RESEARCH ARTICLE

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Key Points:

- Oxidative ratio ($O_2:CO_2$, OR) for the production of aboveground biomass in a Michigan coniferous forest was 1.069 ± 0.014 over 8 years
- OR for aboveground deciduous forest biomass was 1.038 ± 0.006 over the same time period
- Large variations in growing season precipitation and temperature did not show any influence on aboveground biomass OR

Supporting Information:

- Supporting Information S1

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Plant species, not climate, controls aboveground biomass $O_2:CO_2$ exchange ratios in deciduous and coniferous ecosystems

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Abstract The oxidative ratio (OR) is the $O_2:CO_2$ ratio associated with photosynthesis, respiration, and other ecosystem gas exchange processes and can be reported on the scale of an individual leaf, an ecosystem, up to the entire terrestrial biosphere. The OR of the terrestrial biosphere is used to partition anthropogenic CO_2 between oceanic and terrestrial carbon sinks, and the ease of measurement of this property on smaller scales suggests its potential for other uses. However, controls on the natural variation of OR are not understood in either organic matter pools or fluxes, and this lack of basic information limits the use of the tracer. Here we assess the annual variability of the OR of photosynthesis over ~decade for two temperate forests, one coniferous and one deciduous, and show that the photosynthetic OR signature is strongly dominated by plant species. We determined the OR of this flux by measuring the OR of carbon pools that close on annual or shorter timescales (leaves and individual tree rings), via solid-state ^{13}C NMR spectroscopy and elemental analysis. Leaf litter OR is different between coniferous and deciduous forests, but tree bole OR is constant between species. There was no significant change in leaf litter OR with time, nor any correlations between leaf litter OR and temperature or precipitation. During this time growing season precipitation varied by 95% from the time period average, and growing season temperature by 22%, demonstrating that on the decadal scale photosynthetic OR is invariant over significant shifts in these climate parameters.

1. Introduction

Every organic carbon pool has an associated oxidative ratio (OR) value, where OR is defined as the moles of O_2 emitted during photosynthesis per moles of CO_2 fixed (O_2/CO_2) or the moles of O_2 consumed per mole of CO_2 emitted for reverse processes (e.g., respiration and fire). This exchange ratio between O_2 and CO_2 in the biosphere is an Earth system tracer, with a natural span of values ranging from 0 (CO_2) to 2.0 (CH_4). OR values for the atmosphere, terrestrial biomass, and soil samples typically fall in the range of 0.8–1.2 [Severinghaus, 1995; Masiello et al., 2008; Hockaday et al., 2009; Worrall et al., 2013; Clay and Worrall, 2015a; Gallagher et al., 2014; van der Laan et al., 2014].

Like many other organic matter tracers, OR can be both a property of a pool or a flux. This is analogous to $\delta^{13}C$, which can be measured in biomass, litter, soils, sediments, or atmospheric samples. Like the $\delta^{13}C$ signature of the photosynthetic flux, the OR associated with photosynthesis can be constrained through gas measurements or it can be estimated after CO_2 has been fixed by measuring the signature retained in biomass, soils, or sediments.

Measurements of OR values began in the later part of the twentieth century as part of efforts to determine the fate of fossil fuel CO_2 emitted into the atmosphere. Techniques that apportion fossil fuel CO_2 between the land, ocean, and atmosphere by measuring changes in both atmospheric O_2 and CO_2 [e.g., Keeling et al., 1996] require an estimate of the global biosphere's $O_2:CO_2$ exchange ratio, and the first OR measurements were made by Severinghaus [Severinghaus, 1995]. The $O_2:CO_2$ exchange ratio of the global biosphere has been estimated at 1.1 [Severinghaus, 1995] and more recently 1.04 ± 0.03 [Worrall et al., 2013].

As the body of $O_2:CO_2$ exchange ratio measurements grew, it became apparent that this parameter was more complex than a single number characteristic of the entire Earth system. Seibt [2004] measured $O_2:CO_2$ exchange ratios in a number of pools, including canopy air, soil chambers, and branch bags (bags fitted

over branches to measure gas exchange during photosynthesis) and, in this work, convincingly showed that measurements of the $O_2:CO_2$ ratio in canopy air were not necessarily a good proxy for the exchange ratio of net ecosystem fluxes because of the possibility of differences between the $O_2:CO_2$ ratio of CO_2 assimilation and soil respiration. Soon afterward, *Randerson et al.* [2006] explored the possibility that variations in biomass stoichiometry could affect atmospheric O_2 trends, pointing out that because the gross fluxes associated with photosynthesis and respiration were both large, the $O_2:CO_2$ exchange ratio of the net flux could vary significantly due to small offsets in the exchange ratios of the gross fluxes. Here we term the oxidative ratio of photosynthesis OR_{ab} (the oxidative ratio of the atmosphere-biosphere exchange), and the exchange ratio of the return flux OR_{ba} (the oxidative ratio of the biosphere-atmosphere flux) [Gallagher et al., 2014].

A number of factors have been suggested as potential drivers of an offset between OR_{ab} and OR_{ba} , including land use change, changes in plant biochemistry driven by elevated CO_2 , fire-driven changes in ecosystem structure, and fertilizer-driven shifts in ecosystem stoichiometry (all suggested in *Randerson et al.* [2006]). Allocation of net primary productivity (NPP) among plant parts may also affect OR, because plants may respond to changes in temperature and/or moisture by altering the distribution of carbon between leaves, stems, and roots [e.g., *Ryan et al.*, 2010; *Lu et al.*, 2013], each of which have unique OR signatures. Any changes in allocation could then alter the overall OR_{ab} .

Simultaneously, measurements of the $O_2:CO_2$ exchange ratio of forest canopy and boundary layer gas samples expanded our understanding of the controls on the signature of this reservoir [Seibt, 2004; Kozlova et al., 2005; Stephens et al., 2007; Kozlova et al., 2008; Sirignano et al., 2010; Ishidoya et al., 2013]. Many of these were made in the context of the global O_2 cycle as part of calculating atmospheric potential oxygen. Key observations include those showing that transient, high atmospheric $O_2:CO_2$ exchange ratios (>1.4) could be traced using back trajectory analysis to urban areas and fossil fuel combustion [Stephens et al., 2007]. Because more reduced fuels like natural gas have a higher OR (approaching 2) compared to coal or petroleum (OR values closer to 1.3), these results [Stephens et al., 2007] point to the use of atmospheric OR measurements as an indicator of the overall fuel mix in use in a city. Another key study [Ishidoya et al., 2013] supported earlier results [Seibt, 2004] documenting the difference between soil respiration OR (1.11 ± 0.01 , a proxy for OR_{ba}) and branch bag OR (1.02 ± 0.03 , a proxy for OR_{ab}).

The breadth of data on Earth system OR values expanded when it became clear that OR was related to the oxidation state (C_{ox}) of organic carbon, a property that can be easily measured via elemental analysis or calorimetry [Masiello et al., 2008] or solid state nuclear magnetic resonance (NMR) [Hockaday et al., 2009]. In practice, elemental analysis and NMR have proven to be the simpler of the three techniques, with error approaching ± 0.001 units for studies replicated at the ecosystem level [Gallagher et al., 2014]. OR can be measured on solid samples (biomass, litter, and soils), allowing the reconstruction of the OR_{ab} of individual plants when measured in fresh biomass [e.g., Gallagher et al., 2014]. When weighted by net primary productivity (NPP) data, these measurements allow the observation of whole-ecosystem, growing-season averaged OR_{ab} [Clay and Worrall, 2015a; Gallagher et al., 2014; Worrall et al., 2016].

OR can also be measured in soils, and when this is coupled to NPP measurements and data are collected across a disturbance gradient, these measurements can provide $O_2:CO_2$ exchange ratio data not possible through gas phase measurements. For example, it would be expensive and logistically challenging to make real-time measurements of the effects of fire on ecosystem OR. However, when coupled to NPP measurements, time series measurements of soil OR can provide information on the long-term $O_2:CO_2$ exchange ratio associated with fire, land use change, or elevated CO_2 [Hockaday et al., 2009]. Hockaday et al. [2009] provide an example of how to use a gradient in CO_2 exposure to determine the OR associated with elevated CO_2 . Comparisons between OR measurements of pools (biomass, litter, and soils) and fluxes (gaseous CO_2 and O_2 measurements [e.g., Stephens et al., 2007; Ishidoya et al., 2013; Hockaday et al., 2015; Ishidoya et al., 2015, and others]) may also yield new information about ecosystem processes, for example, by helping to partition root and soil respiration in ecosystems.

It may also be possible to use OR to gain insight into the fraction of an ecosystem's respiration coming from roots. While few OR measurements have been made on roots, one study [Hockaday et al., 2015] did find differences between root OR and leaf and bole OR. While the range of OR values from root respiration is not known, constraint of this term may eventually allow its use in partitioning belowground respiration.

Finally, the relationship between OR and C_{ox} opens up the possibility of the use of these tracers in understanding soil and sediment oxidation and reduction reactions. By explicitly including organic carbon C_{ox} in calculations of the free energy of organic matter oxidation [LaRowe and Van Cappellen, 2011], it is possible to show that only more oxidized forms of organic matter are thermodynamically favorable for use as electron donors in respiration under O_2 restricted conditions [Keiluweit *et al.*, 2016] (note that these authors use the equivalent term NOSC for C_{ox}). These results [Keiluweit *et al.*, 2016] predict that more reduced organic matter should accumulate in soils experiencing reduced O_2 content. This suggests the possibility that the relationship between C_{ox} and thermodynamic parameters [Masiello *et al.*, 2008] could be used to determine past microbial terminal electron acceptor usage in soil.

The first steps in development of OR as a useful tracer are to understand the controls on its natural variation. Here we report replicated, time series measurements of the OR of leaves (a proxy for OR_{ab}) at coniferous and deciduous forests in Michigan from 1995 to 2006 (with a data gap in 1996 and 1997). Just as the $\delta^{13}C$ signature of a leaf can be used to infer the $\delta^{13}C$ signature of the gas flux associated with photosynthesis, the OR signatures of leaves can be used to determine the OR of the photosynthetic O_2 and CO_2 fluxes (OR_{ab}). When a pool is only open to gas exchange for a limited period of time, tracer measurements (e.g., OR or $\delta^{13}C$) of that pool can be used to infer the tracer signature of the flux. Because carbon in leaves and tree rings is photosynthesized only over a limited period of time, they are effectively closed pools, holding the chemical and isotopic signatures of the period of their formation. Measurements of OR on these pools can be used to reliably infer the OR of fluxes that created them. On the other hand, when pools are in long-term exchange with other reservoirs (e.g., open pools like soils and the atmosphere), it is more challenging to use pool values to estimate flux values, although this can be done for both isotopes and for OR (see Hockaday *et al.* [2015] for an example of how to use OR measurements of soil pools to infer OR_{ba} values). Here we have made OR measurements on aboveground closed pools (seasonally fixed biomass) to determine the variation in aboveground ecosystem OR_{ab} on seasonal timescales. We used these measurements to test the effects that forest ecosystem type and climate (temperature or precipitation) have on aboveground OR_{ab} values.

2. Site Description

The samples used here were collected from the Kellogg Biological Station (KBS) Long Term Ecological Research (LTER) site in Michigan (42° 24'N, 85°24'W). KBS LTER specializes in the ecological study of land uses, and among its sites are coniferous, deciduous, and successional forests, as well as a range of agricultural sites. Here we collected samples from two of the forest treatments: TCF (coniferous forest treatments) and TDF (deciduous forest treatments). The deciduous forest site is an example of the mature late-successional biome in the region. In contrast, the coniferous forest sites are 40–60 year old abandoned conifer plantations (<http://lter.kbs.msu.edu/research/site-description-and-maps/>). KBS LTER maintains three geographically separated replicate sites for both forest ecosystems (labeled as R1, R2, and R3). The forest sites vary in size from approximately 0.25 ha to 0.75 ha. We collected tree cores from immediately adjacent to these sites and subsampled the LTER biomass archives for leaf litter collected by KBS staff from 1995 to 2006. During this time period, the KBS LTER site's mean annual temperature ranged from 7.7 to 10.6°C (37% variation from the average for the time period, calculated as (maximum T – minimum T)/maximum T) and the growing season (defined as April to November) temperature ranged from 13.0 to 15.8°C (22% variation from the average). The total annual precipitation ranged from 607.9 to 1156.1 mm (90% variation from the average) and the growing season precipitation ranged from 439–858 mm (95% variation from the average) (Figure 1; data from <http://lter.kbs.msu.edu/datatables—LTER Weather Station: Daily Temperature and Precipitation>).

3. Methods

We measured OR using two techniques: elemental analysis and nuclear magnetic resonance (NMR), both described below. We additionally used data collected by KBS staff, including daily temperature and precipitation data and net primary productivity (NPP) data (litter trap biomass and forest woody biomass annual production), all publicly available at <http://lter.kbs.msu.edu/datatables>. All error is reported as standard deviations.

Figure 1. The mean temperature (°C) and total precipitation (mm) both annually and for the growing season (April to November) from 1995 to 2006. The error bars represent the standard deviation.

3.1. Sample Collection

We subsampled the KBS archive in August 2005 and July 2007 for leaf litter samples for the years 1995 and 1998–2006 (no leaf litter samples were available for 1996 and 1997). The KBS LTER collects leaf litter samples from the forest sites each year, removing branches to insure that results reflect leaves only. Two 0.475 m² litter traps are placed in each site, and the litter is collected every 2 weeks during the fall collection season (<http://lter.kbs.msu.edu/protocols/28>). The litter samples from each plot are combined, dried, massed, ground, and stored at room temperature. We stored subsamples

in sealed glass jars at room temperature prior to analysis. Further information on sampling, processing, and storage protocols can be found on the KBS-LTER webpage (<http://lter.kbs.msu.edu/>).

We collected tree cores from coniferous and deciduous trees in November 2009 using a standard three-thread 0.2" (5.15 mm core) Haglof Increment Borer. We stored the cores in narrow 6 mm diameter cardboard tubes at room temperature until analysis. Because coring had the potential to alter tree productivity and/or metabolism, we sampled immediately adjacent to but not within the LTER experimental plots. We were careful to remain less than 25 m from the plot boundaries (often within a few meters) and were always within the ecosystem type. We analyzed eight cores in bulk and dissected one into individual tree rings. The protocol used to estimate aboveground net primary production for the forest sites at KBS can be found at <http://lter.kbs.msu.edu/protocols/111>.

There was insufficient mass in each individual tree ring for annual OR measurements, so the majority of cores were analyzed in bulk. The eight cores were chosen to represent a range of species, and at least one core from each forest replicate was analyzed. Each tree core was ground using liquid nitrogen in a ceramic mortar that had been washed at least three times with 18 MΩ water. Prior to being ground, tree rings were counted and measured. Only growth ring material from between the cambium and the pith was ground for analysis to preclude bias (i.e., if the borer went through the core, this avoided biasing the OR value toward inner ring OR values).

3.2. Sample Analyses

We measured the C, H, N, and O composition of forest litter samples on a Costech ECS 4010 elemental analyzer and then calculated C_{ox} and OR as described in section 3.3. CHN measurements were made simultaneously in combustion mode, and O measurements were made in pyrolysis mode. Oxygen measurement uncertainty dominates the error in C_{ox} calculations based on CHN and O measurements [Masiello *et al.*, 2008], leading us to repeat O measurements until precision reached 3% (typically 3–8 analyses per sample). For both CHN and O measurements we used acetanilide as a standard and phenylalanine, glycine, and/or peach leaf as internal controls to monitor accuracy and precision.

We measured OR of tree bole samples via solid state ¹³C nuclear magnetic resonance (NMR), because the available sample mass was too low for C, H, N, and O analyses. These two techniques to measure OR have been intercompared and shown to agree within 0.017 OR units [Hockaday *et al.*, 2009]. Even switching to NMR did not allow separate measurements of annual rings on most samples, making it necessary to homogenize entire bole samples prior to analyses. However, one core collected from the coniferous forest replicate 1 site (TCF R1) had especially wide rings in the heartwood near the pith, and we were able to core through the center of the pith. This allowed us to double the amount of biomass collected for each tree ring, giving enough sample for ¹³C NMR and providing annual resolution bole OR data for the seven innermost tree rings, which we labeled ring 1 through ring 7, with ring 1 being the innermost (oldest) ring. More recent tree rings

(outermost) were narrower than older tree rings (closer to the core), making it difficult to accurately separate these younger rings, and therefore, we were unable to anchor the tree rings to specific years, although based on the tree size, ring count, and ecosystem history, it is likely that the widest rings in the heartwood grew in the 1960s. We sectioned the core into annual increments on a clean sheet of aluminum foil using a disposable scalpel and ground the rings as described above.

We performed solid-state ^{13}C NMR on the tree core samples using a 200 MHz Bruker NMR spectrometer located within the Shared Equipment Authority at Rice University. We used a cross polarization pulse sequence with a 4 mm magic angle spinning probe at a frequency of 7 kHz, with a 1 ms contact time and 5 second recycle delay. All spectra were corrected for spinning sidebands (though this correction did not change signal amplitudes), and then manually phased and baseline corrected. We used a molecular mixing model (MMM) [Baldock *et al.*, 2004] to estimate C_{ox} from the sample NMR spectra and C and N content. The data were also spin counted relative to an external sample of glycine [Smernik and Oades, 2000a, 2000b], with an average C observability of $80.8 \pm 3.9\%$ (TCF: $82.0 \pm 3.1\%$; TDF: $76.7 \pm 3.5\%$).

3.3. C_{ox} and OR Calculations

C_{ox} can be calculated from the molecular composition of organic matter $\text{C}_x\text{H}_y\text{O}_z\text{N}_w$ as follows:

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

where x , y , z , and w represent molar masses for carbon (C), hydrogen (H), oxygen (O), and nitrogen (N), respectively. These molar masses can be measured directly by elemental analysis [Masiello *et al.*, 2008]. When NMR is used, C_{ox} is calculated as part of the MMM output [Baldock *et al.*, 2004; Hockaday *et al.*, 2009] (see section 3.3). Elemental analysis and NMR techniques for estimating OR have been shown to be intercomparable for biomass to ± 0.017 OR units [see Hockaday *et al.*, 2009, Table 1].

We calculated OR for all samples from C_{ox} assuming atmospheric nitrogen (N_2) as the ecosystem N source, as below:

$$\text{OR} = 1 - \frac{\text{C}_{\text{ox}}}{4} + \left(\frac{3}{4}\right) \cdot \left(\frac{w}{x}\right) \quad (2)$$

It is possible to calculate OR using N_2 , nitrate (NO_3^-), or ammonium (NH_4^+) as ecosystem N sources, or combinations of all three [Masiello *et al.*, 2008; Gallagher *et al.*, 2014]. The ecosystem N source should be chosen as the form of N that crosses the boundaries of the ecosystem being measured (in this case, hectare-scale forests). N_2 is the appropriate assumption for C_{ox} to OR conversions when ecosystems receive the majority of their nitrogen through N fixation, as is the case here. This assumption is robust to errors caused by small inputs of other forms of N; for example, it generates an error of 0.01 OR units if the forest ecosystems are actually receiving 20% of their N as NO_3^- instead of N_2 [Masiello *et al.*, 2008].

3.4. Statistical Analyses

Two-way ANOVAs were performed to determine if an interaction existed between year and treatment for aboveground OR and leaf litter OR (Table S3). One-way ANOVAs were performed to compare coniferous and deciduous leaf litter and aboveground OR values as well as the effect of time on leaf litter and aboveground OR within each treatment. Comparisons were considered statistically significant for p values < 0.05 . Correlations between OR measurements and climate parameters (temperature and precipitation, both annual and growing season averages and totals) were also calculated using the multivariate platform. All statistical analyses were done in JMP Pro 11, and all error is reported as standard deviation.

4. Results

4.1. Photosynthetic OR (OR_{ab}) Is Higher for Coniferous Forests Than for Deciduous Forests

The average OR of leaf litter biomass over time for the coniferous forest (1.102 ± 0.022) was significantly higher than that for the deciduous forest (1.045 ± 0.011) ($p < 0.0001$; Table 1 and Figure 2a). Comparing leaf litter OR values each year between the two forest types, coniferous forest leaf litter OR was always higher than deciduous forest leaf litter OR. Within each forest ecosystem treatment, leaf litter OR did not change with time (TCF: $p = 0.066$; TDF: $p = 0.60$; Figure 2a).

There was no observable difference in tree bole OR for the coniferous forest ecosystem (1.035 ± 0.007) compared to the deciduous forest ecosystem (1.032 ± 0.003) ($p = 0.5336$; Table 1 and Figure 3). There was also no shift in OR over time in the tree rings, whose annual OR values ranged from 1.027 to 1.033. The range of values, 0.006 OR units, is less than NMR measurement uncertainty when benchmarked against elemental analysis (0.017 OR units [Hockaday *et al.*, 2009]).

We calculated an aboveground OR value for the coniferous and deciduous forests at our field site over time using the average bole and seasonal leaf litter OR values and each ecosystem's corresponding NPP data (Figure 2b and Table 1). The coniferous forest ecosystem's aboveground OR is significantly greater than the deciduous forest ecosystem aboveground OR (1.069 ± 0.014 versus 1.039 ± 0.006 , respectively; $p < 0.0001$; Figure 2b), with no other observed factors driving variation (see next section).

4.2. On an Ecosystem Scale, OR_{ab} Does Not Vary With Temperature or Precipitation

We also considered the possibility that OR_{ab} in these forests could change with time (for example, driven by changes in temperature or precipitation) but found that, in general, there was no change over time for either the coniferous or deciduous forests. The only years where biomass OR values were significantly different were 2004 and 2005 for the coniferous forest ecosystem (Figure 2b). Multivariate analysis of total and growing season temperatures and precipitation and leaf litter and aboveground OR values for coniferous and deciduous ecosystems showed no detectable correlations between climate parameters and leaf litter or aboveground OR_{ab} (Figures 2a and 2b and Table S3). Averaging all aboveground temperate forest OR data gives us a 1.053 ± 0.018 estimate for temperate forest aboveground OR_{ab} (Figure 2b).

While the OR of tree boles and litter did not vary individually over time, it is still possible for the OR of a tree to vary over time by allocating more or less biomass to the bole as opposed to the leaves. Even if neither the OR of the leaf litter or the OR of the bole varies, the OR of a tree may still vary significantly if the NPP distribution among plant organs varies significantly. Similarly, in theory the OR of an entire ecosystem could vary from changes in NPP allocation or ecosystem composition. The small fluctuations in OR observed in the coniferous forest treatment in 2004 and 2005 may be attributable to shifts in NPP distribution between leaves and tree bole biomass, which have slightly different OR values (Table 1).

5. Discussion

5.1. Aboveground OR_{ab} Is Stable in Response to Changes in Environmental Conditions

These results support the growing body of evidence that on an annual timescale the OR of photosynthesis (OR_{ab}) is strongly controlled by plant type, with few other factors influencing its values. For example, OR_{ab} has been shown to be highly stable within crop ecosystems [Gallagher *et al.*, 2014], when comparing the OR of crop biomass (grain and stover) for corn, soy, and wheat at KBS. Variation within individual crop ecosystems was very small, with site-level replicates varying by amounts equivalent to or lower than the instrumental detection limit (± 0.001 – 0.003 OR units). However, between plant types there were large differences in OR_{ab} , ranging from 1.135 ± 0.002 for soybean grains to 1.023 ± 0.001 for corn grains. These results are consistent with other work, with another study [Clay and Worrall, 2015a] finding that plant type plays a major role in determining OR in UK peat ecosystems, with the range in their ecosystem defined by shrub biomass OR values (1.10) and sphagnum OR values (0.99).

Elevated CO_2 also does not seem to change OR_{ab} . Hockaday *et al.* [2015] measured the effects of elevated CO_2 on ecosystem OR pools and fluxes at the Free Air CO_2 Enrichment trial at Oak Ridge National Laboratories in eastern Tennessee. While the ecosystem OR as a whole was responsive to elevated CO_2 , OR_{ab} was not. In this study OR_{ab} was not statistically different between forest plots treated with CO_2 (1.036 ± 0.008) and those that were untreated (1.038 ± 0.008), suggesting again that OR_{ab} is insensitive to environmental parameters.

Fertilizer application stands out as one environmental parameter that may be able to drive small changes in ecosystem-scale OR_{ab} on annual timescales, with two studies documenting detectable changes in biomass OR in response to fertilizer application [Gallagher *et al.*, 2014; Worrall *et al.*, 2015]. At KBS, the OR of corn biomass was 0.007 units higher when comparing the no fertilization treatment with application of almost twice the regionally recommended level of inorganic fertilizer [Gallagher *et al.*, 2014]. Treatment with lower levels of fertilizer (including the regionally recommended level) did not yield OR values statistically different from

Table 1. The Weighted Average Aboveground OR Calculation for Coniferous and Deciduous Forests From 1995 to 2006 Based On the Yearly Leaf Litter OR and NPP, Tree Bole NPP, and Average Bole OR^a

		Leaf Litter					Bole			Total Aboveground	
		OR			NPP (kg/(m ² yr))		NPP (kg/(m ² yr))			OR	
Treatment	Year	<i>n</i>	Average	SD	Average	SD	OR Value	Average	SD	Average	SD
TCF coniferous forest	1995	3	1.113	0.012	0.411	0.113	1.035 ± 0.007 (<i>n</i> = 4)				
	1996										
	1997										
	1998	2	1.137	0.006	0.464	0.174					
	1999	2	1.116	0.010	0.460	0.061		0.541	0.132	1.073	0.008
	2000	3	1.112	0.009	0.265	0.091		0.098	0.300	1.073	0.000
	2001	3	1.087	0.005	0.521	0.124		0.408	0.381	1.067	0.009
	2002	3	1.086	0.005	0.385	0.128		0.348	0.117	1.062	0.004
	2003	2	1.090	0.014	0.418	0.149		0.455	0.275	1.060	0.011
	2004	4	1.083	0.005	0.422	0.048		0.677	0.473	1.053	0.006
	2005	3	1.106	0.020	0.474	0.121		0.165	0.023	1.087	0.015
	2006	3	1.108	0.029	0.372	0.076		0.381	0.220	1.073	0.019
	Average over time	28	1.102	0.022	0.412	0.115	Average coniferous forest aboveground OR over time				1.069
TDF deciduous forest	1995	2	1.043	0.002	0.457	0.059	1.032 ± 0.003 (<i>n</i> = 4)				
	1996										
	1997										
	1998	3	1.041	0.005	0.666	0.110					
	1999	3	1.043	0.008	0.465	0.138		0.705	0.415	1.036	0.001
	2000	3	1.049	0.013	0.410	0.076		0.254	0.168	1.043	0.010
	2001	3	1.049	0.005	0.654	0.136		0.714	0.249	1.040	0.003
	2002	3	1.048	0.009	0.533	0.070		0.477	0.257	1.040	0.003
	2003	3	1.036	0.022	0.512	0.060		0.454	0.216	1.033	0.010
	2004	6	1.041	0.029	0.541	0.058		0.938	0.249	1.035	0.004
	2005	3	1.055	0.010	0.532	0.093		0.644	0.497	1.042	0.001
	2006	3	1.047	0.007	0.453	0.067		0.327	0.220	1.040	0.002
	Average over time	32	1.045	0.011	0.500	0.113	Average deciduous forest aboveground OR over time				1.039
Temperate forests OR:										1.053	0.018

^aThe same bole OR value was used for each weighted average because the OR of the bole was shown to not vary significantly over time (Figure 3).

treatment without fertilizer. The observed OR shift at the very highest level of fertilization, from 1.039 ± 0.004 (0 kg N/ha) to 1.046 ± 0.003 (202 kg N/ha) was small, but statistically significant. Worrall *et al.* [2015] considered both inorganic fertilizer and farmyard manure, finding that manure increased biomass OR by 0.05 units, while inorganic fertilizer decreased it by 0.05 units. Both these studies suggest that changing plant nutrient status will alter ecosystem OR, likely by changing the plant N content, although the results of Worrall *et al.* [2015] show an order-of-magnitude larger shift.

Finally, it remains possible that the OR_{ab} of an ecosystem may change due to a parameter not explicitly measured here: a shift in partitioning between aboveground and belowground resources. Evidence suggests that the partitioning of photosynthate between aboveground biomass and fine roots is an important allocation trade-off in forest ecosystems [Malhi *et al.*, 2011]. Temperature has been shown to predict the relative partitioning of tree biomass into roots versus aboveground biomass [Reich *et al.*, 2014]. The implications of this for ecosystem OR_{ab} remain to be explored.

5.2. Globally, Aboveground OR_{ab} of Temperate Forest Photosynthesis Is Between 1.02 and 1.06

Our temperate forest aboveground photosynthetic OR values (OR_{ab}) are consistent with other studies, and when compared with many other measurements, point to a global temperate forest aboveground OR_{ab} value between 1.02 and 1.06, with deciduous forests having slightly lower values than coniferous forests. We can directly compare our forest OR_{ab} values with those measured using the same NMR and elemental analysis techniques, and when this is done we find a high level of consistency. For example, the deciduous forest value measured here (1.039 ± 0.006) is very close to the value of 1.037 ± 0.008 measured for a deciduous

Figure 2. (a) The average leaf litter OR values for coniferous and deciduous forests over time. No interaction effect exists between treatment and year ($p = 0.054$); therefore, Tukey HSD comparisons were not performed. (b) The average aboveground OR values for coniferous and deciduous forests over time. No interaction effect exists between year and treatment ($p = 0.232$); therefore, Tukey HSD comparisons were not performed. However, deciduous forest aboveground OR is significantly lower than coniferous forest aboveground OR ($p < 0.0001$).

we can learn more about the natural range of temperate forest OR values.

A temperate deciduous forest in central Japan [Ishidoya et al., 2013] has a reported OR value of 1.02 ± 0.03 , measured via gas samples collected from branch bags (note that these authors use the term exchange ratio which is equivalent to the term OR used here). This value is the same, within error, as our temperate deciduous forest annual OR_{ab} of 1.039 ± 0.006 .

However, our aboveground ecosystem OR values are not as close to those of Seibt [2004], who reported the first branch bag OR values for both coniferous (*Pinus sitchensis*) and deciduous (*Fagus sylvatica*) trees. In a Scottish *P. sitchensis* plantation, branch bag measurements yielded OR values of 1.18 ± 0.05 , which does not overlap with our mixed coniferous forest leaf OR values of 1.069 ± 0.022 [Seibt, 2004]. Similarly, their branch bag OR measurements of *F. sylvatica* were 1.10 ± 0.06 , a value that overlaps only slightly with our mixed deciduous forest OR (1.039 ± 0.006) [Seibt, 2004].

There are two possible explanations for the difference between our forest ecosystem OR values and those of Seibt [2004]. First, it could simply be that the ecosystems themselves were not similar enough to expect overlapping values. The mixed deciduous forest we studied includes oak, hickory, and maple, with tree boles sampled from wild black cherry (*Prunus serotina*), white oak (*Quercus alba*), and white ash (*Fraxinus americana*). Seibt [2004] sampled European beech (*Fagus sylvatica*). Our mixed coniferous forest was composed of a

forest in Tennessee [Hockaday et al., 2015]. A larger database of measurements measured via similar techniques [Clay and Worrall, 2015b] also finds similar values for deciduous forests (1.03–1.13) and evergreen forests (1.03–1.08), although their database was not limited to temperate forests.

We estimated OR_{ab} through weighting of biomass pool OR values (leaves and bole OR weighted by NPP). These data can be compared to shorter-term OR_{ab} values measured by branch bags and to measurements of the OR of canopy atmospheres (OR_{atmo}), although in making these comparisons it is important to consider the timescale of measurements and other potential drivers of these values. Our weighted pool OR_{ab} values represent an annually averaged OR_{ab} . We do not expect these values to be identical in all cases to branch bag OR measurements and OR_{atmo} measurements, because branch bag OR measurements can reflect shorter-term variations in plant photosynthesis, and the OR of the atmosphere can be influenced by processes occurring outside the canopy (e.g., soil respiration) or outside the ecosystem (e.g., transport of air masses from a nearby city [Stephens et al., 2007]). Nevertheless, by comparing these types of OR measurements

Figure 3. The measured value of OR for the individual sequential rings in a tree core taken from the coniferous forest treatment replicate 1 site. Ring 1 is the innermost measured ring, and six additional tree rings moving away from the core were analyzed. Measurement uncertainty on individual rings is ± 0.017 OR units. The error bars for bulk OR represent the standard deviation between core samples.

[2004], they agree within error: our mixed coniferous leaves had OR values of 1.102 ± 0.022 , compared to 1.18 ± 0.05 measured by Seibt via branch bags [Seibt, 2004], and our deciduous leaves had OR values of 1.045 ± 0.011 , compared to 1.10 ± 0.06 [Seibt, 2004].

It is also possible to compare our OR_{ab} measurements from biomass with measurements of forest canopy atmosphere OR (OR_{atmo}). As with branch bag experiments, OR_{atmo} measurements reflect the exchange ratio of the ecosystem over the period of time that sampling occurred (typically hours-weeks), which is different from the annual timescale recorded in our weighted ecosystem OR_{ab} values. In addition, canopy air masses can be influenced by processes other than local photosynthesis and respiration, and these processes can lead to altered OR_{atmo} values. Nevertheless, it is interesting to compare these two pools to gauge the spread of possible temperate forest OR values.

Measurements of OR_{atmo} are much more variable than biomass OR values, but the range of observed OR_{atmo} values includes the values we have measured. Stephens et al. [2007] measured the O_2 and CO_2 concentrations over a period of a week at a U.S. National Oceanographic Administration tall tower observatory in a mixed forest in Park Falls, Wisconsin (the WLEF television station's tower). Gas sampling involved collection from single inlets at heights of 496 m and 122 m, and two inlets at 30 m. From the top of the tower to the duplicate ports at 30 m, they found OR_{atmo} values of 1.01, 1.06, 1.10, and 1.01. The variation of values within the canopy could result from a number of processes. It is possible that carbon is allocated differently among plant organs with height (e.g., more C to leaves at height and more C to bole at the base of the tree). It is also possible that variations in OR_{atmo} over height reflect variations in the significance of soil respiration. OR_{ba} (the OR associated with the return flux of CO_2 from the biosphere to the atmosphere) is more variable than OR_{ab} (see the next section), and the effects of this flux on the canopy OR signature may vary spatially.

5.3. Very Low $O_2:CO_2$ Ratios (Soil OR_{ba} and Canopy OR_{atmo}) May Indicate Variation in Processes of Soil Respiration

This study joins an accumulating body of literature showing that the OR of vascular plant biomass ($OR_{biomass}$) does not vary outside a small range of about 1.01–1.15 [Worrall et al., 2013; Clay and Worrall, 2015a, 2015b; Worrall et al., 2015, 2016] [Masiello et al., 2008; Hockaday et al., 2009; Gallagher et al., 2014; Hockaday et al., 2015]. Therefore, OR_{atmo} ($O_2:CO_2$ ratios of gas samples) values outside this range are indicative of other processes. Some explanations may be based on atmospheric drivers: for example, high OR_{atmo} values in the boundary layer have been traced via back trajectory to fossil fuel combustion plumes [Stephens et al., 2007] and very high OR values (as much as 1.7) are markers of the temporal and physical disconnection between in the ocean-atmosphere fluxes of O_2 and CO_2 [van der Laan et al., 2014].

pine mix (*Pinus species resinosa*, *abies*, and *strobis*), compared to Seibt's [2004] *Pinus sitchensis* samples. These organisms may simply have different enough biochemistry to drive different $OR_{biomass}$ values.

Another possible explanation is that the branch bag measurements made by Seibt [2004] are reflective of the OR of leaf construction only and do not reflect the long-term production of tree bole, which has an overall lower OR value compared to leaves. Our OR_{ab} results include bole $OR_{biomass}$ data, and as a result, our OR_{ab} values are slightly lower than those of Seibt [2004], which include only leaf data. When we compare only our leaf OR values (Table 1) with those of Seibt

A number of OR_{atmo} measurements are significantly lower than the forest values reported here. Three studies report OR_{atmo} values below 1.00, with an OR_{atmo} of 0.99 for a deciduous forest in Germany [Kozlova *et al.*, 2005], an OR_{atmo} of 0.94 ± 0.01 for a temperate deciduous forest in Japan [Ishidoya *et al.*, 2013], and an OR_{atmo} value of 0.89 ± 0.012 in a Western Russian spruce and birch forest [van der Laan *et al.*, 2014]. In addition, a very low soil respiration (OR_{ba}) value of 0.64 was measured, and then rejected as potentially contaminated, by Seibt [2004]. These values are all lower than a theoretically lowest limit OR value of 1.00, which would arise if plants were fixing only carbohydrates, with no production of more reduced lignin, proteins, lipids, or amino acids. It is difficult to imagine a scenario where a forest's long-term OR_{ab} was significantly lower than 1.0, simply because plants must produce lipids ($OR \sim 1.37$) for cell walls and lignin ($OR \sim 1.13$) for structural rigidity (values from Baldock *et al.* [2004], Randerson *et al.* [2006], and Masiello *et al.* [2008]).

However, at least two possible processes can drive average OR_{ba} values lower than the minimum possible for vascular photosynthesis: respiration of oxidized carbon species, such as those present in dissolved organic carbon, or respiration of any form of organic carbon by microbes using a terminal electron acceptor other than O_2 .

Respiration of highly oxidized organic carbon is one process that could result in lower OR_{atmo} values. Respiration of these compounds would consume little atmospheric O_2 (because oxidized organic molecules themselves are already rich in oxygen). Oxidized forms of organic carbon such as oxalic acid ($C_2O_4H_2$, $C_{ox} = 3$) have correspondingly low OR values ($OR_{oxalic\ acid} = 0.25$). Low OR values observed in northern ecosystems (e.g., 0.89 [van der Laan *et al.*, 2014]) could reflect an ecosystem in disequilibrium, with preferential decomposition of newly defrosted, labile soil carbon with low OR values. If this were the case, it would point to widespread mineralization of dissolved organic carbon, because the only forms of organic carbon with OR values < 0.9 are soluble [Masiello *et al.*, 2008].

Another possible explanation for low OR values is the use of compounds other than O_2 as terminal electron acceptors. When organic matter is oxidized anaerobically (e.g., using NO_3^- , $Fe(OH)_3$, or other non- O_2 terminal electron acceptors) there is no effect on the atmosphere-biosphere O_2 flux because no O_2 is removed from the atmosphere. This leads to an OR_{ba} of 0. There is ample evidence that even in dry upland soils microbial respiration can be driven by the use of terminal electron acceptors other than O_2 [Keiluweit *et al.*, 2016]. It seems likely that this was occurring in the field sites of van der Laan *et al.* [2014], which were boreal forest ecosystems prone to saturated soils.

6. Conclusions

We have shown that within two types of temperate forests in Michigan, small variations in the OR of photosynthesis (OR_{ab}) did not correlate with larger changes in temperature or precipitation, indicating that either aboveground OR does not vary due to environmental factors over the timescale of measurement in this study (approximately a decade), or it varies based on environmental factors for which we do not have data. Combined with data from other publications, it is becoming clear that on the decadal scale, aboveground OR_{ab} is dominantly controlled by the plant types present in an ecosystem, with climate, CO_2 concentration, and fertilizer playing no or very small roles in driving natural variation in this biogeochemical property.

The invariance of the OR signature of photosynthesis over approximately a decade is useful in the development of this tracer because there are large variations in the OR signatures of other pools and fluxes on shorter timescales. Constraining drivers of OR_{ab} variation narrows the potential sources of environmental shifts in OR, allowing the better use of this tracer to track environmental processes. Potential uses include partitioning ecosystem respiration between soils and roots, determining the long-term importance of different terminal electron acceptors in soils, and a better understanding of the potential sources of carbon to soil carbon pools.

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