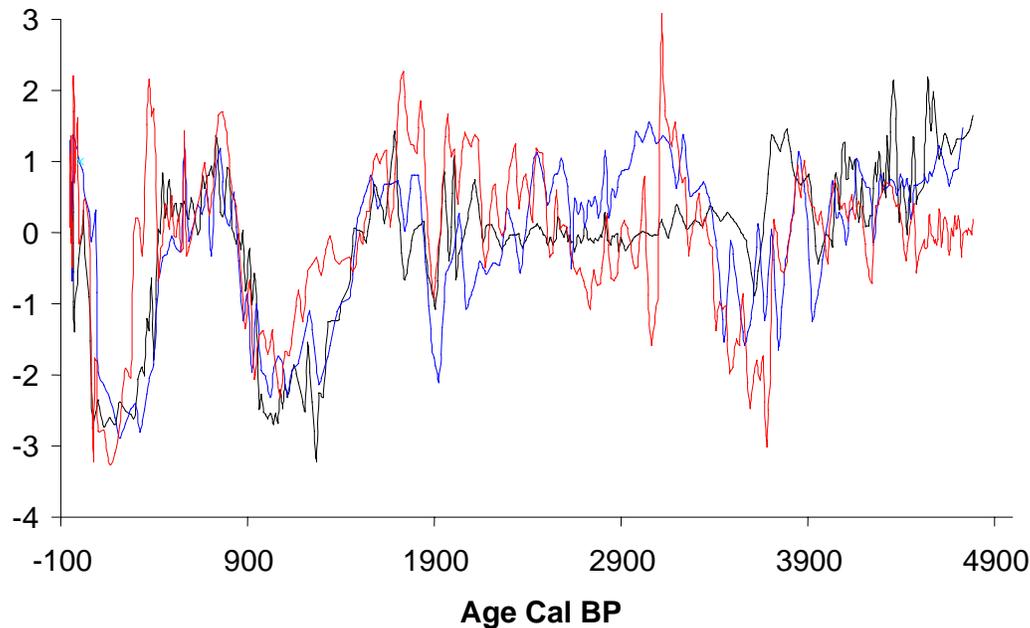


Figure 1. Normalised proxy-climate data, generated during the ACCROTELM project (Chambers 2006), from a core spanning the past five millennia taken from a bog near Ballyduff, central Ireland. Black: plant macrofossil data, in this case using non-metric multidimensional scaling (NMS); blue: estimate of water table depth, based on testate amoebae assemblage data; red: peat humification data. Comparison of the humification, testate amoebae and macrofossil data show a high level of correlation over the last 2,000 years (data courtesy of Professor Fraser Mitchell, ACCROTELM site co-ordinator for Ballyduff). All proxy data are reported as z-scores in which tendencies to wetness are shown by negative shifts in the datasets; positive shifts suggest increasing dryness.

Determination of peat humification therefore provides one putative measure of the climate-influenced breakdown or decomposition of peat. Combined with bulk density and organic matter measurements (see below), these data can be used to interpret past local and regional carbon dynamics in peatlands to put contemporary gas fluxes (e.g. Alm *et al.* 2007) into the context of long-term climate and ecological variation.

3. DETERMINATION OF BULK DENSITY, ORGANIC MATTER AND CARBON

It is clear that the quantification of carbon in peatlands—be it for reconstructing past C-accumulation rates, estimating modern C pools, or quantifying cumulative C over different time periods—requires an accurate determination of peat C content. The total amount of C in peat, like the amount of C in other soils or sediments, is quantified as the product of bulk density (g cm^{-3}) and total C content (gravimetric %) of the

geobiological material. Accurate soil bulk density measurements are crucial for studies requiring the quantification of biogeochemical cycle components, including peatland C palaeostudies. For peat C content, many studies quantify peat organic matter and assume that organic C is about 50% of organic matter by mass. However, the C content of peat organic matter does show variation since it is affected by organic matter quality (e.g. varying amounts of cellulose and lignin-like compounds), and thus ultimately by differences in fossil plant composition (e.g. relative proportions of woody, vascular plant and bryophyte litter inputs) and by changes in organic matter chemistry over time during decomposition.

Down-core differences in organic matter density (i.e. ash-free bulk density) have been used as a simple proxy of the varying degree of total peat decomposition and to infer past surface moisture conditions (e.g. Yu *et al.* 2003). Well-preserved peat tends to be of low organic matter density, and was most likely deposited under productive and/or wet conditions that promote rapid burial of organic

matter. Peat bulk density is variable, but typically 0.05 to 0.2 g cm⁻³ in high-latitude regions (Figure 2) and, it seems, of similar range in tropical peats (e.g. south-east Asia in Page *et al.* 2004, Rieley & Page 2008; Amazonia in Lähteenoja *et al.* 2009), although data from tropical regions are sparse. The organic matter (OM) content of peat is typically estimated gravimetrically by high-temperature loss-on-ignition analysis (e.g. Heiri *et al.* 2001), and represents the material that does not remain as dry ash after combustion (Figure 2). OM content is, of course, high in natural peat-accumulating ecosystems, but can vary substantially as a result of non-organic inputs including exogenous wind-blown and waterborne materials and *in situ* carbonate precipitation in some peatlands. Thus, compared with bulk density, OM density (Figure 3) is more directly related to endogenous ecosystem processes.

Total carbon (TC) in soils and sediments can be measured directly by a number of methods (Nelson & Sommers 1996), but is commonly determined in peat by dry combustion and elemental analysis. Organic carbon (OC) can be measured by dry combustion after removing carbonates by acidification, for example using the acid fumigation method for soils of Harris *et al.* (2001). Inorganic carbon (IC) can be quantified indirectly as the difference between TC and OC (Bisutti *et al.* 2004). An assumption is commonly made that peat contains no IC if it is non-calcareous, particularly under the neutral-acidic conditions typical of many high-latitude and tropical peats.

Ideally, C content is directly measured for all samples. However, many peatland studies, including palaeostudies, estimate peat C indirectly using the relationship between OC and OM content (Ball 1964, Dean 1974). This can reduce some uncertainty since the variation in peat OM content (which is also affected by exogenous inorganic inputs as mentioned above) is greater than the variation in the OC content of OM, and measurement of OM content by loss-on-ignition analysis (Heiri *et al.* 2001, Boyle 2004) is straightforward, fast, and inexpensive. Examples of studies that have directly measured and reported the mean OC content of OM include 51.8% on average by Vitt *et al.* (2000) and 52.6% on average for highly organic peat (>70% OM) by Bauer *et al.* (2006) for high-latitude peat in western Canada. Similarly, the mean OC content of OM for a wide statistical sampling of peat in West Siberia was reported by Beilman *et al.* (2009) to vary between 50.7 and 56.3%, and to be significantly affected by botanical composition (Figure 4). In the absence of direct C measurements in all samples, the measure-

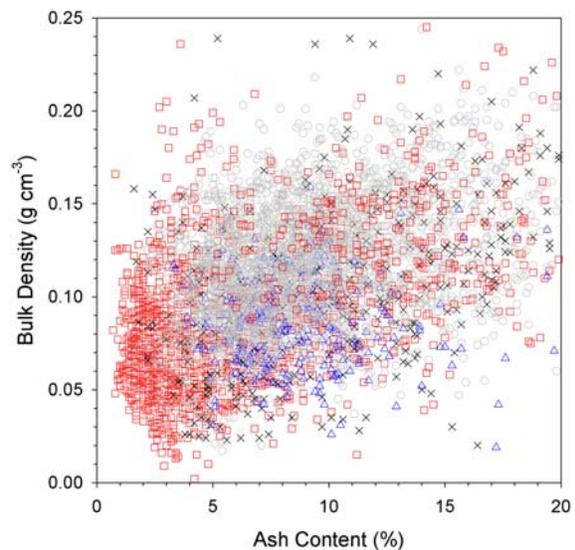


Figure 2. Variation in ash content (non-combustible material at 550°C) and bulk density in a total of 4,923 peat samples of low ash content (<20%) from across western Canada (data from Zoltai *et al.* 2000). Red squares show *Sphagnum* peat, black crosses show forest (sylvic) peat, blue triangles show moss (non-*Sphagnum*) peat and grey circles show sedge peat. Original litter inputs influence peat bulk density and ash content; for example, in western Canada, *Sphagnum*-derived peats are often of the lowest density and lowest ash content (highest organic matter content).

ment of OM in a large number of samples can improve C estimations over using a grand average C content of bulk peat dry mass (e.g. 52% by Gorham 1991 and Clymo *et al.* 1998).

3.1 Peat bulk density and organic matter content

The steps shown in Table 2 can be followed to measure bulk density and estimate organic matter content by loss on ignition (LOI) of small-size (2–10 cm³) peat samples.

3.2 Elemental combustion analysis of peat C

Several systems are available for the autosampling, dry combustion, chromatographic separation and spectroscopic quantification of C against known standards. Each particular system's protocol should be followed for measurement. In general, the accuracy of C analysis can be maximised by packing reactor columns and using reagents specifically for C (and nitrogen) combustion and reduction rather than for measurement of multiple elements (e.g. C, nitrogen, hydrogen, sulphur), but the high C content of peat makes for robust

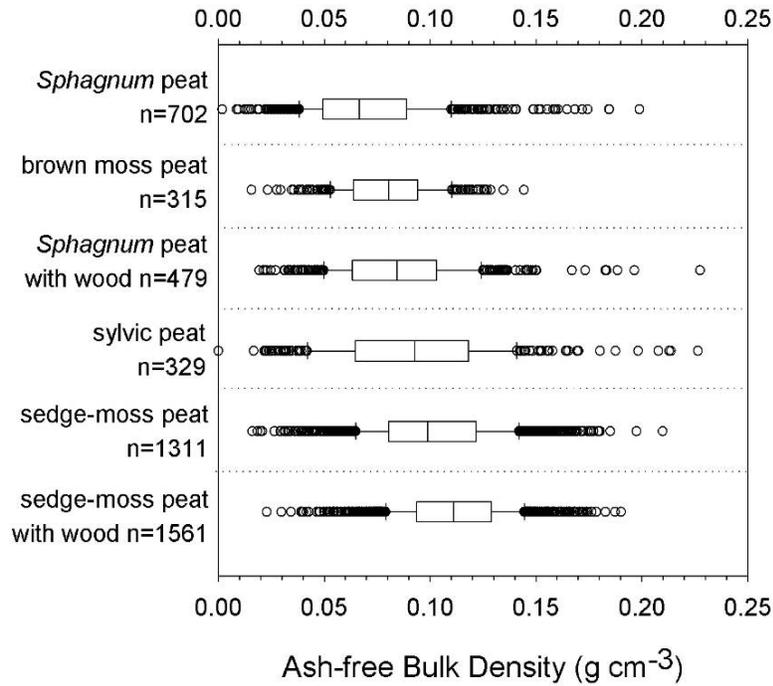


Figure 3. Box plot of ash-free bulk density for different common peat types in western Canada (data from Zoltai *et al.* 2000).

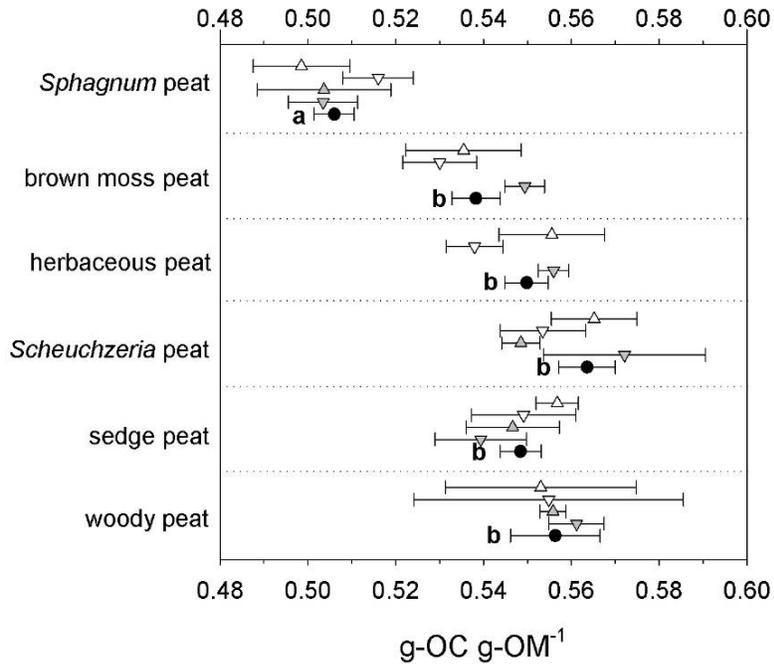


Figure 4. Variation in organic carbon content from 110 statistically-selected peat samples from across the West Siberian Lowland and the influence of peat type (organic carbon data normalised to proportion of organic matter content, i.e. ash-free content; Beilman *et al.* 2009). No significant differences in organic carbon content were found between younger (up triangles) vs. older peat (down triangles) or between northern (open symbols) vs. southern peat (filled symbols); however, mean organic carbon content varies significantly with botanical composition (solid circles), and *Sphagnum* peat is of significantly lower organic carbon content than other peat types.

Table 2. Laboratory protocol for measuring bulk density and organic matter content.

1. Clean and dry as many crucibles (size 10–30 ml) as will fit in a muffle furnace (the volume-limiting step); weigh each crucible on a 0.0001 g balance, and enter crucible number (marked on side and bottom) and weight on LOI sheet.
2. Remove a known-volume sample of peat from the core at first depth of interest (usually at 1–10 cm intervals) either using a volumetric sampler; or, for fibrous peat, saw sample from frozen core and measure by Vernier caliper (see De Vleeschouwer *et al.* 2010); or, for consolidated peat, measure sample volume by displacement in a measuring cylinder. Enter volume of sample on LOI sheet.
3. Place the first sample in a crucible, weigh, and enter the weight on the LOI sheet; collect the next sample and repeat until all the samples have been extracted, weighed and recorded.
4. Place crucibles containing samples in oven at 100°C overnight to evaporate water from the samples.
5. Place crucibles containing dry samples in desiccator cabinet and let cool to room temperature, then weigh each crucible with dry sample and enter the weight on the LOI sheet.
6. When the dry weights have been recorded, place crucibles in the muffle furnace at 550°C for at least 2 hours and preferably for 4 hours to combust all the organic matter.
7. Remove crucibles from the furnace and place in the desiccator cabinet to cool to room temperature, then weigh each crucible with ashed sample and enter the weight on the LOI sheet.

Once all the data have been collected and recorded, the samples can be discarded or archived and the crucibles cleaned.

Calculations are made as follows:

- water content (%): wet weight minus dry weight, divided by wet weight; to express as a percentage, multiply result by 100;
- bulk density (g cm^{-3}): dry weight (g) divided by fresh sample volume (cm^3);
- organic matter content (%): dry weight minus weight after ashing at 550°C, divided by dry weight;
- ash-free bulk density (OM density; g cm^{-3}): bulk density multiplied by organic matter content (%).

measurements overall. High-quality peat C determinations can be promoted by taking a number of important steps beforehand, during sample preparation; including representative sub-sampling, thorough sample homogenisation and careful measurement of known-mass sub-samples (Table 3).

4. PRECISION AND ACCURACY

The use of microbalances and the recording of measurements to several decimal places will mean that data are reported at high precision (i.e. exactness). To maximise the accuracy (i.e. correctness) of peat humification, peat bulk density, organic matter and carbon measurements, we suggest that particular care be taken in a number of key steps of both the field (see De Vleeschouwer *et al.* 2010) and laboratory procedures employed. For determination of peat humification, colorimetric

analysis should be conducted using a batch-sample process in which strict times are adhered to (owing to time-related fading of the resultant extract). Outliers (i.e. an isolated peak or trough) in the data should be checked by repeating the laboratory analysis; spuriously high light-transmission readings (e.g. on *Eriophorum*-rich peat) should be discarded. For bulk density, field collection that does not compact the samples followed by careful measurement of peat volume is critical. For organic matter measurements by loss-on-ignition analysis, it is important to ensure that the crucibles are always weighed at room temperature immediately after cooling in a working dessicator. Analytical reproducibility can be assessed by duplicate analysis of the same homogeneous samples at selected intervals. For measurement of carbon content, we suggest that ground samples must be carefully homogenised to ensure that milligram-sized sub-samples are representative.

Table 3. Considerations for carbon measurement by elemental analysis.

1. Sub-sampling. Representative peat sub-samples (typically 2–10 cm³) are carefully selected from peat profiles and gently dried to constant mass.
2. Homogenising. Because most dry combustion systems use a thermal conductivity detector to measure very small amounts of CO₂-C (typically between 0.5 and 10 mg C, but sometimes as little as 0.1 mg), peat homogenisation is a crucial step. Samples should be ground to fine powder of <250 µm, which can be quickly achieved with a high-frequency ball mill. Continued grinding until the entire sample passes through a 250 µm sieve is recommended to ensure consistency between samples.
3. Measurement. Careful measurement of small amounts (~2–15 mg) of homogenised peat of known mass into combustion vessels (e.g. tin or aluminium, 5 × 9 mm pressed capsules) is achieved using a 0.000001 g microbalance and small-sample manipulation tools.

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Author for correspondence: Professor Frank M. Chambers, Centre for Environmental Change and Quaternary Research, Department of Natural & Social Sciences, University of Gloucestershire, Francis Close Hall, Swindon Road, Cheltenham, Gloucestershire, GL50 4AZ, UK.
 Tel: +44 (0) 1242 714677; Fax: +44 (0) 1242 714826; E-mail: fchambers@glos.ac.uk