



Measurement of soil carbon oxidation state and oxidative ratio by ^{13}C nuclear magnetic resonance

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[1] The oxidative ratio (OR) of the net ecosystem carbon balance is the ratio of net O_2 and CO_2 fluxes resulting from photosynthesis, respiration, decomposition, and other lateral and vertical carbon flows. The OR of the terrestrial biosphere must be well characterized to accurately estimate the terrestrial CO_2 sink using atmospheric measurements of changing O_2 and CO_2 levels. To estimate the OR of the terrestrial biosphere, measurements are needed of changes in the OR of aboveground and belowground carbon pools associated with decadal timescale disturbances (e.g., land use change and fire). The OR of aboveground pools can be measured using conventional approaches including elemental analysis. However, measuring the OR of soil carbon pools is technically challenging, and few soil OR data are available. In this paper we test three solid-state nuclear magnetic resonance (NMR) techniques for measuring soil OR, all based on measurements of the closely related parameter, organic carbon oxidation state (C_{ox}). Two of the three techniques make use of a molecular mixing model which converts NMR spectra into concentrations of a standard suite of biological molecules of known C_{ox} . The third technique assigns C_{ox} values to each peak in the NMR spectrum. We assess error associated with each technique using pure chemical compounds and plant biomass standards whose C_{ox} and OR values can be directly measured by elemental analyses. The most accurate technique, direct polarization solid-state ^{13}C NMR with the molecular mixing model, agrees with elemental analyses to ± 0.036 C_{ox} units (± 0.009 OR units). Using this technique, we show a large natural variability in soil C_{ox} and OR values. Soil C_{ox} values have a mean of -0.26 and a range from -0.45 to 0.30 , corresponding to OR values of 1.08 ± 0.06 and a range from 0.96 to 1.22 . We also estimate the OR of the carbon flux from a boreal forest fire. Analysis of soils from nearby intact soil profiles imply that soil carbon losses associated with the fire had an OR of 1.09_1 (± 0.003). Fire appears to be a major factor driving the soil C pool to higher oxidation states and lower OR values. Episodic fluxes caused by disturbances like fire may have substantially different ORs from ecosystem respiration fluxes and therefore should be better quantified to reduce uncertainties associated with our understanding of the global atmospheric carbon budget.

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1. Introduction

[2] A fraction of the CO_2 entering the atmosphere from fossil fuel emissions does not remain there, and instead is

either transferred to the ocean's dissolved inorganic carbon pool or is taken up by the terrestrial biosphere. Although the amount of fossil fuel CO_2 removed from the atmosphere can be calculated from the difference between emissions and atmospheric loadings, determining how much fossil fuel CO_2 goes separately into the oceans and into the terrestrial biosphere is challenging. Separating these two sinks is important because the correct attribution of contemporary CO_2 emissions to land and ocean sinks is necessary to understand climate-carbon cycle feedbacks and to inform carbon management decisions.

[3] One of the most successful techniques for determining the relative sizes of ocean and terrestrial biosphere sinks of CO_2 is the measurement of changes in the atmosphere's O_2 and CO_2 mixing ratios [e.g., Keeling and Shertz, 1992;

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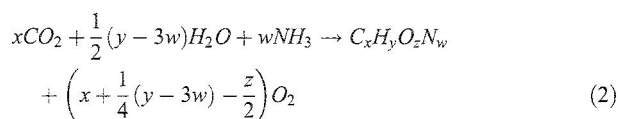
Severinghaus, 1995; Keeling *et al.*, 1996; Langenfelds *et al.*, 1999; Battle *et al.*, 2000]. The use of atmospheric O_2 and CO_2 measurements to track fossil fuel CO_2 sinks requires knowledge of the net oxidative ratio (OR) of the net terrestrial flux, along with similar ratios for ocean exchange and fossil fuel emissions, where OR is defined as the molar ratio of $O_2:CO_2$ fluxes for each budget component.

[4] The OR of the net land flux has been assumed in past studies to have a constant value of 1.10 [Severinghaus, 1995; Keeling *et al.*, 1996; Langenfelds *et al.*, 1999; Battle *et al.*, 2000; Manning and Keeling, 2006]. Few measurements exist, however, to constrain the OR associated with land use change, forest regrowth [Albani *et al.*, 2006], loss of tropical peat lands [Page *et al.*, 2002] and other processes that regulate the magnitude of net terrestrial flux on time-scales of years to decades.

[5] The OR of net terrestrial exchange can be separated into components associated with photosynthesis (OR_{ab}) and the return flux from the terrestrial biosphere to the atmosphere (OR_{ba}). When an ecosystem is in steady state, OR_{ab} should be similar to OR_{ba} . However, when ecosystems are driven out of steady state (e.g., by land use change), OR_{ba} may also reflect the respiration of soil carbon inherited from preexisting ecosystems. Because the gross fluxes of CO_2 due to photosynthesis and respiration are large compared to net ecosystem exchange, small offsets between OR_{ab} and OR_{ba} can introduce uncertainty into estimates of oceanic and terrestrial carbon sinks obtained from analysis of changes in atmospheric O_2 and CO_2 mixing ratios [Randerson *et al.*, 2006b]. Relatively few estimates of OR have been made in terrestrial ecosystems, partly because of the technical difficulty of directly measuring OR. Further, there is no knowledge of how OR of the terrestrial biosphere responds to anthropogenic perturbations such as climate change, fire, land use change, or atmospheric N deposition. Some of these perturbations are challenging to monitor via gas flux techniques either because of environmental conditions during the disturbance (fire) or because of the duration of the disturbance (N deposition, land use change). In this paper we demonstrate a new, NMR-based technique amenable to measuring the OR associated with these kinds of events.

[6] As an alternative to OR estimates derived from atmospheric O_2 and CO_2 mixing ratios in terrestrial ecosystems [Seibt *et al.*, 2004; Sturm and Leuenberger, 2005; Stephens *et al.*, 2007], the OR of terrestrial ecosystem fluxes can be obtained by measuring changes in organic matter content within the ecosystems and the OR of the organic matter pools that are changing. These types of OR measurements rely on the relationship between organic carbon oxidation state (C_{ox}) and OR. C_{ox} can be calculated from organic matter elemental composition ($C_xH_yO_zN_w$) using equation (1). On the basis of the assumption that net photosynthesis follows equation (2), C_{ox} can be related to OR using equation (3). Equation (3) was derived by simplifying the molar ratio of $O_2:CO_2$ from equation (2). Similarly, equations (4) and (5) can be derived using nitrate or N_2 as N sources in equation (2) [Masiello *et al.*, 2008].

$$C_{ox} = \frac{(2z - y + 3w)}{x} \quad (1)$$



$$\text{ammonia : } OR = 1 - \frac{C_{ox}}{4} \quad (3)$$

$$\text{nitrate : } OR = 1 - \frac{C_{ox}}{4} + \frac{2w}{x} \quad (4)$$

$$N_2 : OR = 1 - \frac{C_{ox}}{4} + \frac{3w}{4x} \quad (5)$$

[7] The choice of C_{ox} to OR conversion (equations (3), (4), or (5)) depends on the N cycle characteristics of the ecosystem being studied. For many ecosystems the N_2 -based equation (equation (5)), is the most appropriate choice. However, if an ecosystem receives a substantial fraction (greater than 20%) of its N in the forms of ammonia and nitrate in a ratio that departs from 1:1 ammonia:nitrate, it may be necessary to use equations (3) and (4) in weighted proportions. If an ecosystem receives ammonia and nitrate in equal ratios, the N_2 -based equation gives the same results as a weighted average of the nitrate and ammonia equations, within current experimental error [Masiello *et al.*, 2008].

[8] Equations (1)-(5) ignore the contributions of organic sulfur and phosphorus to organic matter C_{ox} and OR. The potential error associated with this omission is small, and is evaluated in detail in section 3.5.

[9] Terrestrial organic matter is composed of chemicals whose C_{ox} values range from +3 (for some organic acids) to -2 (for some lipids), corresponding to an approximate range of OR values from 0.25 to 1.5. Because there are relatively large differences in chemical composition between plant species, the C_{ox} and OR values of vegetation are sensitive to disturbance history and management practices [Randerson *et al.*, 2006b]. Soil carbon also exhibits distinguishable trends in C_{ox} and OR as a result of organic matter input and diagenesis [Baldock *et al.*, 2004]. Thus, C_{ox} and OR of soil and sediment organic matter may also be useful as proxies of land use history or as an indicator of the vulnerability of organic matter to microbial decomposition (i.e., its chemical recalcitrance). However, to exploit the potential of C_{ox} and OR as carbon cycle proxies, we must first be able to measure these properties.

[10] Measuring the OR associated with changes in soil organic matter is more challenging than measuring the OR associated with changes in plant biomass. Biomass C_{ox} can be measured by traditional elemental analyses of C, H, N, and O, or through bomb calorimetry [Masiello *et al.*, 2008]. On the other hand, soil carbon exists in many forms, including fresh leaf litter, microbial biomass, occluded and free particulates, and mineral-bound soil carbon. Some of these pools are amenable to elemental analysis, particularly leaf litter, but most of them have large enough mineral components to make accurate measurement of organic O and H concentrations impossible. Temperatures used in

Table 1. Elemental Composition and C_{ox} of Selected Organic Matter Samples Determined by Combustion Elemental Analysis and NMR^a

Sample	Elemental Analysis					DP ^{MMM}				CP ^{MMM}				DD ^{DA}	
	H/C	O/C	N/C	C_{ox}	OR ^b	H/C	O/C	C_{ox}	OR ^b	H/C	O/C	C_{ox}	OR ^b	C_{ox}	OR ^b
Pure compounds	1.83 ^c	0.516 ^c	0.111 ^c	-0.463 ^c	1.20	1.38	0.298	-0.455	1.20	1.32	0.289	-0.415	1.19	0.401	1.18
Redwood leaf	1.53	0.617	0.023	-0.226	1.07	1.50	0.580	-0.261	1.08	1.47	0.580	-0.241	1.08	-0.189	1.06
Peach leaf ^d	1.69	0.590	0.054	-0.347	1.13	1.52	0.507	-0.343	1.13	1.52	0.484	-0.389	1.14	-0.199	1.09
Corn grain	1.68	0.773	0.032	-0.034	1.03	1.51	0.677	-0.055	1.04	1.56	0.731	-0.005	1.03	-0.004	1.03
Corn field weeds	1.54	0.732	0.055	0.086	1.06	1.51	0.652	-0.042	1.05	1.49	0.628	-0.067	1.06	0.003	1.04
Organic soil DFTC 2 cm	1.52	0.619	0.016	-0.232	1.05	1.57	0.644	-0.232	1.07	1.55	0.681	-0.143	1.05	-0.080	1.03
Organic soil DFTC 4 cm	1.35	0.459	0.026	-0.351	1.09	1.47	0.524	-0.349	1.11	1.53	0.548	-0.358	1.11	nd	nd
Organic soil DFTC 5 cm	1.34	0.516	0.026	-0.235	1.11	1.44	0.563	-0.238	1.08	1.49	0.523	-0.366	1.11	-0.256	1.08
Mean				-0.225	1.09			-0.247	1.09			-0.248	1.09		
Mean error ^e						-0.073	-0.048	-0.023	0.005	-0.068	-0.045	-0.022	0.005	0.050	-0.013
C_{ox} error contribution ^f						0.073	-0.096			0.068	-0.090				
Measurement uncertainty								±0.026	±0.006			±0.064	±0.017	±0.070	±0.018
Correlation to EA (r)								0.98	0.99			0.88	0.93	0.89	0.94

^aEA, elemental analysis; DP, direct polarization; CP, cross polarization; DD, dipolar dephasing; MMM, molecular mixing model; DA, direct assignment; OR, oxidative ratio; nd, not determined.

^bCalculated using equation (5) assuming N_2 as the source of nitrogen to the ecosystem.

^cTheoretical values for a mixture pure compounds: glycine (35 wt%), glucose (15 wt%), palmitic acid (19 wt%), and vanillyl alcohol (31 wt%).

^dNIST standard reference material 1547.

^eError is defined as the deviation of NMR results from EA results: error = (NMR - EA).

^fThe H/C, O/C, and N/C contributions to C_{ox} error are determined as the terms in brackets in the equation: C_{ox} error = $[-1 \times (H/C \text{ error})] + [2 \times (O/C \text{ error})] + [3 \times (N/C \text{ error})]$.

elemental analyzers (ca. 1000°C) are sufficient to liberate O and H from soil minerals, giving rise to erroneous C_{ox} and OR values. Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy offers a solution to this problem since it can, in theory, quantitatively identify different carbon functional groups in mineral-rich soils [e.g., Wilson, 1987; Baldock et al., 2004].

[11] In this paper we describe methods for calculating C_{ox} and OR values of soils. We test three different solid-state ^{13}C NMR techniques: direct polarization (DP), cross polarization (CP), and dipolar dephasing (DD). We assess error associated with each method and quantify the uncertainty introduced by soil pretreatment with hydrochloric (HCl) and hydrofluoric (HF) acids. Finally, by measuring soil C_{ox} and OR at the site of a boreal forest fire, we estimate the OR of the fire emissions flux. We show that ecosystem disturbances, such as fire, have the potential to influence the OR of the terrestrial biosphere.

2. Methods

[12] Three types of organic samples were used to evaluate accuracy of the three methods. First, the "true" C_{ox} value (± 0.045) was determined for a suite of organic samples and chemical standards by elemental analysis as described by Masiello et al. [2008] and compared to values generated by means of three NMR-based techniques. Second, we used a wide variety of mineral soil samples from Australia to make comparisons between the three NMR methods. The purpose of this was to identify which method(s) were most accurate for mineral soils. Finally, we analyzed a set of mineral soils from boreal forest ecosystems in Alaska by NMR to determine the effects of HF acid treatment on measured C_{ox} and OR values and to explore the effects of fire on soil C_{ox} and OR values.

2.1. Sample Descriptions

[13] All chemical compounds used in the first suite of experiments were purchased from Fisher Scientific at puri-

ties greater than 95%. Glycine, vanillyl alcohol, palmitic acid, and glucose were combined as dry powders in the following proportions (by mass): 35%, 31%, 19%, and 15%, respectively. These compounds were chosen because they generate peaks in the ^{13}C NMR spectrum that coincide with those of the most abundant molecular components of soil organic matter: carbohydrates, lignins, amino acids, and lipids. The mixture was homogenized to a fine powder in a mortar and pestle. The suite of organic samples (listed in Table 1) also included agricultural biomass samples obtained from archives at the Kellogg Biological Station (Hickory Corners, Michigan, USA). These included oven-dried (50°C) corn grain (*Zea mays* L.) and nondominant biomass (a mixture of herbaceous weeds) from three different 1 hectare plots under conventional tillage. Peach leaves were obtained from NIST (standard reference material 1547). Leaves also were obtained from a redwood tree (*Sequoia Endl.*) sampled in 2000 on the campus of the California Institute of Technology, Pasadena, CA, USA.

[14] Boreal forest soils were collected at two locations near Delta Junction, AK, USA, in 2000. The forest stands at the Alaska sites are dominated by black spruce (*Picea mariana*). The soils are derived from wind-deposited loess underlain by glaciofluvial sands and gravels and overlain by organic horizons of varying depth, classified as Typic Eutrocrypts. The site and soils are described in detail by Maines et al. [2004]. One site experienced a stand-replacing fire in 1987 (DF87) and the other remained unburned for approximately 80 years (DFTC).

[15] The Australian samples included mineral soils from 8 different soil types and ecosystems in Australia. These samples were collected, prepared, characterized, and named as described by Skjemstad et al. [1999]: SS6 (Hapludoll, 0-5 cm), SS7 (Kandiustox, 0-5 cm), SS8 (Pellustert, 0-10 cm), ACU 1 (Argixeroll, 0-10 cm), URB-P (Rhodoxeralf, 0-10 cm), B2II (Chromustert, 0-5 cm), Qld (Chromustert, 0-10 cm), Buck (Natixeralf, 0-10 cm). Samples ACU1, Buck, and URB-P originate from the state of South Australia. All other soils were collected in

Table 2. Effect of HF Acid Treatment on C_{ox} of Mineral Soil From Delta Junction, Alaska

Soil Description	Depth (cm)	Percent C	Percent N	N/C (molar)	C_{obs} (%)			C_{ox}		
					DP	CP	DD ^a	DP ^{MMM}	CP ^{MMM}	DD ^{DA}
Donnelly Flats Control Soil										
M horizon: decomposed organic matter	10	40.4	1.24	0.026	90	61	44	-0.151	-0.265	-0.044
after HF treatment		47.4	1.33	0.024	90	70	60	-0.283	-0.271	-0.165
A horizon: decomposed organic matter + charcoal	11	19.5	0.73	0.032	87	44	38	-0.036	-0.120 ^b	-0.189
after HF treatment		37.4	1.29	0.030	106	70	60	-0.051	-0.208 ^b	-0.161
A horizon: dark loam	15	8.39	0.33	0.034	nd ^c	89	70	nd	-0.254	-0.121
after HF treatment		21.7	0.86	0.034	90	57	55	-0.158	-0.226 ^b	-0.185
Donnelly Flats Soil Burned in 1987										
BF horizon: ash, charcoal, and roots	6	13.5	0.68	0.043	nd	33	29	nd	-0.191	-0.128
after HF treatment		30.4	1.53	0.043	80	49	39	-0.120	-0.163 ^b	-0.287
A horizon: gravelly (60%) red/brown loamy sand	12	4.51	0.21	0.040	nd	55	30	nd	-0.248	-0.238
after HF treatment		14.9	0.72	0.041	nd	62	50	-0.113	-0.140 ^b	-0.192
A horizon: light brown silt loam	18	1.79	0.10	0.048	nd	nd	nd	nd	nd	nd
after HF treatment		6.57	0.37	0.048	nd	74	72	nd	-0.155 ^b	-0.283

^a C_{obs} for dipolar-dephasing spectra is defined as the percentage of nonprotonated C observed after the 45 μ s dephasing delay.

^bValues corrected for high charcoal content (>10%) as described in section 3.3. No correction was performed for samples with <10% charcoal.

^cHere nd, not determined.

Queensland. When samples are referenced, the suffix HF indicates that the soil was demineralized with HF acid solution.

2.2. HF Acid Treatment

[16] All mineral soils were demineralized with a 2% HF acid solution according to methods described by *Skjemstad et al.* [1994]. Briefly, mineral soils were treated with 5 aliquots of 2% HF and stirred gently by inversion. Supernatants were removed by centrifugation and decanting between treatments. The demineralized soils were rinsed three times with distilled water, and dried at 60°C

2.3. Elemental Analysis

[17] Concentrations of C, H, and N were measured with a model CEC 440HA catalytic combustion analyzer (Control Equipment Corp., North Chelmsford, MA) at the University of California Santa Barbara. Oxygen content was determined by catalytic pyrolysis on an ESC4010 elemental analyzer at Rice University (Costech Analytical Technologies Inc., Valencia, CA). The elemental concentrations (CHNO) measured using these techniques facilitate the calculation of accurate C_{ox} values (see Table 1). *Masiello et al.* [2008] demonstrate that C_{ox} values based upon the elemental analysis of 15 pure chemical compounds showed 1:1 correlation with theoretical values ($r_2 = 0.996$). The average uncertainty in C_{ox} measurement by elemental analysis is ± 0.045 C_{ox} units (± 0.011 OR units).

2.4. Carbon 13 NMR Spectroscopy

[18] All ^{13}C NMR data for Australian standard soils were obtained from *Smernik and Oades* [2003]. NMR spectra of boreal soil and plant biomass samples were also acquired using the 200 MHz Varian Unity spectrometer with 7 mm Doty Scientific magic angle spinning (MAS) probe described by *Smernik and Oades* [2003]. NMR data for model compounds, agricultural biomass, and untreated boreal forest soils were obtained on a 200 MHz Bruker Avance spectrometer equipped with a 4 mm MAS probe. Direct polarization (DP) spectra were acquired with a single 90 degree ^{13}C pulse, 5 kHz MAS frequency, and 90 s recycle delay. Cross polarization (CP) and dipolar dephas-

ing (DD) experiments were acquired with a 1 ms contact time, at 5 kHz MAS frequency, and recycle delay >2 s. DD experiments employed the pulse sequence of *Harbison et al.* [1985]. A dephasing delay of 45 μ s was used to estimate the contribution of nonprotonated (and mobile methyl) functional groups to the CP or zero-delay DD (DD₀) spectrum. Intensity corrections for the signal loss during the dephasing delay (15% for Sp^2 and 50% for Sp^3 carbon) were applied according to *Smernik and Oades* [2001].

[19] The degree of quantitation of an NMR experiment can be determined by comparing the carbon-normalized signal intensity detected for each sample to the carbon-normalized signal intensity of an external standard, a procedure known as spin counting. Glycine was used as the spin counting standard, following the convention of *Smernik and Oades* [2000a]. For each sample, the percentage of carbon observed in the NMR spectrum (C_{obs}) was calculated using equation (6), where signal intensities have been corrected for signal loss due to $T_{1\rho}$ relaxation [*Smernik and Oades*, 2000a]

$$C_{obs}(\%) = 100 \times \frac{\text{signal intensity per unit carbon for sample}}{\text{signal intensity per unit carbon for glycine}} \quad (6)$$

Measurements of C_{obs} for soils from Delta Junction, Alaska are listed in Table 2. C_{obs} values for the Australian soils have been published previously [*Smernik and Oades*, 2000b] and range 54-100% for CP NMR and from 84-106% for DP NMR. These values are comparable to those found in the literature for CP and DP NMR experiments on whole soils and HF-treated soils [*Keeler and Maciel*, 2003, and references therein].

[20] Standard CP and DD NMR experiments are typically less quantitative (lower C_{obs} values) than DP NMR experiments. This is a well known phenomenon for complex organic mixtures such as soil organic matter. However, CP techniques, which transfer magnetization from ^1H to ^{13}C , are widely used for soil analysis because cross polarization enhances the intensity of ^{13}C magnetic flux by a factor of 4, and also reduces experiment times by 1-2 orders of magnitude. This is often required for mineral soil horizons where the carbon content is typically much less

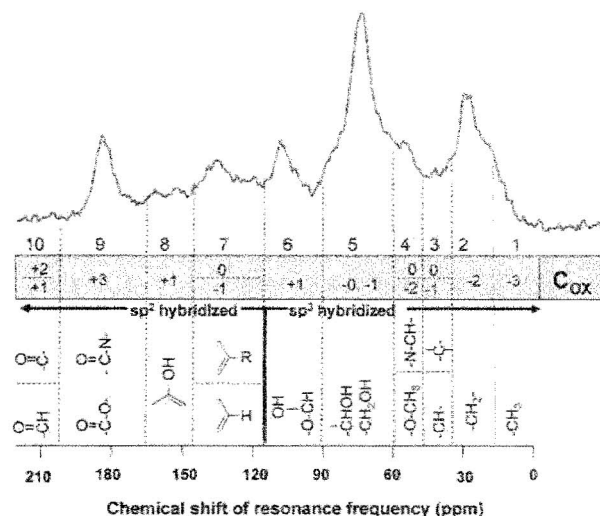


Figure 1. The direct C_{ox} assignment approach (DD^{DA}). Carbon ^{13}C NMR spectra are divided into 10 regions, indicated by vertical lines. Carbon functional groups in each spectral region and their corresponding resonance frequencies are shown along the lower axis. Six of the ten regions contain multiple functional groups. The horizontal dashed lines indicate additional functional group separation gained by applying a 45 μs dipolar dephasing delay (DD NMR).

than 5% by weight. Indeed, DP NMR is sometimes technically infeasible or too time-consuming for mineral soils, hence, the missing DP NMR data in Table 2.

2.5. C_{ox} Calculation via Molecular Mixing Model

[21] The molecular mixing model (MMM) described by Baldock *et al.* [2004] was used to calculate C_{ox} values from both DP and CP NMR data. We denote the application of the molecular mixing model to DP and CP NMR data as Dp^{MMM} and Cp^{MMM} , respectively. The model accepts input in the form of integrated NMR chemical shift regions (i.e., peak areas) and the C/N ratio from combustion elemental analysis. Model output is the best fit linear combination of four terrestrial biomolecule classes (lipid, lignin, carbohydrate, and protein) that describe the observed spectra and C/N ratio. Charcoal and carbonyl C are also included as model components to account for the alteration of organic matter by fire and diagenetic oxidation, respectively. Finally, the user has the option to select model output which excludes one or more of the components. For instance, we excluded charcoal as a potential component for the biomass samples. The individual C_{ox} values of the 6 model components are then weighted by the relative contribution of each component to calculate the C_{ox} value of the soil organic matter as a whole. The C_{ox} values of the 6 model components are given in Table 1 of Baldock *et al.* [2004].

2.6. Direct Assignment of C_{ox} Values to NMR Spectral Regions

[22] A second approach to determining C_{ox} involves direct assignment of C_{ox} values to specified regions of the NMR spectrum. We assigned C_{ox} values to each spectral region based upon the C functional group that it represents, rather than any fundamental relationship between C_{ox} and

chemical shift in the ^{13}C resonance frequency. The chemical shift of a given carbon atom is a function of the electro-negativity and electron environment generated by neighboring atoms, sometimes several bonds removed. On the other hand, oxidation state of a given C atom is determined by the proportions in which valence shell electrons are bonded to C, H, N, and O atoms. Figure 1 illustrates the distribution of C_{ox} values along the chemical shift axis.

[23] Although five of the spectral regions delineated in Figure 1 contain functional groups with more than one C_{ox} value, the C_{ox} value of these spectral regions can be uniquely identified through DD NMR experiments. Regions 3, 7, and 10 contain two types of C atoms (protonated and nonprotonated) that can be separated and quantified by dipolar dephasing experiments. Protonated C resonances dephase through C-H dipolar couplings during the 45 μs delay period, and therefore do not appear in the DD NMR spectrum. Therefore, protonated C in signal regions 3, 7, and 10 (in Figure 1) are quantified as the difference between the signal intensity in the CP and DD spectra (i.e., protonated C is equal to CP peak area minus DD peak area). In region 4, the N-alkyl C and methoxyl C are both protonated. Thus, one would expect both resonances to dephase during the 45 μs delay. However, rotational motion of the methoxyl group weakens the ^{13}C - 1H dipolar coupling, and this signal does not dephase. Therefore, we correct signal remaining in region 4 for intensity lost to T_2 relaxation that occurs during the 45 μs delay, and then use this information to quantify methoxyl C and N-alkyl C, as prescribed by Smelik and Oades [2001]. These approximations allow quantification of carbon oxidation states with nonunique chemical shift values.

[24] Finally, region 5 contains both primary and secondary O-alkyl C with differing C_{ox} values. The dephasing behavior of primary and secondary O-alkyl C is very similar and therefore DD experiments cannot be used to separate these functional groups. To deal with region 5, we assume a generic carbohydrate molecular structure composed of hexose and pentose units (the most abundant carbohydrate subunits in the terrestrial environment). The relative abundance of pentose and hexose units is determined by the ratio of O-alkyl:O₂-alkyl C. For O-alkyl:O₂-alkyl ratios > 5:1, we assume that carbohydrates are composed exclusively of hexose units, and for ratios < 4:1, we assume exclusively pentose units. For ratios > 4:1 and < 5:1, we calculate the appropriate mixture of hexose and pentose units. Each pentose unit contains 3 secondary O-alkyl C atoms, and each hexose unit contains 4 secondary O-alkyl C atoms. Thus the ratio of primary O-alkyl:secondary O-alkyl C can be calculated from the measured O-alkyl:O₂-alkyl ratio, and the corresponding C_{ox} value can be assigned to region 5.

[25] To calculate C_{ox} values from DD spectra, we weighted each of the regions specified in Figure 1 according to the corresponding peak area. We refer to this algorithm for C_{ox} calculation as direct assignment (DA). We use the abbreviation DD^{DA} for the application of the direct assignment algorithm to dipolar dephasing NMR data.

3. Results

3.1. C_{ox} Measurement Accuracy

[26] We evaluate accuracy of NMR methods using biomass samples and organic soils, whose "true" C_{ox} values

were determined using an accurate (± 0.045) combustion elemental analysis method [Masiello *et al.*, 2008]. A detailed analysis of the sources of error associated with each NMR method is provided in sections 3.2 to 3.5. A summary of the error analysis is provided below.

[27] The propagation of errors derived from sample pretreatment, NMR analysis, and C_{ox} calculation yields a total C_{ox} measurement error of ± 0.036 for Dp^{MMM} , ± 0.069 for Cp^{MMM} , and ± 0.103 for DD^{DA} . C_{ox} measurement accuracy using NMR is controlled by four sources of error: (1) algorithm approximations (± 0.026 C_{ox} units), (2) NMR quantitation errors, (3) error introduced by the pretreatment of soil with HF acid (± 0.024 C_{ox} units), and (4) error derived from the omission of organic S and P components in C_{ox} calculations. The most significant of these errors is NMR quantitation error in Cp^{MMM} and DD^{DA} .

[28] Algorithm errors arise from approximations used in the calculation of C_{ox} from NMR data. Both MMM and DA algorithms contain approximations based upon the most common chemical structures found in soil organic matter. NMR quantitation errors occur when a given carbon functional group is overrepresented or underrepresented in the NMR spectrum relative to the "true" functional group distribution in the sample. Errors of this type are referred to as relative quantitation errors. The treatment of soils with a dilute solution of HF acid removes paramagnetic minerals from soil, a prerequisite for quantitative NMR analysis of most mineral soils. However, the organic matter composition (and C_{ox}) of different soils respond very differently to HF treatment, making it impossible to apply a systematic HF correction factor. Therefore, HF acid is an important source of measurement uncertainty. Finally, our calculations of C_{ox} exclude organic S and P, introducing a trivial source of error, which is described in section 3.5.

3.2. C_{ox} Error Introduced by Algorithm Approximations

[29] The molecular mixing model algorithm uses six generic compounds to represent all forms soil organic matter. The accuracy of the calculated C_{ox} values depends upon whether a mixture of only six components (lipid, lignin, carbohydrate, protein, charcoal, and carbonyl C) is adequate to fully describe NMR spectra of soils from a broad range of geographic locations. This issue has been addressed in previous work [Baldock *et al.*, 2004; Nelson and Baldock, 2005]. We note that the model-predicted NMR peak areas for the Australian soils show 1:1 correlations with the measured NMR peak areas (slopes = 0.99-1.00, $r^2 > 0.98$, $p > 0.97$), and also accounted for $100 \pm 4\%$ of the total NMR spectral area [Nelson and Baldock, 2005]. Similar fits are achieved for the Alaska soils used in this paper (data not shown). Therefore, errors in C_{ox} values calculated by the mixing model algorithm do not appear to arise from poor fitting of the NMR data.

[30] Error in the mixing model algorithm (± 0.026 C_{ox} units) can be attributed to the approximate C_{ox} values that are assigned to each of the 6 model components. This is mathematically equivalent to assigning a fixed H/C and O/C ratio to each of the 6 model components. The inherent variability in the elemental composition of biological molecules in nature is not represented by the model, and can be seen influencing model-predicted H/C and O/C ratios in

Table 1. The largest errors in the model-predicted H/C and O/C ratios occur for mixtures of pure chemical compounds. This is not surprising because the molecular mixing model is calibrated with typical elemental compositions of the lipids, lignin, and amino acids found in soils. Vanillyl alcohol, palmitic acid, and glycine are highly simplified representations of lignins, lipids, and amino acids, whose elemental compositions differ from those represented in the mixing model. The elemental composition of glucose is the same as the elemental stoichiometry used to represent carbohydrates in the model ($C_6H_{12}O_6$), and therefore, should not be a source of the NMR-based H/C and O/C error. Despite H/C and O/C errors, the mixing model approach yielded accurate C_{ox} values (Table 1). This is because the model underestimates or overestimates H/C and O/C in a 2:1 ratio. Since the C_{ox} calculation contains the term $-2[O] + [H]$, these errors cancel.

[31] It is possible to estimate the error due to algorithm approximations in the molecular mixing model. We do this using the Dp^{MMM} data in Table 1 because in the case of these samples analyzed using the MMM, all sources of error other than algorithm error can be ruled out (NMR quantitation error, S and P error, and HF acid treatment error). NMR quantitation error can be ruled out because the DP NMR spectra of the organic samples in Table 1 had C_{obs} values between 90-100%. S and P errors can be ruled out because their concentrations are insignificant (<0.5 wt%) in fresh plant biomass. HF acid treatment error can be ruled out because all these samples are organic, and have not undergone HF treatment. Finally, the model-predicted peak areas are an excellent fit to measured NMR peak area distribution, and account for the entirety of the NMR signal area ($\pm 4\%$) [Nelson and Baldock, 2005]. Therefore, Dp^{MMM} measurement error of ± 0.026 C_{ox} units (± 0.004 OR units) can be attributed to algorithm approximations which are intrinsic to the mixing model.

[32] Algorithm error can also occur in the direct assignment approach. This error is minor. We calculated the theoretical NMR signal distribution for a mixture of pure organic compounds (Table 1), and found that the C_{ox} value obtained via direct assignment algorithm (-0.463) is identical to the C_{ox} value obtained from elemental composition. Although the DA algorithm makes an approximation in determining the relative quantities of primary and secondary O-alkyl C, the net C_{ox} value of these functional groups approaches zero for most samples. Therefore, errors introduced by the approximations in the DA algorithm have a negligible C_{ox} magnitude.

3.3. C_{ox} Error Introduced by NMR Quantitation Errors

[33] Direct polarization NMR experiments are intrinsically quantitative. Thus, if paramagnetic soil minerals are efficiently removed during the HF acid treatment, and if a sufficient delay period is allowed between experiment repetitions (to allow complete relaxation of the ^{13}C magnetization), then DP NMR spectra provides a quantitative representation of soil carbon functional groups. Furthermore, spin counting results indicate that the proportion of soil C observed in HF treated soils (C_{obs}) is 80-100% (see Table 2 and Smernik and Oades [2000b]). Therefore, we

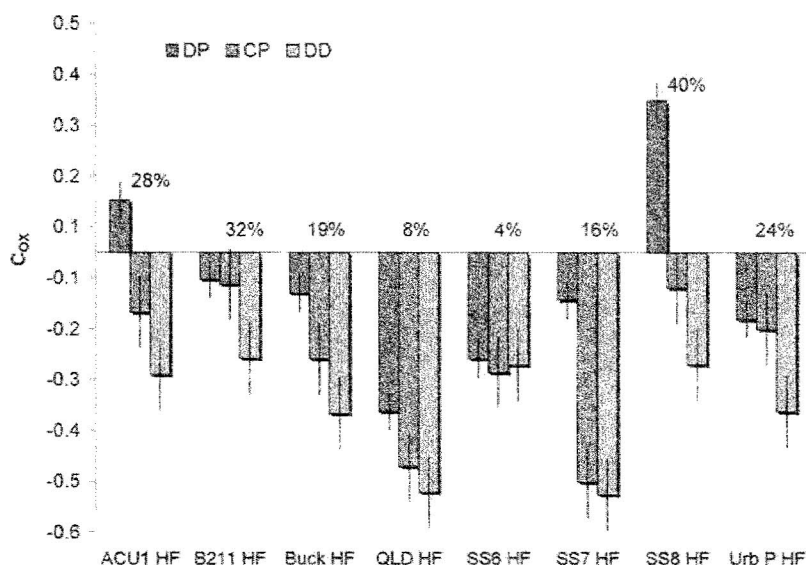


Figure 2. C_{ox} values for eight HF-treated mineral soils from Australia. Three C_{ox} values were calculated for each soil based upon three different ^{13}C NMR techniques: direct polarization (DP), cross polarization (CP), and dipolar dephasing (DD). Numerical values indicate the charcoal carbon content as a percentage of the total soil organic carbon, as determined by DP^{MMM} . Error bars represent the propagated measurement uncertainty introduced by assumptions in C_{ox} calculation algorithm and HF acid treatment.

treat DP NMR data as a "standard" against which to measure CP and DD NMR quantitation errors.

[34] The low observability of aromatic charcoal carbon is likely the most important source of quantitation error in CP NMR of HF-treated mineral soils. The differences between Dp^{MMM} and Cp^{MMM} C_{ox} values are as large as 0.4 C_{ox} units (for sample SS8 in Figure 2). Soils for which the Dp^{MMM} and CP WIMC $_{ox}$ values differ substantially are those with high charcoal content (Figure 2). Figure 3 demonstrates that approximately 93% of the disparity between Dp^{MMM} and Cp^{MMM} C_{ox} values can be explained by differences in the soil charcoal content. Differences in charcoal content predicted by the mixing model are a reflection of differences in the observability of charcoal carbon in CP and DP NMR spectroscopy. CP NMR spectroscopy is able to detect only ~30% of charcoal carbon due to the remote protonation of condensed aromatic carbon atoms and the rapid $T_1\rho$ relaxation of charcoal protons [Smernik *et al.*, 2002]. Conversely, DP NMR routinely detects >90% of charcoal carbon [Skjemstad *et al.*, 1999; Baldock and Smernik, 2002; Smernik *et al.*, 2002].

[35] Nelson and Baldock [2005] showed that Cp^{MMM} underestimates soil charcoal by a factor of 2 relative to the UV photooxidation technique across a broad range of soil charcoal concentrations [Skjemstad *et al.*, 1999], while Dp^{MMM} generates very reasonable charcoal estimates. Therefore, Cp^{MMM} C_{ox} values in Table 2 have been corrected for underestimation of charcoal by applying a factor of 2 multiplication to the model-predicted charcoal content [Nelson and Baldock, 2005, Figure 2]. The charcoal correction brings the Cp^{MMM} C_{ox} values into statistical agreement with Dp^{MMM} C_{ox} values (± 0.026 uncertainty) for the DF87 samples in Table 2.

[36] The DDDA approach requires the acquisition of two NMR spectra: DDo and DD45, which are equivalent to CP NMR spectra with the insertion of a delay prior to data acquisition of 0us and 45us, respectively. Therefore, DD^{DA} is susceptible to greater quantitation error because errors can occur in both CP (i.e., DDo) and DD45 NMR spectra. The C_{ox} values determined by DD^{DA} exhibit a surprising behavior relative to C_{ox} values determined by Cp^{MMM} and Dp^{MMM} a positive bias for organic samples and a negative bias for mineral soils. This discrepancy has its source in NMR quantitation error, and we provide a detailed investi-

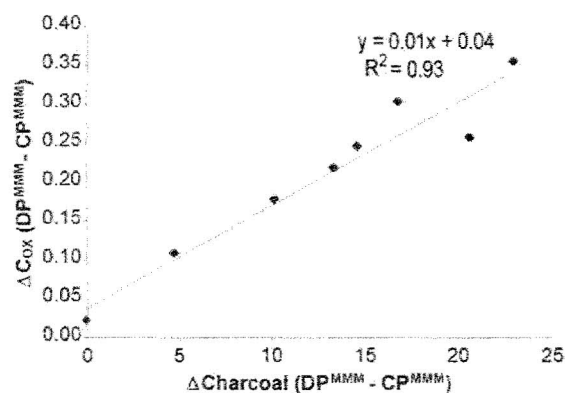


Figure 3. Differences in C_{ox} values determined by Cp^{MMM} and Dp^{MMM} as a function of the differences in percent charcoal carbon. Data are for the Australia soils shown in Figure 2. Differences in the quantity of charcoal detected by CP NMR and DP NMR account for 93% of the variance in C_{ox} values determined by Cp^{MMM} and Dp^{MMM} .

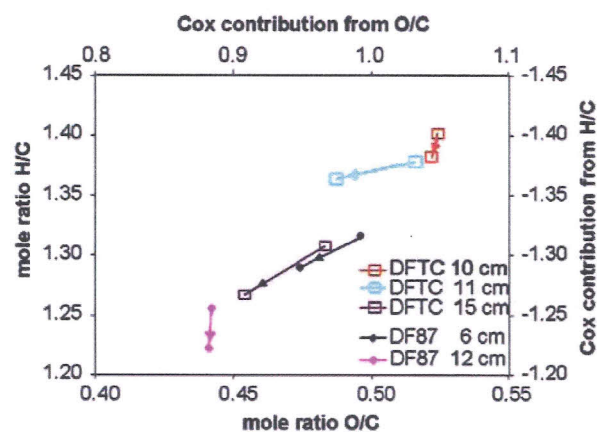


Figure 4. Vector diagram showing the trajectory of changes in C_{ox} and elemental composition caused by HF treatment. Values are calculated from CP NMR spectra by the molecular mixing model. Soil samples correspond to those in Table 2 from near Delta Junction Alaska. Open symbols represent soil from an unburned site (DF8C), and solid symbols represent soil burned in 1987 (DF87). Soil sample depth is indicated in centimeters. HF-induced changes in C_{ox} are the vector sum of the C_{ox} contributions shown on the x axis (C_{ox} contribution equal to $-1 \times (H/C)$) and the y axes (C_{ox} contribution equal to $2 \times (O/C)$). This demonstrates why the magnitude and direction of HF treatment effects on C_{ox} differ for each sample, owing to differential changes in H/C and O/C of the organic matter in mineral soils.

gation of this in the auxiliary material.¹ Our analysis revealed that the underestimation of mobile alkyl structures is a likely source of positive C_{ox} bias in plant biomass and organic soils. In contrast, alkyl structures are overestimated relative to phenolic and carboxylic structures in the mineral soils, all of which contribute to the negative C_{ox} bias.

[37] Therefore, even though the direct assignment algorithm is not a significant source of error, C_{ox} values are very sensitive to the quantitation errors in DD NMR. For mineral soils ($n = 14$), DD NMR quantitation errors introduce measurement uncertainty of ± 0.100 C_{ox} units. Therefore, we recommend the molecular mixing model approaches (Dp^{MMM} and Cp^{MMM}) as more accurate measures of soil C_{ox} .

3.4. HF Acid Treatment Effects

[38] The HF acid treatment serves the dual purposes of removing paramagnetic minerals which interfere with NMR analysis, and concentrating organic matter by removing silicate minerals. Since HF acid treatment is a prerequisite to NMR analysis of most mineral soils, we investigated its effect on measured C_{ox} values. For this experiment, we selected mineral soils from two soil profiles from Alaska because 6 soils from these profiles had a carbon content high enough ($C:4$ wt %) that NMR spectra could be obtained

prior to HF acid treatment. Then we measured C_{ox} before and after HF treatment.

[39] HF acid treatment had the expected effect of increasing the concentrations of C, H, N, and O, while also improving the NMR observability (C_{obs}) of the soil organic carbon (Table 2). The measured C_{ox} values of mineral soils changed significantly upon HF treatment. The HF acid treatment caused increased C_{ox} values in some samples and decreased C_{ox} values in other samples, with no apparent trend dominating. To understand why this is the case, we plotted the H/C and O/C ratios and their corresponding C_{ox} contributions on a van Krevelen diagram in Figure 4. No significant changes were observed in N/C ratio (see Table 2), so N/C is not included in Figure 4. The points represent soil from a discrete depth within the mineral soil profile before and after HF treatment, and the vectors connecting the points indicate the magnitude and direction of the HF treatment effect.

[40] Previous studies suggest that HF-induced changes in the composition of soil organic matter can occur through the removal of acid-soluble or hydrolysable organic molecules, or through structure alteration of (insoluble) organic matter. HF acid hydrolysis/dissolution typically results in the loss of 10–30% of the organic carbon in mineral soils [Skjemstad et al., 1994; Schmidt et al., 1997; Mathers et al., 2002; Rumpel et al., 2002; Goncalves et al., 2003]. The alteration of organic chemical structure by HF is typically less important. Structure changes have been documented for specific monomers of lignin and simple sugars using very sensitive gas chromatography and mass spectrometry (MS) techniques, but bulk techniques such as ^{13}C NMR, infrared spectroscopy, and stable isotope ratio MS do not detect these changes in the HF-treated residue [Keeler and Maciel, 2003; Schmidt and Gleixner, 2005; Rumpel et al., 2006]. On the other hand, analyses of the hydrolyzates (in the HF acid supernatant) show ^{13}C and ^{15}N isotope enrichment and a predominance of carboxylamide, O-alkyl, and N-alkyl signals relative to untreated samples [Dai and Johnson, 1999; Mathers et al., 2002; Schmidt and Gleixner, 2005].

[41] These spectroscopic data from the literature are consistent with the molecular mixing model results. Both suggest a preferential loss of carbohydrates and amino acids during HF acid treatment. The dramatic differences in the slopes of the lines in Figure 4 show that samples respond quite differently in terms of the absolute and relative proportions of carbohydrates and amino acids that are lost during HF treatment. This is probably because the loss of organic matter during HF treatment can be caused by more than one process, e.g., by acid hydrolysis or by the dissolution of organo-mineral complexes and subsequent removal with the HF acid supernatant. Therefore, it is not appropriate to apply a C_{ox} correction factor for HF acid treatment. Henceforth, we handle the HF acid treatment as a source of uncertainty (± 0.024) in the C_{ox} measurement.

3.5. C_{ox} and OR Bias Introduced by the Omission of Sulfur and Phosphorus

[42] Typical SIC and PIC molar ratios in leaf litter are on the order of 11200 and 113000, respectively [e.g., Parton et al., 1988; McGroddy et al., 2004]. Therefore, S and P have negligible contributions to the oxidation state of carbon in fresh biomass inputs. However, the recycling of these

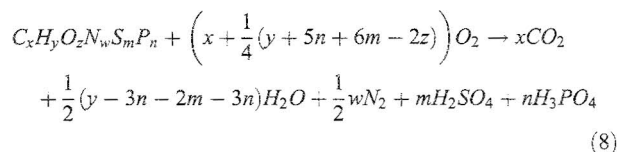
¹Auxiliary materials are available in the HTML. doi:10.1029/2008JG000803.

essential nutrients by soil microorganisms enriches the S/C and P/C ratios of soil organic matter to 1/150 and 1/185, respectively [Wang et al., 2006; Cleveland and Liptzin, 2007]. Organic phosphorus in plant biomass and soil is predominantly in the form of phosphate esters, which have an oxidation state of +5. Organic sulfur in soils has two predominant forms with different oxidation states. Carbon-bonded sulfur ($S_{ox} = -2$) and sulfate esters ($S_{ox} = +6$) occur with a relative abundance of 63:37 [Wang et al., 2006], giving an average S oxidation state of +1. Therefore, the C_{ox} for organic matter containing S and P ($C_xH_yO_zN_wS_mP_n$) takes the form

$$C_{ox} = \frac{y - 2z + 3w - m - 5n}{x} \quad (7)$$

of equation (7), where the variables represent the molar quantities of each element, and the coefficients represent the oxidation states.

[43] On the basis of the typical soil organic matter S/C and P/C ratios, the omission of S results in positive bias of +0.007 C_{ox} units, and the omission of P causes a C_{ox} bias of +0.027. To determine the influence of S and P on the soil OR value, we add the charge-neutral forms of sulfate (H_2SO_4) and phosphate (H_3PO_4) to the balanced equation (8) for organic matter oxidation



$$OR = 1 - \frac{C_{ox}}{4} + \frac{3w}{4x} + \frac{5m}{4x} \quad (9)$$

and relate C_{ox} to OR by substituting equation (7) into the definition of OR (moles O_2 /moles CO_2 released by decomposition). Thus, equation (9) was derived from the ratio of O_2 and CO_2 coefficients in equation (8). According to equation (9), the combined C_{ox} bias from the omission of S and P (+0.034 C_{ox} units) causes an OR bias of -0.0002. Since the S and P bias is negligible with respect to measurement uncertainty (± 0.009 OR units), we do not apply any S or P corrections to the C_{ox} and OR values presented here.

3.6. Patterns in Soil OR

[44] Soil C_{ox} and OR vary nonlinearly with soil depth (Table 3 and Figure 5), irrespective of the measurement technique employed. The DFTC soil profile (Figure 5) shows a striking OR pattern with depth, where OR increases from 1.05 to 1.11 within a 3 cm depth increment (2–5 cm soil depth) corresponding to the transition from the fibric to the humic horizon in organic soil. With increasing depth in the mineral A horizon, the OR gradually returns to a value of 1.07. These changes in OR with depth imply that soil depth and genetic origin may affect C_{ox} and OR and that the depth of burning into the soil organic matter layer may influence the OR of fire emissions.

[45] The mean OR of mineral soils from Alaska (1.07 ± 0.02) and Australia (1.07 ± 0.05) measured in this study were substantially lower than the OR of mineral soils from the Harvard Forest (1.22). These differences suggest that mineral soil OR may vary substantially from ecosystem to ecosystem, potentially reflecting spatially varying differences in tissue chemistry of plant and root inputs and stabilization pathways [e.g., Torn et al., 1997; Randerson et al., 2006b]. It is also important to note, however, that the Harvard mineral soil OR estimate is based on NMR spectra collected from a previous study and more work is needed to systematically explore spatial patterns using standardized techniques.

[46] OR values determined by NMR characterize the OR of the bulk soil C pool, whereas O_2 and CO_2 flux measurements characterize the OR of the active microbial decomposition of soil organic matter. The ORs of soil C pools in Table 3 are generally lower than the ORs of soil respiration fluxes. The average OR of the 14 mineral soil C pools that we measured, for example, is 1.08 ± 0.06 , whereas the average literature value for the OR of soil fluxes is 1.15 ± 0.07 . For comparison, the plant biomass OR values in Table 1 range from 1.03 to 1.13. A mean for net primary production inputs to the soil may be nearer to the lower end of this range based on a synthesis of herbaceous and woody plant chemical composition data (that yielded mean OR values of 1.03 and 1.05 for these two plant functional types, respectively) [Randerson et al., 2006b]. A systematic offset between soil respiration and bulk soil organic matter OR may imply that microbial decomposition preferentially mineralizes organic matter with an oxidation state that differs from residual microbial products that accumulate in the soil. Simultaneous measurements of bulk soil OR and in situ soil respiration OR using gas exchange techniques are needed in future work to quantify these offsets. An important challenge will be to account for O_2 and CO_2 diffusion effects on the OR of soil respiration measurements [Severinghaus, 1995], an issue that has received considerable attention within the stable isotope measurement community [e.g., Amundson et al., 1998]. At Harvard Forest, where the OR values of both the pools and the fluxes were measured, the OR of Harvard forest soil respiration (OR_{ba}) is lower than the OR of the soil C pool. This suggests that the microbial community is consuming more oxidized organic matter (a lower OR value, like carbohydrates) and that more reduced litter compounds and microbial residues are accumulating in the soil. This is consistent with soil NMR data, which show that Harvard forest soil organic matter is highly aliphatic, comprising 27% and 42% lipid C in the O and A horizons, respectively.

4. Discussion

4.1. NMR and Gas Flux Methods Are Different and Complementary

[47] NMR-based OR measurements do not measure properties identical to those measured by gas flux techniques. NMR-based techniques measure the OR of the carbon pool subjected to NMR, while gas flux techniques measure the OR of the soil C mineralized during the period of measurement. In other words, NMR techniques measure the OR of carbon pools, while gas flux measurements measure the OR

Table 3. Oxidative Ratio of Mineral Soils Carbon Pools and Fluxes

Soil Sample	Depth (cm)	Location	Ecosystem	Oxidative Ratio	Uncertainty	Method ^a	Reference ^b
DFTC HF	10	Delta Junction, AK, USA	boreal black spruce forest	1.09	±0.04	DP ^{MMM}	1
DFTC HF	11	Delta Junction, AK, USA	boreal black spruce forest	1.03	±0.04	DP ^{MMM}	1
DFTC HF	15	Delta Junction, AK, USA	boreal black spruce forest	1.06	±0.04	DP ^{MMM}	1
DF87 HF	6	Delta Junction, AK, USA	boreal black spruce forest	1.06	±0.04	DP ^{MMM}	1
DF87 HF	12	Delta Junction, AK, USA	boreal black spruce forest	1.06	±0.04	DP ^{MMM}	1
DF87 HF	18	Delta Junction, AK, USA	boreal black spruce forest	1.10	±0.07	CP ^{MMM}	1
ACU1 HF	0-10	Gumeracha, SA, AU	temperate Mediterranean eucalypt woodland	1.03	±0.04	DP ^{MMM}	1
B211 HF	0-5	Toowoomba, Qld, AU	subtropical eucalypt woodland	1.07	±0.04	DP ^{MMM}	1
BUCK HF	0-10	Kimba, SA, AU	temperate Mediterranean eucalypt woodland	1.08	±0.04	DP ^{MMM}	1
QLD HF	0-10	Chinchilla, Qld, AU	subtropical acacia woodland	1.13	±0.04	DP ^{MMM}	1
SS6 HF	0-5	Tallagalla, Qld, AU	subtropical vine scrub forest	1.11	±0.04	DP ^{MMM}	1
SS7 HF	0-5	Toowoomba, Qld, AU	subtropical eucalypt woodland	1.06	±0.04	DP ^{MMM}	1
SS8 HF	0-10	Toowoomba, Qld, AU	subtropical native grassland	0.96	±0.04	DP ^{MMM}	1
URB P HF	0-10	Urrbrae, SA, AU	temperate Mediterranean planted pasture	1.10	±0.04	DP ^{MMM}	1
Harvard Forest ^c	0-2	Petersham, MA, USA	temperate mixed-hardwood forest	1.15	±0.07	CP ^{MMM}	1
Harvard Forest ^c	2-10	Petersham, MA, USA	temperate mixed-hardwood forest	1.22	±0.07	CP ^{MMM}	1
Harvard Forest 1 ^d	0-2	Petersham, MA, USA	temperate mixed-hardwood forest	1.07	±0.02	O ₂ /CO ₂ flux	2
Harvard Forest 2 ^d	0-2	Petersham, MA, USA	temperate mixed-hardwood forest	1.09	±0.02	O ₂ /CO ₂ flux	2
Harvard Forest 2 ^d	2-5	Petersham, MA, USA	temperate mixed-hardwood forest	1.06	±0.02	O ₂ /CO ₂ flux	2
Harvard Forest 1 ^d	5-10	Petersham, MA, USA	temperate mixed-hardwood forest	1.06	±0.02	O ₂ /CO ₂ flux	2
Harvard Forest 2 ^d	5-10	Petersham, MA, USA	temperate mixed-hardwood forest	1.08	±0.03	O ₂ /CO ₂ flux	2
Merced	0-2	Merced, CA, USA	temperate riparian grassland	1.21	±0.02	O ₂ /CO ₂ flux	2
Merced	2-8	Merced, CA, USA	temperate riparian grassland	1.22	±0.05	O ₂ /CO ₂ flux	2
Hopetown 1	0-16	Hopetown, CA, USA	temperate managed grassland	1.20	±0.02	O ₂ /CO ₂ flux	2
Hopetown 2	0-16	Hopetown, CA, USA	temperate managed grassland	1.17	±0.02	O ₂ /CO ₂ flux	2
Scripps		La Jolla, CA, USA	Mediterranean ornamental planting	1.19	±0.02	O ₂ /CO ₂ flux	2
Bornhöved	0-20	Kiel, Germany	temperate grassland and forest (average)	1.30	±0.05	O ₂ /CO ₂ flux	3
Giessen Grassland	nr ^e	Giessen, Germany	temperate native grassland	1.11		O ₂ /CO ₂ flux + ¹⁵ N tracer	4
						Mean	Standard Deviation of the Mean
Soil-based (pool) measurements						1.08	±0.06
Gas-based (flux) measurements						1.15	±0.07

^aAll NMR-based oxidative ratio (OR) values are calculated from C_{ox} using equation (5) which assumes N₂ as the source of N to the ecosystem. Values from references 3 and 4 were converted from respiratory quotient (moles CO₂ respired/moles O₂ consumed = 1/OR) of soils in the absence of, or corrected for, substrate additions.

^bReferences: 1, this study; 2, *Severinghaus* [1995]; 3, *Dilly* [2001]; 4, *Müller et al.* [2004].

^cSoils were sampled in 1989 at the Prospect Hill site. NMR data were collected by *Dria* [2004].

^dSoils were sampled in 1994 at the DIRT experiment located ~5.5 km from Prospect Hill. The author notes that OR values may be affected by sample storage prior to analysis.

^eHere nr, not reported.

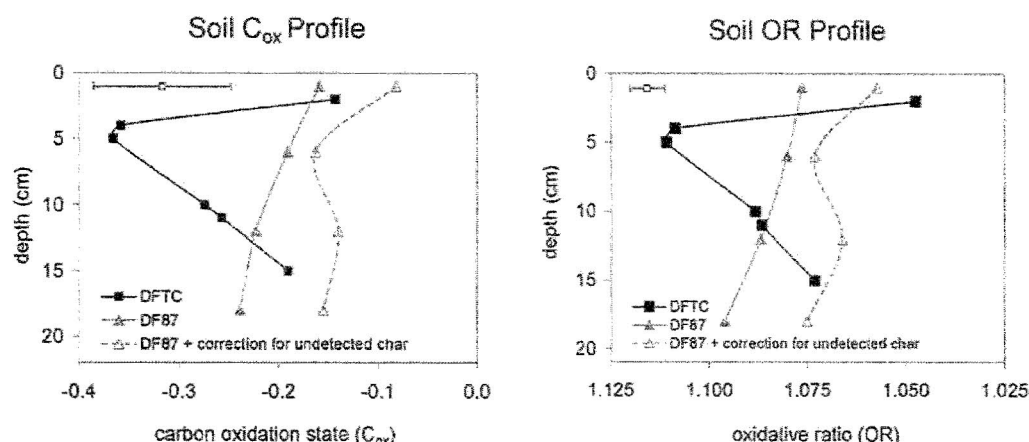


Figure 5. Changes in boreal forest soil (left) C_{ox} and (right) OR with depth at the Donnelly Flats tower control (DFTC) and a nearby site that burned in 1987 (DF87). C_{ox} values were measured by CP^{MMM} . Open symbols represent DF87 soil C_{ox} values that have been corrected to include undetected charcoal C. Measurement uncertainty (± 0.069 C_{ox} units) is shown separately as a bar at the top of each profile. Soil from ≥ 10 cm depth was treated with HF acid to remove paramagnetic interferences.

of carbon fluxes. To use NMR-based measurements to track the OR of a disturbance (e.g., land use change or fire), it is necessary to know both the amount of carbon oxidized by the disturbance and its OR. We demonstrate this type of calculation in section 4.2 (see below). The primary advantages of NMR-based OR measurements are (1) the ability to estimate OR under conditions that are not amenable to deploying gas flux instruments (e.g., during a wildfire), and (2) the ability to estimate the OR of a disturbance event that occurs over long timescales (e.g., decadal-scale respiration of preexisting soil C after a change in land use). Because physical separations can be applied to soil samples before analyses, NMR-based techniques also present the opportunity to measure the ORs of active and passive soil carbon pools separately.

[48] The error in NMR-based OR measurements cannot be directly compared to the OR error in gas flux measurements for the reasons described above. However, it remains valuable to consider measurement uncertainty. The propagation of algorithm error (± 0.026) and NMR quantitation error with uncertainty caused by HF acid pretreatment (± 0.024) gives a total C_{ox} measurement uncertainty of ± 0.036 for Dp^{MMM} and ± 0.069 for Cp^{MMM} , corresponding to soil OR uncertainty of ± 0.009 ($\pm 1\%$) and ± 0.017 ($\pm 2\%$), respectively. Systematic errors in O_2 and CO_2 gas flux measurements are on the order of $\pm 2\%$ to $\pm 3\%$ [Severinghaus, 1995; Seibt et al., 2004; Stephens et al., 2007]. Therefore, although the NMR and gas flux techniques probe different components of the soil C pool and are not likely to yield the same OR values, the accuracy of NMR-based OR values are comparable to the accuracy of flux-based OR values.

[49] The differences in OR between fluxes and pools may derive from differences in the oxidation states of C in actively versus passively cycling pools, whose C_{ox} and OR can be measured separated by NMR-based techniques. Soil organic matter can be physically and/or chemically

separated into fractions that approximately resemble active and passive C pools. Microbial biomass carbon extracts and low density soil fraction (< 1.6 – 2.0 g cm $^{-3}$) are representative of the active C pool with residence times < 10 years, and the high density fraction (> 1.6 – 2.0 g cm $^{-3}$) is a proxy for mineral-associated organic matter in intermediate and passive pools with mean residence times > 25 years [von Lutzow et al., 2006]. Future NMR-based C_{ox} and OR measurements may provide insight to the manner in which soil properties, such as physical structure and mineralogy, influence soil C_{ox} and OR.

4.2. NMR Can Be Used to Calculate the OR of Ecosystem Disturbance

[50] The episodic efflux of C from ecosystems during fire or land use transition can be just as important as natural ecosystem respiration in moving C from the biosphere to the atmosphere [Schimel, 1995]. The difficulties associated with monitoring O_2 and CO_2 during disturbance events, like fires, has limited our ability to estimate the OR associated with large episodic C fluxes. A unique aspect of the NMR technique is the ability to determine the OR of episodic disturbances which alter the size of the soil C pool. For instance, a soil that was recently burned or converted to agriculture may be compared to soils in an adjacent undisturbed site. The net soil C flux resulting from the disturbance can be estimated as $\Delta pool/dt$, where $\Delta pool$ is the change in the size of the soil C pool and dt is the time of the disturbance interval. This approach is described by the following equation:

$$C_{ox}^{disturbance} = \frac{(C_{ox}^{final} \times pool^{final}) - (C_{ox}^{initial} \times pool^{initial})}{(pool^{final} - pool^{initial})} \quad (10)$$

where $C_{ox}^{initial}$ and C_{ox}^{final} are the oxidation states of the soil C before and after the disturbance, respectively, and $pool^{initial}$

Table 4. Molecular Mixing Model Results Corresponding to Soil C_{ox} Profiles in Figure 5^a

Soil (cm)	Percent of Soil Organic Carbon					
	Carbohydrate	Protein	Lignin	Lipid	Carbonyl	Charcoal
DFTC						
2	71	5	15	5	2	2
4	54	8	20	14	0	4
5	44	8	29	14	1	4
10	40	8	33	10	1	8
11	35	10	28	13	3	11
15	33	11	21	14	5	16
DF87						
1	49	15	20	6	4	6
6	33	14	15	16	6	16
12	25	14	12	19	3	27
18	25	16	7	23	6	23

^aBaldock et al. [2004].

and pool^{final} are the total C in the soil before and after the disturbance.

[51] We modified this approach to estimate the OR of soil fire emissions from a boreal forest fire that occurred near Delta Junction, Alaska in 1999 [Randerson et al., 2006a]. Using data in the work of Neff et al. [2005], we estimated the C stored in organic soil horizons before (pool^{initial}) and after the fire (pool^{final}) that occurred at the DFTC site in 1999. Specifically, we assumed that the fire burned to a depth of 7 ± 2 cm in the soil, and accounted for changes in bulk density and carbon content in the soil with depth. Because we do not have samples from the site of the 1999 fire, we made the assumption that the C_{ox} of the upper organic soil horizons consumed in the 1999 fire are the same as those from an intact soil profile in a nearby control forest (DFTC). This is a reasonable assumption given the proximity of the sites and the similarity of the ecosystems. We estimate that this flux of carbon had an oxidation state in the range -0.263 to -0.283, and an OR value in the range 1.088 to 1.093, depending upon the depth of burning into the organic soil layers.

[52] In the above example, we did not account for changes in the remaining SOM pool as a result of the fire (e.g., the accumulation of char on the soil surface) because we do not have samples from the site of the 1999 fire. However, the organic soil residues affected by the 1987 the unburned soil horizons (C_{ox}^{initial} = -0.165). An increase in char in the final SOM pool probably means that our first order estimate of the fire OR (1.06) is too low since charred residues are relatively oxidized in comparison to unburned horizons.

[53] The OR value often used to characterize biosphere-atmosphere exchange does not consider ecosystem disturbance and other processes that regulate the flux on decadal timescales. The above example, albeit approximate, demonstrates that the OR of episodic disturbances may diverge from the OR of ecosystem respiration. The implications of this finding for global C cycle modeling are discussed, in part, by Randerson et al. [2006b] who showed that a disequilibrium between the average OR of photosynthesis (OR_{ab}) and the return flux (OR_{ba}) of 0.0175 results in the incorrect apportionment of 1 Pg C a⁻¹ to the biosphere or to the ocean.

4.3. NMR Can Be Used to Determine Controls on Soil OR

[54] NMR-based methods are able to track how soil C_{ox} and OR are related to organic matter composition. Figure 5 and Table 4 relate changes in the soil organic matter chemistry to changes in soil C_{ox} and OR with depth in the soil profile. A sharp decrease in the DFTC soil C_{ox} (increase in OR) in the soil depth interval from 2 to 5 cm corresponds to a decline in carbohydrates, consistent with a soil horizon transition from O to A. At depths >10 cm, there is a relative accumulation of proteins. Charcoal also accumulates below 10 cm, shifting soil carbon to a higher oxidation state and toward lower OR.

[55] By comparing soils across a burn sequence, it is possible to study the influence of forest fire on soil C_{ox} and OR with depth. Soil samples in 2000 within the perimeter of the 1987 Granite Creek fire (DF87) had higher N/C ratios and a larger soil organic matter pool by ~50% (Table 2) as compared with DFTC. The elevated organic N/C ratios could have several causes. The loss of the fresh litter layer with low N/C ratio would leave behind and older and more decomposed pool of organic matter with a higher N/C ratio. Fire can alter C and N cycling via its effect on soil microbial communities (see review by Knicker [2007]). The recovery of soil microbial biomass C and N to prefire levels can take more than a decade [Fritze et al., 1993]. Charring that occurs during the smoldering stage of a forest fire also alters organic nitrogen chemistry by increasing the aromatic (pyridinic) N content of forest soil organic matter, as shown by ¹⁵N NMR [Knicker et al., 2005].

[56] The DF87 C_{ox} and OR values are also strongly influenced by charcoal formed during the fire. The CP NMR mixing model results indicate that charcoal represents approximately 2–4% of the C pool in the organic soil horizons at the control site (DFTC), and 6% of soil C at the more recently burned (DF87) site (see Table 4). Both the increased N/C ratio and the increased charcoal content of fire-impacted soils result in higher soil C_{ox} and lower soil OR values. We also corrected DF87 soil C_{ox} and OR values for the underestimation of highly condensed aromatic charcoal carbon that occurs in CP NMR spectroscopy. The charcoal-corrected C_{ox} and OR values are shown as open triangles in Figure 5. These OR values likely represent the long-term impact of forest fire on the composition of the atmosphere because they include a form of charcoal (black carbon) which decomposes more slowly than other plant residues [Preston and Schmidt, 2006, and references therein]. Some evidence also suggests that charcoal influences the actively cycling pool, expediting decomposition of soil labile organic matter by stimulating microbial activity [Zackrisson et al., 1996; Wardle et al., 1998; Pietikainen et al., 2000; Warnock et al., 2007; Wardle et al., 2008].

[57] The many ways that disturbance can alter soil OR (e.g., via changes in C_{ox}, microbial respiration rates, plant community structures, etc.), highlight the need for simultaneous NMR and gas flux measurements. The two techniques provide complementary information on the controls of soil OR. For instance, NMR is sensitive to changes in soil C_{ox} and the molecular composition of organic matter in carbon pools with different residence times, but largely insensitive to dynamic microbial activities, and assumptions (or measurements) are required regarding carbon residence

times and nitrogen cycling. On the other hand, gas flux techniques provide a direct measure of microbial respiration and rapidly cycling organic matter, but provide no information about the oxidation state of the stable carbon in soils, and little insight to mechanisms controlling OR. Mechanistic studies would further support modeling activities to predict changes in carbon uptake by the terrestrial biosphere in response to changes in climate and land use.

5. Conclusions

[58] We demonstrate a novel solid-state ^{13}C NMR technique for estimating the oxidation state of soil C pools and the OR of fluxes caused by episodic disturbances like fire and land use change. In a boreal black spruce forest, fire increased the oxidation state of the soil C pool substantially by removing surface layers that were comprised of relatively reduced SOM. Soil C_{ox} and OR values also exhibit substantial variation with soil depth and across ecosystems. NMR-based OR measurements allow the investigation of mechanisms controlling soil OR at the molecular level and across a range of timescales. NMR-based OR data are complementary to O_2 and CO_2 gas flux measurements. Future studies should focus on characterizing the OR of ecosystem disturbances, and seek mechanistic insight to how ecosystem type, soil type, soil moisture, and temperature control C_{ox} and OR. Knowledge of these relationships would facilitate predictive modeling of terrestrial C sink response to climate change.

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