*An inverse model for relating organic carbon thermal reactivity and isotope composition using Ramped PyrOx*

**Abstract**

Serial oxidation coupled with isotope ratio analysis (13C/12C and 14C/12C, expressed as δ13C and Fm) of eluted CO2 is a promising set of techniques for understanding the relationship between chemical composition, source, and residence time of organic carbon (OC) in the environment. However, a general treatment of oxidation kinetics is currently lacking. Here, we develop an inverse model to determine the nonparametric probability density function of OC thermal activation energy (*E*) contained within a given sample, *p0*(*E*), using the Ramped PyrOx (RPO) method. By analyzing a set of test samples representing various environments (fluvial suspended sediments, soils, marine sediments), we show that OC decay follows first-order kinetics and that our model results are independent of experimental conditions such as oven ramp rate. In contrast, decay kinetics of carbonate-rich samples cannot be accurately constrained, likely due to matrix effects and catalysis of CaCO3 decomposition during analysis.

Results indicate that samples with a large spread in δ13C and Fm values between RPO fractions also contain a complex, broad *p0*(*E*) distribution due to the fact that they integrate over multiple OC sources with contrasting chemical and isotopic composition. We therefore propose that *p0*(*E*) is a useful metric for describing OC source and quality. To compare with isotope measurements, we calculate the average *E* value contained in each RPO fraction by determining the temporal evolution of *p0*(*E*) throughout an experiment. For the samples analyzed here, results indicate that δ13C and Fm vary linearly as a function of *E*, suggesting that OC bonding environment (as measured by thermal reactivity) is tightly coupled with isotope composition.

**Introduction**

The balance between organic carbon (OC) synthesis, remineralization to CO2, and burial in soils/sediments exerts a significant control on the global carbon cycle on timescales of years to millions of years (*e.g.* Lasaga et al., 1985; Derry and France-Lanord, 1996; Hayes and Waldbauer, 2006; Galy et al., 2008a). However, OC remineralization is not a straightforward process and depends on multiple complicating factors such as molecular diversity (Kellerman et al., 2015), secondary chemical interactions (Hedges et al., 2000a; Schmidt et al., 2011), physical protection by particles (Mayer, 1994; Mikutta et al., 2006), environmental conditions such as O2 exposure time (*e.g.* Hartnett et al., 1998), microbial diversity (Kramer and Gleixner, 2008; Janssens et al., 2010; Schmidt et al., 2011), and microbial "priming" of recalcitrant material (Bianchi, 2011). The relative importance of these factors is still actively debated and will likely vary depending on environmental conditions (*e.g*. Hedges et al., 2001; Rothman and Forney, 2007; Schmidt et al., 2011; Kellerman et al., 2015), thus hindering our ability to mechanistically understand and interpret the causes of observed heterogeneity in OC decay rates (Boudreau and Ruddick, 1991).

To address this issue, a novel class of analytical techniques, broadly termed "serial oxidation" methods, has recently been developed. Such analyses separate compounds within a bulk sample based on various metrics of lability – that is, susceptibility to remineralization by chemical hydrolysis (Helfrich et al., 2007), *uv* light (Follett et al., 2014), heat (Szidat et al., 2004; Currie and Kessler, 2005; Rosenheim et al., 2008), microbial respiration (Beaupré et al., 2016), etc. – and measure the stable carbon (13C/12C) and radiocarbon (14C/12C) ratios of evolved CO2. By separating evolved CO2 into different "bins," isotopic information can be obtained for groups of compounds exhibiting similar physical and/or chemical properties. Serial oxidation is therefore a promising method to directly probe the relationship between OC molecular composition, source, and environmental residence time.

Like bulk measurements, serial oxidation techniques benefit from the fact that all carbon contained within a sample is analyzed, and results therefore reflect the entire complex OC mixture. This contrasts with compound-specific isotope methods used for tracing carbon source and fate, in which particular biomarkers thought to represent major OC components are analyzed (*e.g.* plant- wax lipids; Hayes et al., 1989; Eglinton et al., 1996; Sessions et al., 1999). However, biomarker compound classes typically constitute <1% of total OC, and significant biases in production rates, preservation, and integration have recently been observed (Garcin et al., 2014; Hemingway et al., 2016b). Furthermore, it has been shown that biomarker classes thought to track similar OC sources (*i.e.* plant-wax lipids and lignin phenols) can display drastically different 14C content (Feng et al., 2013b), thus complicating their use as a tracer for OC residence time. Serial oxidation methods are able to circumvent this issue while still providing information related to the distribution of isotope ratios within a sample that is otherwise lost when considering only bulk averages (Blair and Aller, 2012).

However, a theoretical treatment of serial oxidation kinetics is generally lacking, thus hindering our ability to correlate experimental isotopic results with intrinsic molecular properties and reaction energetics. To address this issue, we develop a framework for relating OC thermal recalcitrance with its corresponding 13C and 14C content during ramped-temperature pyrolysis/oxidation (termed "Ramped PyrOx" or "RPO" analysis). This method, first described by Rosenheim et al. (2008), involves heating a sample at a controlled rate while continuously quantifying and collecting evolved CO2, which is binned over user-defined time windows (termed "fractions") and analyzed for carbon isotope composition. RPO analysis has recently been used in a host of environmental settings including soils (Plante et al., 2013), riverine sediments (Rosenheim and Galy, 2012; Rosenheim et al., 2013b; Schreiner et al., 2014; Bianchi et al., 2015), and marine sediments (Rosenheim et al., 2013a; Subt et al., 2016) to investigate the differences in 13C and 14C composition for various OC components contained within a single sample. Despite these promising initial results, quantitative interpretation has thus far been limited due to the fact that reaction kinetics within the RPO instrument remain unknown.

Here, we describe degradation rates using an inverse implementation of the distributed activation energy model (DAEM) in which OC quality – that is, susceptibility to thermal degradation – is described by activation energy (*E*) (Braun and Burnham, 1987; Burnham et al., 1987; Cramer, 2004). Similar to the isothermal reactive continuum model (Boudreau and Ruddick, 1991; Forney and Rothman, 2012a,b), the DAEM treats OC remineralization as a superposition of parallel first-order decay reactions that are described by a probability density function (pdf) of *E*. In contrast to many previous studies (*e.g.* Lakshmanan and White, 1994; Cai and Liu, 2007; de Caprariis et al., 2012), our implementation does not require that *E* follows a particular parametric form (*e.g.* Gaussian), but rather estimates a nonparametric *E* distribution for unreacted material remaining at any time. Furthermore, because DAEM-derived *E* is an intrinsic property of a given chemical bonding environment (*i.e.* it does not depend on experimental conditions such as temperature ramp rate), thermal recalcitrance can be reasonably viewed as a proxy for OC molecular composition and redox state. Therefore, by calculating a pdf of *E* across each time window in which CO2 was collected, our method aims to directly compare the distribution of OC molecular and isotopic composition contained within a sample.

We emphasize that biogeochemical OC recalcitrance can differ from thermal OC recalcitrance due to the presence of catalysts, extracellular enzymes (Sinsabaugh et al., 2008; Arnosti, 2011), interaction with *uv* light (Spencer et al., 2009), microbial priming (Bianchi, 2011), *etc*. within the environment. It is precisely these differences that offer insight into the biogeochemical mechanisms controlling the carbon cycle. For example, the loss of high-*E*, 14C-free OC across a shale redox front or in a soil profile might represent preferential biological oxidation of highly condensed, rock-derived carbon despite the high chemical and thermal recalcitrance of these compounds (Petsch et al., 2001; Rethemeyer et al., 2004; Marschner et al., 2008). Conversely, the persistence of low-*E* material with steadily decreasing 14C content in aging sediments could indicate physical-chemical protection of otherwise labile compounds (Mayer, 1994; Rothman and Forney, 2007). By comparing activation energy with isotope composition for each RPO fraction in a variety of environmental samples, our method aims to fundamentally address the following question: *How, if at all, do biogeochemical processes decouple observed reservoir ages (as measured by 14C content) from OC recalcitrance as predicted by chemical bonding environment?*

To test our theoretical description of RPO kinetics, we analyzed a set of three samples representing a range of environments: fluvial suspended sediments, marine sediments, and soils. By subjecting these samples to various experimental conditions, we are able to validate the assumptions of our model while also offering insight into potential limitations of this approach. Finally, we compare reaction energetics with RPO-derived isotope composition and interpret these relationships within the context of current carbon cycle knowledge.

**Materials and Methods**

Sample selection and preparation

As a representative fluvial sample, we chose suspended sediments collected from the surface of the Narayani River at the base of the Himalayas (27.70°N, 84.43°E) that has been previously analyzed for bulk OC and plant-wax carbon isotopes (Galy et al., 2008b; Galy and Eglinton, 2011; Galy et al., 2011). Aliquots of this sample, henceforth referred to as "Narayani PB-60," were taken for RPO analysis from freeze-dried archived material and acidified under HCl fumes to remove carbonates as described in Whiteside et al. (2011). Because residual chloride has been shown to interact with the RPO catalyst wire (Hemingway et al., 2016a), acidified aliquots were rinsed 3× in 18MΩ MilliQ water and freeze-dried overnight at −40°C prior to analysis. For consistency and to properly calculate RPO isotope mass balance, organic carbon content (%OC), 13C composition, and 14C composition were re-measured using fumigated and rinsed material following the methods of McNichol et al. (1994a,b).

To represent marine sediments, we chose a carbonate-rich sample collected from the Southern Ocean (60.24°S, 170.19°W) as part of the Joint Global Ocean Flux Study (JGOFS; Sayles et al., 2001). Aliquots were taken from archived core-top material (0 − 0.5 cm, stored at −80°C), freeze-dried overnight at −40°C, and homogenized prior to RPO analysis. Inorganic carbon content (%IC), %OC, and bulk 13C composition were re-quantified following McNichol et al. (1994a). This sample, henceforth referred to as "JGOFS MC-1," was analyzed without acidification in order to investigate the effect of carbonates on RPO results.

Lastly, we analyzed a soil sample overlaying the Pololu lava flow located on the Kohala Peninsula of Hawaii (20.15°N, 155.83°W; Chadwick et al., 2007). Archived material (freeze-dried, 70−90 cm depth) was homogenized and aliquots were taken for RPO analysis. Because this sample, henceforth referred to as "Pololu 4169," overlies igneous bedrock and does not contain petrogenic carbonates, acidification was not required. Bulk %OC and 13C content was measured using a Thermo DeltaV elemental analyzer-isotope ratio mass spectrometer and bulk 14C content was measured following McNichol et al. (1994b).

Ramped PyrOx analysis

The RPO analytical setup has been described in detail previously (Rosenheim et al., 2008; Hemingway et al., 2016a). In summary, a solid sample is loaded into a pre-combusted (850°C, 5 hours) quartz reactor and placed into a two-stage oven (Figure 3.1). The reactor is then sealed and the sample is exposed to an atmosphere of 92:8 He:O2 with a flow rate of 35 mL min-1, resulting in oxidative carbon combustion (*c.f.* pyrolysis as described in Rosenheim et al., 2008). O2 is provided in excess to ensure that degradation kinetics do not depend on O2 concentration. During analysis, the oven surrounding the sample is programmed to heat at a user-defined ramp rate (β, see Table 3.1 for symbol descriptions) and instantaneous temperature within the oven is measured using two thermocouples separated by ≈ 1 cm to monitor temperature homogeneity, which is typically < 5°C. Following standard practice (Rosenheim et al., 2008), a ramp rate of β = 5°C min-1 was used for all experiments in which CO2 gas was collected for isotope analysis. In the second (downstream) oven, eluent gas is passed over a Cu, Pt, and Ni catalyst wire held at 800°C to facilitate oxidation of reduced carbon-containing gases to CO2.

After exiting the second oven, eluent gas is distilled through a water trap and passed into a flow-through infrared gas analyzer (IRGA) to measure CO2 concentration (in parts per million by volume; ppmCO2) with 1-second temporal resolution (resulting ppmCO2 vs. temperature plots are referred to as "thermograms" throughout this study). IRGA measurements are calibrated using a two-point calibration curve before each analysis to account for instrument drift, and are precise to within ± 5 ppmCO2 (Hemingway et al., 2016a). Downstream of the IRGA, eluent gas is passed into one of two switchable traps and CO2 is cryogenically frozen while He and O2 are vented to the atmosphere. Traps are switched at user-defined time points and CO2 is further distilled, quantified, transferred into glass tubes packed with ≈ 100 mg CuO and ≈ 10 mg Ag, and flame sealed.

Isotope measurement, blank correction, and data processing

After recombustion at 525°C for 1 hour to remove trace contaminant gases, isotope composition of CO2 contained in each RPO fraction was analyzed following standard procedures (McNichol et al., 1994b; Pearson et al., 1998), where 13C content is expressed in δ13C per mille (‰) notation relative to Vienna Pee Dee Belemnite (VPDB) and 14C content is expressed in fraction modern (Fm) notation following Stuiver and Polach (1977). RPO fraction masses, δ13C values, and Fm values were corrected for blank carbon contribution, and δ13C was additionally corrected to ensure 13C mass balance as incomplete oxidation to CO2 has been shown to exhibit a small fractionation effect (Hemingway et al., 2016a). Analytical uncertainty was propagated throughout all corrections.

All calculations contained herein were performed using the open-source ’rampedpyrox’ package for Python v.3.5 as described in Hemingway (2016).

**Results**

RPO fraction temperature ranges, CO2 masses, δ13C, and Fm are reported in Tables 3.2–3.4 along with independently measured bulk isotope composition for each sample. The resulting Narayani PB-60 thermogram can be described as a bimodal distribution with peaks at 365°C and 662°C, similar to that observed previously when analyzed in pyrolysis mode (Figure 3.2A; Rosenheim and Galy, 2012). Corresponding RPO fraction Fm values decrease monotonically between 150 and 725°C from 0.891 ± 0.004 (fraction 1) to 0.014 ± 0.002 (fraction 8), followed by a small yet statistically significant increase to 0.042 ± 0.002 in the final fraction. δ13C values display the opposite trend, rising from −29.5 ± 0.2‰ (fraction 1) to −21.8 ± 0.2‰ (fraction 8) followed by a slight decrease to −23.5 ± 0.2‰.

In contrast, the JGOFS MC-1 thermogram is dominated by a single peak with a maximum decay rate at 652°C (Figure 3.2B). This is within the range of previously observed carbonate decomposition temperatures (Plante et al., 2013), consistent with the fact that ≈ 95% of the carbon in this sample is present as calcite (Sayles et al., 2001). While Fm was not measured, δ13C values increase drastically throughout the experiment from −20.1 ± 0.2‰ (fraction 1) to 0.9 ± 0.2‰ (fraction 5). Lastly, carbon contained in Pololu 4169 exhibits the lowest degradation temperatures of all samples studied, with a maximum decay rate at 348°C and < 0.5% of initial carbon remaining unreacted at 600°C (Figure 3.2C). Fm values are remarkably stable across RPO fractions, ranging from 0.278 ± 0.001 (fraction 1) to 0.285 ± 0.001 (fraction 5). Despite this, δ13C values display a significant decrease with increasing temperature, ranging from −25.1 ± 0.3‰ (fraction 1) to −26.9 ± 0.3‰ (fraction 5).

To test if thermogram shapes depend on initial carbon mass (*G0*), we reanalyzed Nayarani PB-60 and JGOFS MC-1 for various values of *G0* while holding all other experimental conditions constant (*i.e.* β = 5°C min-1). Narayani PB-60 thermograms scale linearly with *G0* throughout the experiment, with maximum decay rates ranging from ≈ 0.06µgC sec-1 (*G0* = 268µgC) to ≈ 0.20µgC sec-1 (*G0* = 828µgC; Figure 3.3A). *G0* has no apparent effect on elution temperature for this sample, with maximum decay rates observed at 662.0 ± 0.8°C for all values of *G0*. Similarly, JGOFS MC-1 decay rates scale positively with *G0*, with a maximum decay rate ranging from ≈ 0.10µgC sec-1 (*G0* = 98µgC) to ≈ 0.88µgC sec-1 (*G0* = 951µgC; Figure 3.3B). However, unlike Narayani PB-60, the temperature at which maximum decay rates are observed increases with *G0* from 620.5°C (*G0* = 98µgC) to 652.2°C (*G0* = 951µgC).

Lastly, we analyzed Narayani PB-60 at multiple ramp rates (β = 2, 5, and 10°C min-1) while holding *G0* constant. Here we normalize thermograms by initial mass and ramp rate in order to accurately compare between experimental conditions, resulting in plots of fractional carbon loss per °C (Figure 3.3C). Results indicate a consistent shift toward higher elution temperatures for higher ramp rates, as predicted by parallel first-order kinetics (Braun and Burnham, 1987; Miura, 1995; Miura and Maki, 1998). For example, the temperature at which the maximum decay rate is reached increases from 621.2°C when β = 2°C min-1 to 688.5°C when β = 10°C min-1. This temperature increase is accompanied by a corresponding decrease in decay rate, with maximum values dropping from 3.1 × 10−3 ° C-1 (β = 2°C min-1) to 2.7 × 10−3 °C-1 (β = 10 C min-1).

**Discussion**

To properly interpret RPO thermograms as a function of OC chemical composition, and to relate these results with corresponding δ13C and Fm values, remineralization kinetics during thermal degradation must be fully constrained. The distributed activation energy model (DAEM) is a promising method to do so, as it has long been utilized to describe the non-isothermal decay of complex carbon mixtures such as biomass (*e.g*. Bradbury and Sakai, 1979; White et al., 2011) and fossil fuel precursor OC (*e.g.* Burnham et al., 1987; Braun and Burnham, 1987; Burnham and Braun, 1999; Cramer, 2004; Dieckmann, 2005) during thermogravimetric analysis. Here we derive the DAEM by first considering the case where OC is separated into a finite set of discrete components with unique activation energy values. We then generalize this description to allow for a continuous distribution of OC quality, as has been done previously (see Burnham and Braun, 1999, for review). Finally, following Forney and Rothman (2012a,b), we describe an inverse method to determine the regularized solution of the ill-posed DAEM, and compare resulting reaction energetics with RPO fraction δ13C and Fm values.

Mathematical derivation

*Discrete DAEM*

First, we consider the case where OC is described by a finite set of discrete components associated with unique activation energy values. During OC remineralization, the decay rate of carbon contained in a particular component *i* is often described as as a first-order process with respect to the mass of carbon remaining in component *i* at any time *t*, *gi*(*t*) (Berner, 1980; Braun and Burnham, 1987):

(Equation 3.1)

where *ki*(*t*) is the dynamic first-order rate coefficient associated with component *i* at time *t*. Although OC decay in the environment can additionally depend on oxidant concentration, we omit this dependency here since O2 is provided in excess in our experimental setup. In contrast to the "multi-G" and "reactive continuum" models that are often used to describe environmental OC degradation rates (Westrich and Berner, 1984; Boudreau and Ruddick, 1991; Forney and Rothman, 2012a,b), here we explicitly allow *ki*(*t*) to vary with time. Because rate coefficients are related to temperature and activation energy, *ki*(*t*) can be written as a function of E following the Arrhenius equation so long as temperature is measured at each time point:

(Equation 3.2)

where *k0* is the empirically derived Arrhenius pre-exponential ("frequency") factor, R is the ideal gas constant, *Ei* is the activation energy of carbon contained in component *i*, and *T*(*t*) is the measured temperature of the system at time *t*. For non-isothermal systems, time-dependent (*i.e.* dynamic) decay coefficients can therefore be described by the static property *Ei* and the observed variable *T*(*t*). Although *T*(*t*) is related to *t* by a constant ramp rate β during RPO analysis, we leave this written as is to emphasize that the DAEM is valid for any measured time-temperature history. Substituting Equation 3.2 for *ki*(*t*), first-order decay during a non-isothermal process at time *t* can be written as:

(Equation 3.3)

The mass of carbon remaining in component *i* at time *t* is therefore determined by integrating Equation 3.3 from an initial time, *t0* = 0, to a final time *t*:

(Equation 3.4)

where *gi,0* is the initial mass of carbon contained in component *i* and *t’* is the change-of-variables substituted time variable ranging from *t0* = 0 to *t*. Due to the integration of the Arrhenius equation from *t0* = 0 to *t*, Equation 3.4 states that *gi*(*t*) depends on the entire time-temperature history of the experiment. That is, *dgi*(*t*)/*dt* is governed by a balance between decreasing *gi*(*t*) as OC is remineralized and increasing *ki*(*t*) with increasing *T*(*t*) as the experiment progresses. This balance should result in a predictable shift in RPO thermograms toward higher elution temperatures with increasing β, as is observed (Figure 3.3C; Braun and Burnham, 1987; Miura, 1995; Miura and Maki, 1998).

Furthermore, following the multi-G model of Westrich and Berner (1984), any environmental sample containing a complex OC mixture can be described as a superposition of a finite set of *n* components, each decaying according to a unique *ki*(*t*) and thus corresponding to a unique *Ei* value. The total carbon mass remaining at *t*, *G*(*t*), is therefore the sum of the mass remaining in each component *i* = 1, 2, …, *n* at that time:

(Equation 3.5)

where *G0* is the initial OC mass present in the entire sample, defined as the sum of initial mass contained in each component:

(Equation 3.6)

and *pi,0* is the fraction of total carbon initially contained in component *i*:

(Eequation 3.7)

such that Σ*pi,0* ≡ 1.0. The fraction of OC initially present within each component can therefore be determined by fitting Equation 3.5 to the observed *G*(*t*) profile measured by the RPO instrument. While informative, this discrete description of the DAEM suffers from two major limitations: *(i)* *n* must be set a priori or determined empirically (Boudreau and Ruddick, 1991) and *(ii)* any noise recorded in the data will result in large uncertainty in best-fit *pi,0* and *Ei* values (Forney and Rothman, 2012b). To circumvent these issues, a more general description of non-isothermal first-order decay can be derived that does not assume a finite set of components with unique *Ei*, but rather allows *E* to vary continuously (Burnham et al., 1987; Burnham and Braun, 1999; Cramer, 2004).

*Continuous DAEM*

In this continuous model, the mass of carbon remaining at time *t* that is associated with any activation energy value *E*, *g*(*t, E*), can be determined by substituting *g*(*t, E*) for *gi*(*t*) and *E* for *Ei* in Equation3.4:

(Equation 3.8)

where *g0*(*E*) is the initial mass of carbon associated with activation energy value *E*. The total carbon mass remaining at time *t*, *G*(*t*), can now be defined by replacing the summation over components *i* = 1, 2, …, *n* in Equation 3.5 by an integral over all possible (*i.e.* non-negative) values of *E*:

(Equation 3.9)

where *p0*(*E*)*dE* is now the fraction of total carbon initially associated with the infinitesimal

range of activation energy values about *E* such that:

(Equation 3.10)

That is, the distribution of *p0*(*E*) over all values of *E* describes the initial probability density function (pdf) of activation energy that will lead to the observed OC decay rates when a sample is analyzed in the RPO instrument. Unlike measured thermograms, *p0*(*E*) is not a function of experimental conditions such as ramp rate – rather, it is an intrinsic property of the physical-chemical bonding environment within a particular sample. As RPO analysis proceeds, this pdf must evolve with time to reflect the fact that some carbon has been remineralized to CO2. Therefore, at any time *t* the remaining fraction of total OC initially present in the sample that is associated with any activation energy value, *p*(*t, E*)*dE*, is calculated as *p0*(*E*)*dE* multiplied by a double exponential decay term analogous to Equation 3.8:

(Equation 3.11)

Equation 3.11 implies that the carbon initially remineralized to CO2 must be associated with the lowest values of *E*, as low *E* will lead to a double exponential term that approaches zero most rapidly. Put differently, OC that is described by higher *E* values will resist remineralization until more time has passed and, therefore, higher temperatures have been reached – *i.e.* it is more thermally recalcitrant.

*Parallel first-order verification*

Because the DAEM is a specific case of *n*-order non-isothermal kinetic models (Braun and Burnham, 1987; White et al., 2011), we must verify that carbon degradation in the RPO instrument behaves according to a superposition of parallel first-order reactions (with respect to OC concentration) rather than higher-order processes. Differentiating Equation 3.9 with respect to time, the total rate of carbon remineralization at any time *t* is given by the equation:

(Equation 3.12)

It can be seen that the DAEM describes *dG*(*t*)/*dt* as a linear function of *G0* multiplied by an integral term that depends on *p*(*t, E*) but is independent of *G0*. In contrast, if carbon decomposition within the RPO instrument were to follow a higher-order process, the relationship between *dG*(*t*)/*dt* and *G0* would be nonlinear and evolve as a function of time (*e.g.* Follett et al., 2014). Replacing the integral term in Equation 3.12 by *m*(*t*), the loss of carbon at time *t* as predicted by the DAEM simplifies to:

(Equation 3.13)

Therefore, similar to the isothermal case described in Follett et al. (2014), a superposition of parallel first-order decay reactions will result in a linear relationship between *dG*(*t*)/*dt* and *G0* with a zero intercept and a time-dependent slope equal to:

(Equation 3.14)

where *m*(*t*) can be interpreted as the *G0*-normalized decay rate at time *t*. We verify that OC remineralization within the RPO instrument follows parallel first-order kinetics by assessing the linearity between *dG*(*t*)/*dt* and *G0* at any time *t* across a range of *G0* values using Narayani PB-60 a test sample (Figure 3.3A). We chose Narayani PB-60 because it exhibits the widest range of decomposition temperatures of any sample analyzed here (Figure 3.2). For 4 arbitrarily chosen time points, it can be seen that this relationship is linear with an ordinary least squares R2 ≥ 0.999 (Figure 3.4A), resulting in identical *G0*-normalized thermograms within analytical uncertainty (Figure 3.4B). Therefore, the decay of complex OC mixtures contained in decarbonated samples during RPO analysis can indeed be accurately described by a superposition of parallel first-order reactions.

*A note of caution for carbonates*

While most RPO studies to date have focused on OC analysis by acidifying to remove carbonates (*e.g.* Rosenheim et al., 2008; Rosenheim and Galy, 2012; Rosenheim et al., 2013b; Schreiner et al., 2014; Bianchi et al., 2015), it has recently been argued that acid hydrolysis and/or dissolution of short range order minerals during acid treatment can alter the OC chemical bonding environment and therefore affect thermal stability (Plante et al., 2013). Analyzing raw samples without acid treatment can circumvent these issues, however the effect of carbonates on decay kinetics has not yet been considered. To test if carbonate-rich samples follow parallel first-order kinetics, we similarly analyzed JGOFS MC-1 for a range of *G0* values (Figure 3.3B). Prior to *t* ≈ 4500 sec, when δ13C values of eluted CO2 indicate a predominantly OC source (Figure 3.2B), *dG*(*t*)/*dt* can be accurately described as a linear function of *G0* (R2 ≥ 0.999). However, as carbonate begins to decompose above *t* ≈ 4500 sec, the relationship between *dG*(*t*)/*dt* and *G0* becomes highly nonlinear – that is, the resulting carbonate peak shifts toward higher t with increasing *G0* (Figure 3.4C–D).

To investigate if non-first-order decomposition is an intrinsic property of CaCO3 or if this is due to interactions with other materials within the sample (so-called "matrix effects"), we additionally analyzed a purified Icelandic spar CaCO3 standard at multiple masses (*G0* = 258, 492, 1014µgC, β = 5°C min-1). Results indicate that purified carbonate, unlike JGOFS MC-1, does follow first-order kinetics, with a maximum decomposition rate occurring at 700 ± 6°C independent of *G0* (not shown). Interaction with reduced organic carbon, corresponding hetero-atoms (*e.g.* N, P, S), or trace metals contained within the sample matrix are therefore the likely cause of non-first-order CaCO3 decomposition when analyzing environmental samples. Thus, while avoiding the issues of acid treatment, the analysis of carbonate-containing samples will result in thermograms that cannot be accurately described by the DAEM presented here, and is not recommended when using the RPO instrument to determine reaction energetics.

Solving for *p0(E)* using an inverse method

Following Forney and Rothman (2012a,b), we present a method to invert the DAEM and solve for the pdf of *E* subject to a non-negativity constraint. In contrast to previous DAEM solutions (Lakshmanan and White, 1994; Cai and Liu, 2007; de Caprariis et al., 2012), this approach does not require an a priori assumption about the parametric form of *p0*(*E*). Additionally, because inverse methods are ill-posed and thus highly sensitive to noise, we "smooth" the solution using a Tikhonov regularization to remove this sensitivity (Tikhonov and Arsenin, 1977; Hansen, 1994). To numerically calculate *p0*(*E*), we discretize the continuous variable *t* over the time course of the experiment into a vector **t** containing *nt* nodes, *tj*, such that:

(Equation 3.15)

For generality, and because the DAEM is frequently applied over geologic timescales with non-uniformly distributed time measurements, Equation 3.15 does not require a uniform time step (*i.e.* it is possible that ∆*tj* ≠ ∆*ti*≠*j* ). Similarly, we generate a vector **E** over the range values considered for the model solution (typically 50 − 350 kJ mol-1) that contains *nE* nodes, *El*, such that:

(Equation 3.16)

It can be seen from Equation 3.9 that the DAEM can be separated into two components: *(i)* *p0*(*E*) and *(ii)* a double exponential term that is independent of *p0*(*E*). This term, analogous to the Laplace transform for the isothermal reactive continuum model (Forney and Rothman, 2012b), describes the fraction of carbon initially associated with an activation energy value *E* that has decayed by time *t*. We therefore generate a matrix **A** describing the dynamic disordered kinetics of the system by calculating the value of this term for all combinations of *tj* and *El*:

(Equation 3.17)

Finally, we integrate the measured RPO thermogram and interpolate the resulting fraction of total carbon remaining at each time point, *G*(*t*)/*G0*, onto each discretized time point in **t** to generate a vector of fractional carbon remaining, **g**. The DAEM can thus be written in matrix form as:

(Equation 3.18)

where **p** is a discretized vector of *p0*(*E*) with length *nl* such that each component *pl* is equal to:

(Equation 3.19)

While **p** can be calculated directly by multiplying g by the computed inverse of **A**, it is possible that this will result in negative values of *pl* if **g** contains noisy data (Forney and Rothman, 2012b). However, because *pl* represents a probability, it cannot be negative by definition. We therefore require the solution to be non-negative by solving the constrained least squares problem according to:

(Equation 3.20)

where ||x|| ≡ (Σ*xi*2)0.5 is the vector norm and we require that *pl* ≥ 0. The resulting **p** vector is thus the non-regularized solution to the inverse DAEM.

*Choice of frequency factor*

In order to construct the **A** matrix and solve for **p**, our method requires that the Arrhenius pre-exponential factor *k0* be prescribed *a priori*. There exists significant discussion in the literature on the best choice of *k0*, as multiple values of this parameter can describe laboratory results equally well but will result in drastically different predictions of OC degradation rates over geologic timescales (Braun and Burnham, 1987; Burnham et al., 1987; Lakshmanan et al., 1991; Dieckmann, 2005). Furthermore, it has been argued that *k0* represents the variable change in entropy associated with the decay of specific organic compounds and should therefore be parameterized as a function of *E* (the so-called "kinetic compensation effect" or "KCE"; Tang et al., 2000). A linear increase in *k0* with *E* from ≈ 108 sec-1 (*E* = 175 kJ mol-1) to ≈ 1026 sec-1 (*E* = 400 kJ mol-1) has thus been utilized to better predict petroleum formation rates (Dieckmann, 2005). To circumvent the issue of multiplicity, and to account for the KCE, Miura (1995) and Miura and Maki (1998) developed a method to estimate the best-fit *k0* for each value of *E* by comparing the shift in elution temperatures when a sample is analyzed at multiple ramp rates (*e.g.* Figure 3.3C). However, because this approach is based on large extrapolations in 1/*T* vs. β/*T2* space, it is highly sensitive to noise in temperature and β measurements (Burnham and Braun, 1989).

To select a best-fit *k0*, here we calculate **A** and **p** for a range of *k0* values and determine the root mean squared error (RMSE) between measured *G*(*t*) and that predicted by Equation 3.20. We include the KCE by calculating *k0* as a function of E according to:

(Equation 3.21)

Resulting RMSE values using a range of KCE slope and intercept can be seen in Figure 3.5 for Narayani PB-60 (β = 5°C min-1, *E* ranging from 50 to 350 kJ mol-1). By setting an "acceptable" cutoff of RMSE ≤ 10−4, it can be seen that there exist multiple KCE slope and intercept combinations that can equally fit the observed data. Additionally, we estimate the best-fit *k0* using a range of ramp rates (β = 2, 5, and 10°C min-1) following the method of Miura and Maki (1998) (Figure 3.5, white circle). While this estimate falls outside of the RMSE cutoff range, likely due to noise in temperature and β measurements, it results in a KCE slope near zero and suggests that *k0* is constant during RPO oxidation of this sample. To accurately compare RPO results between samples, we therefore select a constant *k0* value of 1010 sec-1, well within the RMSE cutoff range, for all samples analyzed herein (Figure 3.5, red star). While a different choice of *k0* will shift *p0*(*E*) to higher or lower *E*, we emphasize that it will not affect the distribution of *p0*(*E*), and that only relative changes in *E* should be interpreted. For example, although a shift in *k0* from a constant value of 107 to 1012 sec-1 results in an increase in mean *E* from 150 kJ mol-1 to *E* from 224 kJ mol-1, the resulting relative standard deviation of *p0*(*E*) remains identical at 20%.

*Tikhonov Regularization*

In principle, after choosing a value of *k0* and constructing the **A** matrix, the nonparametric pdf of *E* that best describes an RPO thermogram can be determined using Equation 3.20. However, the inverse DAEM is highly sensitive to noise at the level of RPO instrument precision (*i.e.* approximately ± 5 ppmCO2, ±5°C; Hemingway et al., 2016a), and is therefore ill-posed (Hansen, 1994). To minimize this sensitivity to data uncertainty, we "smooth" the inverse DAEM solution using Tikhonov regularization (Tikhonov and Arsenin, 1977; Hansen, 1994; Forney and Rothman, 2012a,b). This approach is often used to solve constrained inverse problems by calculating an optimal solution that minimizes complexity in *p0*(*E*) (as determined by the intensity of fluctuations, or "roughness") while maximizing solution accuracy. Following Forney and Rothman (2012b), we calculate roughness as the norm of the first derivative of **p**:

(Equation 3.22)

where **R** is the discretized first derivative operator over the range of *E* values considered. The regularized inverse solution can therefore be determined by including this roughness term when solving the constrained least squares:

(Equation 3.23)

where ω is a scalar that determines how much to weight the roughness relative to the residual error. The best choice of ω is often considered to be the value that optimally minimizes the residual error and solution roughness. As described in Hansen (1994), this is equal to the value corresponding to the point of maximum curvature in a log − log plot of the residual error vs. the roughness when allowing ω to range over many orders of magnitude (*i.e.* the so-called "L-curve", Figure 3.6). The best-fit ω value as determined by the L-curve therefore results in a "smoothed" solution of *p0*(*E*) with a residual error that is, in principle, approximately equal to the measurement uncertainty (Forney and Rothman, 2012b).

However, we note that the best-fit residual error for JGOFS MC-1 is ≈ 100-fold higher than for Narayani PB-60 and Pololu 4169 due to the non-first-order decay of CaCO3 that cannot be accurately predicted by the DAEM, as described above (Section 3.5.2, Figure 3.6B). This results in a *G0*-dependent *p0*(*E*) distribution, with the resulting *E* value associated with the carbonate peak in this sample shifting from 211 kJ mol-1 when *G0* = 98µgC to 220 kJ mol-1 when *G0* = 951µgC (not shown). Still, regularized pdfs of *E* for samples containing exclusively OC are nearly identical across all experimental conditions (*i.e.* β, *G0*), supporting the hypothesis that *p0*(*E*) is an intrinsic property of OC contained within a sample. For example, although there exist small differences between individual runs due to measurement uncertainty and variability in best-fit ω values (range of 0.044 − 0.448, *n* = 5), the main features of the pdf of *E* contained within Narayani PB-60 are robust for all conditions considered in this study (Figure 3.7).

Unlike previous studies that assume a Gaussian distribution of *p0*(*E*) during fossil fuel pyrolysis (Lakshmanan and White, 1994; Cai and Liu, 2007; de Caprariis et al., 2012), regularized results calculated here clearly do not follow any parametric form (Figure 3.8, thick black lines). Rather, it can be seen that *p0*(*E*) generally resembles the RPO thermogram shape (Figure 3.2), as would be expected due to the fact that thermally recalcitrant OC is associated with higher values of *E*. Furthermore, observed high-frequency variability in *p0*(*E*) relative to the corresponding thermograms is an intrinsic result of non-isothermal kinetics. That is, during RPO analysis, OC associated with a single *E* value will remineralize over a range of temperatures with an increasing rate coefficient until it has been exhausted, thus resulting in a "smoothed" thermogram relative to the governing *p0*(*E*) distribution. Highly variable, non-parametric *p0*(*E*) observed here likely results from the extreme chemical complexity and range of oxidation states contained in environmental OC samples (*e.g.* Kellerman et al., 2015). In contrast, fossil fuel precursors have undergone various degrees of diagenesis and thermal maturation, potentially resulting in OC mixtures exhibiting more similar chemical properties that can be described by a Gaussian distribution of pyrolysis *E* values (Braun and Burnham, 1987). This proposed relationship between *p0*(*E*) complexity and chemical diversity is additionally supported by the differences between samples analyzed herein. For example, fluvial suspended sediments such as Narayani PB-60 integrate a range of OC sources (*e.g*. recently fixed biomass, pre-aged soils, and eroded rock-derived material; Blair and Aller, 2012) and would therefore be expected to contain a broader and more variable *p0*(*E*) distribution than that contained in a single soil OC sample such as Pololu 4169, as is observed (Table 3.2, 3.4; Figure 3.8A, C). We therefore propose that variability in the pdf of *E* is a potentially useful metric for comparing relative OC chemical complexity between samples.

*Determining p0*(*E*) *contained in each RPO fraction*

To further understand how the distribution of OC molecular structure relates with source and reservoir age, we compare the average *E* value corresponding to CO2 contained within each RPO fraction with its corresponding isotope composition. To do so, we first calculate the subset of the pdf of *E* that is contained within an RPO fraction *f* by taking the difference between *p*(*t, E*) at the initial and final time points for each fraction. Because A*j,l* describes the relative amount of carbon initially associated with *El* remaining at time *tj*, it can be seen from Equation 3.11 that the discretized *p*(*tj, E*) values can be calculated by multiplying each *pl* in **p** by the corresponding element in the *j*th row of **A**. The pdf of *E* corresponding to the CO2 contained in each RPO fraction is therefore equal to *p*(*t0,f*, *E*) − *p*(*tf,f*, *E*) for each value of *E*, where *t0,f* and *tf,f* are the initial and final time points, respectively, for RPO fraction *f*. Resulting distributions are typically non-parametric and highly overlapping, reflecting the fact that CO2 isotope composition for each RPO fraction is itself a weighted average of multiple sources (Figure 3.8, gray shaded regions). Average *E* values and corresponding variance can thus be calculated as the first and second moments, respectively, of each distribution (Table 3.2–3.4).

Relationships between isotopes and reaction energetics

*Kinetic isotope fractionation*

While not necessary for Fm because it is fractionation-corrected by definition (Stuiver and Polach, 1977; Santos et al., 2007), we must correct for any kinetic isotope effects occurring within the RPO instrument before interpreting δ13C as a carbon source tracer (Hemingway et al., 2016a). If kinetic fractionation is large, as has been observed both during thermogenic methane formation (Tang et al., 2000; Cramer, 2004) and dissolved OC oxidation by *uv* light (Oba and Naraoka, 2008), then this effect could overprint carbon source δ13C signals. However, when directly measured using single- compound standards, Hemingway et al. (2016a) concluded that 13C fractionation within the RPO instrument must be smaller than ≈ 1 − 2‰. Still, we correct the measured δ13C values of each RPO fraction using the ratio of carbon-normalized 13C and 12C decomposition rates at each time point:

(Equation 3.24)

Where we have added a preceding 12 or 13 superscript to specify isotope-specific variables. Following the Arrhenius equation, 13/12r(*t*) can be described as a function of the difference in *E* between 13C- and 12C-containing molecules:

(Equation 3.25)

Although 13–12∆*E* is likely not identical for all compounds due to differences in the entropy and enthalpy of isotope substitution (Tang et al., 2000), the estimated range of values for RPO analysis is small (0.3 − 1.8 × 10−3 kJ mol-1; Hemingway et al., 2016a). We therefore assume 13–12∆*E* = 1.8 × 10−3 kJ mol-1 for all RPO fractions, noting that a choice of 0.3 × 10−3 kJ mol-1 would result in δ13C values that are identical to those calculated here within analytical uncertainty.

13/12r(*t*) can be determined using the ratio of carbon-normalized, isotope-specific decay rates as calculated in Equation 3.12 by substituting *p0*(12E) and *p0*(13E) for *p0*(*E*). Because carbon is present as ≈ 99% 12C, we set *p0*(12E) = *p0*(*E*) such that *d12G*(*t*)/*dt* = *dG*(t)/*dt*. Corresponding *d12G*(*t*)/*dt* can then be determined using *p0*(13E) = *p0*(E + 13−12∆*E*). That is, 13C-containing molecules decay at rates governed by a pdf of *E* that is identical to *p0*(*E*) but has been shifted to the right by 1.8 × 10−3 kJ mol-1. We then correct the measured δ13C values of each RPO fraction *f* for kinetic isotope fractionation by dividing by the average 13/12r(*t*) value over the time of collection:

(Equation 3.26)

For the samples analyzed here, 13/12r(*t*) is initially ≈ 0.999, indicating slightly faster decay of 12C at low temperatures, and gradually increases to ≈ 1.002 when *G*(*t*) << 0.01*G0*, as has been described previously (Cramer, 2004; Hemingway et al., 2016a). Resulting kinetic fractionation corrections are near or within analytical uncertainty, with absolute δ13C values for all RPO fractions shifted by < 0.2‰ (δ13Cf,corrected − δ13Cf: maximum = +0.16‰, Pololu 4169 fraction 1; minimum = −0.10‰, Narayani PB-60 fraction 9).

Comparing *p0*(*E*), 13C content, and 14C content

In addition to exhibiting the narrowest and least complex pdf of E, Pololu 4169 displays a much smaller spread in both δ13C and Fm values than does Narayani PB-60 (Figure 3.9A–D), supporting the idea that highly complex *p0*(*E*) distributions reflect the integration of multiple OC sources with variable chemical structures and reservoir ages. Pololu 4169 Fm values are low and stable for all RPO fractions (Figure 3.9B), indicating that low-*E* OC can become significantly pre-aged given the proper environmental conditions (Schmidt et al., 2011). Furthermore, Fm values for this sample display a small yet statistically significant increase with increasing *E*, opposite of what would be expected if thermal recalcitrance were a major driver of reservoir age (0.0001 [kJ mol-1]-1, R2 = 0.895, *p*-value = 1.5 × 10−2). Coincident with increasing Fm, δ13C decreases slightly with increasing *E* (Figure 3.9A), suggesting a second source of 13C-depleted, 14C-enriched OC at higher *E* values, potentially due to downward percolation of surface dissolved OC (Chadwick et al., 2007). Still, the fact that Fm does not decrease with increasing *E* is consistent with the current paradigm of soil carbon dynamics, which interprets reservoir age as an ecosystem property rather than a function of OC chemical structure (Mikutta et al., 2006; Janssens et al., 2010; Schmidt et al., 2011).

Pololu 4160 *p0*(*E*) distribution is concentrated at low *E* values and is dominated by a large, broad peak despite the fact that soil OC contains a mixture of humic material, lipids, carbohydrates, lignin, *etc*. (Figure 3.8C; Helfrich et al., 2007). RPO analysis therefore does not result in a separation of complex OC mixtures into individual, highly resolved peaks representing individual compounds or compound classes. Combined with a relatively homogenous δ13C and Fm values between RPO fractions, a narrow *p0*(*E*) distribution indicates that individual compound classes contained in environmental samples are not separable based on thermal lability despite the unique decomposition temperatures when analyzed as purified compounds (Williams et al., 2014). In contrast, it is well known that individual biomarkers such as lignin and *n*-alkanoic acids contained in soils can display drastically different 14C content (see Schmidt et al., 2011, for review). The fact that this is not observed between RPO fractions is strong evidence that OC decomposing at any given *E* value does not correspond to a single compound or simple set of compounds, but is rather derived from a mix of sources. This can be achieved, for example, if relatively oxidized functional groups decompose at low *E* independent of their chemical source, while the remaining aromatic and aliphatic core structures resist degradation until higher temperatures have been reached. This interpretation indicates that RPO analysis separates OC based on the redox state and bonding environment of individual carbon atoms rather than properties of whole molecules, analogous to the process by which enzymes degrade OC in the environment (Sinsabaugh et al., 2008).

Still, significant trends in isotope composition with increasing *E* can be observed. For example, the relationship between δ13C and E for Narayani PB-60 RPO fractions 1–8 is remarkably linear, with a slope of 0.07‰ (kJ mol-1)-1 (R2 = 0.954, *p*-value = 3.2 × 10−5; Figure 3.9C). The ≈ 4‰ 13C depletion in fraction 9 relative to what would be expected based on this trend is at least partially due to charring, as has been described previously during non-isothermal OC pyrolysis (Williams et al., 2014). Charring can result in an apparent shift toward higher *E* values for labile OC compounds, as free-radical formation and subsequent condensation of aromatic material will increase thermal stability. However, because it has been shown that processes occurring in series can still be treated as a superposition of parallel reactions (Forney and Rothman, 2014), charring does not violate the kinetic model developed here. Rather, this simply results in a small inclusion of otherwise labile OC within the highest-temperature RPO fraction.

Nonetheless, fractions 1–8 strongly suggest that Narayani PB-60 OC contains 2 end members that mix linearly as a function of *E*. Importantly, although the isotope composition of CO2 contained in each RPO fraction represents a weighted average of OC associated with a particular *E* range, this does not inherently require a linear mixing trend between fractions. For example, mixing two theoretical end members with identical *p0*(*E*) distributions but contrasting 13C compositions will shift each RPO fraction along the δ13C axis (*i.e.* vertically in Figure 3.9C) according to the relative contribution of each end member, resulting in a "flat" δ13C relationship with *E*. In contrast, a mixture of two end members containing drastically different chemical structures and thus non- overlapping *p0*(*E*) distributions would lead to two "clusters" of RPO fraction results in a plot of δ13C vs. *E*. A linear trend like that observed here therefore indicates two end members with unique 13C compositions yet complex, overlapping *p0*(*E*) distributions. That is, it requires a decrease in the contribution by a 13C-depleted end member and a corresponding increase in the contribution by a 13C-enriched end-member with increasing *E*.

This is further evidenced by the similarly linear trend in Fm with increasing *E* for RPO fractions 1–8 [slope = −0.01 kJ mol-1)-1, R2 = 0.987, *p*-value = 7.6 × 10−7)]. Unlike Pololu 4169, ≈ 20 ± 5% of OC contained in Narayani PB-60 is derived from the erosion of OC-rich bedrock in this catchment (OCpetro; Galy et al., 2008a; Rosenheim and Galy, 2012). Because this material is 14C-free by definition, and because it has been shown that similarly condensed "black" carbon pyrolyses above ≈ 650°C (Williams et al., 2014), OCpetro is the likeliest source of high-*E* OC in this sample. However, strong linear trends in both δ13C and Fm with increasing *E* require that a fraction of this material has been incorporated into lower *E* OC, as the pyrolysis temperatures observed in Williams et al. (2014) correspond to an *E* value of ≈ 200 kJ mol-1. Similar to the observation in Pololu 4169 that biospheric OC (OCbio) generally contains low *E* values, previously measured δ13C and Fm values of long-chain *n*-alkanoic acids extracted from Narayani PB-60 are consistent with the lowest *E* RPO fractions (Figure 3.9C–D; Galy and Eglinton, 2011; Galy et al., 2011).

Therefore, a binary mixture combining biospheric OCbio described by relatively homogenous Fm near that of *n*-alkanoic acids and an *E* distribution similar to Pololu 4169 with unaltered OCpetro described by *E* ≥ 200 kJ mol-1 would result in "clustered" RPO fractions in Figure 3.9D due to the lack of significant *E* overlap. This is clearly not observed. Rather, the linear trends observed for both δ13C vs. *E* and Fm vs. *E* require the presence of two end members with unique isotope compositions but overlapping *p0*(*E*). This further indicates that each "peak" in *p0*(*E*) (*e.g.* Figure 3.8A) does not represent an isotopically unique signal derived from a specific class of compounds. Instead, this implies that each end-member contains carbon atoms described by similar chemical bonding environments and redox states. RPO analysis is therefore ideally suited for separating isotopically unique yet chemically overlapping OC sources.

Lastly, we note that JGOFS MC-1 RPO δ13C values are driven by a sharp increase in CaCO3 contribution at higher *E* values, with fraction 5 perfectly matching the independently measured calcite value (Figure 3.9E; Sayles et al., 2001). Although carbon in this sample is present as ≈ 95% calcite, the δ13C value of fraction 1 is near that expected for phytoplankton biomass OC in this region (Rau et al., 1989), suggesting that RPO analysis can sufficiently separate low-*E* OC from CaCO3 despite the potential for catalysis, matrix effects, and non-first-order kinetics. Still, we emphasize that non-first-order kinetics and mass-dependent CaCO3 elution temperatures do hinder our ability to interpret changes in OC isotope composition for carbonate-containing samples. We therefore do not recommend quantitatively interpreting RPO isotope and *p0*(*E*) results from carbonate-rich samples without independent constraints on end-member composition.

**Conclusion**

Serial oxidation techniques such as RPO are a promising new class of methods for relating OC chemical composition, isotopic composition, and environmental residence times. To better interpret these data, we develop an inverse kinetic model that determines the underlying distribution of activation energy required to thermally degrade OC. Unlike previous implementations of this model, our description does not require any *a priori* assumptions about the shape of the pdf of *E* values, *p0*(*E*), but rather determines the regularized non-parametric solution. By analyzing Narayani PB-60 using a range of oven ramp rates and initial masses, we show that the underlying *E* distribution is independent of experimental conditions and is therefore an intrinsic property of the OC chemical bonding environment. In contrast, results from a CaCO3-rich sample, JGOFS MC-1, indicate that inorganic carbon degradation rates cannot be predicted by our model, likely due to catalytic reactions and matrix effects during RPO analysis.

To compare reaction energetics with measured δ13C and Fm values, we describe the temporal evolution of *p0*(*E*) during an RPO experiment and calculate the average *E* value corresponding to the carbon contained in each fraction. After correcting δ13C values for kinetic isotope fractionation, plots of δ13C vs. *E* and Fm vs. *E* indicate that activation energy is strongly correlated with OC isotope composition for the samples analyzed herein. RPO results are consistent with hypothesized controls on OC source and residence time as determined by bulk and compound-specific isotope measurements. We therefore suggest that paired kinetic and isotopic measurements as determined by RPO analysis can offer novel insight into a range of carbon cycle processes.

**Table and Figure Captions**

Table 1: List of mathematical symbols used throughout this study.

Table 2: Narayani PB-60 measured RPO temperature ranges, CO2 masses, δ13C, Fm, and modeled *E* values for each fraction. Also included are mass-weighted averages [Σ(1–9)] and independently measured bulk isotope values.

Table 3: JGOFS MC-1 measured RPO temperature ranges, CO2 masses, δ13C, and modeled *E* values for each fraction. Also included are mass-weighted averages [Σ(1–5)] and independently measured bulk isotope values.

Table 4: Pololu 4169 measured RPO temperature ranges, CO2 masses, δ13C, Fm, and modeled *E* values for each fraction. Also included are mass-weighted averages [Σ(1–5)] and independently measured bulk isotope values.

Figure 1: Schematic of the RPO instrumental setup. User-defined inputs are printed in blue, while resulting observed measurements are printed in red (See Table 1 for symbol definitions).

Figure 2: RPO mass-normalized thermograms (gray shaded region, units not shown), δ13C values (white circles, left axis), and Fm values (transparent bars, right axis) for **(A)** Narayani PB-60, **(B)** JGOFS MC-1 (Fm not measured), and **(C)** Pololu 4169. Width of Fm bars corresponds to the temperature range of collection for each RPO fraction. Where visible, δ13C error bars represent propagated analytical uncertainty (Fm uncertainty not visible).

Figure 3: Testing the effects of initial mass (*G0*) and ramp-rate (β) on RPO thermograms: **(A)** Narayani PB-60 and **(B)** JGOFS MC-1 analyzed for multiple *G0* values, **(C)** Narayani PB-60 analyzed for multiple β values. Decay rates in panel **(C)** are normalized by *G0* and β in order to properly compare between each analysis.

Figure 4: First-order kinetic assessment: **(A)** decay rate vs. *G0* relationships at four arbitrarily chosen time points for Narayani PB-60, including best-fit regressions (dashed lines); **(B)** mass- normalized decay rates for each analysis used in **(A)**; **(C)** decay rate vs. *G0* relationships at four arbitrarily chosen time points for JGOFS MC-1, including best-fit regressions (dashed lines); and **(D)** mass-normalized decay rates for each analysis used in **(C)**. Linear relationships and nearly identical normalized decay rates in panels **(A-B)** confirm the first-order nature of OC decay, while non-linear relationships and a shifting carbonate peak in panels **(C-D)** indicate non-first-order CaCO3 decay kinetics.

Figure 5: Model residual RMSE using a range of KCE slopes and intercepts for Narayani PB-60 (β = 5°C min-1). Each pixel represents the best-fit result using Equation 3.20 for a given *k0* as determined by Equation 3.21. "Acceptable" fits with residual RMSE ≤ 10−4 are contained within the red dotted line. Estimated result using the method of Miura and Maki (1998) for 3 ramp rates (β = 2, 5, 10°C min-1) is plotted as a white circle, while the point corresponding to *k0* = 1010 sec-1 (the value chosen for all samples in this study) is plotted as a red star.

Figure 6: Tikhonov Regularization L-curves for all samples analyzed (β = 5°C min-1): **(A)** Narayani PB-60, **(B)** JGOFS MC-1, and **(C)** Pololu 4169. White circle corresponds to the point of maximum curvature – *i.e.* the best-fit ω value. Note the ≈ 100× higher residual error for JGOFS MC-1 due to the presence of carbonates.

Figure 7: Mean (black line) and standard deviation (gray shaded region) of regularized *p0*(*E*) distributions for Narayani PB-60 analyzed using a range of *G0* and β values (*n* = 5), indicating that DAEM results are largely independent of experimental conditions for decarbonated samples.

Figure 8: Regularized *p0*(*E*) distributions (black line) and the corresponding subset of *p0*(*E*) that is contained in each RPO fraction (gray shaded region): **(A)** Narayani PB-60, **(B)** JGOFS MC-1, and **(C)** Pololu 4169. Overlapping distributions are a result of the fact that OC described by a single *E* value decays over a range of temperatures.

Figure 9: Plots of resulting *E* vs. fractionation-corrected δ13C or Fm for each sample analyzed: Pololu 4169 [**(A)** δ13C, **(B)** Fm], Narayani PB-60 [**(C)** δ13C, **(D)** Fm], and JGOFS MC-1 [**(E)** δ13C]. Error bars in δ13C and Fm represent propagated analytical uncertainty, while error bars in *E* are the standard deviation contained within each fraction. Blue lines in panel **(C)** and **(D)** are the ΣLC24–32 *n*-alkanoic acid δ13C and Fm values, with shaded regions representing the reported ±1σ uncertainty (Galy and Eglinton, 2011; Galy et al., 2011).