2001-2006 Mission
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The Oxidation State of Soil Organic Carbon: A New Proxy for Carbon Storage Mechanisms and Land Use Change

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Summary

The two most fundamental organic chemical reactions on the Earth, photosynthesis and respiration, act to cycle carbon between redox states: photosynthesis transforms carbon from the +4 oxidation state (CO_2) to energy-rich compounds with carbon oxidation states close to 0 (e.g., glucose); respiration reverses the reaction. With each cycle of photosynthesis and respiration, a very small fraction of organic carbon survives to enter the Earth's soil and sedimentary organic carbon reservoirs (Hedges and Keil 1995). This slow leak out of active cycling is responsible for the formation of the Earth's 1,500-2,300 Gt (Amundson 2001) soil organic carbon pool.

Although it is understood that this oxidation-reduction cycle is fundamental to the large-scale existence of soil organic carbon, almost nothing is known about smaller-scale fluctuations in oxidation state of carbon within soils and larger ecosystems. Soil carbon oxidation state is a basic property likely influenced by soil forming factors. At a fundamental level, C_{ox} describes the energy stored within an organic moiety, and in this way connects ecosystem energetics to soil properties. In this grant we made the first measurements of carbon oxidation state (C_{ox}) in soils and in other Earth system carbon pools, and developed language to connect measurements of C_{ox} to ecosystem thermodynamics measurements. Additionally, we identified 1) extent of organic matter decomposition, and 2) chemical structure of original biomass as the two properties most strongly influencing organic matter C_{ox} values.

The techniques and theory developed in this grant lay the foundation for future work using C_{ox} to understand soil processes.

Objectives

The goal of this project was to explore the use of soil C_{ox} in understanding soil carbon cycle processes.

In the 24 months covered by this proposal, we focused on four areas of research:

1) Development of math and language for reporting C_{ox} data and comparing with existing thermodynamic data;

2) Development of two techniques to measure C_{ox} in soils, both based on solid state ^{13}C NMR;

3) General C_{ox} measurements to outline the C_{ox} values of Earth system carbon pools and map out potential large-scale controls;

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4) Specific C_{ox} measurements in a pair of soil profiles to understand more detailed controls C_{ox} within the soil system.

Development of Math and Language

Making and writing about measurements of C_{ox} has required that we develop some language. We have tried to be simple about this, and most of what we have done in terms of language has involved anchoring our new variable (C_{ox}) within the existing chemical language of oxidation and reduction, as well as anchoring it to the existing language of ecosystem thermodynamics. We defined C_{ox} via the most simple chemical expression for natural organic matter, $C_xH_yO_zN_w$. For any given natural organic mixture, the oxidation state of carbon (C_{ox}) is:

$$C_{ox} = \frac{2\bullet[O]-[H]+3\bullet[N]}{[C]} \qquad \text{equation (1)}$$

where [O], [H], [N], and [C] represent the molar fractions of oxygen, hydrogen, nitrogen, and carbon present.

We can relate C_{ox} to the ecosystem variable *oxidative ratio*: the ratio of moles of O_2 released to moles of CO_2 fixed via photosynthesis. OR is related to C_{ox} as:

$$OR = 1 - \frac{C_{ox}}{4} \qquad \text{equation (2)}$$

This relationship is derived by balancing the stoichiometric equation for photosynthesis and then ratioing mols O_2/mols CO_2. The relationship between C_{ox} and OR is important because the OR of biospheric materials is a currently approximated variable in estimations of the size of the ocean's carbon sink (Keeling, Piper et al. 1996). Small variations in ecosystem OR can cause large variations in fossil fuel sink apportionment (Randerson, Masiello et al. 2005). Techniques developed as part of this grant to measure C_{ox} in soils have spill-over applications in improving accuracy of fossil fuel CO_2 sink apportionment.

We have also derived a relationship between OR and the commonly measured ecosystem parameter construction cost, typically determined via bomb calorimetric measurement of ΔH_c (heat of combustion) and EA_c (percent organic carbon):

$$\left(0.06968\ \text{Å\textasciitilde}\ \Delta H_C - 0.065\right)\ \text{Å\textasciitilde}\ \frac{0.4}{EA_c} = OR \qquad \text{equation (3)}$$

This anchors the concept of carbon oxidation state to the thermodynamics of plant carbon fixation, and may allow the conversion of existing biomass ΔH_c data to C_{ox} and OR data. A complete derivation of this equation is attached as Appendix 1.

Techniques to Measure C_{ox} Introduction to Solid State ^{13}C NMR

Solid state ^{13}C NMR has proved to be one of the few techniques permitting chemical characterization of a significant fraction of soil organic carbon (OC). Sometimes ^{13}C NMR can detect all the carbon in an OC sample, with the right choice of NMR technique and sample (Smernik and Oades 2000a; Smernik and Oades 2000b). ^{13}C NMR of complex natural mixtures produces a characteristic 'mountain range' spectrum, analyzed by identifying peaks according to functional group. ^{13}C NMR spectra of natural samples are divided into five or six regions, usually described as carbonyl, O-aromatic, aromatic, di-O-alkyl, O-alkyl, N-alkyl, and alkyl (Baldock and Skjemstad 2000). A typical spectrum is shown in figure 1 and table 1 shows representative functional groups associated with each spectral region.

Table 1. *Adapted from (Baldock and Skjemstad (2000)*

chemical shift	types of carbon
0-45	alkyl carbon
45-65	N-alkyl and methoxyl C
65-95	O-alkyl C
95-110	di-O-alkyl C
110-145	aromatic/unsaturated C
145-160	phenolic C
160-190	amide/carboxyl C
190-220	aldehyde/ketone C

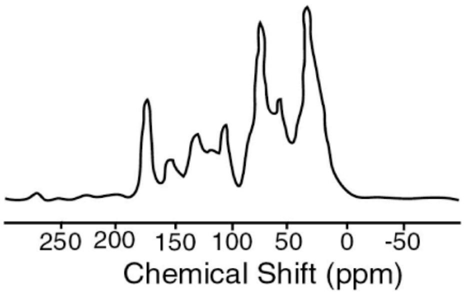

Figure 1. Adapted from (Baldock and Skjemstad 2000)

We have made preliminary C_{ox} measurements via a technique that combines ^{13}C NMR spectroscopy and molecular modeling. We have also considered a simpler but potentially more error-prone approach: direct calculation of C_{ox} from ^{13}C NMR spectra. Because C_{ox} measurements are new and because NMR spectroscopy of soils is complex, we spent a significant amount of time developing techniques.

Molecular Modeling Based on ^{13}C NMR Spectra

In this approach, we took advantage of a modeling technique recently developed to deconvolve ^{13}C NMR organic matter spectra into a simple combination of bulk chemical classes (Hedges, Baldock et al. 2002; Nelson and Baldock 2005). By inputting ^{13}C NMR spectra into an iterative model, it is possible to reproduce solid state ^{13}C NMR spectra of complex organic matter as a mixture of spectra of six model biomolecules (e.g., standardized spectra of carbohydrates, lignins, proteins, lipids, charcoal, and carbonyl functionality). The errors associated with this model are surprisingly small: most soil spectra can be reproduced with less than 9% difference between the actual and modeled spectra; in the case of samples run for this study, all spectra were reproduced within 5% and many within 2%. One output of this model is an estimation of sample molar C, H, N, and O contents, which can be used to calculate C_{ox} via equation (1).

Because the molecular model of Nelson and Baldock (2005) is relatively well validated and comes with accompanying error estimates, we chose to use this approach in our initial soil measurements. We also considered a second technique, direct calculation of C_{ox} from ^{13}C NMR spectra.

Direct Calculation of C_{ox} From ^{13}C NMR Spectra

The simplest approach to calculating C_{ox} from a ^{13}C NMR spectrum would be to directly assign carbon oxidation states to individual regions of a spectrum and then calculate a total sample C_{ox} value via weighted average of spectral intensity. The challenge associated with this approach is the problem of multiple oxidation states in a number of spectra regions: for example, the 65-95 ppm region of the spectrum contains carbon with Cox values of both –1 and 0. A number of spectral regions suffer from this problem. Over the course of this grant, we identified assumptions that need testing before this approach can be used. For example, returning to the 65-95 ppm region, this area is dominated by natural sugars, which have limited structural variation. It may be possible to assume that the majority of the carbon within this region is composed of five or six carbon sugars, allowing an average C_{ox} value of ~ -0.2 to be assigned to the region. The errors caused by these types of assumptions remain to be tested.

Quantitation of ^{13}C NMR Spectra

Because accurate measurement of C_{ox} via NMR depends on how well a ^{13}C NMR spectrum represents the original sample, we invested significant time in understanding how good our NMR spectra were. The basic issue is that not all ^{13}C nuclei within a natural OC sample produce NMR signals of equal intensity, and this can reduce the observability of some types of carbon. In particular, ^{13}C NMR experiments are vulnerable to nonuniform signal intensity due to paramagnetic materials (especially Fe) present in the host matrix, large distances between ^{13}C nuclei and ^{1}H nuclei (for example in the conjugated aromatic rings of charcoal), and high molecular mobility (Kinchesh, Powlson et al. 1995; Smernik and Oades 2000).

Although it was not possible to guarantee that all the spectra generated for this grant detected 100% of the carbon present, we did determine the percent carbon measured (C_{obs}) via ‘spin counting’ experiments (Smernik and Oades 2000). In these experiments, the amount of signal detected per mass carbon in a known standard is compared to the amount of signal detected per mass of the sample, allowing an estimation of how much carbon was ‘seen’ by the NMR spectrum.

The Natural C_{ox} scale and C_{ox} Values Within the Earth System

Natural environmental biochemicals range from C_{ox} values of about –2 to about +1 (*see fig. 2*). However, a range of measurements made as part of this grant suggest that natural organic carbon does not span this range; instead, natural biomass seems to stay very close to the carbon oxidation state of glucose (*fig 3*).

One goal of this study was to convert previously existing ^{13}C NMR spectra into C_{ox} measurements. We used previous ^{13}C NMR measurements made on organic matter decomposition sequences to produce a first estimate of how C_{ox} changes through decomposition. The first set of samples we examined was a corn litter bag decomposition sequence. Litter bags that measured 15 x10 cm were filled with corn litter, buried at 15 cm either in the loam soil on which the corn was grown or in an adjacent forest soil of the same provenance, and allowed to decompose for 0, 34, 193, 231, 306, and 582 days (*fig. 4a,b*). For comparison, we have included a marine decomposition sequence from the Equatorial Pacific. In this sequence, (*fig. 5*), organic matter was intercepted as it fell to the sea floor at 500 m and 3,000 m, and the spectra of sinking materials were compared to those of phytoplankton and sedimentary organic carbon.

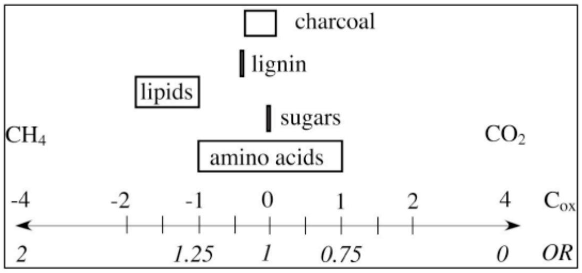

Figure 2. Cox and OR values for common biochemicals.

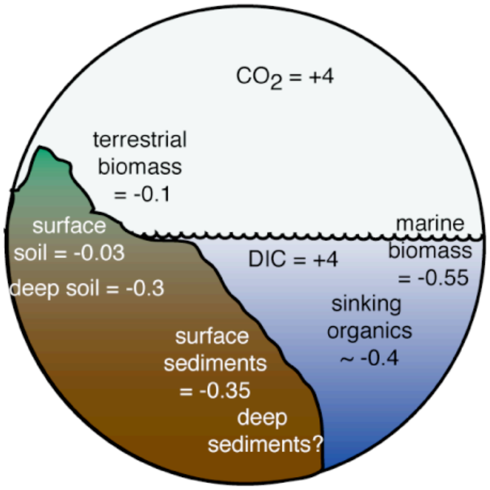

Figure 3. A first estimate of the organic carbon oxidation states of natural organic matter.

These figures show that as terrestrial decomposition progresses, C_{ox} values oscillate, but in general tend toward more negative (reduced) values. A second trend clear from comparing figures 4 and 5 is the distinction between marine organic carbon and terrestrial organic carbon, with marine organic matter being significantly more reduced than terrestrial organic matter. Modeling results suggest that this is due to the higher percentage of lipids ($C_{ox} \leq -1$) in marine samples, and simultaneous higher percentage in structural carbohydrates (e.g., cellulose, $C_{ox} = 0$) present in terrestrial organic matter. Interestingly, decomposition appears to bring both marine and terrestrial organic carbon closer to a common value, ~ $C_{ox} = -0.2$.

Detailed C_{ox} Measurements Within a Soil Profile

Finally, we measured two soil C_{ox} profiles at a pair of field sites, one burned in 1987 and a control site unburned for at least 100 years. These sites were located within a long-term ecological field station in Alaska, south of Fairbanks close to Delta Junction.

Our results from the Delta Flats field sites (*fig. 6*) show soil C_{ox} values varying both with depth and with a combination of fire frequency and ecology. The unburned site is dominated by mature black spruce and moss, while the burned site is a mixture of deciduous trees (aspen and willow) and young black spruce, with a less pronounced moss layer. These first soil C_{ox} profile suggest that soil C_{ox} values are influenced both by the parent organic matter and by the degree of decomposition (both profiles show more reduced carbon with depth.) Interestingly, these soils have 'remembered' the C_{ox} values of their forest floor down to at least a depth of 10 cm.

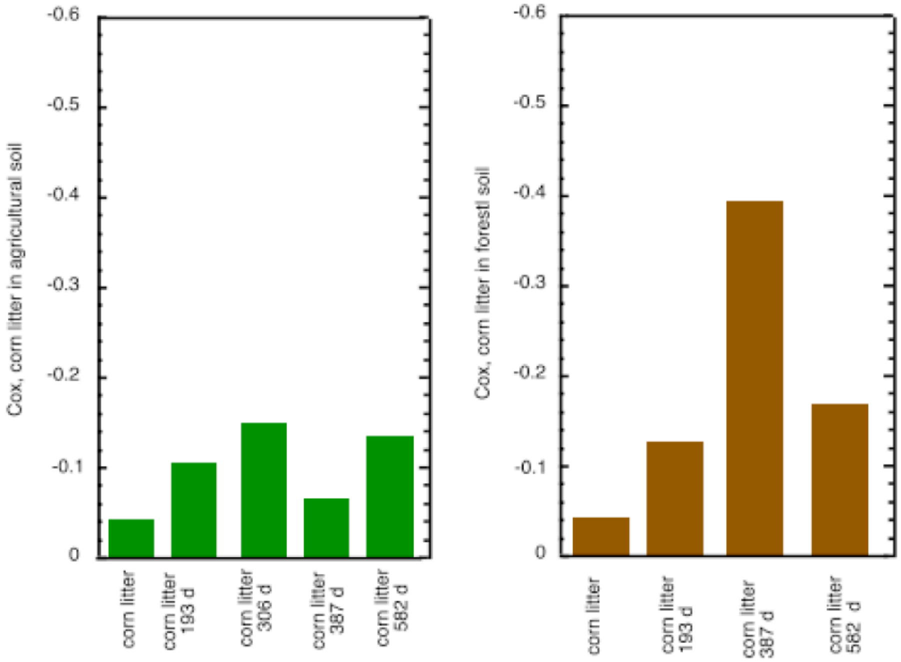

Figure 4a, b. Variations in C_{ox} as corn litter decomposes in two different solid environments: left, in an agricultural loam soil (where litter grew) and right, in adjacent uncultivated forest soil.

The trend toward a common C_{ox} value in both marine and terrestrial systems is thought-provoking: is this due to conversion of complex, relatively unique precursor organic matter into chemically uniform decomposer biomass? Alternately, is the final C_{ox} value of a decomposing system constrained by the thermodynamics of respiration: at some point, is it no longer energetically valuable to convert biomass to CO_2? If the second scenario is the case, C_{ox} should be influenced by the local thermodynamic conditions of decomposition – in particular a microbe's choice of terminal electron acceptor. One novel application possible under this scenario is the use of C_{ox} as a marker for the presence of oxygen both in recent and ancient sediments.

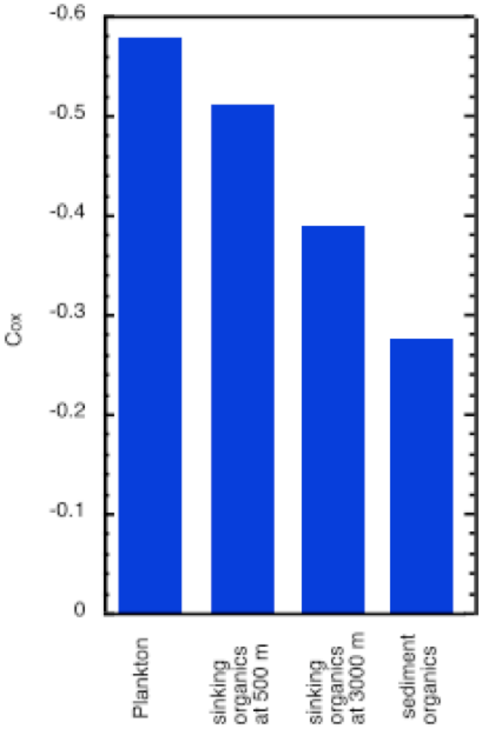

Figure 5. Changes in C_{ox} as OC decomposes in the equatorial Pacific. Data for figures 4 and 5 are from Nelson and Baldock, 2005.

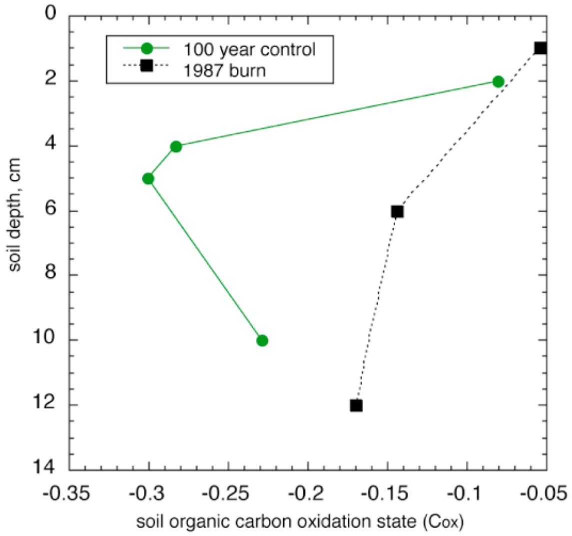

Figure 6. C_{ox} soil profiles.

Appendix 1

Derivation of the Relationship Between OR and ΔHC

This derivation begins with definitions and equations from the paper:

Williams, K., F. Percival, J. Merino, H.A. Mooney. 1987. Estimation of tissue construction cost from heat of combustion and organic nitrogen content. *Plant, Cell, and Environment* 10: 725-734.

This paper is abbreviated as Williams PCE 87. When equations are from Williams PCE 87, they are denoted as W PCE 87 (equation number), in which the equation number matches that in the original paper. Equations unique to this derivation begin with equation 7.

From Williams PCE 87:

The term ‘glucose equivalent’ is abbreviated as *GE* and is defined as the number of moles of glucose required to provide the carbon and electrons contained in one mole of product:

$$GE = \frac{c}{6} + \frac{h - 2x + kn + ms}{24} \qquad \text{W PCE 87(1)}$$

where *GE* units are mols glucose/mols product, c, h, x, n, and s are moles of carbon, hydrogen, oxygen, nitrogen, and sulfur in a mole of product (or 100g of dry plant matter), k is –3 (ammonium) or +5 (nitrate), and s is –2 (sulfide) or +6 (sulfate).

Changing units from moles to grams,

GE’ = glucose equivalents of a substance expressed in grams glucose/grams material

GE_0’ = GE’ calculated using values for k and m

next, Williams PCE 87 derives a direct expression for GE_0’:

$$GE_0' = GE' \times \frac{glucose,g/glucose,mol}{product,g/product,mol} = GE' \times \frac{molecmass,glucose}{molecmass,\ product}$$

Williams PCE 87 calculate an empirical relationship between GE_0’ and ΔH_c, where ΔH_c is the heat of combustion of a biomass sample (‘product’) in kJ/g. They do this by:

- calculating GE for a number of individual biochemicals using equation 1,
- converting GE to GE_0’ using known molar masses of glucose and individual compounds,
- regressing against measured ΔH values in kJ/g, to get:

$$\Delta H_C = 14.352\ {}^{\circ}\text{—} GE_0' + 0.929 \qquad \text{W PCE 87 (4)}$$

where $r^2 = 0.992$.

Moving back to the term GE_0’, when the molecular mass of glucose is 180.15 g/mol and the molecular mass of the product is MW g/mol, this equation becomes:

$$GE_0' = \left[\frac{c}{6} + \frac{h - 2x - 3n + 6s}{24}\right] \times \frac{180.15}{MW} \qquad \text{WPCE 87 (5)}$$

GE_0' can also be expressed in terms of ΔH_c (rearranging equation (4)):

$$GE_0' = 0.06968 \times \Delta H_C - 0.065 \qquad \text{W PCE 87 (6)}$$

using the nomenclature of Williams PCE 87, and the assumption of charge neutrality for biomass, organic carbon oxidation state is defined as:

$$\begin{aligned} 0 &= C_{ox} \cdot c - 2 \cdot x + h - 3n + 6s \\ C_{ox} &= \frac{2x - h + 3n - 6s}{c} \end{aligned} \qquad (7)$$

Factoring out c/6, equation (5) becomes:

$$GE_0' = \frac{c}{6}\left[1 + \frac{h - 2x - 3n + 6s}{4c}\right] \times \frac{180.15}{MW} \qquad (8)$$

inserting $-C_{ox}$,

$$GE_0' = \frac{c}{6}\left[1 - \frac{C_{ox}}{4}\right] \times \frac{180.15}{MW} \qquad (9)$$

rewriting,

$$GE_0' = \frac{c}{MW}\left[1 - \frac{C_{ox}}{4}\right] \times \frac{180.15}{6} \qquad (10)$$

working with c/MW:

$$\frac{c}{MW} = \frac{molC / molproduct}{gproduct / molproduct} = \frac{molC}{gproduct} \qquad (11)$$

define EA_c as the percent carbon results from an elemental analyzer, as

$$EA_c = \frac{gcarbon}{gproduct} \qquad (12)$$

then, converting g carbon to mol carbon:

$$EA_c \times \frac{molC}{12.011gC} = \frac{gcarbon}{gproduct} \times \frac{molC}{gcarbon} = \frac{molC}{gproduct} \tag{13}$$

and therefore:

$$\frac{c}{MW} = \frac{EA_c}{12.011} \tag{14}$$

substituting into eqn (10),

$$GE_0' = \frac{EA_c}{12.011} \times \left[1 - \frac{C_{ox}}{4}\right] \times \frac{180.15}{6} \tag{15}$$

setting eqn (6) equal to eqn (15),

$$0.06968 \times \Delta H_C - 0.065 = \frac{EA_c}{12.011}\left[1 - \frac{C_{ox}}{4}\right] \times \frac{180.15}{6} \tag{16}$$

setting OR = 1 – C_{ox}/4 and solving for OR,

$$\left(0.06968 \times \Delta H_C - 0.065\right) \times \frac{6}{180.15} \times \frac{12.011}{EA_c} = OR \tag{17}$$

reducing constants, this becomes:

$$\left(0.06968 \times \Delta H_C - 0.065\right) \times \frac{0.4}{EA_c} = OR \tag{18}$$

Heat of combustion (ΔH_C) is also proportional to tissue construction cost, as

$$construction\cos t = \frac{GE}{E_G} \qquad \text{W PCE 87 (3)}$$

where EG is tissue growth efficiency, and is an estimated parameter.

References

Amundson, R. 2001. The carbon budget in soils. *Annual Review of Earth and Planetary Sciences* 29: 535-562.

Baldock, J.A., and J. O. Skjemstad. 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. *Organic Geochemistry* 31: 697-710.

Hedges, J.I., J.A. Baldock, et al. 2002. The biochemical and elemental compositions of marine plankton: A NMR perspective. *Marine Chemistry* 78: 47-63.

Hedges, J.I., and R.G. Keil.1995. Sedimentary Organic Matter Preservation – an Assessment and Speculative Synthesis. *Marine Chemistry* 49: 81-115.

Keeling, R.F., S.C. Piper, et al. 1996. Global and hemispheric CO_2 sinks deduced from changes in atmospheric O_2 concentration. *Nature* 381: 218-221.

Kinchesh, P., D.S. Powlson, et al. 1995. 13C NMR studies of organic matter in whole soils: I. Quantitation possibilities. *European Journal of Soil Science* 46: 125-138.

Nelson, P., and J. Baldock. 2005. Estimating the molecular composition of a diverse range of natural organic materials from solid-state 13C NMR and elemental analyses. Biogeochemistry 72: .1-8.

Randerson, J.T. C. Masiello, C. Still, T., Rahn, and C.B. Field. 2006. Is the oxidative ratio of the terrestrial biosphere changing? Implications for trends in atmospheric O2. Global Change Biology, 12: 260-271.

Smernik, R.J., and J.M. Oades. 2000. The use of spin counting for determining quantitation in solid state 13C NMR spectra of natural organic matter 1. Model systems and the effects of paramagnetic impurities. *Geoderma* 96: 101-129.

Smernik, R.J., and J.M. Oades. 2000. The use of spin counting for determining quantitation in solid state C-13 NMR spectra of natural organic matter 2. HF-treated soil fractions. *Geoderma* 96: 159-171.

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