The impact of fertilizer management on the oxidation status of terrestrial organic matter.

Fred Worrall[1*], Gareth D. Clay[2], & Andy Macdonald[3]

1. Dept. of Earth Sciences, University of Durham, Durham, DH1 3LE, UK.
2. Geography, School of Environment, Education and Development, University of Manchester, Manchester, M13 9PL, UK.
3. Sustainable Soils and Grassland Systems Department, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK.

***Corresponding author:** email Fred.Worrall@durham.ac.uk

Running title: Fertiliser management and oxidation status

Summary

The oxidative ratio (the ratio of moles of O_2 produced per mole CO_2 sequestered - OR) of the organic matter in the terrestrial biosphere governs the ability of the terrestrial biosphere to uptake CO_2. The value of OR is known to vary between environments, but it would also be expected to vary with management. This study measured the OR of plant and soil samples from the long-term grassland plots on the Park Grass experiment at Rothamsted (SE England). The selected plots included those with different fertilizer inputs, including Farmyard manure or inorganic fertilizers and an unfertilized control, each with and without lime. The measurements show that:

i) Use of inorganic fertilizer caused the OR of soil organic matter to increase.

ii) Farmyard manure (FYM) caused OR of the soil to increase but that of the vegetation decreased.

iii) Liming had the effect of decreasing OR and counteracting effects of fertilizer.

iv) The OR of the ecosystem increased with FYM application but decreased with inorganic fertilizer application.

The global pattern in the use of organic amendments and inorganic fertilizers suggest that the likely impact of the predicted increase in global inorganic fertilizer use will result in a net decrease in the OR of the organic matter of the terrestrial biosphere, and an increase in its ability to act as a carbon sink. Corresponding increases in global FYM use and its impact upon global OR are unlikely to be large enough to counteract this effect.

Keywords: grassland; soil organic matter; soil organic carbon; oxidative ratio

Introduction

Keeling and Shertz (1992) proposed that the magnitude of global sinks of carbon could be estimated from the relative changes of oxygen (O_2) and carbon dioxide (CO_2) concentrations in the atmosphere and this approach has been used widely (Prentice et al., 2001). The approach uses the following formula:

$$f_{land} = -\frac{CS}{OR_{terra}^{global}} f_{fuel} + \frac{1}{k_1 k_2 OR_{terra}^{global}} \frac{d\left(\frac{O_2}{N_2}\right)}{dt} \qquad \text{(i)}$$

Where: f_x= the annual flux of CO_2 (Gt C/yr) with x= land, fuel or cement; (O_2/N_2) = the molar ratio of atmospheric O_2 and nitrogen (N_2); CS = the combustion stoichiometry (1.43 - Battle et al., 2000); OR_{terra}^{global} = the oxidative ratio of the global terrestrial biosphere; constants K_1 and K_2 = converts ppm to per meg (0.471 and 4.8 respectively). Battle et al. (2000) refer to OR_{terra}^{global} as the photosynthetic stoichiometry and this term is also known as α_B in the calculation of the atmospheric potential oxygen (APO – Stephens et al., 1998). The oxidative ratio (OR) represents the ratio of moles of O_2 produced per mole CO_2 sequestered by the terrestrial biosphere. The OR of organic matter can be directly related to the oxidation state of that organic matter (Masiello et al., 2008).

Steinbach et al. (2011) considered the influence of varying CS on the estimation of f_{land} but what about the value of OR_{terra}^{global}? The value of OR stated in numerous studies is 1.1 (Battle et al et al., 2000, Langenfelds et al., 1999, IPCC, 2007), but this value was based upon a study of the Biosphere 2 experiment (Severinghaus, 1995) that did not set out to measure an OR value applicable to the global terrestrial biosphere. It is easy to demonstrate that changes in the value of OR can have consequences for the estimation of the terrestrial and oceanic carbon sinks (Randerson et al., 2006). An approximate 10% variation in the estimate of the OR value leads to a 10% change in the value of f_{land}.

Given that Steinbach et al. (2011) have shown the impact of varying CS on global C fluxes, is a value of 1.1 for the global OR appropriate? When OR is measured for individual ecosystems, values are rarely 1.1 (e.g. Gallagher et al., 2014). However, it is not possible to extract a globally-meaningful value from any one ecosystem study. Worrall et al. (2013) compiled literature for whole soil and vegetation data from across the globe to provide a flux-weighted estimate of global OR of 1.03 ± 0.03. However, Worrall et al. (2013) had to assume that the major control on OR was the differences between global soil types or biomes and could not consider difference due to, for example, the role of management or land use change. Worrall et al. (2013) had to make do with the data that were available rather than with data from experiments designed to consider oxidation state and so had no data where it was possible to compare different carbon reservoirs (e.g. soil organic matter vs. biomass) within a single environment. Clay and Worrall (2015a and b) have conducted focused experiments to infill the gaps identified in Worrall et al. (2013). But, no attempt has been made to consider impact of management upon OR. Therefore, this study considers the oxidation status of the vegetation and soil.

Approach and Methodology

A value of OR can be calculated from a carbon oxidation state (C_{ox} - Masiello et al. (2008):

$$C_{ox} = \frac{2[O]-[H]+3[N]}{[C]} \qquad \text{(ii)}$$

Where: [X] = molar concentration of C, H, N or O. The OR value is then:

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

Equation (ii) assumes that there is no contribution to the C_{ox} from S or P, an assumption shown to be negligible compared to instrumental error (Hockaday et al., 2009). This error was added to the final calculation made in this study. Equation (iii) is stated assuming the ultimate source of N was N_2: in the majority of cases the OR error of the N_2 assumption would be not more than 0.008 (Hockaday et al., 2009).

The Park Grass Experiment

Started in 1856, the Park Grass is the oldest ecological experiment on permanent grassland in the world (Silvertown et al, 2006). The Park Grass experiment is located at the Rothamsted Experimental Station, Harpenden UK (N51^{o}48'15" W0^{o}22'29"). The annual 30-year mean (1981 to 2010) for rainfall is 718.4mm and the average monthly air temperature is 10.2°C. The Park Grass experiments currently comprises plots with an average size of 300 m^2 subject to more than 20 different fertilizer treatments (Silvertown et al, 2006). In this study we chose plots to study four agro-ecological factors in a complete factorial design. The factors considered were:

Nutrient application – three treatments were chosen: no application; organic manures (farmyard manure (FYM) and poultry manure (PM)); and inorganic fertilizers only (Table 1).

Sample type – both soil (bulked from 0-23 cm depth, dried and ground material) and vegetation (dried, unground) were analysed. The Park Grass vegetation consists largely of grasses and herbs. The analysed archived samples (chopped and dried at 80^{o}C) were from cuts made in mid-June to make hay.

Liming – both limed and unlimed plots were chosen for study (Table 1).

Year – the archived plant and soil samples used were collected in 2005 and 2008, including material from the beginning and end of the FYM/PM application cycle (Table 1).

Sample Analysis

About 1g of vegetation and 5g of soil were taken for analysis. Triplicate samples of soil and vegetation were dried to 105°C and milled to a sub-mm powder using a Spex 6770 Freezer Mill. The soils were pre-treated using a 2% hydrofluoric acid (HF) acid solution based on the method of Mathers et al. (2002). 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. The HF treatment was used to remove mineral matter and concentrate organic matter, but this process was not always complete and a sub-sample of each treated sample was analysed for its residual ash content by burning at 550°C overnight. Similarly, sub-samples of vegetation were also ashed at 550°C. Pre-treated soils were than subject to CHN and O analysis on a Costech ECS 4010 Elemental combustion system with pneumatic autosampler (DataApex Ltd, Prague, Czech Republic) and using acetanilide as the standard. All samples, both soil and vegetation, were corrected for their measured ash content.

The data for each element was analysed, but because the oxygen content was analysed on a separate sub-sample from the analysis of C, H and N it was not possible to calculate the OR for each sample. Instead each possible combination of samples for which C, H and N; and O were analysed was considered, i.e. 9 values of OR for each treatment.

The OR of the ecosystem , $(\mathrm{OR}_{\mathrm{ecosystem}})$ will be a weighted average based upon the residence time of carbon in vegetation and in soil. Therefore, $(\mathrm{OR}_{\mathrm{ecosystem}})$ is given by:

$$\mathrm{OR}_{\mathrm{ecosystem}} = \phi_{\mathrm{soil}}\mathrm{OR}_{\mathrm{soil}} + \phi_{\mathrm{veg}}\mathrm{OR}_{\mathrm{veg}} \qquad \text{(iv)}$$

Where: ϕ_x= the proportion of the ecosystem C annual flux that is due to either soil or vegetation; and OR_x = the oxidative ratio with x = soil or vegetation. The comparative sizes of the soil and vegetation reservoirs was estimated from Eswaran et al. (1993) and Olsen et al. (2001), the proportion of carbon in the vegetation reservoir was 0.28 and in the soil reservoir as 0.72. The average soil carbon residence time was taken as between 20 and 40 years based upon a study by Jenkinson and Rayner (1977) for the same soil type as the study site though under arable production. It is likely that the average carbon residence time under grassland would be at the upper end of this range as there is no disturbance from regular ploughing, but values for the grassland are not available. The average carbon residence time for vegetation was taken as between 2 and 5 years (e.g. Gaudinski et al, 2000). No data yet exist comparing soil pool and flux OR values. Given the average residence times and the proportion of the C storage terrestrial biosphere represented by soils and vegetation above then ϕ_{soil}= 0.27 and ϕ_{veg}= 0.73.

Statistical analysis

The analysis of variance (ANOVA) was performed on the soil and vegetation samples separately, and on the values of OR for the ecosystem as derived from Equation (iv). The ANOVA was performed including the mass of the sample as a covariate in case there was a fractionation effect with the sample size given the nature of the HF extraction. The magnitude of the effects, in this case generalized ω^2 (Olejnik and Algina, 2003), of each significant factor and interaction were calculated. Post-hoc testing of the results was made for pairwise comparisons between factor levels using the Tukey test. The Levene test was used to assess homogeneity of variance with respect to the factors in ANOVA. The Anderson-Darling test was used to ensure that the data were normally distributed. If either test was failed the data were log-transformed and re-tested. To avoid type I errors all probability values are assessed as significant if the probability of difference from zero is greater than 95%, but if the probability is close to this value then it is reported.

A power analysis was used to assess the minimum effect size that could be detected within this design. The study was fully factorial with respect to each of 4 factors, 1 centre point was assumed; the standard deviation was estimated as the square root of the mean square difference; and the required experimental power was set at 80%.

Results

The compositions of the ash-corrected samples are shown in Table 2 and Figures 1, 2 and 3. The values of OR are greater than 1 irrespective of the factor or level of each factor, with the highest median values being for vegetation samples; the biggest difference between factor levels being for the sample factor, i.e. between soil and vegetation. The difference in OR between soil and vegetation samples is reflected in the lower median C_{ox} values of vegetation and the higher proportion of carbon and hydrogen in the vegetation samples. The difference between treatment levels for the other factors is considerably less than that due to differences between sample types and would suggest that this one large difference might be dominating the other factors.

Analysis of sample OR

No transformation was necessary for any of the variables for the ANOVA of the OR as calculated for the samples of soil and vegetation. The power analysis suggested that this design was capable of detecting a difference of 0.066 in the OR and 0.1 in C_{ox}. The results of the ANOVA for OR show that there was no significant effect of the sample weight, and therefore there was no evidence that difference in the extraction was leading to a fractionation in the extraction process. The largest effect on the data was the difference between sample types with the soils having an OR = 1.12 ± 0.01 to that of vegetation 1.04 ± 0.01 – values given as mean and standard error. The difference between soil and vegetation samples reflects significant differences for each element considered and for the C_{ox}. The soil organic matter is more oxidised than that found in the vegetation with a lower C/N ratio than found in the vegetation (Table 1) with the difference in C/N being due to both decreased C and increased N content in the soil organic matter.

No other single factor was found to have a significant affect upon OR. However, a number of interactions were found to explain considerable proportions of the original variance in the data. The second highest proportion of the variance was explained by the interaction between sample and fertilizer treatment – explaining 24.3% of the original variance in the dataset (Table 2). For vegetation samples the effect of fertilize, in any form, was to reduce the value of OR with the greatest effect being from the addition of FYM rather than inorganic fertilizer. In contrast, for the soil samples the impact of inorganic fertilizer was to reduce OR by 0.01 but FYM causes an average 0.09 increase in soil OR over the unfertilized control and it should be remembered that the power analysis suggested that the experimental design was only capable of discerning greater than 0.066 change.

The second most important interaction was that between the year of sampling and the fertilize treatment (19.5% - Table 2). Where no fertilizer was applied there was a decline in OR between the sampling years (a decline of 0.061) and there was no change in OR where FYM was applied, but there was an increase in OR between 2005 and 2008 of 0.067 when inorganic fertilizer was applied.

The third most important interaction was that between sample types between the years the samples were collected (7.6% - Table 2) with the difference in OR between vegetation and soil samples being significantly greater in 2008 (an average difference of 0.1) than in

2005 (an average difference of 0.05). The sampling years were chosen for inclusion in this study to cover the cycle of organic manure application used on Park Grass; samples collected in 2005 were taken just after the application of FYM whilst those collected in 2008 were taken before a further application. Consequently, the difference observed in the soil increased over the application cycle, despite the application of poultry manure in 2007 (Table 1).

The least important, but still significant, interaction was that of the changing impact of fertilize with liming (0.7% - Table 2). Liming had the impact of lowering the OR both when FYM and inorganic fertilizer were applied with the greatest effect for liming on inorganic fertilizer - an effect of 0.06.

The error term represented 10.3% of the original variance. The error term includes all unexplained variance and can be made up of factors and interactions not considered in the experimental design as well as sampling and measurement error which itself could be a range of things including spatial heterogeneity within each treatment plot.

Ecosystem OR

The range of OR of the ecosystem ($OR_{ecosystem}$) values for each treatment is shown in Fig.3. The $OR_{ecosystem}$ values varied between 0.84 and 1.32. When the $OR_{ecosystem}$ was considered then the sample weight was a significant covariate, with $OR_{ecosystem}$ increasing with sample weight. Both the effect of fertilizer and liming are significant single factors (Table 3 – explaining 5.8 and 3.9% of the original variance respectively). The effect of liming was to decrease $OR_{ecosystem}$ by 0.02. For the fertilizer factor the impact of FYM was to increase $OR_{ecosystem}$ by 0.05 while the effect of inorganic fertilizer was to decrease $OR_{ecosystem}$ by 0.05. By far the largest effect was that of the interaction between year and fertilizer treatments. Examining this interaction shows that in 2005 both FYM and inorganic fertilizer had smaller $OR_{ecosystem}$ values than untreated plots by 0.04 and 0.09 respectively, but in 2008 both FYM and inorganic fertilizer treated plots had larger $OR_{ecosystem}$ values than untreated plots by 0.076 and 0.047 respectively. The second most important interaction was between fertilizer and liming treatment (8.9% - Table 3). Liming had the effect of decreasing $OR_{ecosystem}$ when there was no fertilizer (decrease in OR of 0.05) and for when there was treatment with inorganic fertilizer (decrease in OR of 0.03), but not for when there was treatment with FYM where it caused an increase in $OR_{ecosystem}$ (OR increased by 0.02). The least important interaction (7.4% - Table 3) was that between year and liming factors with liming causing a decrease in $OR_{ecosystem}$ of 0.04 in 2005, but there was no effect of liming in 2008.

When $OR_{ecosystem}$ was judged relative to the unfertilized, unlimed plot in 2005 then, although each single factor was found to be significant, by far the most important was the difference between sampling years (80.6% of the original variance explained – Table 3). Relative to the unlimed, unfertilized plot in 2005, the $OR_{ecosystem}$ of the same treatment plot in 2008 increased by 20% (92% of untreated plots in 2005 to 112% untreated plots in 2008). The plots receiving nutrients all had larger $OR_{ecosystem}$ values than the untreated plots with the greatest difference being for FYM (4.5% greater) and lowest effect being for the inorganic fertilizer (1% greater). Liming had the effect of reducing the OR by 2%. The most important interaction was that between sampling year and fertilizer treatment

Discussion

The soils of the Park Grass field are classified as Aquic or Typic Hapludalf or Paleudalf on the USDA soil taxonomy. Worrall et al. (2013) have listed Alfisols as having an OR of 1.10 (range =1.07 to 1.12) based upon 1 study and 4 soil samples; this would now be replaced as 1.12 (range = 1.08 to 1.19) based upon 2 studies and 2 soils but 84 samples. Combining this new estimate with others for other USDA soil taxonomic groups and weighted as for the proportion of the global soil organic carbon content (Eswaran et al., 1993) gives a new value of OR^{global}_{soil} = 1.06 ± 0.01, where error is given as the inter-quartile range (IQR). For grasslands, Worrall et al. (2013) had listed 2 studies with 12 samples giving an OR of 0.97 (range = 0.73 to 1.02). This can now be updated for 3 studies and 111 samples to give an OR of 1.02 (range = 0.73 to 1.05). Including these updated estimates gives an OR^{global}_{veg}= 1.040 ± 0.005 (error as IQR). Given the estimated size and turnover in the global soil and vegetation reservoirs used above (Equation iv) a new residence time weighted global OR estimate for the terrestrial biosphere (OR^{global}_{terra}) of 1.055 (IQR = 1.053 to 1.057) can be calculated; the previous value of OR^{global}_{terra} was 1.04 ± 0.03.

This study has been able to show that fertilizer management can alter the OR of an ecosystem. An increase of 0.02 in the global OR results in an increase in the overall sink of 0.005 Tg C. In the UK, inorganic fertilizer use peaked in the 1987 at 1650 Gg N/yr, since when it has declined (Worrall et al., 2009). The inorganic fertilizer application rate used on the study plots was akin to the average inorganic fertilizer use on grassland in the UK in 1964 (Worrall et al., 2009). At the global scale, the average application rate was 127 kg (N+P+K)/ha/yr in 2007 (FAO, 1961 through 2012); this is less than that used on our plots and the average used in the UK but global rates are increasing. In the UK, the cattle population peaked in 1978 at 13.7 million head (MAFF, 1963 to 2000, DEFRA, 2001 to 2011). Based upon the mass-flow model of Webb and Misselbrook (2004) and the survey of manure use by Smith et al. (2001), at peak cattle population the UK was using FYM at a rate nearer 48 Mg/ha/yr (estimated from. for all UK grassland (temporary + permanent but excluding rough grazing) but by 2010 this was back to 36 Mg/ha/yr. At the global scale the population of cattle has risen by an average of 9 million head per year since 1961 to the present level of 1391 million head – that is a 47% increase since 1961 (FAO, 1961 through 2012). The above values for the production of FYM per head and the global area of grassland (Olson et al., 2001) suggest that the present global average FYM loading would be 2.9 Mg/ha/yr. Although the global loading is much smaller than that expected for the intensive cattle raising in the UK it should be noted that this loading is increasing and that each form of livestock for which figures are reported by FAO the livestock have increased, i.e. loading of low C/N ratio manures to land have been increasing and could be expected to increase further and thus driving up the OR of the ecosystem. Galloway et al. (2004) has pointed out that globally reactive N production (i.e. conversion from N_2 to other forms of nitrogen) since 1860 has increased by 30% from 125 to 163 Tg N/yr and was set to increase by a further 38% by 2050. Howden et al. (2010) have shown that reactive nitrogen has been accumulating in the terrestrial biosphere of the UK and if nitrogen is accumulating then it is probable that carbon is accumulating. But this implies decreasing C/N ratios and therefore increasing oxidation status of the accumulating organic matter. Although with respect to OR the question is whether agricultural management changes composition and not concentration and that is what this paper has done for the first time.

The above discussion relies on understanding the position of equilibrium in the terrestrial biosphere, but not the disequilibrium represented by changes in the terrestrial OR. This study has found that inorganic fertilizer would decrease the OR of the environment thus cause a disequilibrium in the environment leading to increase in the terrestrial carbon sink. Given that inorganic nitrogen fertilizer has been available for approximately 100 years this study was able to compare plots with and without inorganic fertilizer and found a decrease of 0.05 in the ecosystem OR. In the 2000s, 14.8 x 10^8 ha of cultivated land existed across the globe, which received an average 71 kg N/ha (74% of that used on the plots in this study) over an area that represents 9.8% of the global terrestrial land area. Therefore the global change over the last 100 years would be a 0.003 decrease in the OR value. This means that there would be a disequilibrium effect of 0.03 Tg C/yr increase in global terrestrial carbon sinks (Randerson et al., 2006) and it could be predicted that a similar scale change would occur in the next 50 years.

It should be noted that this discussion of the impact of nutrient addition, be it by FYM or inorganic fertilizer, is above and beyond the impact that these have on greenhouse gas emissions from land in their own right.

Liming addition counteracted the impact of fertilizer with an average decrease in OR upon addition of lime of 0.011. From 1983 to 1997 lime use in the UK rose, although average application rate remained constant at 2.5 Mg CaO/ha the area receiving an application increased from 10% and 4% of arable and grassland respectively in 1983 to 12% and 7% of arable and grassland in 1997 (Chalmers, 2001). However, this scale of liming will not have been able to counteract OR change caused by fertilize, at least in the UK context.

Conclusions

This study of a long-term grassland experiment in the UK showed that nutrient management had a significant impact upon the oxidation status of a grassland environment. At this site, the impact of nutrient addition (organic and inorganic) led to the development of more oxidised organic matter in vegetation and lower OR values, while for soil the opposite was true: decreased C_{ox} and increased OR. The impact of inorganic fertilizer on the whole ecosystem was to decrease OR, with this being greater in limed soils, but organic fertilizer had the reverse effect, leading to a more reduced ecosystem. Given the global rates and trends in fertilisation of agricultural soils, this study provides further evidence to suggest that the terrestrial biosphere is being progressively oxidised and that this leads to increased flux of carbon to land.

Acknowledgements

We thank the Lawes Agricultural Trust for access to archived plant and soil samples. The Rothamsted Long-term Experiments National Capability is supported by the UK Biotechnology and Biological Research Council and the Lawes Agricultural Trust.

References

Battle, M., Bender, M.I., 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.

Chalmers, A.G. 2001. A review of fertilizer, line and organic manures use on farm crops in Great Britain from 1983 to 1987. *Soil Use and Management,* **17**, 254-262.

Clay, G. D. & Worrall, F. 2015a. Estimating the oxidative ratio of UK peats and agricultural soils. *Soil Use and Management*, **31**, 1, 77-88.

Clay, G.D. & Worrall, F. 2015b. Oxidative ratio (OR) of Southern African soils and vegetation: Updating the global OR estimate. *Catena*, **126**, 126-133.

DEFRA 2001 through 2011. Agriculture in the United Kingdom. Department of Environment, Food and Rural Affairs, HMSO, London.

Eswaran, H., Van den Berg, E. & Reich, P. 1993. Organic carbon in soil of the World. *Soil Science Society of American Journal,* **57**, 192-194.

FAO 1961 and 2012. Yearbook – production. United Nations Food and Agriculture Organisation, Rome.

Gallagher, M. E., Masiello, C. A., Hockaday, W. C., Baldock, J. A., Snapp, S., McSwiney, C. P. 2014. Controls on the oxidative ratio of net primary production in agricultural ecosystems. *Biogeochemistry,* **121**, 3, 581-594.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.E. & Vorosmarty, C.J. 2004. Nitrogen cycles: past, present and future. *Biogeochemistry*, **70**, 153-226.

Gaudinski, J.B., Trumbore, S.E., Davidson, E.A. & Zheng, S. 2000. Soil carbon cycling in a temperate forest: radiocarbon based estimates of residence times, sequestration rates and partitioning fluxes. *Biogeochemistry*, **51**, 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 C-13 nuclear magnetic resonance. *Journal of Geophysical Research – Biogeosciences*, **114**, G02014.

Howden, N.J.K., Burt, T.P., Worrall, F. & Whelan, M.J. 2010. Nitrate concentrations and fluxes in the River Thames 1868 to 2008: the long-term impact of land-use change. *Hydrological Processes*, **24**, 18, 2657-2662.

IPCC 2007. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor, and H.L. Miller (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jenkinson, D.S. & Rayner, J.H. 1983. Turnover of soil organic matter in some of Rothamsted classical experiments. *Soil Science*, **123**. 5, 298-305.

Keeling, R.F. & Shertz, S.R. 1992. Seasonal and interannual variations in atmospheric oxygen and implications for the global carbon cycle. *Nature*, **358**, 723-727.

Langenfelds, R.L., Francey, R.J., Steele, L.P., Battle, M., Keeling, R.F. & Budd, W.F. 1999. Partitioning of the global fossil CO_2 sink using a 19 year trend in atmospheric O_2. *Geophysical Research Letters*, **26**, 13, 1897-1900.

MAFF 1963 – 2000/ Agriculture in United Kingdom. Ministry of Agriculture Fisheries and Food, HMSO, London.

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**, G3, G03010.

Mittikalli, N.M. & Richards, K.S. 1996. Estimation of Surface Water Quality Changes in Response to Land Use Change: Application of The Export Coefficient Model Using Remote Sensing and Geographical Information System. *Journal of Environmental Management,* **48**, 263–282.

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.

Prentice, I.C. et al. (2001) The carbon cycle and atmospheric carbon dioxide, in Climate Change 2001, The Scientific Basis, Contributions of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. J.T.Houghton et al., Cambridge Univeristy Press, Cambridge, UK.

Randerson, J.T., Masiello, C.A., Still, C.J., Rahn, T., Poorter, H. & Field, C.B. 2006. Is carbon within the global terrestrial biosphere becoming more oxidized? Implications for trends in atmospheric O_2. *Global Change Biology,* **12**, 260-271.

Severinghaus, J.P. 1995. Studies of the terrestrial O_2 and carbon cycles in sand dunes gases and in Biosphere 2. PhD thesis Columbia University.

Silvertown, J., Poulton, P., Johnston, E., Edwards, G., Heard, M. & Biss, P.M. 2006. The Park Grass Experiment 1856-2006: Its contribution to ecology. *Journal of Ecology,* **94**, 801-814.

Smith, K.A., Brewer, A.J., Crabb, J. & Dauven, A. 2001. A survey of the production and use of animal manures in England and Wales. III. Cattle manures. *Soil Use and Management,* **17**, 77-87.

Stephens, B.B., Keeling, R.F., Heimann, M., Six, K.D., Murnane, R. & Caldeira, K. 1998. Testing global ocean carbon cycle models using measurements of atmospheric O_2 and CO_2 concentration. *Global Biogeochemical Cycles,* **12**. 2, 213-230.

Steinbach, J., Gerbig, C., Rodenbeck, C., Karstens, U., Minejima, C. & Mukai, H. 2011. The CO_2 release and oxygen uptake from fossil fuel emission estimate (COFFEE) dataset: effects from varying oxidative ratios. *Atmospheric Chemistry and Physics,* **11**, 6855-6870.

Webb, J. & Misselbrook, T.H. 2004. A mass flow model of ammonia emissions from UK livestock production. *Atmospheric Environment* 38, 2163-2176.

Worrall, F., Burt, T.P., Howden, N.J.K. & Whelan, M.J. 2009. The Flux of Nitrate from Great Britain 1974 – 2005 in the context of the terrestrial nitrogen budget of Great Britain. *Global Biogeochemical Cycles,* **23**, GB3017.

Worrall, F., Clay, G.D., Masiello, C.A. & Mynheer, G. 2013. Estimating the oxidative ratio of terrestrial biosphere carbon - global carbon sinks have been underestimated. *Biogeochemistry,* **115**, 1-3, 23-32.

Table 1. Details of manure, lime and fertilizer applications to the Park Grass experimental plots (Harpenden, Hertfordshire, England) selected for this study.

Plot	Liming	Fertilizer manure applications
3b	4 t/ha of limed applied every 4 years from 1903 to 1964; limed to pH 6 (approx) as necessary from 1990.	Unmanured since 1856.
3d	Unlimed	
13/2b	4 t/ha of limed applied every 4 years from 1903 to 1964; limed to pH 6 (approx) as necessary from 2003.	N,P,K,Na &Mg (and straw till 1897) from 1856 to 1904; 96 kg N/ha (ammonium sulphate), 35 kg P/ha (superphosphate), 225 kg K/ha (potassium sulphate), 15 kg Na/ha (sodium sulphate) and 10 kg Mg/ha (magnesium sulphate). Then, from 1905, cattle manure (FYM) and fish guano (Guano) applied alternately every four years, two years apart (i.e. FYM in 1905 and Guano 1907 etc); Guano was replaced with poultry manure (PM) in 2003. FYM was applied at 35 Mg/ha (fresh wt), Guano at 0.75 Mg/ha and PM at 2 Mg/ha. FYM applied in February 2005 and December 2008 supplied approximately 240 kg N, 45 kg P, 350 kg K, 25 kg Na, 25 kg Mg, 40 kg S and 135 kg Ca. PM applied in April 2003 and February 2007 contained about 65 kg N/ha in each application.
13/2d	Unlimed	
14/2b	4 t/ha of limed applied every 4 years from 1920 to 1964; no lime since. pH 6.2 in 2011.	N,P,K,Na & Mg applied annually in spring since 1858; 96 kg N/ha (sodium nitrate), 35 kg P/ha (superphosphate or triple superphosphate), 225 kg K/ha (potassium sulphate), 15 kg Na/ha (sodium sulphate) and 10 kg Mg/ha (magnesium sulphate).
14/2d	Unlimed	

Table 2. Summary of results from soil and vegetation (composition of organic matter samples, ash-corrected) by treatment factor with values given as mean (standard error).

		%C	%H	%N	%O	C/N	C_{ox}	OR
Sample	Soil	43.0 (2.6)	3.9 (0.6)	3.4 (0.3)	30.5 (2.6)	15.1 (6.6)	-0.05 (0.1)	1.12(0.06)
	Grass	43.2 (0.2)	3.9 (0.4)	1.2 (0.3)	39.3 (0.5)	43.9 (1.1)	-0.29 (0.21)	1.03 (0.03)
Year	2005	42.5 (4.3)	4.6 (0.7)	2.1 (0.7)	38.0 (8.4)	35 (17)	-0.09 (0.14)	1.04 (0.05)
	2008	43.0 (5.0)	4.8 (0.9)	1.7 (1.3)	38.2 (9.6)	34 (13)	-0.15 (0.16)	1.06 (0.05)
Liming	limed	43.0 (5.3)	4.7 (0.8)	1.5 (0.6)	38.2 (9.4)	33 (14)	-0.13 (0.13)	1.05 (0.05)
	unlimed	42.5 (3.6)	4.6 (0.6)	2.3 (1.5)	27.3 (8.4)	39 (16)	-0.14 (0.22)	1.06 (0.07)
Fertilize	None	43.1 (4.4)	4.5 (0.7)	1.4 (0.6)	24.8 (9.1)	33 (11)	-0.17 (0.14)	1.07 (0.05)
	FYM	42.4 (4.9)	4.6 (0.5)	1.4 (0.8)	38.1 (10.2)	39 (37)	-0.11 (0.25)	1.05 (0.08)
	NPKNaMg	43.1 (6.1)	4. 7 (1.1)	1.3 (0.7)	39.0 (7.8)	40 (16)	-0.11 (0.11)	1.04 (0.02)

Table 3. The proportion of variance explained by all significant ($p < 0.05$) factors and interactions for all samples.

	%C	%N	%H	%O	C/N	C_{ox}	OR
Sample wt	0.9	1.5	-	0.4	-	-	-
Sample	77.9	83.4	62.3	92.5	86.4	9.3	25.0
Year	1.1	1.4	-	-	0.7	-	-
Fertilizer	-	-	-	-	1.9	-	-
Liming	1.8	3.5	-	0.8	0.5	-	-
Sample * Year	3.4	-	-	-	1.8	9.6	7.6
Sample * Fertilize	-	-	13.8	-	6.7	26.5	24.3
Sample * Liming	4.7	3.4	-	-	-	-	-
Year * Fertilize	-	0.5	-	-	-	23.9	19.5
Year * Liming	-	0.2	-	-	-	-	-
Fertilize * Liming	4.8	0.8	-	3.7	-	0.3	0.7
Error	2.6	1.2	13.7	2.0	1.9	13.0	10.3

Table 4. The proportion of variance explained by all significant ($p< 0.05$) factors and interactions for the ecosystem judged both in absolute and relative terms.

	Ecosystem OR	Ecosystem OR relative
Sample wt	18.5	3.8
Year	-	80.6
Fertilizer	5.8	1.1
Liming	3.9	0.6
Year * Fertilize	54.2	10.6
Year * Liming	7.4	1.3
Fertilize * Liming	8.9	1.7
Error	1.2	0.2

Fig. 1. The distribution of soil sample OR values by treatment factor. The Whisker presents the range of the data, the box represents the 5^{th} to 95^{th} percentile range and horizontal line through the box is the median value.

Fig. 2. The distribution of vegetation sample OR values by treatment factor. The Whisker presents the range of the data, the box represents the 5^{th} to 95^{th} percentile range and horizontal line through the box is the median value.

Fig. 3. The distribution of ecosystem OR values by treatment factor. The Whisker presents the range of the data, the box represents the 5^{th} to 95^{th} percentile range and horizontal line through the box is the median value.