

## RESEARCH ARTICLE

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## Key Points:

- Soil carbon oxidation state increased with atmospheric CO<sub>2</sub> concentration
- The carbon oxidation state is sensitive to disequilibria in O<sub>2</sub> and CO<sub>2</sub> fluxes
- Hardwood forests may be an atmospheric O<sub>2</sub> sink at high CO<sub>2</sub> levels

## Supporting Information:

- Supporting Information S1

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Forest soil carbon oxidation state and oxidative ratio responses to elevated CO<sub>2</sub>

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**Abstract** The oxidative ratio (OR) of the biosphere is the stoichiometric ratio (O<sub>2</sub>/CO<sub>2</sub>) of gas exchange by photosynthesis and respiration—a key parameter in budgeting calculations of the land and ocean carbon sinks. Carbon cycle-climate feedbacks could alter the OR of the biosphere by affecting the quantity and quality of organic matter in plant biomass and soil carbon pools. This study considers the effect of elevated atmospheric carbon dioxide concentrations ([CO<sub>2</sub>]) on the OR of a hardwood forest after nine growing seasons of Free-Air CO<sub>2</sub> Enrichment. We measured changes in the carbon oxidation state (C<sub>ox</sub>) of biomass and soil carbon pools as a proxy for the ecosystem OR. The OR of net primary production, 1.039, was not affected by elevated [CO<sub>2</sub>]. However, the C<sub>ox</sub> of the soil carbon pool was 40% higher at elevated [CO<sub>2</sub>], and the estimated OR values for soil respiration increased from 1.006 at ambient [CO<sub>2</sub>] to 1.054 at elevated [CO<sub>2</sub>]. A biochemical inventory of the soil organic matter ascribed the increases in C<sub>ox</sub> and OR to faster turnover of reduced substrates, lignin and lipids, at elevated [CO<sub>2</sub>]. This implicates the heterotrophic soil community response to elevated [CO<sub>2</sub>] as a driver of disequilibrium in the ecosystem OR. The oxidation of soil carbon pool constitutes an unexpected terrestrial O<sub>2</sub> sink. Carbon budgets constructed under the assumption of OR equilibrium would equate such a terrestrial O<sub>2</sub> sink to CO<sub>2</sub> uptake by the ocean. The potential for climate-driven disequilibria in the cycling of O<sub>2</sub> and CO<sub>2</sub> warrants further investigation.

## 1. Introduction

An increasing number of studies document the declining effectiveness of the biosphere in removing anthropogenic carbon dioxide (CO<sub>2</sub>) from the atmosphere [e.g., *Canadell et al.*, 2007; *Finzi et al.*, 2006; *Gill et al.*, 2006, 2002; *Hungate et al.*, 1997; *King et al.*, 2004; *Kool et al.*, 2007; *Le Quere et al.*, 2013; *Norby et al.*, 2010b; *Polley et al.*, 2006; *Raupach et al.*, 2008]. Some modeling studies suggest that the rapid rise of atmospheric CO<sub>2</sub> levels could trigger carbon cycle-climate feedbacks that drive nonlinear change [*Hansen et al.*, 2007]. Predicting future climate change and the associated carbon (C) cycle feedbacks requires an understanding of the ecological and biogeochemical responses to increasing atmospheric CO<sub>2</sub> concentrations ([CO<sub>2</sub>]). After several decades of CO<sub>2</sub> fertilization research in terrestrial ecosystems, a growing body of evidence points to the primary importance of soil processes in the C cycle response to elevated [CO<sub>2</sub>] [*Billings et al.*, 2010; *Calfapietra et al.*, 2010; *Norby and Zak*, 2011; *Pendall et al.*, 2004]. Indeed, whether an ecosystem will act as a net source or sink of atmospheric C under increasing [CO<sub>2</sub>] depends upon the availability of soil water and nutrients to support greater net primary production (NPP), as well as the degree to which soil organic matter (SOM) is protected against biological oxidation [*Baldock and Skjemstad*, 2000]. However, most terrestrial biosphere models do not yet include process-based algorithms for predicting trends in soil C cycle responses to climate [*Le Quere et al.*, 2009]. Improving climate-C cycle model predictions will require mechanistic studies of soil C cycling processes and their responses to climate change variables at the ecosystem scale [*Luo et al.*, 2011; *Sulman et al.*, 2014; *Zaehle et al.*, 2014].

In this study we examined changes in the chemistry of aboveground and belowground C pools in response to elevated [CO<sub>2</sub>]. We focus on the ecosystem oxidative ratio (OR) and the related parameter carbon oxidation state (C<sub>ox</sub>), because these terms are important in estimating the size of the terrestrial C sink. Large variations in OR values in ecosystem carbon pools may also be an indicator of ecosystem response to environmental change.

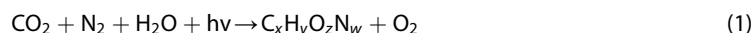
In the terrestrial biosphere, the sources and sinks of atmospheric CO<sub>2</sub> are coupled to the sources and sinks of atmospheric oxygen (O<sub>2</sub>) by the chemical reactions that create and destroy organic matter: photosynthesis,

respiration, decomposition, and combustion. The coupled nature of the  $O_2$  and  $CO_2$  biogeochemical cycles has led to the use of high-precision measurements of atmospheric  $[CO_2]$  accumulation and  $[O_2]$  depletion for the purpose of calculating the relative magnitude of the land and ocean C sinks [Battle et al., 2000; Bender et al., 1998, 2005; Keeling et al., 1996; Langenfelds et al., 1999; Manning and Keeling, 2006; Manning et al., 2003; Seibt et al., 2004; Stephens et al., 1998; Sturm et al., 2006; Valentino et al., 2008].

### 1.1. The Utility of Oxidative Ratio in C Sink Apportionment

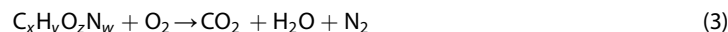
Globally averaged  $O_2/CO_2$  ratios have been given the term “alpha” ( $\alpha$ ) [after Keeling and Shertz, 1992] and are also known as an oxidative ratios (OR) [after Masiello et al., 2008; Randerson et al., 2006]. A commonly used estimate of the OR for fossil fuel combustion ( $\alpha_F$  or  $OR_{ff}$ ) is 1.39 ( $\pm 0.04$ ) [e.g., Bender et al., 1998; Keeling, 1988; Keeling et al., 1996; Keeling and Shertz, 1992], with uncertainties arising from variability in the global mixture of fossil fuel consumption (e.g., natural gas, coal, and petroleum) [Manning and Keeling, 2006; Steinbach et al., 2011]. The OR of the global biosphere ( $\alpha_B$ ) can be depicted as two fluxes in opposing directions. The atmosphere-to-biosphere  $CO_2$  flux ( $F_{ab}$ ) is driven by NPP (equation (1)) and has an associated oxidative ratio ( $OR_{ab}$ ) defined by equation (2). The  $CO_2$  flux returning from the biosphere ( $F_{ba}$ ) is controlled by processes driving organic matter mineralization (equation (3)), such as heterotrophic respiration, deforestation, and fire. The oxidative ratio ( $OR_{ba}$ ) of processes controlling  $F_{ba}$  is represented in equation (4). The net OR for the biosphere ( $OR_{net}$ ) is the sum of  $OR_{ab}$  and  $OR_{ba}$  weighted by the magnitude of the fluxes  $F_{ab}$  and  $F_{ba}$ , as shown in equation (5). The sign convention for  $CO_2$  fluxes is such that withdrawals from the atmosphere ( $F_{ab}$ ) have positive values, and return fluxes ( $F_{ba}$ ) are negative.

Net photosynthetic fixation, NPP:



$$OR_{ab} = \frac{\text{moles } O_2 \text{ produced by NPP}}{\text{moles } CO_2 \text{ consumed by NPP}} \quad (2)$$

Heterotrophic respiration:



$$OR_{ba} = \frac{\text{moles } O_2 \text{ consumed by respiration}}{\text{moles } CO_2 \text{ produced by respiration}} \quad (4)$$

$$OR_{net} = \frac{OR_{ab} \cdot F_{ab} + OR_{ba} \cdot F_{ba}}{F_{ab} + F_{ba}} \quad (5)$$

Due to the intractability of making direct measurements of  $O_2$  and  $CO_2$  fluxes for the global biosphere,  $OR_{net}$  is not calculated from measured quantities. Rather,  $OR_{net}$  and  $\alpha_B$  are based on the elemental stoichiometry (C:H:N:O) of plant biomass. Typical values used for global C budgeting are 1.05 [Keeling and Shertz, 1992] and 1.10 [Battle et al., 2000; Keeling et al., 1996; Manning and Keeling, 2006]. These values are based on a small number of plant and soil chamber measurements made by Severinghaus [1995]. Carbon sink apportionment calculations reported for the last two decades have consistently used the same biosphere OR value ( $\alpha_B$ ) [e.g., Intergovernmental Panel on Climate Change (IPCC), 2007]. The use of a constant  $OR_{net}$  across multiple decades implies that  $OR_{net}$  has remained constant (within uncertainty) over that time period. The assumption of constancy is based upon the relatively narrow range of stoichiometry (elemental ratios, C:H:N:O) observed in woody biomass, and the (steady state) assumption that heterotrophic respiration is in equilibrium with NPP [Severinghaus, 1995].

It is now recognized that  $OR_{net}$  ( $\alpha_B$ ) is unlikely to remain constant and is a property of the biosphere that is susceptible to change on decadal timescales. Biome-scale and ecosystem-scale OR values differ broadly [Worrall et al., 2013] and are susceptible to changes due to land management [Gallagher et al., 2014], fire [Hockaday et al., 2009], and other disturbances, including climatic changes [Randerson et al., 2006]. For example, terrestrial ecosystem responses to rising atmospheric  $[CO_2]$  include changes in plant community structure, plant C allocation patterns, tissue chemistry, nitrogen (N) cycling, soil microbial abundance and activity, and a cascade of changing trophic interactions [Billings et al., 2010; Billings and Ziegler, 2008; Calfapietra et al., 2010; Drigo et al., 2008; Norby and Zak, 2011; Pendall et al., 2004]. Randerson et al. [2006]

demonstrated that modest changes in plant tissue chemistry, C allocation among plant tissues, and/or the spatial extent of biomes could alter the global  $OR_{net}$  and affect the global C and Oxygen (O) budgets.

The need for ecosystem level OR measurements is well recognized [e.g., *Ishidoya et al.*, 2015, 2013; *Keeling et al.*, 1993; *Manning and Keeling*, 2006; *Worrall et al.*, 2013]. Nevertheless, measuring OR at the ecosystem scale is challenging. The concentrations of  $O_2$  and  $CO_2$  are simultaneously influenced by competing natural processes and even at relatively remote sites can be complicated by weather events and meteorological processes [Seibt et al., 2004; Stephens et al., 2007; Sturm et al., 2006; van der Laan et al., 2014]. In this study, we used a novel approach to overcome the challenges of gas exchange measurements by instead determining the mass and oxidation state ( $C_{ox}$ ) of ecosystem C pools as a proxy for ecosystem OR. Here we quantified the differences in aboveground and belowground C pools and  $C_{ox}$  of hardwood forest plots previously exposed to ambient and elevated  $[CO_2]$  for nine growing seasons. The cumulative aboveground and belowground C fluxes were calculated from NPP data and changes in soil C pools. We then used the differences in  $C_{ox}$  of the C pools to estimate the OR of C fluxes.

## 1.2. The Carbon Oxidation State Proxy for Ecosystem Oxidative Ratio

The OR of natural organic matter from any source with a measurable elemental composition ( $C_xH_yO_zN_w$ ) can be estimated from its  $C_{ox}$  once the major sources of nitrogen (N) are known (i.e., biological N fixation versus reactive N deposition). In this study, no reactive N (fertilizer) was applied to the ecosystem; hence, equation (6) is the appropriate expression for relating  $C_{ox}$  values to OR values [Masiello et al., 2007]. The  $C_{ox}$  of plant biomass can be calculated, via equation (7), from the molar concentrations of C, hydrogen (H), N, and O measured by elemental analyses [Masiello et al., 2007]. For soils where minerals interfere with the measurement of organic H and O, the data for equation (7) can be generated by solid-state  $^{13}C$  nuclear magnetic resonance ( $^{13}C$  NMR) and a molecular mixing model (MMM) [Baldock et al., 2004; Hockaday et al., 2009].

$$OR = 1 - C_{ox}/4 + 3[N]/4[C] \quad (6)$$

$$C_{ox} = (2[O] - [H] + 3[N])/[C] \quad (7)$$

The objective of this study was to determine the OR of the net C flux caused by elevated atmospheric  $[CO_2]$  and whether the ecosystem OR of the  $CO_2$ -enriched plots differed from that of the ecosystem OR at ambient  $[CO_2]$ . We used  $^{13}C$  NMR spectroscopy to conduct a biochemical inventory of the leaf litter, fine roots, and soil organic matter. These data provide insight into the biogeochemical processes that drive changes in  $C_{ox}$  and OR in response to elevated  $CO_2$ .

## 2. Methods

### 2.1. Site Description

Samples were collected at the Oak Ridge National Laboratory (ORNL) Free-Air  $CO_2$  Enrichment (FACE) experiment located in eastern Tennessee, USA. Soils at the site are classified as fine, mesic, semiactive, and thermic Aquic Hapludults (soil survey) [Staff, 1967]. A monoculture population of 1 year old sweetgum (*Liquidambar styraciflua* L.) seedlings was planted in spring 1988. The  $CO_2$  fumigation began in April 1998. The ORNL FACE experiment consisted of two elevated  $[CO_2]$  and three ambient  $[CO_2]$  plots. Each plot was 25 m in diameter with 24 perforated vent pipes suspended vertically with 3.3 m spacing between pipes. One ambient plot had no vent pipes and was not used in this analysis. The two elevated plots were amended with  $CO_2$  at a target atmospheric concentration of 565 ppm within the plot. The two ambient plots received ambient air ( $\sim 390$  ppm  $CO_2$ ). The mean annual temperature and precipitation were 14.2°C and 139 cm, respectively. The site is described in detail by Norby et al. [2001], and the FACE system design is described by Hendrey et al. [1999]. More information about the ORNL FACE site can also be found at <http://face.ornl.gov/>. Our study evaluates samples collected in 2006, after nine growing seasons of elevated  $[CO_2]$ . The ORNL FACE experiment was concluded in 2009 after 12 growing seasons of  $CO_2$  enrichment.

### 2.2. Sampling: Soil, Leaf Litter, and Fine Roots

Soil cores (5.1 cm diameter) were collected on 16 September 2006 to a soil depth of 15 cm. Two cores were collected at random locations within each treatment plot (four total plots and eight total soil cores),

sectioned into two depth increments (0–5 cm and 5–15 cm), and then pooled (to reduce subsampling bias) for subsequent elemental and NMR analyses. Soils were sieved to < 2 mm to remove coarse debris. Gravel and woody debris (>2 mm) was negligible in the upper 15 cm. Fine roots (<1 mm diameter) were manually removed from the soil with stainless steel forceps and sorted into living and dead (detrital) fractions based upon color, texture, and elasticity criteria given in *Robertson et al.* [1999]. Fine roots were removed from soil samples prior to the elemental analysis; thus, soil C and N are reported on a root-free basis. Leaf litter was collected during the autumn senescence by placing seven mesh baskets in the understory of each plot. Root and soil samples were air dried to constant weight in the laboratory and subsequently milled to a particle size <250  $\mu\text{m}$ . The contents of the seven litter baskets per plot were oven dried at 70°C, pulverized, and analyzed separately by elemental analyses and  $^{13}\text{C}$  NMR. An inventory of all samples (leaves, roots, and soils) and analyses performed on each sample is provided (Table S1 in the supporting information).

### 2.3. Elemental Analyses and Stock Estimates

The concentrations of C, H, and N were measured by catalytic combustion and subsequent chromatographic separation and detection of  $\text{CO}_2$ , water ( $\text{H}_2\text{O}$ ), and nitrogen ( $\text{N}_2$ ) gases by a Costech ECS 4010 instrument located at Rice University. After switching to pyrolysis mode, the same instrument was used to measure sample organic O concentrations.

Organic C and N in soils were measured after the manual removal of fine roots and following carbonate removal by hydrochloric (HCl) acid fumigation in open-top silver capsules following the procedure of *Harris et al.* [2001]. Organic O and H concentrations in SOM are not reported because of interference from soil minerals and the potential presence of hygroscopic water. All measurements of C, H, N, and O were made in duplicate such that the estimated concentrations for each biomass and soil sample represent the mean of two determinations (see Table S2 in the supporting information for a summary of subsampling and analytical replication).

The NPP ( $\text{g C/m}^2$ ) was estimated for the major sweetgum tissues (leaves, roots, and boles) by taking the product of the NPP (grams dry weight/ $\text{m}^2$ , data from <http://www.ornl.cdiac.gov>) and C concentration in each tissue (Table S2). We calculated cumulative NPP as the sum of NPP during the study period 1998–2006. Soil C and N stocks were calculated by using the product of concentration (%C or %N, respectively) and soil bulk density for the specified depth interval. Soil bulk density was  $1.24 \pm 0.01 \text{ g/cm}^3$  in the Ap horizon (upper 5 cm) and  $1.36 \pm 0.01 \text{ g/cm}^3$  in the Bt1 horizon (5–15 cm) (J. Jastrow, Argonne National Laboratory, personal communication, 2007).

### 2.4. Soil Demineralization

Due to the relatively low C content and high Fe and Mg levels in Oak Ridge soils, it was necessary to demineralize the soils prior to conducting NMR experiments. Demineralization serves the dual purposes of concentrating SOM and removing magnetic minerals that interfere with quantitative detection of C in  $^{13}\text{C}$  NMR spectroscopy. Soils were first treated with 10% (by volume) HCl to remove carbonate minerals. All mineral soils were subsequently demineralized with a mixture containing 10% hydrofluoric acid (HF) and 10% HCl according to methods described in *Gelinas et al.* [2001]. Briefly, 2–5 g of mineral soils were treated sequentially with three aliquots (30 mL each) of HCl/HF mixtures and placed on a rotary shaker at room temperature overnight between treatments. Supernatants were removed by centrifugation and decanted between treatments. The demineralized soils were rinsed 3 times with ultrapure (18 M $\Omega$ ) water and placed in an oven at 50°C until dry.

### 2.5. $^{13}\text{C}$ Nuclear Magnetic Resonance

NMR experiments were conducted on a Bruker Avance 200 MHz solid-state NMR (50 MHz  $^{13}\text{C}$  frequency). The spectrometer was equipped with 4 mm magic angle spinning probe (MAS) and operated at a rotor spinning frequency of 5 kHz. We used two different  $^{13}\text{C}$  NMR pulse sequences: direct polarization and cross polarization (abbreviated DPMAS and CPMAS, respectively). DPMAS spectra were acquired by applying a 30°  $^{13}\text{C}$  excitation pulse and continuous wave proton decoupling. A 5 s recycle delay was used between scans (sufficient for complete  $^{13}\text{C}$  signal relaxation). CPMAS spectra were acquired by applying a 90°  $^1\text{H}$  pulse, a 1 ms  $^{13}\text{C}$  contact pulse, composite-pulse proton decoupling, and a 2 s recycle delay.

The CPMAS pulse sequence is more commonly used for soil samples because its signal-to-noise ratio is more than 4 times greater than DPMAS, but CPMAS can sometimes be less quantitative [Alemany *et al.*, 1983a, 1983b; Smernik *et al.*, 2002a, 2002b]. To assess the relative quantitation in CPMAS NMR, we applied DPMAS NMR to a subset of fine roots ( $n = 12$ ), leaf litter ( $n = 10$ ), and root necromass samples ( $n = 4$ ). The spin counting approach of Smernik and Oades [2000] was used to determine how much of the C in each sample was observed in the NMR spectrum ( $C_{\text{obs}}$ ). In our spin counting experiments, CPMAS and DPMAS NMR experiments both observed an average of  $75 \pm 10\%$  of the organic carbon in the leaf litter and fine root samples. Although there were minor differences in NMR peak area distribution, the trends in biomolecule concentrations across samples and treatments did not differ between CPMAS and DPMAS. The observability of soil C in the CPMAS NMR spectra was  $70 \pm 11\%$  in soil from 0 to 5 cm and  $80 \pm 12\%$  in soil samples from 5 to 15 cm depth. Although DPMAS NMR was viable for litter and roots, the DPMAS technique was not sufficiently sensitive for application to mineral soil samples, which had  $\leq 5\%$  organic C after HF treatment. Thus, CPMAS NMR data are used throughout this investigation because of the sensitivity advantage and because the high  $C_{\text{obs}}$  values suggest that the spectra are reasonably accurate representations of the soil C pool.

## 2.6. Calculating Biomolecule Concentrations Using the Molecular Mixing Model

The molecular mixing model (MMM) of Baldock *et al.* [2004] was applied to the  $^{13}\text{C}$  NMR data to estimate the concentrations of four major classes of biochemical: carbohydrate, lignin, lipid, and protein. Carbonyl C was included as a component in the model for samples affected by decomposition/oxidation, and charcoal was included for soil samples. In this study we modified the lignin peak area distributions to reflect the syringyl and guaiacyl monomer distribution found in sweetgum biomass [Winston *et al.*, 1986]. We judged the model as successfully fitting the NMR spectra when the differences of the sum of squares were less than 5% of the total peak area. This criterion was met for all samples. Finally, we corrected the model-predicted biomolecule for organic matter losses that occurred during the soil demineralization step (see supporting information for details). Concentrations determined by the model are expressed as grams of each molecular component per 100 g of total organic matter.

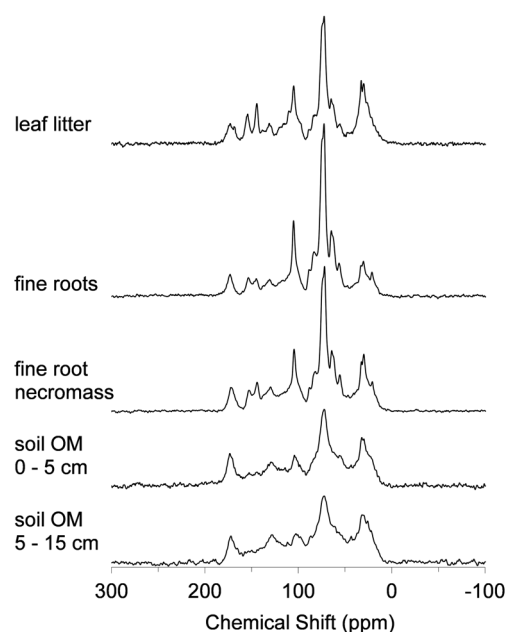
## 2.7. Carbon Oxidation State and Oxidative Ratio

The  $C_{\text{ox}}$  and OR of the plant biomass and necromass were calculated from the molar concentrations of C, H, N, and O measured by combustion elemental analysis, using equations (7) and (6), respectively [Masiello *et al.*, 2008]. Soil  $C_{\text{ox}}$  and OR were calculated from  $^{13}\text{C}$  NMR data and the soil N/C ratio using the MMM [Baldock *et al.*, 2004] following the procedures in Hockaday *et al.* [2009]. Hockaday *et al.* [2009] showed that the  $C_{\text{ox}}$  values measured by the elemental analysis method and the  $^{13}\text{C}$  NMR method are statistically indistinguishable from one another. Therefore, we make direct comparisons between  $C_{\text{ox}}$  and OR measurements made by these techniques.

## 2.8. Statistical Analyses

For the purposes of our experiment, the elevated and ambient  $[\text{CO}_2]$  treatments were replicated twice ( $n = 2$ ) at the ORNL FACE site. All numerical values are reported as the mean and standard error for two replicates of each  $\text{CO}_2$  treatment. The standard error of the sample mean is not an unbiased estimate of error in cases where the sample size is small ( $n < 20$ ). Therefore, we applied the correction factors derived by Gurland and Tripathi [1971] to make an unbiased estimate of the standard error ( $\text{SE} = 1.25 \times s \times n^{-0.5}$ ).

The comparison of means for ambient and elevated  $[\text{CO}_2]$  treatments was made by a directional  $t$  test. The low treatment replication ( $n = 2$ ) and inherently high natural variability in soils conspire to cause large treatment responses to have low probability of significance ( $P > 0.05$ ). A sensitivity analysis indicated that a 50% change in  $C_{\text{ox}}$  (and OR) for the 0–5 cm soil layer would have a  $P$  value  $> 0.10$ . Moreover, Randerson *et al.* [2006] demonstrated that a 1% change in  $\text{OR}_{\text{ab}}$  (0.01 OR units) is relevant for C budgeting. A change of 0.01 OR units would have a probability of significance of  $P = 0.20$  at the ORNL FACE site. Thus, the customary  $P < 0.05$  may be an inappropriate criterion (where  $n = 2$ ) because ecologically significant changes occur at ORNL FACE at higher  $P$  values [e.g., Norby *et al.*, 2002, 2004]. In this study, treatment effects on  $C_{\text{ox}}$  and OR were considered significant at  $P < 0.10$ .



**Figure 1.** Carbon-13 CPMAS NMR spectra of organic inputs (leaf litter and fine roots) and the bulk soil organic matter. Spectra are averages from the ambient  $[\text{CO}_2]$  plots, scaled to equal magnitudes for quantitative comparison.

root in the elevated  $[\text{CO}_2]$  treatment [Iversen *et al.*, 2012, 2008; Norby *et al.*, 2004], changes in tissue biochemistry were modest (Table S3). Leaf litter composition showed no differences in biomolecule concentration with  $\text{CO}_2$  concentration. Fine roots showed a 23% lower protein concentration in the elevated  $[\text{CO}_2]$  treatment ( $P = 0.05$ , Table S3).

### 3.2. $^{13}\text{C}$ NMR of Soil Organic Matter: The Decomposition Continuum

The progression from litter to SOM (Figure 1) represents a decomposition sequence across which O-substituted alkyl C decreased (65–95 ppm), while alkyl C (0–45 ppm) and carboxyl/amide-C increased (165–185 ppm) in relative abundance. The alkyl:O-alkyl ratio can be used as an index for the extent of organic matter decomposition, where higher ratios indicate greater decomposition [Baldock *et al.*, 1997]. At ORNL the alkyl:O-alkyl ratios increased from a value of 0.3 in fine root biomass to 0.5 in fine root necromass and reached 0.7 in SOM (Table S4 in the supporting information). However, the alkyl:O-alkyl index was insensitive to the effects of elevated  $[\text{CO}_2]$ , as we did not detect  $[\text{CO}_2]$  treatment effects within the leaf, root, or soil C pools (Table S4).

### 3.3. The Response of Soil C Oxidation State to Elevated $[\text{CO}_2]$

The oxidation state of soil C was higher in the elevated  $[\text{CO}_2]$  treatment at both the 0–5 cm and 5–15 cm soil layers (Table 1). The magnitude of the elevated  $[\text{CO}_2]$  effect on soil  $\text{C}_{\text{ox}}$  was large ( $\sim 40\%$  increase) and exceeded the effect of soil depth on  $\text{C}_{\text{ox}}$  ( $\sim 20\%$ ). The magnitude of the  $[\text{CO}_2]$ -induced increase in soil  $\text{C}_{\text{ox}}$  was greater at 5–15 cm soil depth than at 0–5 cm. In the 5–15 cm soil layer,  $\text{C}_{\text{ox}}$  increased by 47% in response to elevated  $[\text{CO}_2]$ , from  $-0.213 (\pm 0.042)$  to  $-0.112 (\pm 0.006)$ , whereas in the 0–5 cm layer  $\text{C}_{\text{ox}}$  increased by 30% from  $-0.176 (\pm 0.020)$  at ambient  $[\text{CO}_2]$  to  $-0.124 (\pm 0.043)$  at elevated  $[\text{CO}_2]$  (Table 1).

### 3.4. The Oxidative Ratio of Carbon Pools and Fluxes

To estimate the OR value for C cycling in different compartments of the ecosystem, we used the  $\text{C}_{\text{ox}}$  values in Table 1 to solve equation (6). This generated three different types of OR values (as described in Gallagher *et al.* [2014, Table 1]): (1) flux OR values,  $\text{OR}_{\text{ab}}$  and  $\text{OR}_{\text{ba}}$ , which characterize cumulative fluxes (e.g., NPP); (2) OR values that characterize soil organic C stocks ( $\text{OR}_{\text{SOC}}$ ); and (3) disturbance flux OR values calculated from the differences between elevated and ambient  $[\text{CO}_2]$  treatments,  $\text{OR}_{\text{ab}}^{\text{disturb}}$  and  $\text{OR}_{\text{ba}}^{\text{disturb}}$ . The OR values in Table 2, for plant biomass, provide a direct estimate of the  $\text{O}_2$  and  $\text{CO}_2$  fluxes driven by NPP ( $\text{OR}_{\text{ab}}$ ). The OR values for

## 3. Results

### 3.1. Leaf Litter and Fine Root Biochemistry After Nine Growing Seasons of Elevated $[\text{CO}_2]$

The  $^{13}\text{C}$  CPMAS NMR spectra of leaf litter, fine roots, and fine root necromass (Figure 1) all contain a dominant signal at 60–110 ppm, generated by O-substituted alkyl C, the main constituent of carbohydrates such as cellulose and hemicellulose. Fine roots and root necromass contained substantially less aromatic (110–140 ppm) and phenolic C (140–165 ppm) than leaf litter, indicating that roots had lower lignin concentrations than leaf litter. This is confirmed by the MMM results (Table S3 in the supporting information), which showed that average lignin concentrations in ambient  $[\text{CO}_2]$  leaf litter (39%) were greater than in fine roots (29%) and fine root necromass (35%). Despite the enhanced production rates of leaf litter and fine

**Table 1.** Elemental Composition (Mole Ratio) and Carbon Oxidation State of Organic Matter Pools Sampled in 2006 (After 9 Years of Atmospheric CO<sub>2</sub> Enrichment)<sup>a</sup>

|                              | CO <sub>2</sub> Treatment | H/C           | N/C           | O/C           | C <sub>ox</sub> |
|------------------------------|---------------------------|---------------|---------------|---------------|-----------------|
| Leaf litter                  | Elevated                  | 1.426 ± 0.019 | 0.010 ± 0.001 | 0.660 ± 0.005 | −0.076 ± 0.008  |
|                              | Ambient                   | 1.421 ± 0.021 | 0.011 ± 0.001 | 0.651 ± 0.005 | −0.087 ± 0.014  |
|                              | <i>P</i>                  | 0.45          | 0.26          | 0.17          | 0.28            |
| Fine roots, 0–5 cm           | Elevated                  | 1.521 ± 0.022 | 0.018 ± 0.003 | 0.635 ± 0.009 | −0.196 ± 0.029  |
|                              | Ambient                   | 1.450 ± 0.016 | 0.020 ± 0.001 | 0.549 ± 0.020 | −0.285 ± 0.024  |
|                              | <i>P</i>                  | 0.07          | 0.22          | 0.05          | 0.07            |
| Fine roots, 5–15 cm          | Elevated                  | 1.532 ± 0.018 | 0.008 ± 0.000 | 0.664 ± 0.009 | −0.184 ± 0.001  |
|                              | Ambient                   | 1.518 ± 0.025 | 0.010 ± 0.001 | 0.669 ± 0.005 | −0.152 ± 0.018  |
|                              | <i>P</i>                  | 0.35          | 0.04          | 0.31          | 0.12            |
| Fine root necromass, 0–5 cm  | Elevated                  | 1.489 ± 0.029 | 0.018 ± 0.001 | 0.636 ± 0.026 | −0.157 ± 0.016  |
|                              | Ambient                   | 1.440 ± 0.015 | 0.019 ± 0.001 | 0.682 ± 0.043 | −0.017 ± 0.099  |
|                              | <i>P</i>                  | 0.15          | 0.38          | 0.23          | 0.19            |
| Fine root necromass, 5–15 cm | Elevated                  | 1.533 ± 0.076 | 0.011 ± 0.005 | 0.778 ± 0.171 | 0.053 ± 0.280   |
|                              | Ambient                   | 1.479 ± 0.019 | 0.007 ± 0.001 | 0.651 ± 0.007 | −0.156 ± 0.036  |
|                              | <i>P</i>                  | 0.30          | 0.29          | 0.29          | 0.29            |
| Soil organic matter, 0–5 cm  | Elevated                  | na            | 0.062 ± 0.002 | na            | −0.124 ± 0.043  |
|                              | Ambient                   | na            | 0.065 ± 0.003 | na            | −0.176 ± 0.020  |
|                              | <i>P</i>                  |               | 0.27          |               | 0.21            |
| Soil organic matter, 5–15 cm | Elevated                  | na            | 0.071 ± 0.002 | na            | −0.112 ± 0.006  |
|                              | Ambient                   | na            | 0.076 ± 0.001 | na            | −0.213 ± 0.042  |
|                              | <i>P</i>                  |               | 0.11          |               | 0.12            |

<sup>a</sup>Reported values are the mean ± standard error ( $SE = 1.25 \times s \times n^{-0.5}$ ),  $n = 2$ ; na = not available due to interference from soil minerals. *P* values are for directional *t* test,  $n = 2$ .

C pools have the most relevance to atmospheric O<sub>2</sub> and CO<sub>2</sub> fluxes when the turnover time of the C pool is known (analogous to the <sup>13</sup>C and <sup>14</sup>C isotopic signature of a C pool). In the case of ephemeral pools such as leaf and fine root necromass, which are effectively mineralized to CO<sub>2</sub> and return to the atmosphere in 10 years or fewer [Riley *et al.*, 2009], it is a reasonable approximation to assume that OR<sub>pool</sub> = OR<sub>flux</sub>. In contrast, SOM is

**Table 2.** NPP, Soil C Stocks, and Oxidative Ratio Measured in 2006—After Nine Growing Seasons of Free-Air CO<sub>2</sub> Enrichment<sup>a</sup>

| Primary Products                   | NPP: 2006 (g C/m <sup>2</sup> )             |                       | Oxidative Ratio, OR <sub>ab</sub> (O <sub>2</sub> /CO <sub>2</sub> )  |                            |
|------------------------------------|---------------------------------------------|-----------------------|-----------------------------------------------------------------------|----------------------------|
|                                    | Ambient                                     | Elevated              | Ambient                                                               | Elevated                   |
| Leaf litter                        | 193 ± 5 <sup>b</sup>                        | 220 ± 11 <sup>b</sup> | 1.030 ± 0.004                                                         | 1.025 ± 0.001              |
| Bole + branches + coarse root      | 385 ± 84 <sup>b</sup>                       | 383 ± 39 <sup>b</sup> | 1.040 ± 0.004 <sup>d</sup>                                            | 1.040 ± 0.004 <sup>d</sup> |
| Fine root (0–15 cm)                | 15 ± 3 <sup>b</sup>                         | 32 ± 1 <sup>b</sup>   | 1.069 ± 0.006                                                         | 1.058 ± 0.007              |
| Annual total                       | 592 ± 84                                    | 634 ± 41              | OR <sub>ab</sub> = 1.038 ± 0.008                                      | 1.036 ± 0.008              |
| Elevated [CO <sub>2</sub> ] effect |                                             |                       | OR <sub>ab</sub> <sup>disturb</sup> = 1.007 ± 0.157                   |                            |
| 2006 Soil Organic C Fractions      | Soil C Stocks in 2006 (g C/m <sup>2</sup> ) |                       | Oxidative Ratio, OR <sub>SOC</sub> (O <sub>2</sub> /CO <sub>2</sub> ) |                            |
|                                    | Ambient                                     | Elevated              | Ambient                                                               | Elevated                   |
| Fine root necromass (0–15 cm)      | 13 ± 3 <sup>c</sup>                         | 19 ± 10 <sup>c</sup>  | 1.042 ± 0.008                                                         | 1.059 ± 0.005              |
| Soil organic C (0–5 cm)            | 1305 ± 9                                    | 1330 ± 9              | 1.093 ± 0.009                                                         | 1.077 ± 0.011              |
| Soil organic C (5–15 cm)           | 1892 ± 29                                   | 1699 ± 165            | 1.106 ± 0.015                                                         | 1.085 ± 0.003              |
| Total C stock                      | 3210 ± 51                                   | 3048 ± 181            | OR <sub>SOC</sub> = 1.101 ± 0.019                                     | 1.081 ± 0.012              |
| Elevated [CO <sub>2</sub> ] effect |                                             |                       | OR <sub>ba</sub> <sup>disturb</sup> = 1.477 ± 0.095                   |                            |

<sup>a</sup>Reported values are the mean and standard error ( $SE = 1.25 \times s \times n^{-0.5}$ ). Subscript ab denotes movement of C from the atmosphere to the biosphere. Subscript ba denotes movement of C from the biosphere to the atmosphere. OR<sub>ab,ba</sub><sup>disturb</sup> is the change in oxidative ratio for a disturbance flux, as defined in equation (8).

<sup>b</sup>NPP values from <http://cdiac.ornl.gov/> [Norby *et al.*, 2010a].

<sup>c</sup>Fine root necromass production values 0–15 cm from Iversen *et al.* [2008, Table 2].

<sup>d</sup>Oxidative ratio of nonresinous wood from Severinghaus [1995] and Randerson *et al.* [2006].

known to contain several subpools of C with different residence times that range from decades to millennia [Trumbore, 2000]. Therefore, we refrained from making assumptions regarding soil C turnover time. Instead, we use the differences in the OR of soil C stocks ( $OR_{stock}^{elevated}$  and  $OR_{stock}^{ambient}$ ) in equation (8) to calculate the OR of the disturbance flux ( $OR_{ab}^{disturb}$ ) caused by elevated  $[CO_2]$  treatment. Similarly,  $OR_{ab}^{disturb}$  characterizes the enhancement in NPP caused by elevated  $[CO_2]$ . Equation (8) yielded  $OR_{ba}^{disturb} = 1.477 (\pm 0.095)$  for soil respiration and  $OR_{ab}^{disturb} = 1.007 (\pm 0.157)$  for NPP.

$$OR_{ab,ba}^{disturb} = \frac{(OR_{stock}^{elevated} \cdot C_{stock}^{elevated}) - (OR_{stock}^{ambient} \cdot C_{stock}^{ambient})}{(C_{stock}^{elevated} - C_{stock}^{ambient})} \quad (8)$$

## 4. Discussion

Several lines of evidence suggest that belowground C cycling under elevated  $[CO_2]$  may not be a simple case of enhanced throughput—wherein heterotrophic microorganisms in the soil mineralize the additional inputs from enhanced NPP [Billings *et al.*, 2010; Lukac *et al.*, 2009; Sulman *et al.*, 2014]. We discuss new evidence that elevated  $[CO_2]$  altered the soil C cycling processes. These lines of evidence include the changes in ecosystem OR (section 4.1), soil biochemical stocks and residence times (section 4.2), and the increased oxidation state of the soil C pool (section 4.3). Section 4.4 discusses the implications of these findings for global C budgeting.

### 4.1. The OR of C Cycle Disturbances Caused by Elevated $[CO_2]$

The disparate values obtained for  $OR_{ab}^{disturb}$  and  $OR_{ba}^{disturb}$  (Table 2) indicate that NPP and soil respiration respond differently to elevated  $[CO_2]$ . The  $OR_{ab}^{disturb}$  value of  $1.007 (\pm 0.157)$  is approaching the OR of glucose synthesis (1.00), which suggests that the elevated  $[CO_2]$  treatment caused enhanced production of carbohydrates (e.g., celluloses and hemicelluloses). In contrast, the  $OR_{ba}^{disturb}$  value of  $1.477 (\pm 0.095)$  for soil respiration is substantially higher than OR for the turnover of carbohydrates and plant tissues like leaf litter ( $1.025 \pm 0.001$ ) and fine roots ( $1.058 \pm 0.007$ ) (Table 2). Therefore, the  $OR_{ba}^{disturb}$  value of 1.477 probably indicates that elevated  $[CO_2]$  treatment has enhanced the respiration of reduced compounds (such as lipid or lignin) in the soil.

To further evaluate of this interpretation of  $OR_{ba}^{disturb}$ , sections 4.2 and 4.3 analyze the biochemical inventory and the  $C_{ox}$  of the soil C pool, respectively. The biochemical inventory provides a direct accounting of reduced compounds like lipids while the  $C_{ox}$  value is an aggregated measurement of biochemical components and the degree to which they have undergone partial oxidation (i.e., decomposition).

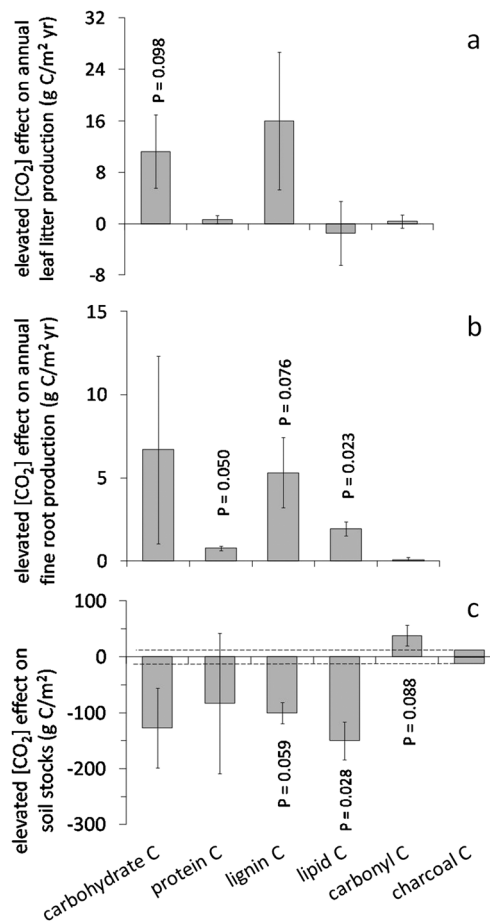
### 4.2. The Response of Soil Biochemical Pools and Residence Times to Elevated $[CO_2]$

The additional C entering the soil as leaf litter and fine roots in the elevated  $[CO_2]$  treatment (the sum of values in Figures 2a and 2b) is dominated by carbohydrate C ( $19 \text{ g C/m}^2 \text{ yr}$ ) and lignin C ( $22 \text{ g C/m}^2 \text{ yr}$ ). The elevated  $[CO_2]$  treatment had elevated ( $P \leq 0.05$ ) fine root protein C ( $2 \text{ g C/m}^2 \text{ yr}$ ) and lipid C ( $3 \text{ g C/m}^2 \text{ yr}$ ) inputs to the soil C pool (Figure 2b).

Despite the greater inputs, the soil C stocks in the elevated  $[CO_2]$  treatment had 15% lower lignin C ( $-100 \text{ g C/m}^2$ ) and 26% lower lipid C ( $-150 \text{ g C/m}^2$ ) than the ambient  $[CO_2]$  treatment (Figure 2c). After nine growing seasons of FACE, the biochemical discrepancies between soil C inputs and soil C stocks suggest a change in the rate and/or mechanism of SOM decomposition. The mean residence times can be calculated as the ratio stock/flux (assuming steady state). We used C flux and C stock values (Figures 2a–2c) in equation (9) to calculate the change in residence time for the bulk soil C and each of the four major biochemical classes. The values in Table 3 suggest that elevated  $[CO_2]$  alters the soil residence time of some classes of biochemicals more than others. The mean residence time of carbohydrate decreased ( $-4 \pm 1$  year), concomitant with changes in the bulk soil organic C pool. The residence time of lignin also decreased slightly ( $-2 \pm 0.5$  year). Elevated  $[CO_2]$  had the largest and most variable effect on the calculated mean residence times of soil lipid ( $-5 \pm 3$  years) and protein ( $-25 \pm 19$  years). Due to the higher variability in

$$\Delta \text{residence time}^i = \left( \frac{\text{elevated } [CO_2] \text{ stock}^i}{\text{elevated } [CO_2] \text{ flux}_{ab}^i} \right) - \left( \frac{\text{ambient } [CO_2] \text{ stock}^i}{\text{ambient } [CO_2] \text{ flux}_{ab}^i} \right) \quad (9)$$

lipid and protein residence times, the changes were not significant ( $P = 0.16$  and  $P = 0.19$ , respectively).



**Figure 2.** Effect of elevated  $[CO_2]$  on the annual biochemical production by (a) leaf litter and (b) fine roots 0–15 cm. (c) Cumulative (1998–2006) effects of elevated  $[CO_2]$  on soil biochemical stocks 0–15 cm. Error bars are the standard errors and represent natural variability within treatments. (c) Measurement uncertainties in bulk soil C stocks ( $\pm 20$  g C/m<sup>2</sup>) are shown as dashed lines and were estimated by using soil charcoal as a conservative tracer—unaffected by  $[CO_2]$  treatments on experimental timescales. Significant  $CO_2$  effects are shown for  $P < 0.10$ .

The observed changes in biochemical residence times are consistent with other studies at Oak Ridge FACE that document changes in the soil microbial biomass and/or activity. For instance, *Iversen et al.* [2012] found that microbial biomass N was ~15% lower in elevated  $[CO_2]$  treatment (0–15 cm soil;  $P = 0.02$ ) after 11 growing seasons. The lower protein C stocks (–26%, Figure 2c) and shorter protein residence times (–25 years, Table 3), observed in this study, may be related to the decline in microbial biomass N observed by *Iversen et al.* [2012]. In addition, studies have shown that soil C mineralization rates have increased in response to elevated  $[CO_2]$ . During the first four growing seasons of elevated  $[CO_2]$ , *King et al.* [2004] reported a 104 g C/m<sup>2</sup> yr increase in total soil respiration (root + heterotrophic respiration), and similar values were measured at the Duke Forest FACE site [*Bernhardt et al.*, 2006]. At ORNL FACE, *Iversen et al.* [2012] found generally higher initial C mineralization rates in the laboratory incubation of surface soils (0–15 cm) from the elevated  $[CO_2]$  treatment ( $P = 0.10$ ). Carbon mineralization rates are an index of substrate availability—faster rates indicate greater availability of soil C to microbial utilization—consistent with the shorter mean residence time of soil C in the elevated  $[CO_2]$  treatment (Table 3).

In addition to elevated rates of soil microbial activity in elevated  $[CO_2]$  treatments, there is some evidence of altered microbial community metabolism. *Dunbar et al.* [2012] showed that the relative abundances of Acidobacteria in the soil (0–15 cm) decreased by a factor of 2 in the elevated  $[CO_2]$  treatment at ORNL FACE. The Acidobacteria are oligotrophic microorganisms (*K* strategists), whose relative abundances decrease with increasing substrate availability, due to competitive pressure from copiotrophic microbes (*r* strategists) [*Fierer et al.*, 2007]. The altered resource ratios in the elevated  $[CO_2]$  treatment are another factor that plays a role in microbial activity and community structure. The elevated  $[CO_2]$  plot at Oak Ridge FACE is a N-limited system [*Iversen and Norby*, 2008]. Nitrogen limitation under elevated  $[CO_2]$  may promote a

**Table 3.** Changes in the Mean Residence Time of Soil Biochemical Stocks Over Nine Growing Seasons of Elevated  $[CO_2]$  Treatment<sup>a</sup>

|                                | Bulk Soil Organic C | Carbohydrate | Lignin       | Protein      | Lipid      |
|--------------------------------|---------------------|--------------|--------------|--------------|------------|
| $\Delta$ residence time (year) | $-4 \pm 1$          | $-4 \pm 1$   | $-2 \pm 0.5$ | $-25 \pm 19$ | $-5 \pm 3$ |
| <i>P</i>                       | 0.05                | 0.07         | 0.02         | 0.19         | 0.16       |

<sup>a</sup>Values are calculated by applying equation (9). Reported uncertainty (due to natural variation in soil) is derived by propagation of the standard errors in stocks and fluxes. *P* values are for a directional *t* test,  $n = 2$ .

microbial community with enzymatic capacities for oxidation of more recalcitrant forms of organic matter (e.g., actinomycetes and fungi), which mineralize more N and release more CO<sub>2</sub> from the soil [Billings and Ziegler, 2008; Ziegler and Billings, 2011].

Considering the previously reported changes in microbial community structure and activity together with our evidence for differential changes in the mean residence time of biochemical substrates (Table 3), it is plausible that changes in the microbial community contribute to the high OR<sub>ba</sub><sup>disturb</sup> value (Table 2). Changes in microbial community function also provide a parsimonious explanation for the increased oxidation state of the soil C pool in response to elevated [CO<sub>2</sub>] (discussed below).

### 4.3. The increase in Soil C<sub>ox</sub> With Elevated [CO<sub>2</sub>]

The C<sub>ox</sub> in the upper 15 cm of the soil was 40% higher than the ambient [CO<sub>2</sub>] treatment ( $P=0.10$ ) after only nine growing seasons of elevated [CO<sub>2</sub>] treatment. Such a rapid change of this magnitude is somewhat surprising given the large size of the soil C pool (3.5 kg C/m<sup>2</sup>). The increase in soil C<sub>ox</sub> cannot be directly attributed to changes in the quality of the organic matter entering the soil C pool because the C<sub>ox</sub> of the leaf and fine root litters remained unchanged or declined (became more negative) in response to elevated [CO<sub>2</sub>] (Table 1). We find evidence of at least two other factors contributing to the rapid increase in soil C<sub>ox</sub>: (1) changes in biochemical composition of the soil C pool (described above in section 4.2) and (2) greater in situ oxidation of SOM.

The elevated [CO<sub>2</sub>] plots exhibited lower abundances of soil lipid and lignin (Figure 2c). Lignin and lipids are reduced chemical compounds with C<sub>ox</sub> values ranging from −0.2 to −0.5 and −1 to −2, respectively [Masiello et al., 2008]. The loss of reduced compounds (with low C<sub>ox</sub>) creates a more oxidized residue with a higher (more positive) C<sub>ox</sub> value in the elevated [CO<sub>2</sub>] treatment. Since the litter quality has not changed (Table S3), the lower lignin and lipid stocks can only be attributed to translocation deeper into the soil profile or complete mineralization via microbial respiration. Incomplete oxidation of soil C has also contributed to the higher soil C<sub>ox</sub>. The SOM from elevated [CO<sub>2</sub>] treatments had a greater relative abundance of carbonyl C (carboxylic acid, aldehyde, and ketone) than the ambient [CO<sub>2</sub>] soils ( $P=0.08$ , Figure 2c). Carbonyl C is a minor component of terrestrial plant biomass, and the increasing abundance of carbonyl C with decomposition is widely used as an indicator of organic matter oxidation [Baldock et al., 2004; Hedges et al., 1988]. The carboxylic acid group has a C<sub>ox</sub> = +3 and while aldehyde C<sub>ox</sub> = +1 and ketone C<sub>ox</sub> = +2. Due to the large positive magnitude of these C<sub>ox</sub> values, the higher carbonyl C in the soil pool (+40 g/m<sup>2</sup>) has contributed to the higher in soil C<sub>ox</sub> in the elevated [CO<sub>2</sub>] treatment.

### 4.4. Carbon and Oxygen Cycle Implications of OR Disequilibrium

Atmospheric [CO<sub>2</sub>] is currently rising at a rate of 2.9 ppm yr<sup>−1</sup> (4.3 Pg C yr<sup>−1</sup>), the highest rate of increase since global record keeping began in 1984 [Hartmann et al., 2013; World Meteorological Organization, 2014]. Global C budgets that were based upon the rates of atmospheric CO<sub>2</sub> rise and O<sub>2</sub> depletion for the 1990s suggest that the rate of C uptake by the land and ocean sinks was 1.4 (±0.8) Pg C yr<sup>−1</sup> and 2.0 (±0.8) Pg C yr<sup>−1</sup>, respectively [Battle et al., 2000, 2006; IPCC, 2007]. Recent C sink estimates (for years 2002–2011) are 2.6 and 2.5 (±0.8) Pg C yr<sup>−1</sup> for the land and ocean sinks, respectively [Le Quéré et al., 2013]. It remains uncertain how the magnitude of future biosphere and ocean C sinks will respond as atmospheric [CO<sub>2</sub>] rise continues. Part of the uncertainty in predicting future sinks is related to the need for information about soil C cycle processes operating under conditions of elevated atmospheric [CO<sub>2</sub>] [Carney et al., 2007; Jastrow et al., 2005].

Our data for the ORNL FACE site provide an opportunity to assess whether elevated atmospheric [CO<sub>2</sub>] could affect the OR of terrestrial ecosystems. To address this question for the hardwood forest at ORNL, we used the OR values from Table 2 to estimate the ecosystem-scale OR values (see Table 4). Since we do not have direct measurements of annual fluxes and OR<sub>ab</sub> values, several assumptions were required. (1) We used the accounting of annual NPP to estimate F<sub>ab</sub> and cumulative change in soil C stocks to estimate cumulative carbon fluxes, F<sub>ba</sub>. (2) We assumed that values measured in the ninth season are representative of the mean OR<sub>ab</sub> for all nine growing seasons, 1998–2006. (3) Finally, we assumed that the OR of the soil C pool in the control plot (ambient [CO<sub>2</sub>]) did not change significantly over 9 years. Regarding the final assumption, published data on soil OR are sparse and insufficient to assess whether OR is variable on annual timescales. The mathematical expressions and the calculated OR values are shown in Table 4 and depicted as a vector diagram in an ecosystem context in Figure 3.

**Table 4.** Estimated C Fluxes and Ecosystem Oxidative Ratios for the Period 1998 to 2006 at Oak Ridge FACE<sup>a</sup>

|                                   | Ambient [CO <sub>2</sub> ] | Elevated [CO <sub>2</sub> ] | Equations                                                                                                                                                                                                                                   |
|-----------------------------------|----------------------------|-----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Fluxes (g C/m<sup>2</sup>)</i> |                            |                             |                                                                                                                                                                                                                                             |
| F <sub>ab</sub>                   | 7685 ± 961                 | 8377 ± 478                  | $F_{ab} = \sum_{1998}^{2006} \text{wood NPP} + \text{fine root NPP}^c + \text{leaf NPP}$                                                                                                                                                    |
| F <sub>ba</sub>                   | −1364 ± 158                | −1824 ± 237                 | $F_{ba} = -(\text{SOC}_{\text{inputs}} - \text{SOC}_{\text{accrued}})^d$                                                                                                                                                                    |
| F <sub>net</sub>                  | 6320 ± 974                 | 6553 ± 534                  | $F_{\text{net}} = F_{ab} + F_{ba}$                                                                                                                                                                                                          |
| <i>Oxidative Ratios</i>           |                            |                             |                                                                                                                                                                                                                                             |
| OR <sub>ab</sub>                  | 1.039 ± 0.002 <sup>b</sup> | 1.037 ± 0.001 <sup>b</sup>  | $\text{OR}_{ab} = \frac{(\text{OR}_{\text{wood}} \cdot \text{wood NPP} + \text{OR}_{\text{root}} \cdot \text{fine root NPP} + \text{OR}_{\text{leaf}} \cdot \text{leaf NPP})}{F_{ab}}$                                                      |
| OR <sub>ba</sub>                  | 1.006 ± 0.001 <sup>b</sup> | 1.054 ± 0.007 <sup>b</sup>  | $\text{OR}_{ba} = \frac{(\text{OR}_{\text{leaf}} \sum_{1998}^{2005} \text{leaf litter} + \text{OR}_{\text{root}} \sum_{1998}^{2005} \text{fine root} - \text{OR}_{\text{SOC}}^{\text{accrued}} \cdot \text{SOC}_{\text{accrued}})}{F_{ba}}$ |
| OR <sub>net</sub>                 | 1.046 ± 0.002 <sup>b</sup> | 1.033 ± 0.002 <sup>b</sup>  | $\text{OR}_{\text{net}} = \frac{\text{OR}_{ab} \cdot F_{ab} + \text{OR}_{ba} \cdot F_{ba}}{F_{\text{net}}}$                                                                                                                                 |

<sup>a</sup>All fine root NPP and fine root mortality data are from the Carbon Dioxide Information Analysis Center database [Norby *et al.*, 2010a]. Uncertainties are standard error of the mean ( $n = 2$ ). Calculated quantities not shown here (SOC<sub>inputs</sub>, SOC<sub>accrued</sub>, etc.), can be found in Table S5 in the supporting information.

<sup>b</sup>Significant differences,  $P < 0.10$ .

<sup>c</sup>Only includes data for 0–5 and 5–15 cm soil depths for which we have C<sub>ox</sub> and OR values.

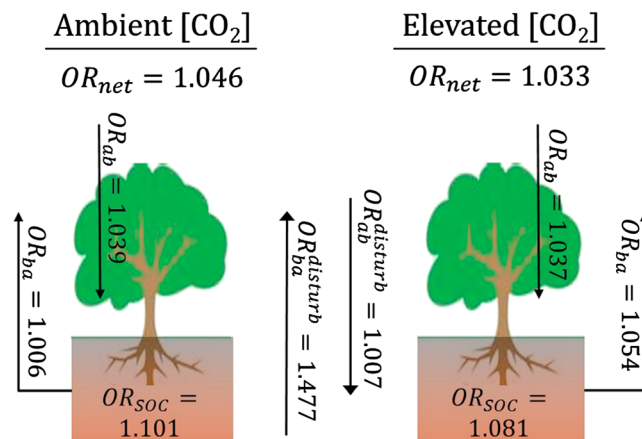
<sup>d</sup>F<sub>ba</sub> is an estimate of changes in C stock, and therefore, represents the total potential heterotrophic respiration, not a measured C flux. It assumes that transport losses are negligible (i.e., particle eluviation and dissolved organic C leaching) [Tipping *et al.*, 2012].

<sup>e</sup>Assumes that leaf and root necromass from the sampling year (2006) have not contributed to soil respiration or accrual of SOC.

<sup>f</sup>SOC<sup>1998</sup> stocks for 0–15 cm soil depth from Jastrow *et al.* [2005]. OR<sup>1998</sup><sub>SOC</sub> is assumed to be equal to OR<sup>2006</sup><sub>SOC</sub> of the ambient [CO<sub>2</sub>] plot.

Several of the elevated [CO<sub>2</sub>] responses shown in Figure 3 are particularly noteworthy. First, the OR of hardwood forest NPP (OR<sub>ab</sub>) did not change with elevated [CO<sub>2</sub>], but the inferred OR of soil respiration (OR<sub>ba</sub>) was substantially higher. Second, forests under both ambient and elevated [CO<sub>2</sub>] exhibited substantial OR disequilibria (OR<sub>ab</sub> ≠ OR<sub>ba</sub>). Third, the ecosystem switched from OR<sub>ab</sub> > OR<sub>ba</sub> at ambient [CO<sub>2</sub>] to OR<sub>ba</sub> > OR<sub>ab</sub> at elevated [CO<sub>2</sub>].

The implications of OR disequilibria in the terrestrial biosphere were discussed extensively by Randerson *et al.* [2006]. These authors demonstrated that various ecosystem disturbances (climate change, land use change,



**Figure 3.** The oxidative ratios (O<sub>2</sub>/CO<sub>2</sub>) of net primary production (OR<sub>ab</sub>), cumulative biosphere-atmosphere flux (OR<sub>ba</sub>), and net ecosystem exchange (OR<sub>net</sub>) are shown for the (left) ambient [CO<sub>2</sub>] and (right) elevated [CO<sub>2</sub>] treatments at Oak Ridge FACE. The elevated [CO<sub>2</sub>] disturbance is shown as vectors labeled with the corresponding OR<sup>disturb</sup> values. The values OR<sup>disturb</sup><sub>ab</sub> and OR<sup>disturb</sup><sub>ba</sub> are for the fluxes caused by elevated [CO<sub>2</sub>].

fire, etc.) are likely to promote OR disequilibria. Equation (10) describes the rate of atmospheric O<sub>2</sub> depletion by the terrestrial C sink (F<sub>net</sub>) and includes the disequilibrium forcing term, (OR<sub>ba</sub> − OR<sub>ab</sub>)F<sub>ba</sub> [Randerson *et al.*, 2006]. By convention, the sign of F<sub>ba</sub> is positive. Therefore, if OR<sub>ba</sub> > OR<sub>ab</sub>, the disequilibrium term (OR<sub>ba</sub> − OR<sub>ab</sub>)F<sub>ba</sub> has a positive value and constitutes a sink of atmospheric O<sub>2</sub>. Conversely, OR<sub>ba</sub> < OR<sub>ab</sub> generates a disequilibrium term with a negative magnitude and a source of atmospheric O<sub>2</sub> coming from the biosphere. At the ORNL FACE site, the OR disequilibrium (OR<sub>ba</sub> − OR<sub>ab</sub>) has a value of −0.033 (±0.002) at ambient [CO<sub>2</sub>] and 0.017 (±0.007) at elevated [CO<sub>2</sub>]. The transition from negative

OR disequilibrium to positive OR disequilibrium suggests that elevated  $[\text{CO}_2]$  has generated a terrestrial sink of  $\text{O}_2$ .

$$\frac{d\text{O}_2}{dt} = -(\text{OR}_{\text{ab}} \cdot F_{\text{net}} + (\text{OR}_{\text{ba}} - \text{OR}_{\text{ab}})F_{\text{ba}}) \quad (10)$$

Small OR disequilibria have the potential to generate large atmospheric  $\text{O}_2$  fluxes because the OR disequilibrium is multiplied by the gross C flux,  $F_{\text{ba}}$  (equation (10)) [Randerson *et al.*, 2006]. At ORNL FACE, the  $\text{O}_2$  sink is small because  $F_{\text{ba}}$  is small relative to  $F_{\text{net}}$  in the context of a fast-growing hardwood forest. However, this is not the case for the global biosphere. The NPP ( $F_{\text{ba}}$ ) of the terrestrial biosphere is approximately  $60 \text{ Pg C yr}^{-1}$  [Cramer *et al.*, 1999], while estimated values for  $F_{\text{net}}$  are  $1.4$  to  $2.6 (\pm 0.8) \text{ Pg C yr}^{-1}$  [Battle *et al.*, 2006; Le Quéré *et al.*, 2013]. To illustrate the implications of our findings, we will assume a hypothetical scenario in which the global biosphere exhibits the OR disequilibria measured at the ORNL FACE site. A global OR disequilibrium of  $-0.033$  would generate a terrestrial  $\text{O}_2$  source on the order of  $-2 \text{ Pg O}_2 \text{ yr}^{-1}$  ( $-0.033 \cdot 60 \text{ Pg C yr}^{-1} = -1.98 \text{ Pg C yr}^{-1}$ ), with an additional land C sink of equal magnitude. Accounting for this in global C budgets would require moving  $\sim 2 \text{ Pg C}$  from the ocean sink to the land sink. The OR disequilibrium established under conditions of elevated  $[\text{CO}_2]$  has interesting C cycle implications as well. If the global biosphere responded to rising  $[\text{CO}_2]$  with an OR disequilibrium of  $0.017$ , this would generate an annual  $\text{O}_2$  sink of  $1 \text{ Pg}$  ( $60 \text{ Pg C yr}^{-1} \cdot 0.017 = 1.02 \text{ Pg C yr}^{-1}$ ) within the terrestrial biosphere. Carbon budgets constructed under the assumption of OR equilibrium would ascribe a terrestrial  $\text{O}_2$  sink to a reduction in the terrestrial C uptake (reduced  $F_{\text{net}}$ ) and subsequently partition  $\sim 1 \text{ Pg C yr}^{-1}$  to the ocean C pool. These global OR disequilibrium scenarios are not entirely realistic because the deciduous forest monoculture at ORNL is not an appropriate model for the entire global biosphere. Nevertheless, the magnitude of the OR disequilibrium measured at ORNL illustrates the potential shortcomings of the equilibrium OR approximation and the implications for global C budgets.

## 5. Conclusions

We used a novel method to estimate ecosystem OR from measurements of the oxidation state of C pools in soils and plant biomass. We applied this approach to a temperate hardwood forest at Oak Ridge FACE after nine growing seasons. Elevated atmospheric  $[\text{CO}_2]$  had no effect on the oxidative ratio of NPP. In contrast, we found evidence for several significant changes in the soil C cycle processes. (1) The oxidation state of the soil C pool increased by 40%, which is related to a lower abundance of reduced organic compounds (e.g., lignin and lipid) in the soil under elevated  $[\text{CO}_2]$ . (2) The higher oxidation state of the soil C pool at elevated  $[\text{CO}_2]$  contributed to disequilibrium in the ecosystem OR. More specifically, the effect of elevated  $[\text{CO}_2]$  was the creation of a terrestrial  $\text{O}_2$  sink—an  $\text{O}_2$  flux unaccompanied by a  $\text{CO}_2$  flux. The potential for decoupling (i.e., disequilibrium cycling) of terrestrial carbon and oxygen fluxes deserves further investigation, as it has important implications for the land and ocean C budgets.

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