Estimating the oxidative ratio of UK peats and agricultural soils

Gareth D. Clay[1*], Fred Worrall[2]

1. Geography, School of Environment, Education and Development, University of Manchester, Oxford Road, Manchester, M13 9PL, UK
2. Department of Earth Sciences, Durham University, Science Laboratories, South Road, Durham, DH1 3LE, UK

Abstract

Organic matter in the terrestrial biosphere has a fundamental role in moderating the exchange of CO_2 between the atmosphere and the biosphere. One important property of organic matter is its oxidative ratio (OR); that is the ratio of moles O_2 released per mole CO_2 sequestered through photosynthesis i.e. the lower the OR, less O_2 is released per mole of CO_2 fixed. In global assessments of CO_2 partitioning, the failure to account for changes in OR could lead to an underestimate of terrestrial carbon sequestration. It is known that OR can vary between environments and management, but what other factors could be playing a role in controlling OR?

This study measured the OR of a range of peat (Histosols) and mineral soils (Inceptisols) under similar management from ~~the~~ across the United Kingdom to investigate how OR varies within and between material types. The study shows that OR values varied significantly between material types (median peat OR = 1.10, median vegetation OR = 1.03

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[*] Corresponding author: Email: gareth.clay@manchester.ac.uk; Tel: +44 (0)161 306 6426; fax: +44 (0)161 306 6677

and median mineral soil OR = 1.14) and they also varied between study sites. Furthermore there were no significant differences in OR with peat depth.

Given the results from this study we can suggest that future sampling strategies should include sampling of the major carbon pools (i.e. vegetation, litter and soil) and that, as a first approximation, OR can be examined on the basis of these carbon pools alone. The values measured in the study give a new residence time weighted global OR estimate for the terrestrial biosphere (OR_{terra}^{global}) of 1.056 ± 0.02.

Keywords

Terrestrial carbon cycle; elemental composition; organic matter; peatlands~~;~~

1. Introduction

In 2011 anthropogenic emissions of CO_2 reached 9.5 ± 0.5 PgC yr^{-1} (Le Quéré et al., 2013) and in May 2013 atmospheric concentrations of CO_2 surpassed 400 ppm for the first time in human history (Jones, 2013). By~~U~~ using a carbon budget approach (e.g. Le Quéré et al., 2013), and assessing changes in atmospheric O_2 and CO_2 concentrations (Keeling et al., 1996), atmospheric carbon ~~is~~ can be partitioned between the carbon pools in the atmosphere, oceans and terrestrial biosphere (residual sink). Battle et al. (2000) used changes in atmospheric levels of O_2 and N_2 in order to calculate the sizes of annual sinks between reservoirs and for the CO_2 flux to the land proposed the following equation:

$$f_{land} = -\frac{OR_{ff}}{OR_{terra}} f_{fuel} + \frac{1}{(4.8\times 0.471\times OR_{terra})}\frac{d\left(\frac{O_2}{N_2}\right)}{dt} \qquad \text{Equation 1}$$

where: f_{fuel}= flux of CO_2 due to fossil fuel combustion; $\frac{d\left(\frac{O_2}{N_2}\right)}{dt}$ is rate of change of molar ratio of atmospheric O_2 and N_2; OR_{ff} ~~is~~ = the combustion stoichiometry; OR_{terra} ~~is~~ = the oxidative ratio of the terrestrial biosphere.

Within equation 1 there are two terms that play an important role in understanding the partitioning of atmospheric carbon, the oxidative ratio (OR) which is the molar ratio of O_2 and CO_2 fluxes associated with either fossil fuel combustion (OR_{ff}) or photosynthesis (OR_{terra}). ~~This ratio is an important component in equation 1 and so research has considered the relative changes in O_2 and CO_2 in the atmosphere as a means of understanding the relative magnitude of global sinks and sources of CO_2 (Keeling and Shertz, 1992; Keeling et al., 1996).~~ Until recently there has only been one estimate of OR for the terrestrial biosphere (OR_{terra}) that has been used with equation 1. This ~~and that~~ came from Severinghaus (1995) who estimated the value to be 1.1. This value of 1.1 has been commonly adopted ~~through~~ in several global studies (e.g. IPCC, 2007). However, more recent work has shown that this value may not be appropriate ~~for equation 1~~. In a recent meta-analysis of global OR values, Worrall et al. (2013) showed that, whilst within the range of natural occurrence, the commonly used value of 1.1 is probably not the most accurate value. Worrall et al.~~-~~ (2013) ~~showed~~ suggested that 1.04 ± 0.03 was a more appropriate choice and that adopting this value meant that the sink of carbon to the land has been underestimated by up to 14%.

Direct ~~atmospheric~~ measurement of OR is possible through simultaneous measurements of atmospheric O_2 and CO_2 (e.g. Seibt et al., 2004), however several technical challenges have been noted with this method (see discussion in Masiello et al., 2008). An alternative and complementary approach is to directly measure the OR of biomass pools in the terrestrial biosphere. Masiello et al. (2008) detail the mathematical linkage between the oxidation state of organic carbon (C_{ox}) and OR ~~and another fundamental properties of the carbon cycle that of the oxidation state of organic carbon (C_{ox})~~. It is possible to calculate C_{ox}, and therefore OR, for the carbon pools (e.g. aboveground biomass) of an ecosystem using elemental analysis of %C, %H, %N and %O.

Worrall et al. (2013) based their global estimate of OR on a weighted average of OR values for different soil orders and global-scale biomes. The assumption was ~~but in so doing assumed~~ that the major control on differences in OR was ~~indeed~~ the differences between carbon pools (e.g. soil vs. vegetation) and between soil orders and between vegetation biomes, i.e. that the greatest control on OR variation was a difference between, for example, Inceptisols and Mollisols, or between savannah and boreal forest. This assumption was a necessity arising from the limited amount of data available for individual environments across the globe. Additionally no studies were included where both soil and vegetation were analysed for the same site ~~were included~~ and given the limited number of studies targeted at OR this assumption could not be tested.

~~Therefore, t~~This study aims to assess ~~and understand~~ the variation in OR between two biomes and two soil orders across one country. ~~Thus in this study w~~We aim to test whether the assumption that OR is controlled by differences between soil orders and biomes is true by assessing the magnitude of variation between soil orders and vegetation ~~biomes~~ types in comparison to other possible sources of variation. ~~In this study we will consider the variation between organic matter types in comparison to the variation within a soil order and the site at which we find a soil and its associated vegetation.~~

2. Materials and Methods

The approach of this study was to consider the variation in two soil types – peat soils (Histosols) and minerals soils (Inceptisols) across a north-south transect through the United Kingdom. For each site all the possible organic matter types were sampled with the view of comparing the between site, between organic matter types and within site variation in the values of OR.

2.1. Sampling sites and methodology

In~~During the~~ summer~~ of~~ 2011 peat cores were taken from ~~8 ~~eight sites across ~~a climatic gradient through ~~the UK (Figure 1; Table 1). Within each peat site the actual location of sampling was chosen as being the least disturbed location available i.e. little or no visible land management in recent years. At each ~~sampling ~~location two peat cores of up to 1 m depth were taken using a 70 mm diameter gouge auger. Each core was subdivided into 50 mm sections in the field and placed into sealed plastic ~~sample ~~bags. ~~ In addition to collecting two peat cores at each site,~~ R~~r~~epresentative samples of dominant vegetation types and surface litter were also collected at each site. The exact vegetation composition varied amongst sites (Table 1) but typically at each site the following were sampled: mosses (e.g. *Sphagnum* spp.); sedges (e.g. *Eriophorum* spp.) and shrubs (e.g. *Calluna vulgaris*). The vegetation data were split into seven functional groups – shrubs, grasses, sedges, *Sphagnum* mosses, non-*Sphagnum* mosses, cropland vegetation, and litter.

~~To act as a comparative sample f~~For each peat site, two nearby locations ~~not on peat soils ~~were also selected for sampling – both~~ locations were~~ on mineral soils,~~ but~~ one ~~was chosen ~~under arable and the other under pasture land use. For these comparator sites, soils, litter, and vegetation were sampled. In mineral soils no profile samples were taken because of the paucity of organic carbon at depth ~~in most mineral soils ~~but soil samples were taken from the upper 100 mm~~ using a trowel~~, whilst litter and vegetation were sampled in the same way as for the peatland sites. ~~For the purposes of analysis and reporting, ~~T~~the vegetation data were split into seven functional groups – shrubs, grasses, sedges, *Sphagnum* mosses, non-*Sphagnum* mosses, cropland vegetation, and litter.~~

All samples were dried at 105°C for 48 hours prior to further analysis. Peat ~~B~~bulk density was then calculated on a dry weight basis using the volume of the core section and mass of dry soil solids. The mineral soils were pre-treated using a 2% HF acid solution based on the methods of Mathers et al. (2002) and Skjemstad et al. (1994). ~~Approximately 5g of mineral soils were treated with five 50mL aliquots of 2% HF acid and shaken. Supernatants were centrifuged and decanted between treatments. Soils were then rinsed with deionised water at least 3 times and then dried at 75°C.~~ HF-treated soils, peat, litter and vegetation samples were all ground using a Spex 6770 Cyromill.

For comparative purposes, three standard, naturally-occurring organic materials were considered: lignin, humic acid, and cellulose. The lignin and humic acid were ~~from~~ supplied by Aldrich and the cellulose was taken from ash-free paper. ~~The standards were analysed for their elemental composition (C, H, N and O) and their energy content (gross heat value, ΔH_c).~~

2.1.1. CHNO Analysis

~~All samples were analysed for CHNO elemental content.~~ Samples were analysed for their carbon, hydrogen and nitrogen (CHN) and oxygen (O) concentration on a Costech ECS 4010 Elemental combustion system with pneumatic autosampler. Oxygen concentrations were analysed on a separate set up to the CHN set up. ~~It was set up for CHN analysis where Reactor 1 consisted of chromium (III) oxide/Silvered cobaltous-cobaltic oxide catalysts @ 950°C and Reactor 2 consisted of reduced high purity copper wires @ 650°C. Helium was used as the carrier gas at a flow rate of 95 ml min^{-1}. This was filtered for hydrocarbons upstream of the instrument. A packed 3m GC column was used for separation of the gases. A thermal conductivity detector (TCD) was used to calculate the signal of each sample. For oxygen (O) concentration, the Costech ECS was also used but was set up for O analysis. Reactor 1 consisted of a nickelised carbon/ silica chips/nickel wool pyrolysis tube @ 1060°C~~

~~whilst Reactor 2 was left empty. Helium was used as the carrier gas at a flow rate of 130 ml min^{-1} but no oxygen was used. A 2m packed oxygen GC column was used for separation of the gases. Chloropentane vapour was added to the carrier gas to enhance decomposition of the oxygen compounds and to reduce possible memory effects from previous samples (Kirsten, 1977).~~

~~Computer software used was EAS Clarity (DataApex Ltd, Prague, Czech Republic).~~ For both CHN and O setups a calibration curve of $r^2 > 0.999$ were created using acetanilide as the standard. Samples of acetanilide were included within each run as unknown samples to act as internal quality control checks. Each sample (peat, soil or vegetation) was analysed in triplicate.~~, i.e. three times on the CHN setup and a further three times on O set up, and a mean calculated for C, H, N and O.~~

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2.1.2. *Calorimetry*

Energy content, as gross heat value (ΔH_c), was measured for all peat, vegetation and litter samples. Masiello et al. (2008) has shown that it is possible to derived C_{ox} values (and therefore OR values) from calorimetry data. Analysis was performed on a 6200 Isoperibol Calorimeter (0.1% Precision Classification, Parr Instrument Company, Illinois, USA) with 1108(P) Oxygen Bomb. Calibration was performed as a rolling average of 10 measurements using benzoic acid standards. ~~Samples were placed in crucibles and compressed to stabilise the peat surface and weighed following compression, with a weight of approximately 0.8 g used. Where sample amount was deficient, a benzoic acid spike was used. Following analysis, fuse corrections were performed by measuring the length of fuse wire remaining, measured in calories and converting to MJ/Kg. The difference was taken away from the energy content recorded during analysis.~~ Limited organic matter content~~sample~~ meant that gross heat values could not be calculated for mineral soils.

2.1.3. C_{ox} and oxidative ratio (OR) calculation

A value of OR can be calculated from the carbon oxidation state (C_{ox}) which in turn can be calculated from elemental compositions of organic matter as follows (Masiello et al., 2008):

$$C_{OX} = \frac{2[O]-[H]+3[N]}{[C]} \qquad \text{Equation 2}$$

Where: [X] = molar concentration of C, H, N or O, and assuming the majority of organic nitrogen exists as amine groups in amino acids. Furthermore, sulphur is not included in this equation as it is assumed to form < 0.25% of biomass (Charlson et al., 2000).

As C_{ox} and OR are related through the balancing of organic matter synthesis, the OR value is calculated as the ratio of O_2 and CO_2 coefficients (for further details see Masiello et al., 2008). ~~(for further details see Masiello et al. (2008)).~~ Simplified, it is ~~then~~ calculated as:

$$OR = 1 - \frac{C_{ox}}{4} + \frac{3[N]}{4[C]} \qquad \text{Equation 3}$$

Equation 3 assumes that there is no contribution to the C_{ox} from S or P, and it has been shown that the error in the OR of making such an assumption would be only ± 0.002 (Hockaday et al., 2009). This equation also assumes that the nitrogen source in carbon fixation is N_2. ~~There are two further possible nitrogen (N) conversions (Masiello et al., 2008):~~

~~Ammonia (NH_3):~~ $\cancel{OR = 1 - \frac{C_{ox}}{4}}$ ~~Equation 4~~

~~Nitrate (HNO_3):~~ $\cancel{OR = 1 - \frac{C_{ox}}{4} + \frac{2[N]}{[C]}}$ ~~Equation 5~~

~~For the purposes of this paper, Equation 3 was used as N_2 is the dominant form in the peatland ecosystem.~~ The agricultural soils will likely received N in other forms in addition to N_2, but no data were available for these sites and other studies have shown minimal changes in OR when using alternative assumptions ~~for the reason that [N]/[C] is always likely to be < 0.1~~ (Gallagher et al., in review).

As a quality control check, OR values were only calculated for those samples that had measured data for C, H, N and O; if one of these data were missing (e.g. lost sample), no OR value was calculated.

2.2. Statistical analysis

2.3. Analysis of Variance (ANOVA)

The design of the study allows for a several statistical comparisons to be made using an ANOVA approach. Firstly, one-way ANOVA was used to test whether there were significant differences within the organic matter types being considered. This set of ANOVA could be sub-divided into ~~several~~ separate ANOVA: the difference in OR for mineral soils under arable and under pasture; the difference in OR between vegetation functional groups ~~where the factor levels were: shrubs, grasses, sedges, Sphagnum mosses, non-Sphagnum mosses, cropland vegetation, and litter~~. The second set of ANOVA that could be performed was for the peat soils only. A two-way ANOVA was used to determine the statistical significance of the factors – site and peat depth. Finally, the variation in organic matter types between sites was examined. All types of ~~the~~ organic matter ~~types~~ were considered and all sites but for comparison with the mineral soil samples~~,~~ only the surface samples of peat soils from each site were included (depth < 20 cm). Further, for reasons of cross-classification between the factor levels the depth in the peat profile, the vegetation functional group, and the land use of the mineral soil were not considered as a separate factor.

Response variables used were energy content, C_{ox}, and OR. If necessary t~~T~~he response variables were log-transformed to ensure data normality prior to ANOVA. ~~tested for normality prior to ANOVA using the Anderson-Darling test; if the response variable failed the test it was log-transformed and re-tested. Further transformation to ensure normality of the response variable did not prove necessary.~~ The ~~*Post hoc* testing of the results was performed using the~~ Tukey test at 95% level was used to determine significant differences between levels of any factor. The magnitude of the effects of each significant factor and interaction were calculated using the generalized ω^2 (Olejnik and Algina, 2003). ~~Response variables used were: C/N ratio, H/C ratio, O/C ratio, energy content, C_{ox}, and OR.~~

~~A power analysis was used to assess the minimum effect size that could be detected within this latter comparison of organic matter type and site. The study was fully factorial with respect to each of 2 factors, 3 centre points were assumed; the standard deviation was estimated as the square root of the mean square difference; and the required experimental power was set at 80%.~~

2.4. OR and ΔH_c

Masiello et al. (2008) used ΔH_c from calorimetry to calculate C_{ox} values for a range of standard materials. If ~~then~~ there is a relationship between C_{ox} and ΔH_c it might be reasonable to expect there to be a relationship between ΔH_c and OR values from this study. If so it may be able to shed some light on underlying mechanisms leading to OR variation ~~and also provide a simpler method of calculating OR for many materials~~. Therefore, ΔH_c values were plotted against OR values for the peat soils and vegetation ~~along~~ together with the standard

Comment [F1]: 80% is like 95% in stats test – I SHALL TRY AND FIND A REFERENCE OTHERWISE I WILL USE A FOOTNOTE

Comment [GC2]: R2: can you justify this percentage

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Comment [GC4]: Think we just cut this to save on space materials – cellulose, lignin and humic acid. Mineral soils were excluded from this analysis as no ΔH_c values could be calculated.

2.5. Global OR values

The data from this survey can be used to update the estimation of global OR made by Worrall et al. (2013). Worrall et al. (2013) have proposed a weighted average approach:~~based upon the residence time of carbon in vegetation and in soil as this gives a greater importance to the faster turnover of carbon in the vegetation pool. Therefore~~:

$$OR_{terra}^{global} = f_{soil}^{terra} OR_{soil}^{global} + f_{veg}^{terra} OR_{veg}^{global} \qquad \text{Equation } \cancel{6}4$$

Where: OR_{terra}^{global} = the oxidative ratio of the global terrestrial biosphere; OR_{soil}^{global} = the oxidative ratio of the global soils; OR_{veg}^{global} = the oxidative ratio of the global vegetation; f_{soil}^{terra} = the proportion of the terrestrial biosphere C annual flux that is due to soils; and f_{veg}^{terra} = the proportion of the terrestrial biosphere C annual flux that is due to vegetation.

The annual flux from the soils or vegetation was based upon the size of reservoir divided by the average residence time. The comparative sizes of the soil and vegetation reservoirs ~~was~~ were estimated from Eswaran et al. (1993) and Olson et al. (2001), where the proportion of carbon in the vegetation reservoir was 0.28 and in the soil reservoir ~~as~~ 0.72. The average carbon residence time for soils was taken as between 20 and 40 years based upon a study by Jenkinson and Rayner (1977). Mills et al. ~~(2014)~~(2014)~~-~~ examined radiocarbon results for 133 UK~~,~~ soils and found that the carbon turnover was best modelled as two pools – a fast pool with 20 year residence time and a slow, 1000 year turnover pool. ~~–~~The average carbon residence time for vegetation was taken as between 2 and 5 years (e.g. Gaudinski et al., 2000). We recognize that the OR of soil fluxes and soil pools may not be identical (just as the carbon isotopic values of the bulk soil carbon pool rarely match the carbon isotopic values of the soil CO_2 flux). However, we must start with the assumption of equivalence between soil OR flux values and OR pool values because no data yet exist comparing soil pool and flux OR values. Given the above approach the values of $f_{soil}^{terra} = 0.27$ and $f_{veg}^{terra} = 0.73$. The value of OR_{veg}^{global} is the weighted average of the expected values of the 16 global biomes where the weighting is the area of each biome (Loveland and Belward, 1997). ~~and~~ OR_{soil}^{global} is the weighted average of the expected values of each of the soil orders of USDA soil taxonomy where the weighting is the organic carbon content of each order (Eswaran et al., 1993). Since in both cases the data for any one biome or soil order ~~is~~ are scarce, then the expected value is taken as the median of each biome or soil order.

Worrall et al. (2013) applied Equation ~~6~~ 4 based upon the classifying organic material samples into one of either the 12 USDA soil orders (~~although~~ Gelisols and Histosols were combined into one~~ because of lack of data~~) or 16 global biomes (Loveland and Belward, 1997). The soil samples collected as part of this study were classified as either Inceptisols or Histosols. The vegetation samples can be classified as~~in one of the following~~: Grassland, Cropland, Shrubland or Permanent wetland.

3. Results

Overall OR could be calculated for 251 peat samples, 49 vegetation samples and 14 mineral soil samples. Table 2 shows the data ~~for the individual elemental concentrations~~ for each of the material types whilst Tables 3 and 4 show the parameters for vegetation and peat soils respectively. ~~and t~~The composition of the standard materials ~~, naturally-occurring organic types~~ are listed in Table 5. None of the datasets needed to be transformed prior to ANOVA.

Of the three material types, vegetation samples had the lowest OR values followed by peat soils; mineral soils showed the highest OR values (Table 2). The values for mineral soils are within the range of previously reported values (Hockaday et al., 2009) though the peat and vegetation means were lower than 1.1 ~~though~~ but still within the range of results reported by Worrall et al. (2013).

3.1. Variation within organic matter type

Mineral soils

Within the mineral soil dataset it was possible to determine whether there was a significant difference between land uses, i.e. between soils under arable and soils under pasture. Results from the one-way ANOVA shows that there were no significant differences in ~~elemental ratio data (C/N, O/C, and H/C),~~ C_{ox} or OR between mineral soils (n = 14) under different land uses.

Vegetation types

Within the vegetation data, there were significant ~~($p < 0.05$)~~ differences in ~~all elemental ratios,~~ energy content ($p < 0.001$), C_{ox} ($p < 0.018$) and OR ($p < 0.001$) values between vegetation functional groups ~~(Table 6)~~ from the one-way ANOVA. The *post hoc* testing showed ~~a great deal of variation in where the significant differences lay. For example, for C/N ratio the only difference lay between non-*Sphagnum* mosses and arable crops and grasses (Table 6) with grasses and crops having the lower C/N ratio (Table 3). However, for other elemental ratios and energy content there were a number of significant differences between functional groups (Table 6). T~~the highest OR values were found in shrubs (e.g. *Calluna vulgaris*, *Erica tetralix*) whilst the lowest values were found in mosses, both *Sphagnum* and non-*Sphagnum* (Table 3). The reverse of this ~~is~~ was true for C_{ox} values where the lowest values were found on shrubs and the highest on *Sphagnum*. ~~However, the variation within these functional groups meant that only certain combinations of groups were statistically different from one another (Table 6).~~

Peat Soils

In the ANOVA model, site and peat depth were included but due to a collinearity between site and depth, the interaction term could not be plotted.

For all the measured parameters, the site factor was found to be significant (Table ~~7~~6). *Post hoc* testing ~~of site factor for each parameter showed considerable variation between sites (Table 8). For example, with C/N ratios, Auchencorth and Forsinard showed the lowest values whilst the highest values were found on Westhay Moor (Table 4). The extremes for O/C ratios were found between Whixhall and Dartmoor, whilst for H/C ratios the largest ratios were found on Forsinard and the lowest on Thorne (Table 4).~~showed ~~T~~ the lowest energy contents were found at Auchencorth with the highest at Dartmoor (Table 4). There were significant differences between peatland sites in terms of C_{ox} and OR values explaining 21% and 39% of the variation in the data respectively (Table ~~7~~6). ~~*Post hoc* testing (Table 8) showed that~~ The lowest OR values ~~gave some similar patterns to the elemental data with~~were found at Thorne ~~resulting in the lowest OR values~~whilst ~~and~~ Forsinard and Bodmin ~~having~~ had the highest OR values.

Depth was a significant factor for ~~all parameters~~bulk density and energy content although not significant for ~~with the exception of~~ C_{ox} and OR (Table ~~7~~6). ~~In terms of down core profiles generally t~~ There were increases in ~~C/N ratio and~~ energy content down the core ~~and decreases in O/C and H/C ratios with depth (Figure 2). These profiles were as would be expected as the peat becomes more carbon rich with depth.~~ whilst t~~T~~he observed data for bulk density ~~was~~ were more complex~~ and~~; ~~across all cores the~~ bulk density ~~generally~~ increased in the upper 30 centimetres before decreasing with depth (Figure 2).

3.2. Organic matter type vs. site

It was possible to analyse the differences between surficial peat (0 – 20 cm), vegetation and surface mineral soils across all the sites considered in study for ~~the element ratios and~~ OR. Bulk density and energy content were not considered because neither could be measured for all sample types. ~~but because the analysis covered all the organic matter types being considered then it was not possible to include the bulk density and the energy content.~~ The analysis was performed with the caveats that the above analysis found the following significant differences: there were significant differences in the OR between vegetation functional group; there were no significant differences between OR for different land uses on the mineral soils; and there were no significant differences with depth for the OR of peat soils. ~~The power analysis shows that this design was capable of detecting a difference of 0.02 in the OR at the 80% probability.~~

When comparing all organic matter types and all sites for elemental ratios and OR there were significant differences between the site and organic matter type factors (Table ~~9~~7). For the OR values the most important of the factors was the difference between organic matter types (explaining 70% of the original variance) and *post hoc* testing showed that there were significant differences between all the organic matter types considered. The highest OR values given by the mineral soils (1.15 ± 0.01) followed by peat soils (1.079 ± 0.006) with the lowest OR represented by vegetation (1.037 ± 0.007) where the values are given as the estimated marginal means (averages accounting for all other factors and covariates) and the uncertainty in each is given as the standard error. The variation between organic matter types was greater than the variation between sites with the site factor explaining only 21% of the original variance. The *post hoc* testing between the individual sites shows that only one site, Thorne, was significantly different from all the others ~~Thorne was significantly lower than all other sites~~ (Figure 3~~; Table 10~~). When the data for Thorne were removed then Auchencorth was found to have OR values significantly higher than all other sites: there were no other significant differences between any other sites considered in this study.

~~When the elemental ratios were considered then the first observation was that there were no significant differences between the sampled sites for the O/C ratio (Table 9); this study could find no evidence that O/C varied across the UK. For both the C/N and H/C ratios, and as for the OR values, the organic matter type factor was more important than the site factor (Table 9). *Post hoc* testing of the elemental ratios shows two distinct patterns. For the C/N ratio the *post hoc* testing shows that the significant difference between sites was been Westhay (C/N = 43 ± 3) and the samples from both Dartmoor (C/N = 26 ± 3) and Bodmin (C/N = 24 ± 4). For the H/C ratio the significant difference between sites lay between Thorne and Auchencorth, Bodmin, Dartmoor, Forsinard and Whixall with Thorne having significantly lower values. The pattern of the differences in the H/C ratios is distinctly closer to that observed for the OR values than the pattern observed for C/N ratios and thus implying that the difference at the Thorne site was due to the H/C ratios and not due to differences in O or N.~~

There was no significant interaction between the site and organic matter type factors for any of the parameters considered in this study. The lack of significant interaction between the two factors means that the difference between organic matter types does not vary with site suggesting that Inceptisol is different from a Histosol regardless of the position within the UK and as such there is a fixed relationship between the organic matter types. Regressing the mean vegetation OR and mean bulk peat OR for each of the eight sites shows no significant relationship between them.

3.3. Variation in Organic Matter Composition

A comparison of OR and ΔH_c ~~with respect to~~in peat soil samples and ~~the~~ vegetation samples show several possible patterns (Figure 4a and b). Given the result of Masiello et al. (2008) it would be expected that OR would increase with ΔH_c and this is true for ~~such a relationship is discernible in these plots as the line between~~ the organic material standards (humic acid, cellulose and lignin). With respect to the sampled peat sites some of the soils sampled from Thorne, Westhay and the Peak District plot below this line with lower ΔH_c values than would be expected for their OR values (Figure 4a). Conversely, all the samples from all the other sites (Auchencorth, Bodmin, Dartmoor, Forsinard and Whixall) plot above this line with OR greater than the equivalent mix of the organic matter standards. Furthermore, it could be proposed that majority of samples form a 3 end- member triangle the end-members of which are marked by humic acid, lignin and a high OR end-member represented by peat soil samples from Forsinard (Figure 4a). The latter end-member with high OR represents organic matter that is as reduced as the lignin standard but does not have the calorific value. Some soil samples plotted at even lower values of ΔH_c than those from Forsinard and these come from Auchencorth. One ~~possible~~ explanation of this is that the peat soil at Auchencorth ~~has received inputs of mineral matter.~~ had visible evidence of trace mineral matter in the field. Inputs of fine silt or clay into the peat would not alter the measurement of OR as it is based on a ratio of elements in the organic matter but it would ~~in effect~~ dilute ~~out~~ the energy value of any sample.~~,~~ It ~~it~~ may also explain large values of bulk density observed for this site which contribute to inflexion in the bulk density profile (Figure 2).

Comment [GC5]: R2: Surely you could test this through lab analyses

Comment [F6]: We can confirm by ash content if you want?

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~~With respect to vegetation type m~~Most vegetation types were better constrained within the line defined by the organic matter standards (Figure 4b) and the peat samples from Forsinard than was observed for the peat soils (Figure 4a). The end-member represented by the peat soils of Forsinard plots close to the samples of *Erica tetralix*. Some samples of both grass and *Sphagnum* plot at lower values of OR than would be expected from a combination of the organic matter standards~~ and this type of plant material which is more oxidised than any combination of the standards but at similar calorific value.~~.

4. Discussion

This study has ~~been able to comment on~~evaluated the oxidation status of peat (Median OR = 1.10) and agricultural soils (Median OR = 1.14) across a latitudinal transect across the United Kingdom. Significant differences were found in the oxidative ratio of major terrestrial carbon pools (soil and vegetation). This is perhaps not unsurprising given the different processes operating in each carbon pool but this study has been able to quantify the difference with respect to OR.

Within-group variation of OR generated a number of interesting results. Firstly, soils under agricultural management did not vary significantly in their OR value. One might expect that different management practices, and different vegetation types, would influence soil processes ~~that ~~which would, in turn, affect OR. ~~Even from this study i~~It is possible to see the large contrast between cellulose and lignin, and so woody vegetation ~~that is woodier ~~would be expected to have a higher OR than non-woody types. Furthermore, N is a component of the oxidation state of an ecosystem ~~(Equation 2) ~~and so diversity of N inputs and N sources (e.g. organic wastes vs. inorganic fertiliser) might be expected to shift the OR of an environment. ~~However, this result is for the ecosystem and not the components of that ecosystem (e.g. soil or vegetation) and the lack of observed difference between grasslands and croplands in this study may reflect totality and not just processes that affect vegetation alone.~~

Secondly, OR did not vary significantly with depth and there was ~~not~~ no consistent OR depth profile ~~variation~~ between ~~across~~ all the sites. ~~Peat depth was an important factor in the elemental ratio datasets and many of these trends can be used to identify structural changes in the peat and to infer changes in the peat decomposition process. The C/N ratio can be used to infer decomposition rates, specifically the loss of mass, whilst the H/C and O/C ratio are commonly used to infer humification rates . Decreases in H/C ratios and O/C ratios are specifically linked to dehydrogenation and decarboxylation respectively .~~ The lack of ~~a~~ significant OR trends is perhaps one of the more unusual observations from this study. Given the classical explanation of peat formation it would be expected that C_{ox} would decline with depth as the peat profile becomes more anaerobic, and therefore an increase in OR would be expected. ~~The classical explanation of peat soils is that they rapidly become anaerobic~~ In peat soils anaerobic conditions result from ~~due to~~ excess organic matter and slow ingress of air due to persistent waterlogged conditions, leading to successive use and exhaustion of redox couples (Reddy and D'Angelo, 1994). However, this succession is noted mainly for inorganic chemical species (e.g. Fe(III)) in soil water and for peat soil water concentrations are often low compared to mineral soils giving them very little buffering capacity with respect to redox conditions meaning that species in solutions are rapidly transformed, e.g. Fe(III) to Fe(II). However, the concentration of inorganic redox species that can be reduced is very low~~amount of reduction occurring is minimal~~ in comparison to the amount of organic matter that could be oxidised and so therefore dramatic changes in inorganic soil solution chemistry are not reflected in the substrate.

~~Thirdly, when examining the variation in organic matter composition a number of samples plotted outside of the 3 end member space delineated by humic acid, lignin and the high OR end-member (Figure 4a). Of particular note is that many of the samples of peat soil from the site at Auchencorth plot at values of ΔH_c outside this triangle and this is probably~~

~~due to inputs of silt. Furthermore, some of the vegetation samples also plotted at lower than expected OR values. Whatever component lends low OR values to these grass and *Sphagnum* samples may be part of the explanation of low OR peat samples from the sites at Thorne, Westhay and the Peak District. Equally, the composition of the proposed third end-member is not known.~~

By considering natural organic carbon reservoirs in a structured survey, this study has examined the variation in OR and it can assess whether the global assessment of OR proposed by Worrall et al. (2013) was appropriate. Firstly, the study has shown that it ~~is~~ was possible to distinguish between the naturally-occurring organic matter types (e.g. soil and vegetation) when looking at OR; that is there ~~is~~ are ~~a~~ significant differences between organic carbon pools that are easy to sample and model over large scales.

Secondly, Worrall et al. (2013) could only use the data that ~~was~~ were available in the published literature so had little control on the soils orders available and their geographic distribution. This study has been able to take one soil order (Histosols) and compare the OR across a range of locations under similar management in order to assess the relative source of variation in OR. ~~However, the study can show~~It has shown that there is more variation between carbon pools than between sites but that the variation between carbon pools is independent of the changes between sites~~, i.e.~~. ~~t~~This is good statistical justification to support the approach used in Worrall et al. (2013) that as a first approximation, and at large scales, OR is better understood by considering soil and vegetation separately. Thirdly, the study has shown a significant difference between the two soil orders considered by this study (Histosols and Inceptisols) which is an underlying assumption of Worrall et al. (2013) and of the calculation of OR_{soil}^{global}. ~~But as discussed above there was no significant difference but two of the biomes directly analysed, i.e. grassland and croplands.~~

Fourthly, we can start to address the question of what kind of sample is truly representative of the OR of an environment. Results from the study showed that if OR does not vary with peat depth, then it may be appropriate just to sample the surface peat rather than coring. If peat can be simplified to ~~just~~ surface samples, can the OR of the environment be simplified to ~~just~~ one sample? This study has ~~looked at the vegetation associated with the soils and has~~ shown that vegetation and peat samples were not significantly similar to each other to warrant only one sample being taken. Therefore future sampling should concentrate on sampling both the vegetation and soil~~s~~ carbon pools.

Finally, this study is a continuation of earlier work (Worrall et al., 2013) ~~that calculated a global OR value from a database of OR values.~~ and ~~T~~this study is able to ~~add to that database and is able to~~ update the global OR figure accordingly. The peat soils from this study are classified as Histosols ~~in the USDA soil taxonomy.~~ and in Worrall et al. (2013) list Histosols as having an OR of 1.03 (range 0.92 – 1.11) based on 8 studies, 23 locations and 345 samples - updating these values with data from this study gives an OR for Histosols of 1.08 with an interquartile range of 1.05 - 1.11, based now on 9 studies, 31 locations and 596 samples. The mineral soils for this study could be classed as Inceptisols. ~~i~~In ~~the review of~~ Worrall et al. (2013) the Inceptisols had an OR of 1.07 (range 1.03 to 1.10) based on 2 soils from 1 study this can now be updated to be from 2 studies and 7 sites with 18 samples to give a median value of 1.15 (IQR 1.10 to 1.15). Coupling this information with new estimates for Alfisols (Worrall et al., in review) that updated the OR of 1.10 (range = 1.07 to 1.12) based upon 1 study and 4 soil samples to that of 1.12 (range = 1.08 to 1.19) based upon 2 studies and 2 soils but 84 samples. This would give a new value of OR_{soil}^{global} = 1.084 ± 0.002, where the uncertainty is interquartile range. Similarly, the values for vegetation types can be updated, for grassland the new estimate would be 1.02 with an interquartile range of 1.00 – 1.05; croplands would now have a median value of 1.00 (0.99 – 1.05); shrublands with a median value of 1.10 (1.08 to 1.12); and permanent wetlands with a median of 1.02 (0.99 – 1.04). This gives an OR_{veg}^{global} = 1.049 ± 0.2 (error as IQR). A new residence time weighted global OR estimate for the terrestrial biosphere (OR_{terra}^{global}) of 1.056 (IQR = 1.054 to 1.058) can be calculated; the previous value of OR_{terra}^{global} was 1.04 ± 0.03.

This study has been able to validate the sampling approach of Worrall et al. (2013) but ~~i.e. sampling the major carbon pools, but this work is able to now suggest that this can be improved further. B~~by increasing the level of sophistication and characterising individual components of the carbon pools e.g. lignin vs. cellulose components, active vs. passive soil organic matter, or dissolved organic carbon (DOC) fractions, it may be possible to elucidate the underlying controls on OR in the terrestrial environment. Future research should therefore explore these factors.

5. Conclusion

This study has shown that there are significant differences in oxidative ratio ~~(OR)~~ between mineral soils, peats and vegetation. Whilst there were significant differences in OR between different sites and material types, there was no significant interaction between the factors. ~~Furthermore there was no significant difference in OR with peat depth.~~ This suggests, on a large scale at least, that it the most important factor in OR variation is material type rather than site location and the OR of an environment can be determined by simple sampling of the major terrestrial carbon pools.

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References

Battle, M., Bender, M.L., Tans, P.P., White, J.W.C., Ellis, J.T., Conway, T., Francey, R.J., 2000. Global carbon sinks and their variability inferred from atmospheric O-2 and delta C-13. Science, 287(5462): 2467-2470.

Charlson, R.J., Anderson, T.L., McDuff, R.E., 2000. The sulfur cycle. In: Jacobson, M.C. (Ed.), Earth System Science: From Biogeochemical Cycles to Global Change. Elsevier, San Diego, pp. 343-359.

Eswaran, H., Vandenberg, E., Reich, P., 1993. Organic carbon in soil of the World. Soil Science Society of America Journal 57(1): 192-194.

Gallagher, M.E., Masiello, C.A., Hockaday, W.C., Baldock, J.A., Snapp, S.S., Mcswiney, C.P., in review. Controls on the Oxidative Ratio of Agricultural Ecosystems. Global Biogeochemical cycles.

Gaudinski, J., Trumbore, S., Davidson, E., Zheng, S., 2000. Soil carbon cycling in a temperate forest: radiocarbon-based estimates of residence times, sequestration rates and partitioning of fluxes. Biogeochemistry, 51(1): 33-69.

Hockaday, W.C., Masiello, C.A., Randerson, J.T., Smernik, R.J., Baldock, J.A., Chadwick, O.A., Harden, J.W., 2009. Measurement of soil carbon oxidation state and oxidative ratio by 13C nuclear magnetic resonance. Journal of Geophysical Research: Biogeosciences, 114(G2): G02014.

IPCC, 2007. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Jenkinson, D.S., Rayner, J.H., 1977. Turnover of soil organic matter in some of Rothamsted classical experiments. Soil Science, 123(5): 298-305.

Jones, N., 2013. Troubling milestone for CO2. Nature Geoscience, 6: 589.

Keeling, R.F., Piper, S.C., Heimann, M., 1996. Global and hemispheric CO2 sinks deduced from changes in atmospheric O-2 concentration. Nature, 381(6579): 218-221.

Le Quéré, C., Andres, R.J., Boden, T., Conway, T., Houghton, R.A., House, J.I., Marland, G., Peters, G.P., van der Werf, G.R., Ahlström, A., Andrew, R.M., Bopp, L., Canadell, J.G., Ciais, P., Doney, S.C., Enright, C., Friedlingstein, P., Huntingford, C., Jain, A.K., Jourdain, C., Kato, E., Keeling, R.F., Klein Goldewijk, K., Levis, S., Levy, P., Lomas, M., Poulter, B., Raupach, M.R., Schwinger, J., Sitch, S., Stocker, B.D., Viovy, N., Zaehle, S., Zeng, N., 2013. The global carbon budget 1959–2011. Earth Syst. Sci. Data, 5(1): 165-185.

Loveland, T.R., Belward, A.S., 1997. The International Geosphere Biosphere Programme Data and Information System global land cover data set (DISCover). Acta Astronautica, 41(4–10): 681-689.

Masiello, C.A., Gallagher, M.E., Randerson, J.T., Deco, R.M., Chadwick, O.A., 2008. Evaluating two experimental approaches for measuring ecosystem carbon oxidation state and oxidative ratio. Journal of Geophysical Research: Biogeosciences, 113(G03010).

Mathers, N.J., Xu, Z., Berners-Price, S.J., Senake Perera, M.C., Saffigna, P.G., 2002. Hydrofluoric acid pre-treatment for improving ^{13}C CPMAS NMR spectral quality of forest soils in south-east Queensland, Australia. Soil Research, 40(4): 665-674.

Mills, R.T.E., Tipping, E., Bryant, C.L., Emmett, B.A., 2014. Long-term organic carbon turnover rates in natural and semi-natural topsoils. Biogeochemistry, 118(1-3): 257-272.

Olejnik, S., Algina, J., 2003. Generalized Eta and Omega Squared Statistics: Measures of Effect Size for Some Common Research Designs. Psychological Methods, 8(4): 434-447.

Olson, J.S., Watts, J.A., Allison, L.T., 2001. Major world ecosystem complexes ranked by carbon in live vegetation: a database., Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory.

Reddy, K.R., D'Angelo, E.M., 1994. Soil processes regulating water quality in wetlands. In: Mitsch, J.W. (Ed.), Global Wetlands: Old World and New. Elsevier, New York, pp. 309-324.

Seibt, U., Brand, W.A., Heimann, M., Lloyd, J., Severinghaus, J.P., Wingate, L., 2004. Observations of O2:CO2 exchange ratios during ecosystem gas exchange. Global Biogeochemical cycles, 18(4): GB4024.

Severinghaus, J.P., 1995. Studies of the terrestrial O2 and carbon cycles in sand dunes gases and in Biosphere 2. PhD thesis Columbia University.

Skjemstad, J.O., Clarke, P., Taylor, J.A., Oades, J.M., Newman, R.H., 1994. The removal of magnetic materials from surface soils - a solid state ^{13}C CP/MAS NMR study. Soil Research, 32(6): 1215-1229.

Worrall, F., Clay, G.D., Masiello, C.A., Mynheer, G., 2013. Estimating the oxidative ratio of the global terrestrial biosphere carbon. Biogeochemistry, 115(1): 23-32.

Worrall, F., Clay, G.D., MacDonald, A., in review. The impact of fertiliser management on the oxidation status of a long-term grassland in SE England. Biogeochemistry.

Figure 1. Location of study sites within Great Britain.

Figure 2. Interval plot of elemental ratios, energy content (MJ/kg), bulk density (g/cm^3) and Oxidative Ratio with peat depth. 95% confidence interval of the mean

Figure 3. Interval plot of OR for surficial peat samples for each site. 95% confidence interval of the mean

Figure 4. Plot of OR vs. ΔHc. a) Peat soil samples highlighted; b) Vegetation samples highlighted.

Table 1. Location information for each of the sites

Table 2. Median values (inter-quartile range in parentheses) for each measured or derived variable for the three material types.

Table 3. Median values (inter-quartile range in parentheses) for each measured or derived variable for functional plant groups

Table 4. Median values (inter-quartile range in parentheses) for each measured or derived variable for peat soils by site.

Table 5. Median values (inter-quartile range in parentheses) for each measured or derived variable for the three standards

~~Table 6. One-way ANOVA within vegetation. p = probability of factor being zero, *post hoc* testing where ≠ denotes a significant difference between levels.~~

Table 6. ANOVA for peat samples energy content, C_{ox} and OR values, and bulk density. df = degrees of freedom, p = probability of factor being zero, ω^2 = generalized proportion of variance explained

~~Table 7. ANOVA for peat samples elemental ratios and OR values, energy content and bulk density. df = degrees of freedom, p = probability of factor being zero,~~ ~~ω^2~~ ~~= generalized proportion of variance explained~~

~~Table 8. *Post hoc* testing of site factor from Table 7 (ANOVA) where ≠ denotes a significant difference between levels~~

Table ~~9~~7. ANOVA for the comparison of surficial peat and mineral soils with vegetation. df = degrees of freedom, p = probability of factor being zero, ω^2 = generalized proportion of variance explained

~~Table 10. *Post hoc* testing of site factor from Table 9 (ANOVA) where ≠ denotes a significant difference between levels.~~

Field Code Changed