Oxidative ratio (OR) of southern African soils and vegetation: updating the global OR
 estimate

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11 Abstract

Oxidative ratio (OR) is the ratio of moles O_2 released per mole CO_2 sequestered through photosynthesis in the terrestrial biosphere. The lower the OR value the more CO_2 an environment can potentially sequester. It is this property of the organic matter that plays a role in models of CO_2 partitioning between the atmosphere and the biosphere. Recent studies have shown that the accepted value of OR (1.1±0.05) may not be appropriate but that there are a number of research gaps before a full account of global OR values can be carried out.

This study aims to fill some of the research gaps by carrying out a targeted sampling campaign in southern Africa. Vegetation, litter and soil samples were taken from a range of soil orders and biomes across a series of locations in South Africa and Swaziland. From these samples this study has been able to update a recent meta-analysis and show that although there were significant differences between some sites and vegetation types, there was no

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significant difference between the soil orders or biomes sampled. This study has also been able to update the global OR_{terra} estimate to 1.06 ± 0.06 .

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26 Keywords

27 Oxidative ratio; <u>T</u>errestrial carbon cycle; elemental composition; organic matter; Swaziland;
28 South Africa

29

30 1. Introduction

Battle et al. (2000) used changes in atmospheric levels of oxygen (O₂) and nitrogen (N₂) to calculate the sizes of annual sinks between global reservoirs and for the carbon dioxide (CO₂) flux to the land proposed the following equation:

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$$f_{land} = -\frac{OR_{ff}}{OR_{terra}} f_{fuel} + \frac{1}{(k_1 k_2 OR_{terra})} \frac{d(\frac{O_2}{N_2})}{dt}$$
 Equation 1

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where: f_{fuel} is flux of CO₂ due to fossil fuel combustion; $\frac{d\left(\frac{O_2}{N_2}\right)}{dt}$ is rate of change of the molar ratio of atmospheric O₂ and N₂; OR_{ff} is the combustion stoichiometry; OR_{terra} is the oxidative ratio of the terrestrial biosphere; and k₁ and k₂ are conversion factors (0.471 and 4.8 respectively).

Within equation (1) the relative partitioning of atmospheric carbon is denoted by the important term – oxidative ratio (OR). The OR is the molar ratio of oxygen (O_2) and carbon dioxide (CO_2) fluxes, associated with either fossil fuel combustion (OR_{ff}) or photosynthesis (OR_{terra}). Given the importance of OR in equation 1 to the estimation of global carbon fluxes research has focussed on the relative changes of atmospheric O_2 and 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, which came from Severinghaus (1995) who estimated the value to be 1.1 ± 0.05 and this value has been commonly adopted through several global studies (Battle et al., 2000; IPCC, 2007).

50 In a recent meta-analysis of global terrestrial OR values, Worrall et al. (2013) showed that, whilst within the range of natural occurrence, the commonly used value of 1.1 was 51 probably not the most appropriate value. Worrall et al (2013) showed that 1.04 ± 0.03 was a 52 53 more appropriate choice and that adopting this value meant that the terrestrial carbon sink has been underestimated by up to 14%. However, in their analysis, Worrall et al. (2013) found 54 55 that there were several soil orders and global biomes (Loveland and Belward, 1997) that were under sampled or for which no OR value existed. They showed that there were only single 56 studies that had sufficient elemental data to calculate OR values for Alfisols, Andisols, 57 58 Aridosols, Oxisols, and Vertisols and that there were no values for Ultisols. For global 59 biomes there was only one study under woody savannah and no studies with data for shrublands and savannahs; permanent wetlands; or for urban biomes. 60

This study aimed to tackle these some of these knowledge gaps through a targeted sampling campaign in South Africa and Swaziland, an area where a number of the undersampled soil orders and biomes were located. Furthermore, the review of Worrall et al. (2013) had to re-calculate available data but found no studies which had sampled different carbon pools at the same site, i.e. no study was available that sampled vegetation and its underlying soil, and so therefore it was impossible to judge whether OR was governed by its location, its vegetation or by its soil orders.

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69 **2. Methods**

The approach of this study was to consider the variation in OR across multiple soil orders and
multiple biomes. For each site all the possible organic matter pools were sampled with the

view of comparing OR values between sites, organic matter material types and assess
variability within sites.-

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75 2.1. Field sampling

Sampling was carried out in January 2012 and in total 30 sites were visited across the 76 Gauteng, Mpumalanga and Limpopo provinces of South Africa, as well as in Swaziland 77 (Figure 1; Table 1). At each site, soil, vegetation, and litter were sampled whenever present. 78 This method of compartmentalising the major carbon pools has been found to be a suitable 79 80 sampling strategy for other OR focussed studies (Clay and Worrall, in press). Soils were sampled from the upper 5 cm using a trowel whilst vegetation was carefully removed using 81 secateurs. Sampling locations were selected so the greatest possible range of USDA soil 82 83 orders and biomes were visited. For all but Lithosol, each soil order was sampled at two separate locations under distinct biomes. Similarly, each biome was sampled at more than 84 one location; however, it was not possible to perform a complete factorial approach with 85 86 respect to soil order and biome simply because each possible combination does not exist in nature. Sites were classified into one of 15 biomes based on the IGBP land cover classes 87 (Loveland and Belward, 1997; Loveland et al., 2000) and into one of 12 soil orders of the 88 USDA soil taxonomy. Furthermore, the dominant plant functional type (PFT) was sampled at 89 each site along with any co-dominant PFTs. All samples were bagged in the field and air 90 91 dried to reduce the moisture content and the possibility of oxidation prior to international shipping. 92

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94 **2.2. Laboratory analysis**

Upon arrival in the United Kingdom (UK) all samples were dried at 105°C for 48 hours prior
to further analysis. For soils any root matter present was removed and bulk density was then

97 calculated on a dry weight basis. The soils were pre-treated using a 2% hydrofluoric (HF)
98 acid solution based on the methods of Mathers et al. (2002) and Skjemstad et al. (1994).
99 Approximately 5g of soils were treated with five 50mL aliquots of 2% HF acid and shaken.
100 Supernatants were centrifuged and decanted between treatments. Soils were rinsed with
101 deionised water at least 3 times and then dried at 75°C. In the final stage of sample
102 preparation, the HF-treated soils, along with all litter, and vegetation samples were all ground
103 using a Spex 6770 Cyromill.

For comparative purposes, three standard, naturally-occurring organic biochemical compounds were analysed: lignin, humic acid, and cellulose. The lignin and humic acid were from Aldrich and the cellulose was supplied by Whatman.

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108 *Carbon (C), hydrogen (H), nitrogen (N), oxygen (O) (CHNO) analysis*

All samples were analysed for CHNO elemental content. Samples were analysed for their 109 carbon, hydrogen and nitrogen concentration on a Costech ECS 4010 Elemental combustion 110 system with pneumatic autosampler. It was set up for CHN analysis where Reactor 1 111 consisted of chromium (III) oxide/Silvered cobaltous-cobaltic oxide catalysts at 950°C and 112 Reactor 2 consisted of reduced high purity copper wires at 650°C. Helium was used as the 113 carrier gas at a flow rate of 95 ml min⁻¹ and oxygen at a flow rate of \sim 30 ml min⁻¹. This was 114 filtered for hydrocarbons upstream of the instrument. A packed 3m gas chromatograph (GC) 115 column was used for separation of the gases. A thermal conductivity detector (TCD) was 116 used to calculate the signal of each sample. For oxygen concentration, the Costech ECS was 117 also used but was set up for O analysis. Reactor 1 consisted of a nickelised carbon/silica 118 chips/nickel wool pyrolysis tube at 1060°C whilst Reactor 2 was left empty. Helium was 119 used as the carrier gas at a flow rate of 130 ml min⁻¹ but no oxygen was used. A 2m packed 120 oxygen GC column was used for separation of the gases. Chloropentane vapour was added to 121

the carrier gas to enhance decomposition of the oxygen compounds and to reduce possiblememory effects from previous samples (Kirsten, 1977).

124 The computer software used was EAS Clarity (DataApex Ltd, Prague, Czech 125 Republic). For both CHN and O setups calibration curves of $r^2 > 0.999$ were created using 126 acetanilide as the standard. Samples of acetanilide were included within each run as unknown 127 samples to act as internal quality control checks. Each sample (litter, soil, or vegetation) was 128 analysed in triplicate (i.e. three times in one run on the CHN setup and a further three times in 129 one run on O set up), and a mean value calculated for C, H, N and O for each sample

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131 *Calorimetry*

Energy content, as gross heat value (ΔH_c), was measured for all vegetation and litter samples. 132 Even after HF treatment the mineral content of the soil samples was still too high for an 133 134 energy content to be measured. Masiello et al. (2008) has shown that it is possible to derive carbon oxidation state (Cox) values (and therefore OR values) from calorimetry data. Analysis 135 136 was performed on a 6200 Isoperibol Calorimeter (0.1% Precision Classification, Parr 137 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 138 in crucibles and compressed to stabilise the surface and weighed following compression, with 139 140 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 141 of fuse wire remaining, measured in calories, and converting to kJ/g using a constant. The 142 correction value was subtracted from the energy content recorded during analysis. 143

144 Masiello et al. (2008) used ΔH_c from calorimetry to calculate C_{ox} values for a range of 145 standard materials. If there is a relationship between C_{ox} and ΔH_c , it might be reasonable to 146 expect there to be a relationship between ΔH_c (measured using calorimetry) and OR values 147 (calculated from elemental analysis) from this study. Clay and Worrall (in press) were able to 148 plot ΔH_c and OR values to identify unusual observations that may explain the variation in OR 149 between sites in a study of OR variation across UK peatlands. Therefore, ΔH_c values were 150 plotted against OR values for the vegetation and litter along with the standard materials – 151 cellulose, lignin and humic acid. Only vegetation and litter samples were included in this 152 analysis.

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154 C_{ox} and oxidative ratio (OR) calculation

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

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

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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).

163 As C_{ox} and OR are related through the balancing of organic matter synthesis, the OR 164 value is calculated as the ratio of O_2 and CO_2 coefficients (for further details see Masiello et 165 al. (2008)). Simplified it is then calculated as:

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$$OR = 1 - \frac{C_{ox}}{4} + \frac{3[N]}{4[C]}$$
 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 from this assumption would be only ± 0.002 (Hockaday et al., 2009). This

equation also assumes that the nitrogen source in carbon fixation is N₂. There are two further
possible nitrogen conversions (Masiello et al., 2008):

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174 Ammonia (NH₃):
$$OR = 1 - \frac{C_{OX}}{4}$$
 Equation 4

175 Nitrate (HNO₃):
$$OR = 1 - \frac{C_{OX}}{4} + \frac{2[N]}{[C]}$$
 Equation 5

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For the purposes of this paper, Equation 3 was used as N_2 is the dominant form in the ecosystem. The agricultural soils will likely have received N in other forms in addition to N_2 , but no fertilizer 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., 2014).

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.

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186 2.3. Statistical analysis

187 The design of the study allows for several statistical comparisons to be made using an 188 ANOVA approach. Firstly, one-way ANOVA was used to test whether there were 189 significant differences in OR between the organic matter material types being considered, i.e. 190 soil vs vegetation vs litter. Secondly, this set of ANOVA could be sub-divided into separate 191 ANOVA: the difference in OR between soil orders; the difference between biomes; the 192 difference in vegetation functional groups.

Finally, the variation in organic matter material types (soil, vegetation and litter) between sites was examined. In this final ANOVA, it was possible to analyse the differences between soils, vegetation and litter across the sites considered in study for the element ratios and OR. However it was not possible to include energy content as no values were measured for soils. Furthermore, as litter was not collected on all sites it was not possible to compare all sites directly, with respect to organic matter material type. Sites without litter were removed from the ANOVA and only those sites with soil, vegetation, and litter were included in further analysis (n = 14).

The response variables (C/N ratio, H/C ratio, O/C ratio, energy content, Cox, and OR) 201 were tested for normality prior to ANOVA using the Anderson-Darling test. If the response 202 variable failed the test it was log-transformed and re-tested – it did not prove necessary to 203 204 further transform the data. Post-hoc testing of the results was performed using the Tukey test at 95% level to determine significant differences between levels of any factor. The 205 magnitude of the effects of each significant factor and interaction were calculated using the 206 207 generalized ω^2 (Olejnik and Algina, 2003). All results are reported to a significance level of p<0.05. 208

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210 2.3.1. Global OR values

The data from this survey is used to update the estimation of global OR made by Worrall et al. (2013). Worrall et al. (2013) used a weighted average based upon the residence time of carbon in the vegetation and soil carbon pools as this better reflected the faster turnover of carbon in the vegetation pool compared to the soil carbon pool. Therefore, OR_{terra}^{global} is:

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$$OR_{terra}^{global} = f_{soil}^{terra} OR_{soil}^{global} + f_{veg}^{terra} OR_{veg}^{global}$$
Equation 6

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218 Where: OR_{terra}^{global} = the oxidative ratio of the global terrestrial biosphere; OR_{soil}^{global} = the 219 oxidative ratio of global soils; OR_{veg}^{global} = the oxidative ratio of 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.

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The proportion of annual global flux from the soils or vegetation (f_{soil}^{terra} and f_{veg}^{terra} respectively) were based upon the size of each carbon pool divided by the average residence time of the carbon in that pool:

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$$1 = f_{soil}^{terra} + f_{veg}^{terra}$$
 Equation 7

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$$f_{soil}^{terra} = \frac{\varphi_{soil}^{terra}}{t_{soil}^{terra} \left(\frac{\varphi_{soil}^{terra}}{t_{soil}^{terra}} + \frac{\varphi_{veg}^{terra}}{t_{veg}^{terra}}\right)}$$
Equation 8

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Where: φ_x^{terra} = the proportion of the terrestrial carbon pool that is in x, with x either soil or 231 vegetation; and t_x^{terra} = average residence time of carbon in the terrestrial carbon pool 232 represented by x, with x as either soil or vegetation (years). The comparative sizes of the soil 233 and vegetation carbon pools were estimated from Eswaran et al. (1993) and Olson et al. 234 235 (2001) where the proportion of carbon in the vegetation pool was 0.28 and in the soil pool as 0.72. The average carbon residence time for soils was taken as between 20 and 40 years 236 based upon a study by Jenkinson and Rayner (1977). The average carbon residence time for 237 vegetation was taken as between 2 and 5 years (e.g. Gaudinski et al., 2000). Given the above 238 approach the values of $f_{soil}^{terra} = 0.27$ and $f_{veg}^{terra} = 0.73$. 239

The value of OR_{veg}^{global} was calculated as the weighted average of the expected value of OR for each of 15 global biomes (Loveland and Belward, 1997) where the weighting factor was the area of each biome:

$$OR_{veg}^{terra} = \frac{1}{A_{total}} \sum_{1}^{15} A_n E(OR_n)$$
 Equation 9

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246 Where: A_n = the area of biome n (km²); A_{total} = the total area of all n biomes (km²); and 247 E(OR_n) = the expected value of the OR of biome n. Given the lack of data for most biomes 248 the median was taken as the expected value.

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Similarly, OR_{soil}^{global} was calculated as a weighted average of the expected values of the OR for each of the 12 USDA soil taxonomy soil orders (although Gelisols are treated as equivalent to Histosols) where the weighting factor was the total carbon content of each soil order as estimated by Eswaran et al. (1993) – note that such estimates of carbon content are not available for separate global biomes.

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- 256 257

 $OR_{veg}^{terra} = \frac{1}{OC_{total}} \sum_{1}^{11} OC_n E(OR_n)$ Equation 10

Where: OC_n = the organic carbon content of soil order n (Pg C); OC_{total} = the total organic carbon content of all n soil orders (Pg C); and E(OR_n) = the expected value of the OR of soil order n. As above, given the lack of data for most soil orders, the median was taken as the expected value.

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3. Results

In total 42 vegetation samples, 14 litter samples and 30 soil samples were analysed. Table 2 shows the elemental concentration data, energy content, C_{ox} and OR values for each of the material types whilst Tables 3, 4 and 5 show the data for each of the soil orders, biomes and main vegetation types respectively. None of the datasets needed to be transformed prior to ANOVA. Of the three material types, soils had the lowest OR values (highest C_{ox} values, i.e. the most oxidised) compared to vegetation or litter, which appear to have very similar values

- 276
- 277 3.1. Variation within organic matter material type

278 Soil orders

The one-way ANOVA of the soils data (Supplementary Table 1) showed no significant
differences in elemental ratios, C_{ox}, or OR between soil orders.

- 281
- 282 Biomes

A one-way ANOVA of the data divided into biomes (Supplementary Table 2) showed no significant differences in elemental ratio, ΔH_c , C_{ox} or OR.

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286 *Vegetation functional groups*

There were significant differences between vegetation functional groups in the elemental 287 ratios. For C/N ratio, the significant difference (p = 0.003) lay between tree branches and all 288 289 other vegetation types, with the exception of sugar cane samples. The highest C/N ratios were found in tree branch samples (Supplementary Table 3). The O/C ratio showed a significant 290 differences (p = 0.005) where the difference lay between tree leaves and crops, grass and tree 291 292 branches. In this instance the lowest O/C ratios were found in tree leaves whilst the higher values of O/C were found in crop, grass and tree branch (Supplementary Table 3). For H/C 293 ratios the only significant difference (p = 0.005) was found between tree leaves and grass. 294

There were no significant differences in the ANOVA models for ΔH_c (p = 0.668), C_{ox} (p = 0.100) or OR (p = 0.053). However, within the post-hoc testing for OR there were significant differences between tree branches and leaves, no matter where the leaves came from. In this case the OR of the tree branches was significantly lower than that of the leaves (Supplementary Table 3).

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301 3.2. Organic matter material type vs. site

302 Site was a significant factor for both O/C and H/C ratios (Table 3) explaining 31% and 17% of the variation in the data respectively. Post-hoc testing showed that the highest 303 ratios were found on sites 18, 19 and 21 which were those sites under sugar cane plantations 304 305 (Table 1). Specifically for O/C ratios, site 18 was different from sites 3, 7, 10, 26, and 28 whilst site 19 was different from site 7. The sites 3, 7, 10, 26, and 28 are predominately those 306 with trees or 'woody' stems (Table 1). For H/C ratios, site 18 was different from sites 3, 5, 7, 307 11, 26, 28, and 29 whereas site 19 was different from 3, 5, 7, 11, 12, 26, 27, 28, and 29 whilst 308 site 21 was different from site 7. Again the differences appear to be between sugar cane 309 310 plantations and those with the presence of trees. There were no significant differences (p > p0.05) between the OR, or Cox of sites. 311

Material type was a significant factor in the case of H/C ratio, C_{ox} and OR explaining 36%, 42% and 30% of the variation in the data respectively (Table 3). Post-hoc testing showed than_that_in the case of H/C ratio soil samples had a lower value than for either vegetation or litter. For C_{ox} , soil had a higher value than either vegetation or litter, whilst OR had the opposite pattern with soil having a lower OR value than either vegetation or soil. This echoes the general pattern in material types seen in Table 2.

There were significant interactions between site and material types for O/C and H/C ratios explaining 47% and 40% of the variation in the datasets respectively (Table 3). In the O/C data soils generally had lower or very similar values to vegetation and litter but for sites 18, 19 and 21 there is a pronounced switch with these sites having higher soil OR values than either vegetation and litter. The pattern was similar for the H/C ratio where soil had lower H/C values than litter and vegetation but on sites 18 and 19 soil OR values were greater than litter or vegetation. No significant interactions were found for C/N ratio, C_{ox} or OR.

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326 3.3. Variation in organic matter composition

Masiello et al. (2008) showed a positive correlation between OR and ΔH_c , and indeed, for the 327 data from this study a correlation can be observed between the OR and ΔH_c of the organic 328 material standards (humic acid, cellulose and lignin; Figure 2; note this correlation is for the 329 standards only and that none exist for the samples). All the vegetation and litter samples plot 330 on or above this line with lower ΔH_c values than would be expected for their OR values. 331 Furthermore, all samples except one appear to lie between three end-members where the end-332 members are lignin, humic acid and a third end-member of unknown composition (Labelled 333 'A' on Figure 2). This third end-member lies at a low ΔH_c value whilst having an OR value 334 of approximately 1.11 and similar to lignin. Clay and Worrall (in review) have also 335 336 identified a similar end-member (high OR with a relatively low ΔH_c) in their assessment of OR values in UK peats. It is possible that soil samples had had an input of mineral matter 337 such as silt effectively reducing the energy value whilst retaining an overall OR signal – OR 338 is a ratio and so not diluted by the presence of mineral matter which is removed through HF 339 digestion. However, in this mixing diagram, only vegetation and litter were considered. 340 341 Instead the end-member may be a specific compound in certain samples that is in a reduced form but has a low calorific value. For reference, nearby litter and vegetation samples came 342 from a mixed forest and grasslands, respectively. 343

In Worrall et al. (2013) the value of OR_{veg}^{global} was based on an analysis of 32 samples, this can 346 now be updated to include 213 samples (Table 4) and the biome area weighted value of 347 $OR_{veq}^{global} = 1.07 \pm 0.02$, where the uncertainty is the inter-quartile range. Similarly, OR_{soil}^{global} 348 was based upon 33 samples and this can now be updated to include analysis of 490 samples 349 (Table 5) and gives an organic carbon content weighted value of $OR_{soil}^{global} = 1.06 \pm 0.04$. 350 Given the updated values, the residence time weighted value of OR_{terra}^{global} (Equation 6) can 351 now be estimated as 1.06 \pm 0.06. By applying this new value of OR_{terra}^{global} to equation (1), the 352 new value of f_{land} will be 1.45 Gt C/yr. 353

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355 4. Discussion

This study was specifically designed to fill in some of the data gaps identified from Worrall 356 et al. (2013) and as such is able to present OR data for the first time for Ultisols and to 357 358 expand the database of OR values for Alfisols, Entisols, Histosols, Oxisols and Vertisols. 359 This work is also able to report, for the first time, OR values for savannahs and to increase the database for woody savannahs, evergreen, deciduous, and mixed forest, and grasslands. 360 Furthermore, recent additional studies (Clay and Worrall, in press; Worrall et al., in review) 361 have also presented OR data from a range of soil and vegetation types since the original 362 meta-analysis. 363

This study has shown that it is possible to distinguish between major carbon pools i.e. between vegetation and soils or between litter and soil, but that vegetation and litter are not significantly different from each other. Vegetation and plant litter can be described as lying on a decay continuum (Melillo et al., 1989; Fang et al., 2011) and as such it may be that the samples chosen are closer on this continuum that than they are to the underlying mineral soil. In this study, the soils were found to have significantly (p = 0.001) lower OR values (Median 370 = 1.02) than vegetation (Median = 1.07) which is the reverse of that found in Worrall et al. (2013) where vegetation OR was generally lower than soil OR. However, that previous study 371 could not consider, nor had very few samples of, the soil orders sampled here, in particular 372 373 for Oxisols and Ultisols. Oxisols and Ultisols are typified by old and oxidised soils compared to the other soil orders and therefore might be considered to have highly oxidised organic 374 content. It should also be noted that this study found higher values of vegetation OR than 375 376 previously reported, and for all the global biomes that could be considered in this work, for which a previous estimated was made, this study found higher values of OR. 377

378 The significant difference between soil and vegetation pools justifies the method of Worrall et al. (2013) as expressed in Equation 6. However, this approach was based on the 379 idea that OR_{veg}^{global} and OR_{soil}^{global} could be understood from the combination of results for a 380 range of global biomes and soil orders. This was partly out of necessity as it was only 381 possible to classify results from disparate literature studies into broad, globally-applicable 382 383 classes. However, this study found no significant difference between soil orders. Clay and Worrall (in review) did find a significant difference between Histosols and Inceptisols from 384 eight locations across the UK, while the present study could find no difference between any 385 386 soil orders. It may therefore be that not all soil orders are different and that the biggest difference is between organic-rich and mineral-rich soils. Similarly this study could not find a 387 significant difference between the biomes. 388

There may not have been significant differences between the sampled soil orders and biomes but there were significant differences between vegetation functional groups with respect to elemental composition and possibly OR. This perhaps suggests that the control on differences in OR are due to varying proportions of biochemical compounds between vegetation, sites or biomes. For example, the comparison of OR and ΔH_c (Figure 2) shows that variation in the OR of vegetation and litter samples is bracketed by the reduced lignin 395 and the comparatively oxidised cellulose and so variation in these plant components could control the OR of the biome. This study was careful to be the first to sample all major organic 396 matter material types wherever available on any site but it did not quantitatively recover the 397 398 biomass and so variation between biomes may just reflect the choice of sample within a biome rather than the whole biome. Additionally, this study did not look at the role of roots in 399 with respect to OR. Roots represent a large global carbon store (~268Pg, Robinson, 2007) 400 401 and given their significant role in soil respiration (Hanson et al., 2000; Bond-Lamberty et al., 2004), it may be that for complete ecosystem OR estimates, the OR of roots will need to be 402 403 calculated. Therefore, there is a need in the future to quantitatively recover and sample the biomass within a biome. The difference found between sites within this study may well 404 reflect this difference between biochemical compounds and hence the difference between 405 406 sugar cane plantations and sites with woody vegetation.

407 By assessing the OR of each carbon pool at a sampling site, this study is able comment on what kind of sample may be representative of the OR of the environment. In 408 409 terms of O/C and H/C ratios there was a significant interaction between site and material types indicating that organic matter material types varied amongst site. This might imply that 410 classification by material types is site-dependent and not suitable for large-scale surveys. 411 However this was only seen for two ratios and the lack of an interaction between site and 412 413 material type for OR suggests that the difference between organic matter material types is 414 independent of the change between sites and as such it is appropriate to sample all the major carbon pools in OR assessments. 415

This study, along with other studies, can now dramatically increase the amount and diversity of data that can be used to calculate the <u>global OR estimate</u> - OR_{terra}^{global} . It is interesting to note that values have converged and not diverged, with OR_{soil}^{global} and OR_{veg}^{global} no longer significantly different from each other. There is still a large variation in the density 420 of sampling of OR of both biomes and soil orders, but given the findings of this study there 421 are key contrasts that should now be focused on. For example, there is still only one study 422 each of Aridisols and Andisols but it may be more important to consider contrasts between 423 organic-rich and organic-poor sub-orders rather than between the orders themselves. Equally, 424 the contrast in biomes may be particularly pronounced during land use change and given the 425 results here we suggest that the contrast between forest and grassland might be particularly 426 large.

427

428 5. Conclusions

This study showed that there were significant variations in oxidative ratio (OR) between organic matter material types and that OR values were lowest in the soils relative to vegetation and litter. The analysis suggests that OR variation may be controlled by varying proportions of organic matter biochemical compounds such as lignin and cellulose. This study has also been able to add new data to the expanding literature on OR and can update the global OR_{terra} estimate to 1.06 ± 0.06 .

435

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438 (FERA) licence and handled under FERA approved guidelines in the Department of
439 Geography, Durham University.

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520 Figure 1. Location map of sampling sites in South Africa and Swaziland

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Figure 2. Plot of OR and ΔHc values for vegetation and litter. Standard materials (cellulose,
lignin and humic acid) are included for comparative purposes. Linear trend line fitted to
standard materials (cellulose, lignin, humic acid). For the purposes of the mixing diagram,
humic acid and cellulose form two end-members, whilst the approximate position of the third
end-member, labelled A, is of unknown composition.

528 Table 1. Site location with major descriptive information and samples collected.

529

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

532

Table 3. ANOVA for site and material types for elemental ratios, C_{ox} and OR values. df = degrees of freedom, p = probability of factor being zero, ω^2 = generalized proportion of variance explained.

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Table 4. The range and median values of OR for each of the global biomes. Additional datafrom [1] Worrall et al. (in review), and [2] Clay and Worrall (in review).

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Table 5. The range and median values of OR for each of the USDA global soil orders,
(Gelisols included with Histosols). Additional data from [1] Worrall et al. (in review), and [2]
Clay and Worrall (in review).

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545 Supplementary Table 1. Median values (inter-quartile range in parentheses, where applicable)546 for each measured or derived variable for soil orders.

547 Supplementary Table 2. Median values (inter-quartile range in parentheses, where applicable)548 for each measured or derived variable for biomes.

549 Supplementary Table 3. Median values (inter-quartile range in parentheses, where applicable)

550 for each measured or derived variable for the main vegetation functional groups