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Evaluating two experimental approaches for measuring ecosystem carbon oxidation state and oxidative ratio

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[1] Degree of oxidation of organic carbon (C_{ox}) is a fundamental property of the carbon cycle, reflecting the synthesis and decomposition of natural organic matter. C_{ox} is also related to ecosystem oxidative ratio (OR), the molar ratio of O_2 to CO_2 fluxes associated with net ecosystem exchange (NEE). Here we compare two methods for measuring C_{ox} and OR: (1) %C, %H, %N, and %O elemental analysis, and (2) heat of combustion (ΔH_c) measured by means of bomb calorimetry coupled with %C elemental analysis (hereafter referred to as calorimetry). Compared with %C, %N, %H, and %O elemental analysis, calorimetry generates C_{ox} and OR data more rapidly and cheaply. However, calorimetric measurements yield less accurate C_{ox} and OR data. We additionally report C_{ox} and OR data for a pair of biomass standards and a suite of biomass samples. The OR values we measured in these samples were less variable than OR data reported in the literature (generated by simultaneous measurement of ecosystem O_2 and CO_2 gas mixing ratios). Our biomass OR values had a mean of 1.03 and range of 0.99–1.06. These estimates are lower than the OR value of 1.10 that is often used to partition uptake of fossil fuel CO_2 between the ocean and the terrestrial biosphere.

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1. Introduction

[2] The oxidation state of organic carbon (C_{ox}) is a fundamental property of the Earth's carbon cycle. C_{ox} can vary from -4 to $+4$, although most organic compounds span a narrower range between -2.2 and $+3$ (Figure 1). C_{ox} values contain information about organism and ecosystem biogeochemistry, including tissue composition, biochemical synthesis pathways, and environmental conditions at the time of growth. In soils and sediments C_{ox} may provide insight into a carbon pool's decomposition and diagenetic history. In addition, C_{ox} is mathematically related to ecosystem oxidative ratio (OR), a parameter used to track the fate of fossil fuel CO_2 .

[3] Photosynthesis, biosynthesis and respiration are important drivers of organic matter C_{ox} , shifting carbon between $+4$ (CO_2) and 0 (glucose) oxidation states on timescales of seconds to years. Other known drivers include geologic-timescale diagenesis, which has generated pools of

relatively reduced carbon: a large pool of kerogen (refractory geologic organic matter) with a C_{ox} value of ~ -1.15 (calculated from Mann *et al.* [1991]) as well as a relatively small pool of petroleum, with a C_{ox} value of ~ -1.90 (calculated as an average of short-chain saturated and longer chain unsaturated hydrocarbons).

[4] Although we have some understanding of trends in organic matter C_{ox} on very short (seconds) and very long (geologic) timescales, we know less about what controls C_{ox} on timescales from months to centuries. Understanding controls on C_{ox} over intermediate timescales is important in understanding how terrestrial ecosystems influence atmospheric O_2 levels. We show in this paper that C_{ox} is mathematically related to ecosystem oxidative ratio (OR), the molar ratio of O_2 and CO_2 fluxes associated with net ecosystem exchange (NEE). Shifts in ecosystem OR change the rate of ecosystem O_2 production, and may influence regional atmospheric O_2 mixing ratios. Variation in atmospheric O_2 mixing ratios can be used to track the environmental fate of fossil fuel CO_2 [Keeling *et al.*, 1996; Keeling and Shertz, 1992; Prentice *et al.*, 2001], and because of this, accurate measurements of terrestrial ecosystem OR are important.

[5] Based largely on gas exchange measurements of OR in a range of temperate soils [Severinghaus, 1995], the OR associated with terrestrial biosphere exchange often has been assumed to be constant at approximately 1.10 [e.g., Battle *et al.*, 2000; Keeling *et al.*, 1996; Langenfelds *et al.*, 1999; Manning and Keeling, 2006]. However, the natural biochemical variation in OR is large (Figure 1) [Keeling, 1988; Seibt *et al.*, 2004]. Plant compounds vary in their OR

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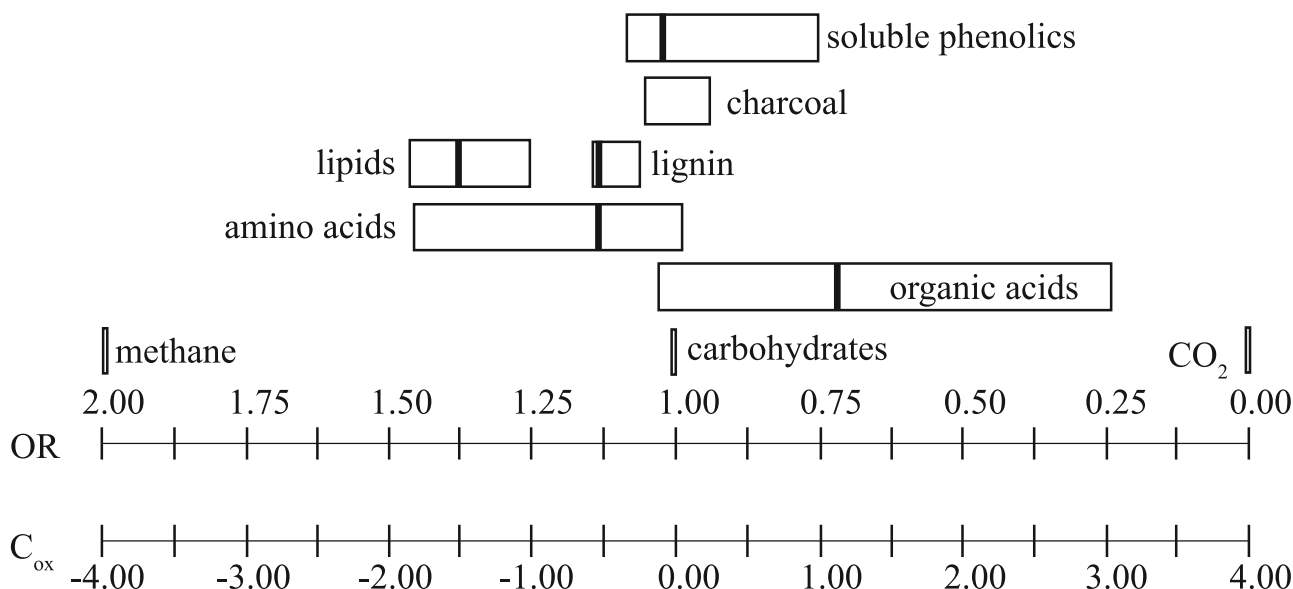


Figure 1. OR (upper axis) and C_{ox} (lower axis) of naturally occurring environmental compounds. The upper axis oxidative ratio line is idealized to an ecosystem using N₂ as the sole N source. Use of other forms of N (ammonia or nitrate) cause small shifts in the C_{ox} to OR conversion for N-containing compounds (e.g., amino acids, see text for discussion). “Carbohydrate” here represents both structural and nonstructural carbohydrates. The solid lines within the amino acid, lipid, and organic acid classes represent the most common natural values for proteins (1.13), lipids (1.37), and organic acids (0.68), as reported by *Baldock et al.* [2004] and *Randerson et al.* [2006]. We have added a solid line at 1.13 as a most common OR for lignin, recognizing that this may be subject to uncertainty introduced in the chemical extraction (Kraft process) used to separate lignins from biomass. The lower bound for soluble phenols is set to the OR of gallic acid (0.75), a tannin, and the upper bound at phenol itself (1.16), with ferulic acid (1.05) chosen as a representative of the most common plant phenolics [*Randerson et al.*, 2006].

value from 0.25 for highly oxygenated, short-chain organic acids like oxalic acid, to 1.44, for lipids like palmitic acid. Other important classes of plant compounds include lignin and protein, which have OR values that are substantially greater than cellulose or other structural and nonstructural carbohydrates (Figure 1).

[6] If the terrestrial biosphere’s OR is changing, as might be the case with expansion of agriculture and increasing levels of disturbance in terrestrial ecosystems, it may affect carbon sink partitioning calculations that are based on changing rates of atmospheric O₂ and CO₂ [*Randerson et al.*, 2006]. For example, were terrestrial ecosystems to synthesize proportionally less lignin (OR ~ 1.13) or more cellulose (OR = 1.00), less O₂ would be released per mol CO₂ fixed during net primary production. Failing to account for such a trend could lead to an underestimation of the size of the terrestrial carbon sink. Although shifts in the terrestrial biosphere OR (and ensuing disequilibria between the OR of photosynthesis and ecosystem respiration) are likely to be small at a global scale, they represent a source of uncertainty in sink partitioning calculations that is approximately the same as uncertainties introduced from our lack of understanding of the mean terrestrial OR (i.e., whether the mean OR of the terrestrial sink is 1.05 or 1.10) [*Randerson et al.*, 2006].

[7] The OR of ecosystem exchange has been measured experimentally in several studies. One approach involves

simultaneous measurements of atmospheric concentrations of O₂ and CO₂ [*Seibt et al.*, 2004; *Stephens et al.*, 2007; *Sturm et al.*, 2005]. O₂:CO₂ measurements yield high-resolution data with the potential to give detailed information on a number of natural and anthropogenic carbon cycle processes. Direct O₂:CO₂ measurements are typically taken at fine time steps (from days [*Seibt et al.*, 2004; *Sturm et al.*, 2005] to less than an hour [*Stephens et al.*, 2007]). *Seibt et al.* [2004] have shown that at high temporal frequency, O₂:CO₂ values can vary substantially. For example, the O₂:CO₂ of net assimilation can vary over more than a factor of 2, from 0.7 to 1.6, with both high and low values occurring during midafternoon on the same day [*Seibt et al.*, 2004]. Soil respiration O₂:CO₂ was measured by *Seibt et al.* [2004] as 0.94. Finally, both *Seibt et al.* [2004] and *Stephens et al.* [2007] provide evidence that canopy measurements of O₂:CO₂ ratios can be strongly influenced by air mass history, with canopy measurements rising to values as high as 1.53 in the presence of plumes influenced by fossil fuel combustion [*Stephens et al.*, 2007]. *Sturm et al.* [2005] report even higher O₂:CO₂ values of 2.1 ± 0.2 and 2.2 ± 0.2 at continental European mountain sites that primarily sample air masses from the free troposphere.

[8] Fossil fuel CO₂ apportionment calculations require accurate ecosystem OR data at annual timescales, but as demonstrated above, deriving these data from direct gas phase O₂:CO₂ measurements is challenging. An alternate

approach is to directly measure the OR of the biomass pools that are increasing or decreasing in size. This approach complements estimates of OR derived from gas exchange studies because (1) it uses a completely different set of measurement techniques and (2) it involves measurement of ecosystem organic matter pools rather than gas fluxes.

[9] In this paper we relate C_{ox} to OR and report two methods for measuring the C_{ox} of an ecosystem's above-ground biomass: (1) elemental analysis of %C, %H, %N, and %O (an approach described earlier [Keeling, 1988; Severinghaus, 1995]) and (2) calorimetry coupled with elemental analysis of %C, which we will refer to hereafter as calorimetry. We show that elemental analysis produces the most accurate data over a broad range of biomass C:N ratios. Although calorimetry can provide highly precise C_{ox} measurements for some samples, its accuracy for N-rich samples is not as high as elemental analysis. We report both C_{ox} and OR for a suite of biomass samples. C_{ox} is not sensitive to the source or loss form of N, in contrast to OR, which can vary if the N in organic matter enters or leaves the system as N₂, ammonia, or nitrate. We also discuss ecosystem N source/sink assumptions necessary for the conversion of C_{ox} to OR, and estimate the error associated with these assumptions.

2. Relationship Between C_{ox} and OR

[10] The oxidation state of organic carbon in a charge-neutral organic matter sample can be defined as:

$$C_{ox} = \frac{2[O] - [H] - k[N] - m[S]}{[C]} \quad (1)$$

where [O], [H], [N], [S], [C] are the molar concentrations of elements in the organic compound, and the multipliers correspond to the oxidation state of the elements within the compound, with k equal to -3 for ammonia or +5 for nitrate and m equal to -2 for sulfide or +6 for sulfate. Equation (1) can be derived by assuming that the sum of the oxidation states of all elements in a organic matter sample is the net charge on that sample (zero):

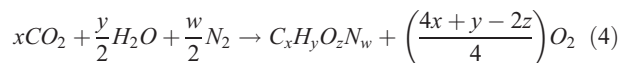
$$C_{ox}[C] - 2[O] + [H] + k[N] + m[S] = 0 \quad (2)$$

[11] We simplify this equation by eliminating sulfur, as it is on the order of 0.25% of biomass [Charlson *et al.*, 2000]. This simplification holds for living biomass, but C_{ox} measurements on organic pools with higher concentrations of S (e.g., some marine sediments) may require accounting for S. We additionally simplify equation (2) by assuming that the oxidation states of noncarbon species are the following: H_{ox} = 1, O_{ox} = -2, and N_{ox} = -3. For biomass C_{ox} measurements, the assumption N_{ox} = -3 is equivalent to assuming that the majority of organic N exists as amine groups in amino acids. Thus for any organic molecule C_xH_yO_zN_w, the definition of C_{ox} simplifies to:

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

[12] Molar percentages of C, H, N, and O can all be measured on a flash combustion elemental analyzer.

[13] Deriving the relationship between C_{ox} and OR requires balancing the equation for organic matter synthesis (or oxidation) and then calculating the ratio of O₂/CO₂ fluxes. For example, we can balance the equation for carbon fixation using N₂ as the N source:



[14] OR can be calculated as the ratio of the O₂ and CO₂ coefficients in equation (4) (e.g., $\frac{4x+y-2z}{4x}$). Equation (5) can be derived by substituting equation (3) for C_{ox} in this O₂:CO₂ ratio and reducing, giving the relationship:

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

3. Nitrogen Source Error Assessment

[15] There are 2 additional possible conversions between C_{ox} and OR: one beginning with NH₃ and another beginning with HNO₃ as the ecosystem N source:

$$\text{Ammonia} : \quad OR = 1 - \frac{C_{ox}}{4} \quad (6)$$

$$\text{Nitrate} : \quad OR = 1 - \frac{C_{ox}}{4} + \frac{2w}{x} \quad (7)$$

[16] Both of these equations are derived from O₂:CO₂ ratios calculated from balanced synthesis equations, as in equation (4). In the cases of both ammonia and nitrate, the charge-neutral state of the molecule is used in calculations to preserve the C_{ox} assumption of charge neutrality.

[17] When organic matter contains N, OR values can change significantly depending on the C_{ox} to OR conversion equation used. We explored the variation in OR values using a suite of 9 biomass samples collected from 5 temperate ecosystems, including a coniferous forest, a deciduous forest, a grassland which was burned annually, and a cornfield. All sites were part of the Kellogg Biological Station Long Term Ecological Research Station (42° 24' N, 85° 24' W, lter.kbs.msu.edu). The largest range of possible OR values occurred for red clover (*Trifolium pratense* L.), the sample with the most N. Depending on the assumption of N source, red clover OR values ranged from 0.99 to 1.07. Conversely, a poplar (*Populus* sp.) tree bole with low N had OR values ranging from 1.05 to 1.07 (Table 1).

[18] There are conditions under which each of the three C_{ox} to OR conversion equations is the appropriate choice for estimation of the OR of ecosystem biomass. The N₂-based conversion equation is likely to be the appropriate choice for ecosystems where N₂ fixation supplies the N needed for an increase in biomass or organic matter (equation (5)). Because the C_{ox} to OR conversion equations are similar, this N₂-only assumption can tolerate moderate inputs of ammonia or nitrate without significant loss of accuracy. For example, OR values shift by about 0.01 units

Table 1. C_{ox} and OR Data for a Range of Temperate Plants as Measured Using Elemental Analysis^a

Biomass	Molar Ratio Relative to C				OR Assuming Varying N Sources				
	C	H	N	O	C _{ox}	OR, N ₂	OR, NH ₃	OR, HNO ₃	50% NH ₃ + 50% HNO ₃
Corn grain	1	1.61	0.0339	0.850	0.189	0.978	0.953	1.021	0.987
Corn stover	1	1.48	0.0098	0.723	-0.008	1.009	1.002	1.022	1.012
NPP-weighted weed mixture	1	1.57	0.0314	0.715	-0.048	1.036	1.012	1.075	1.043
Deciduous leaves	1	1.41	0.0169	0.636	-0.083	1.034	1.021	1.055	1.038
Coniferous Leaves	1	1.43	0.0179	0.594	-0.186	1.060	1.047	1.082	1.064
<i>Solidago canadensis</i> L. (goldenrod)	1	1.49	0.0221	0.676	-0.068	1.034	1.017	1.061	1.039
<i>Phleum pratense</i> L. (timothy)	1	1.52	0.0109	0.736	-0.017	1.013	1.004	1.026	1.015
<i>Trifolium pratense</i> L. (red clover)	1	1.47	0.0387	0.692	0.031	1.021	0.992	1.070	1.031
Poplar tree bole (no bark)	1	1.58	0.0094	0.676	-0.197	1.056	1.049	1.068	1.059

^aUpper bound measurement error on C_{ox} data is ±0.11 and on OR data is ±0.028, calculated as the average error on peach leaf and redwood standards. All samples are from Kellogg Biological Station, and were harvested in 2004. The corn grain, stover, and NPP-weighted weed mixture were harvested from Treatment 1, Replicate 1 (conventional agriculture), the coniferous and deciduous leaves were harvested from Replicate 1 of the coniferous and deciduous forest treatments, and the goldenrod, timothy, and red clover were harvested from Replicate 1 of Treatment 7, an annually burned grassland. The poplar bole was harvested in 1997.

when an ecosystem receives 20% of its N as nitrate (calculations based on Table 1).

[19] Another situation where the N₂-based C_{ox} to OR conversion equation may be appropriate is when accumulation of ecosystem biomass draws upon internal ecosystem reservoirs of N. This may occur, for example, if a carbon sink within an ecosystem is driven by climate warming that stimulates decomposition and a transfer of N from soil organic matter (with a low C to N ratio) to woody biomass (with a high C to N ratio) [Field *et al.*, 1992]. For many ecosystems, recycled N from decomposition (and subsequent plant uptake) has been shown to account for 90% or more of the N required for net primary production [e.g., Chapin *et al.*, 2002]. Given that the ultimate source of this recycled N is atmospheric N fixation, the transfer of some of this recycled N into additional carbon storage corresponds to the N₂ case. Equation (5) also is appropriate for use in estimating OR when organic matter is accumulated in an N-free form (i.e., cellulose).

[20] In contrast with the case of internal N cycling, terrestrial carbon storage driven by deposition of nitric acid would require use of equation (7) and would yield higher OR values (Table 1). Ecosystem NEE driven by the application of ammonium nitrate in plantation or agricultural systems would require the use of equations (6) (ammonia) and (7) (nitrate) in fractions representing ecosystem uptake of each N source. Assuming that N associated with carbon storage originates from ammonia and nitrate at a 1:1 molar ratio yields OR values slightly higher than the N₂ case (Table 1). For all samples in Table 1, the difference between the N₂ and the 50% ammonia/50% nitrate cases (a difference of 0.002 to 0.008 OR units) is smaller than the accuracy of measurement reported below for our most accurate OR measurement technique (±0.011 OR units).

4. Materials and Methods

[21] C_{ox} values of organic matter can be measured in 3 ways: (1) directly from a sample's %C, %H, %N, and %O; (2) via bomb calorimetric measurement of ΔH_c (heat of combustion) and %C, and (3) via ¹³C nuclear magnetic resonance (NMR) [Baldock *et al.*, 2004; W. C. Hockaday *et al.*, The measurement of soil carbon oxidation state and oxidative ratio by ¹³C nuclear magnetic resonance, submit-

ted to *Journal of Geophysical Research*, 2008, hereinafter referred to as Hockaday *et al.*, submitted manuscript, 2008]. The first two techniques are only appropriate for samples very low in mineral concentration, such as biomass. We focus on the first two techniques here; the third technique, ideal for soil C_{ox} measurements, is the subject of another publication [Hockaday *et al.*, submitted manuscript, 2008].

[22] The simplest measurement of C_{ox} is the direct measurement of %C, %H, %N, and %O within a sample via elemental analysis. Once the stoichiometry of an organic mixture C_xH_yO_zN_w is known, C_{ox} can be calculated using equation (3). For biomass and litter samples, %C, %H, %N, and %O can be measured via elemental analysis and then inserted into equation (3) to calculate C_{ox}. This approach has been used previously by Keeling [1988] to estimate the OR of terrestrial biomass, but without the inclusion of %O data. Note that storage of samples under perfectly dry conditions is not necessary for accurate OR measurements because one additional mole of H₂O has a net zero effect on the oxidation state of a sample. However, our most accurate results were obtained from samples stored in a reproducible manner, which included storage at constant temperature and moisture conditions.

[23] C_{ox} can also be calculated by bomb calorimetric measurement of ΔH_c coupled to %C measurements. This is possible because the heat of combustion of a sample is related to its C_{ox} value by the approximation:

$$C_{ox} = 4 - \frac{1.6}{EA_c} (0.06968\Delta H_c - 0.065) \quad (8)$$

[24] This relationship can be derived from the literature on the construction cost of organic matter (see Appendix A).

4.1. Elemental Analysis

[25] All %C, %H, %N and %O measurements were made via high-temperature combustion followed by chromatographic separation of the gases and detection by thermal conductivity (TCD). Samples of each standard were sent to the UC Santa Barbara Marine Sciences Institute Analytical Lab for CHN analyses, where they were run on a Perkin-Elmer 2400 Series CHNS/O elemental analysis system. We made %O measurements on a Costech ECS 4010 Elemental Combustion system at California Institute of Technology

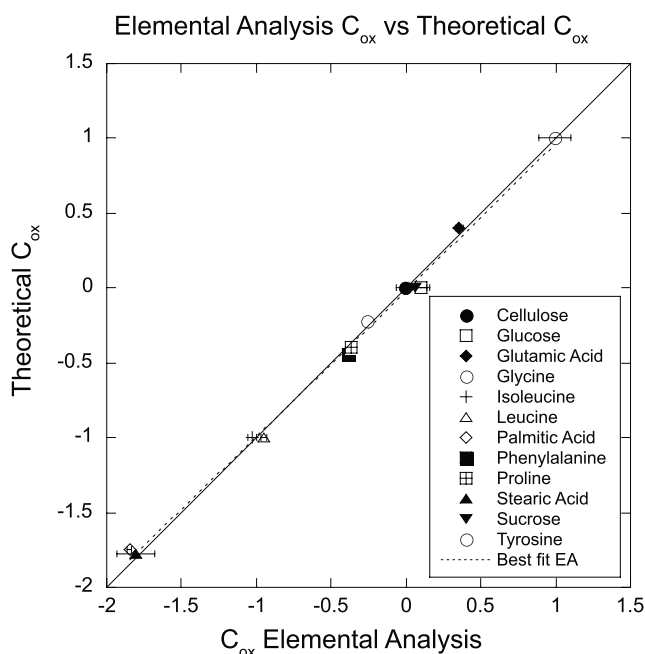


Figure 2a. C_{ox} values calculated from elemental formulas for the standards given in Tables 2a and 2b as compared with values measured by means of elemental analysis. The 1:1 line is solid and the best fit is dashed with an $R^2 = 0.996$.

and a second ECS 4010 at Rice University. Additionally, some of our %O measurements were made on a FlashEA 1112.

4.2. Calorimetry

[26] We made calorimetric measurements at Rice University using a Parr 6200 Calorimeter with a semimicro oxygen bomb (model 1107). We calibrated the calorimeter using benzoic acid to determine the average energy equivalent (EE), which was 542.5863 ± 2.1002 cal/°C. We combusted pellets of each standard in 440 psi of O_2 inside the bomb, limiting pellet mass to 0.07–0.23 g to prevent the release of greater than 1200 cal. We calculated the heat of combustion (cal/g) of each standard by monitoring the temperature rise of the water surrounding the bomb. We inspected each run for signs of an incomplete combustion (i.e., soot), and discarded these data. We performed a minimum of 3 complete combustions for each standard and corrected each sample for heat liberated due to the combustion of the fuse wire and formation of nitric acid. To account for heat released due to the formation of nitric acid from the N_2 gas in the bomb chamber, we applied a nitric acid correction of 1.5 cal [Parr-Instrument-Company, 2004] to all runs on samples which did not contain N. When samples did contain N, we determined the nitric acid correction by titrating the bomb washings with ~ 0.07 N Na_2CO_3 [Parr-Instrument-Company, 2004]. None of the standards used contained sulfur, so no sulfur correction was necessary.

4.3. Samples

[27] We purchased all chemical standards from commercial suppliers (Fisher Scientific or VWR International). All chemicals used were at least 98% pure; most were at least

99.5% pure. We stored standards and biomass samples in a desiccator when not in use. We put aliquots of standards and biomass in a drying oven at 50°C overnight prior to analysis.

5. Results and Discussion

[28] We measured 12 pure chemical standards via both techniques (cellulose, glucose, sucrose, glutamic acid, glycine, isoleucine, leucine, tyrosine, phenylalanine, proline, stearic acid, and palmitic acid). We calculated theoretical C_{ox} values for the pure standards and compared these values to those generated by elemental analysis (Figure 2a and Table 2a) and by calorimetry (Figure 2b and Table 2b).

[29] Elemental analysis yielded the most accurate C_{ox} values. EA-derived C_{ox} values fell along a 1:1 line when plotted versus theoretical C_{ox} values (Figure 2a) with an average error of ± 0.045 C_{ox} units and an average error of $OR \pm 0.011$ units (independent of C_{ox} to OR conversion equation used). There was no systematic bias in the elemental analysis data: measurements did not tend to generate values higher or lower than the theoretical value for samples (Table 2a). The average standard deviation on measurements (0.047 C_{ox} units) was very similar to the absolute error on measurements (0.045 C_{ox} units). Accuracy in elemental analysis-based C_{ox} measurements was limited by the accuracy in EA %O data (approximately 4%).

[30] Measurements made by calorimetry yielded C_{ox} data that was less accurate than that measured by elemental analysis. However, calorimetry proved slightly more precise in terms of the repeatability of measuring a single compound, opening up the possibility that with more methods development, calorimetry may prove as strong a technique

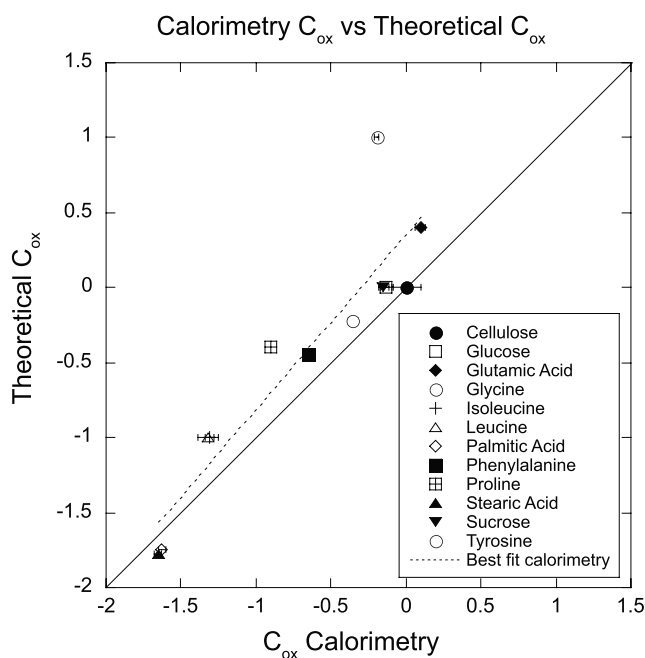


Figure 2b. Theoretical C_{ox} values calculated from elemental formulas as compared with measured via calorimetry. The 1:1 line is solid and the best fit is dashed with an $R^2 = 0.836$.

Table 2a. Accuracy and Precision in Measurements of C_{ox} Made Using Elemental Analysis (EA)^a

Standard	C _{ox} Measured	Std. Dev.	Actual	Error	Abs. Error
Cellulose	-0.0023	0.0661	0.0000	0.0023	0.0023
Glucose	0.1040	0.0580	0.0000	-0.1040	0.1040
Glutamic acid	0.3576	0.0247	0.4000	0.0424	0.0424
Glycine	0.9958	0.1085	1.0000	0.0042	0.0042
Isoleucine	-1.0297	0.0294	-1.0000	0.0297	0.0297
Leucine	-0.9529	0.0208	-1.0000	-0.0471	0.0471
Palmitic acid	-1.8457	0.0129	-1.7500	0.0957	0.0957
Phenylalanine	-0.3892	0.0116	-0.4444	-0.0552	0.0552
Proline	-0.3639	0.0236	-0.4000	-0.0361	0.0361
Stearic acid	-1.8030	0.1247	-1.7778	0.0252	0.0252
Sucrose	0.0612	0.0752	0.0000	-0.0612	0.0612
Tyrosine	-0.2552	0.0036	-0.2222	0.0330	0.0330
Peach Leaf	-0.3472	0.1442	NA		
Redwood Leaf	-0.2275	0.0730	NA		
Mean	chemical biomass	0.0466 0.1086		-0.0059	0.0447

^aThe Error column indicates the difference between theoretical and measured values, and the Absolute Error column indicates the absolute value of the Error column.

as elemental analysis. Calorimetric measurements of C_{ox} on pure standards had an average standard deviation of ± 0.034 units (OR ± 0.0085 units) compared with the C_{ox} average standard deviation measured by EA of ± 0.047 units. However, calorimetrically measured C_{ox} deviated from a 1:1 line when all the compounds were plotted versus their theoretical C_{ox} (Figure 2b). This resulted in a decreased overall accuracy for calorimetric C_{ox} data, as seen in the large absolute error, ± 0.29 C_{ox} units (compared to EA absolute error of ± 0.045 C_{ox} units). The difference between calorimetric C_{ox} measurements and theoretical C_{ox} values was a function of sample C:N ratio, with samples rich in N yielding the least accurate measurements. Indeed, C:N ratio correlated with error, with an R² value of 0.75 for the 7 samples measured that contained nitrogen (calculations not shown). C_{ox} values for N-free samples (sugars and lipids) had a much smaller error, ± 0.10 C_{ox} units (0.02 OR units), with the most accurate measurements resulting from measurements on the most common biomolecule, cellulose (± 0.0036 C_{ox} units, or 0.001 OR units).

[31] The error associated with calorimetric C_{ox} measurements may be caused by tuning biases in the relationships used by Williams *et al.* [1987] to derive equation (A2) (see Appendix A). Equation (A2) relies on an empirical relationship between heat of combustion (ΔH_c , measured via calorimetry) and glucose equivalents [Williams *et al.*, 1987]. Glucose equivalents is a plant biophysical parameter that is logically equivalent to C_{ox} (see derivations in Appendix A). Although the relationship between glucose equivalents and heat of combustion observed by Williams *et al.* [1987] is highly linear for hundreds of compounds, it deviates from linearity for compounds rich in N (see Figure 1 by Williams *et al.* [1987]). This bias significantly impacts measurements made on our N-rich pure amino acid standards, but for biomass samples the C:N ratio may be low enough that calorimetry may eventually prove to have an accuracy equal to or greater than elemental analysis.

[32] Along with measurements on pure chemical standards, we also made repeated C_{ox} measurements on 2 biomass standards (NIST SRM 1547 peach leaves, and an

internal redwood needle (*Sequoia* sp.) standard generated at CalTech). The C_{ox} standard deviations for peach leaf and redwood repeated elemental analysis measurements are ± 0.14 and ± 0.07 , respectively, significantly larger than the error associated with pure chemicals. The peach leaf and redwood elemental analysis C_{ox} data set reported in Tables 2a and 2b is a combination of data generated on two flash elemental analyzers, a Costech ECS 4010 at CalTech and a Flash EA 1112 used through a subcontract to CE Elantech, and this data set contains variability associated with initial method development. Therefore error bars associated with these measurements should be viewed as upper bounds.

[33] For these biomass standards, calorimetry compares favorably with elemental analysis. C_{ox} measured using these two techniques generate data that are not statistically different. Peach leaf C_{ox} values are particularly close (-0.347 ± 0.144 by EA versus -0.338 ± 0.063 by calorimetry). The calorimetric C_{ox} technique performs best with low N samples, and the NIST peach leaf standard is low in N.

[34] We converted C_{ox} values for these samples to OR values using equation (5). Peach leaf OR was 1.134 ± 0.036 (elemental analysis) and 1.098 ± 0.016 (calorimetry). Redwood leaf OR was 1.077 ± 0.018 (elemental analysis) and 1.049 ± 0.023 (calorimetry).

[35] We also generated C_{ox} and OR data for a pool of biomass samples (Table 1). Although these biomass samples are not a comprehensive ecosystem survey, we can use this data to get a sense of the appropriate OR value for key pools that are likely to be important in biomass accumulation. The average of all our N₂-based OR values was 1.03, with a high of 1.06 for coniferous needles and a low of 0.98 for corn grain. Our poplar tree bole sample had an OR of 1.056 ± 0.011 . This value was slightly higher than the mean OR of wood 1.043 ± 0.023 reported by Randerson *et al.* [2006] based on 29 observations of elemental ratios for different tree species compiled by Gaur and Reed [1995]. Taken together, these data suggest that the OR of pools that are important for C storage in many ecosystems is closer to 1.05 than to the often-used value of 1.10.

Table 2b. Accuracy and Precision in Measurements of C_{ox} Made Using Bomb Calorimetry^a

Standard	C _{ox} Measured	Std. dev.	Actual	Error	Abs. Error	C/N
Cellulose	0.0036	0.0957	0.0000	-0.0036	0.0036	...
Glucose	-0.1311	0.0439	0.0000	0.1311	0.1311	...
Glutamic acid	0.1001	0.0337	0.4000	0.2999	0.2999	5
Glycine	-0.1958	0.0130	1.0000	1.1958	1.1958	2
Isoleucine	-1.3097	0.0297	-1.0000	0.3097	0.3097	6
Leucine	-1.3181	0.0195	-1.0000	0.3181	0.3181	6
Palmitic acid	-1.6274	0.0231	-1.7500	-0.1226	0.1226	...
Phenylalanine	-0.6462	0.0211	-0.4444	0.2017	0.2017	9
Proline	-0.9007	0.0317	-0.4000	0.5007	0.5007	5
Stearic acid	-1.6532	0.0232	-1.7778	-0.1246	0.1246	...
Sucrose	-0.1475	0.0370	0.0000	0.1475	0.1475	...
Tyrosine	-0.3517	0.0334	-0.2222	0.1295	0.1295	9
Peach leaf	-0.3378	0.0632	NA		NA	
Redwood needle	-0.1150	0.0934	NA		NA	
Mean	pure compounds	0.0338		0.2486	0.2904	
	biomass	0.0783				

^aThe Error column indicates the difference between theoretical and measured values, and the Absolute Error column indicates the absolute value of the Error column.

[36] We define an individual ecosystem's OR as the sum of photosynthesis and respiration, excluding anthropogenic industrial practices (fossil fuel combustion and fertilizer production). *Ciais et al.* [2007] have showed that if fertilizer production and its accompanying O₂ fluxes are included as part of global ecosystem OR, it is necessary to further lower the estimate used in carbon sink partitioning calculations.

6. Ecosystem OR Measurements

[37] The net ecosystem OR can be estimated as the mass-weighted sums of the ORs of the carbon pool increments within the ecosystem:

$$OR_{net}\Delta M = OR_{abi}\Delta M_{abi} + OR_{rbi}\Delta M_{rbi} + OR_{si}\Delta M_{si} \quad (9)$$

where OR_{abi} represents the OR of the aboveground biomass increment, OR_{rbi} represents the OR of the root biomass increment, and OR_{si} represents the OR of the soil, litter, and coarse woody debris organic matter increment, and each of the ΔM terms represents the mass increment of each pool over a given time interval.

[38] Over an annual timescale, OR_{abi}, OR_{rbi}, and some parts of OR_{sbi} can be directly measured using the techniques described here. The measurement of the C_{ox} (and OR) of the carbon increment within mineral soils is more challenging, but may be accomplished via solid state ¹³C NMR [*Baldock et al.*, 2004; Hockaday et al., submitted manuscript, 2008]. It may be possible to approximate OR_{net} in some ecosystems by measuring just a few key pools that are known to comprise the bulk of ecosystem carbon storage (e.g., the bole, coarse woody debris, and litter increments in many forest ecosystems on decadal timescales). The use of these approximations will need to be assessed on an individual ecosystem basis.

7. Conclusions

[39] It is possible to accurately measure biomass carbon oxidation state (C_{ox}) using both elemental analysis and bomb calorimetry. Over a broad range of sample C:N values, elemental analysis generates data that is higher in accuracy but lower in precision than bomb calorimetry. The main source of error in elemental analysis is uncertainty associated with %O measurements. The major source of error associated with calorimetry is natural variation in the relationship between C_{ox} and ΔH_c (heat of combustion). Both sources of error can be expected to drop with further method development.

[40] C_{ox} measurements can be directly transformed into ecosystem OR values through simple algebraic relationships. The exact choice of C_{ox} to OR conversion equation depends on the major source of N to the ecosystem. The generation of the most accurate OR data will require some knowledge of the balance between nitrogen fixation, ammonia and nitrate as N sources to the ecosystem. OR data accurate to ± 0.01 units can be generated if ecosystem N sources can be constrained $\pm 20\%$. Data on a suite of biomass samples from 5 temperate ecosystems yield OR values ranging from 0.98 to 1.06, with an average of 1.03. These data suggest that the OR value of 1.10 currently used in carbon sink apportionment calculations may be too high.

[41] The types of annually integrated measurements we describe here complement existing OR measurement techniques like real-time O₂:CO₂ gas measurements [*Seibt et al.*, 2004; *Stephens et al.*, 2007; *Sturm et al.*, 2005], and both techniques would benefit from methods intercomparison.

Appendix A: Derivation of the Relationship between C_{ox} and ΔH_c

[42] This derivation begins with definitions and equations from the paper:

[43] *Williams, K., Percival, F., Merino, J., Mooney, H.A.* (1987), Estimation of tissue construction cost from heat of combustion and organic nitrogen content, *Plant, Cell, and Environment*, 10, 725–734.

[44] This paper is abbreviated as Williams PCE 87. All equations in this appendix are numbered sequentially beginning with (A1). When equations are from Williams PCE 87, they are additionally denoted as W PCE 87 (equation number), where the equation number matches that in the original paper.

[45] From Williams PCE 87:

[46] 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:

$$GE = \frac{c}{6} + \frac{h - 2x + kn + ms}{24} \quad (A1) \text{ (W PCE 87(1))}$$

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 100 g of dry plant matter), following the Williams PCE 87 notation. In addition, k is –3 (ammonium) or +5 (nitrate), and s is –2 (sulfide) or +6 (sulfate).

[47] Changing units from moles to grams,

[48] GE' = glucose equivalents of a substance expressed in grams glucose/grams material, and GE'₀ = GE' calculated using values for k and m.

[49] Williams PCE 87 derive a direct expression for GE'₀:

$$\begin{aligned} GE'_0 &= GE \times \frac{\text{glucose.g}}{\text{product.g}} \bigg/ \frac{\text{glucose.mol}}{\text{product.mol}} \\ &= GE \times \frac{\text{molecmass, glucose}}{\text{molecmass, product}} \end{aligned}$$

[50] 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. This is done by first, calculating GE for a number of individual biochemicals using equation (A1), then converting GE to GE'₀ using known molar masses of glucose and individual compounds, and finally regressing against measured ΔH values in kJ/g, to get:

$$\Delta H_c = 14.352 \times GE'_0 + 0.929 \quad (A2) \text{ (W PCE 87(4))}$$

where R² = 0.992.

[51] Returning 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:

$$GE'_0 = \left[\frac{c}{6} + \frac{h - 2x - 3n + 6s}{24} \right] \times \frac{180.15}{MW} \quad (\text{A3}) \quad (\text{W PCE 87(5)})$$

[52] GE'_0 can also be expressed in terms of ΔH_c (rearranging equation (5)):

$$GE'_0 = 0.06968 \times \Delta H_c - 0.065 \quad (\text{A4}) \quad (\text{W PCE 87(6)})$$

[53] Using the nomenclature of Williams PCE 87, and the assumption of charge neutrality for biomass $C_cH_hO_xN_nS_s$, organic carbon oxidation state is defined as:

$$\begin{aligned} 0 &= C_{ox} \cdot c - 2 \cdot x + h - 3n + 6s \\ C_{ox} &= \frac{2x - h + 3n - 6s}{c} \end{aligned} \quad (\text{A5})$$

[54] Factoring out $c/6$, equation (A5) becomes:

$$GE_0 = \frac{c}{6} \left[1 + \frac{h - 2x - 3n + 6s}{4c} \right] \times \frac{180.15}{MW} \quad (\text{A6})$$

inserting $-C_{ox}$,

$$GE_0 = \frac{c}{6} \left[1 - \frac{C_{ox}}{4} \right] \times \frac{180.15}{MW} \quad (\text{A7})$$

rewriting,

$$GE'_0 = \frac{c}{MW} \left[1 - \frac{C_{ox}}{4} \right] \times \frac{180.15}{6} \quad (\text{A8})$$

modifying x/MW :

$$\frac{c}{MW} = \frac{\frac{\text{molC}}{\text{gproduct}}}{\frac{\text{molproduct}}{\text{molproduct}}} = \frac{\text{molC}}{\text{gproduct}} \quad (\text{A9})$$

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

$$EA_c = \frac{\text{gcarbon}}{\text{gproduct}} \quad (\text{A10})$$

then, converting g carbon to mol carbon:

$$EA_c \times \frac{\text{molC}}{12.011\text{gC}} = \frac{\text{gcarbon}}{\text{gproduct}} \times \frac{\text{molC}}{\text{gcarbon}} = \frac{\text{molC}}{\text{gproduct}} \quad (\text{A11})$$

and therefore:

$$\frac{c}{MW} = \frac{EA_c}{12.011} \quad (\text{A12})$$

substituting into equation (A10),

$$GE'_0 = \frac{EA_c}{12.011} \times \left[1 - \frac{C_{ox}}{4} \right] \times \frac{180.15}{6} \quad (\text{A13})$$

setting equation (A6) equal to equation (A13),

$$0.06968 \times \Delta H_c - 0.065 = \frac{EA_c}{12.011} \left[1 - \frac{C_{ox}}{4} \right] \times \frac{180.15}{6} \quad (\text{A14})$$

solving for C_{ox} ,

$$\begin{aligned} 4 \times \left(1 - \left((0.06968 \times \Delta H_c - 0.065) \times \frac{12.011}{EA_c} \times \frac{6}{180.15} \right) \right) \\ = 4 - \frac{1.6}{EA_c} (0.06968 \Delta H_c - 0.065) = C_{ox} \end{aligned}$$

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

$$\text{construction cost} = \frac{GE}{EG} \quad (\text{A15}) \quad (\text{W PCE 87(3)})$$

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

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