Impacts of Drying and Rewetting on the Radiocarbon Signature of Respired CO2 and the Implications for Incubating Archived Soils

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Key Points:

* ∆14C of CO2 measured in incubations of archived soils provides additional time points for constraining soil carbon models
* Air-drying and rewetting soils shifted the ∆14C of respired CO2 by 10 to 20‰, while the duration of storage had no significant impact
* The direction and magnitude of the shift in ∆14C of CO2 depended on sampling year, and the effect was stronger in grassland soils than forest soils

Abstract

Measuring the radiocarbon signature of respired CO2 (∆14C-CO2) in laboratory incubations is a useful technique for quantifying the rate of soil carbon cycling, as this flux integrates the contribution to respiration from soil carbon pools with a range of intrinsic decomposition rates. Soil archives have the potential to provide multiple observations of ∆14C-CO2 at the same location over time, reducing uncertainty of soil carbon models by providing additional constraints for parameterization. However, air-drying, storage, and subsequent rewetting associated with archiving may lead to changes in the relative contribution of slower and faster cycling soil carbon pools to microbial respiration. We assessed the effects of air-drying, rewetting, and storage duration on 14C-CO2 observed in incubations of soil samples from forests and grasslands collected over the past two decades. We found that air-drying and rewetting led to significant (*p* < 0.05) changes in ∆14C-CO2, with mean differences smaller in forests (12.1‰) than in grasslands (21.4‰), relative to the controls. The duration of storage did not have a significant effect. The ∆14C-CO2 of the rewetting pulse was not significantly different from what was measured during equilibrium respiration, suggesting that air-drying and rewetting induces lasting effects in laboratory incubations, either by making enough substrate available to fuel respiration beyond the rewetting pulse or by promoting microbial recycling. While the shift in ∆14C-CO2 induced by air-drying and rewetting is significant, the differences observed are equivalent to the changes in atmospheric radiocarbon over three to five years (e.g. from 2000 to 2020), indicating that this technique could be a valuable tool for improving soil carbon model uncertainty in the future, but may not be able to resolve subdecadal carbon cycling rates.

**Plain Language Summary**

Soils play a key role in the global carbon cycle by sequestering carbon from the atmosphere for decades to millennia. However, it is unclear if they will continue to provide this ecosystem service as the climate changes. Microbial decomposition of soil carbon returns carbon back to the atmosphere, and radiocarbon dating of this returning CO2 can be used to quantify how long the carbon has been in the soil, proving an estimate for sequestration potential. Incubating archived soils would provide multiple observations of the change in radiocarbon over time, a key insight into the rate at which carbon cycles in and out of the soil. However, air-drying, time spent in archives, and subsequent rewetting of soils may affect the balance of younger versus older carbon leaving the soil, biasing the estimates of soil carbon sequestration potential. We compared the radiocarbon signature of CO2 from soils incubated with and without air-drying and archiving, and found that the air-dried soils appeared to release slightly older carbon than soils that had never been air-dried. The amount of time the soils were archived did not appear to have an effect. However, the effect of air-drying on the age of soil carbon decomposed by microbes was small: increasing the apparent storage time of carbon in soils by four years in forests and eight years in grasslands. These results suggest that incubating archived soils is a promising technique for improving our ability to model near-term soil carbon sequestration and our understanding of global climate change.

1 Introduction

The laboratory soil incubation is a commonly used technique for understanding soil carbon dynamics. Soil carbon is a heterogeneous mixture of organic matter, some components of which persist in the soil for months or years, while others persist for centuries or millennia. The persistence of soil carbon can be understood through the concept of different “pools” of carbon, each defined by the mechanism by which carbon is stabilized in the soil and characterized by a distinct probability distribution of ages (Carlos A. Sierra, Hoyt, He, & Trumbore, 2018).

Natural abundance radiocarbon provides information about carbon ages on centennial or millennial scales, while insight into decadal scale dynamics can be gained from tracing the pulse of radiocarbon introduced into the biosphere from nuclear weapons testing (“bomb-C”) in the mid-20th century (Trumbore, 2009). The bomb-C pulse peaked in the atmosphere in the 1960s, but due to differential rates of biological processing, the peak is lagged in time and dampened in soils. The relative enrichment of bomb-C in different pools of soil carbon is a useful tool for inferring the rate at which carbon enters and leaves, and for a homogenous pool it is functionally equivalent to the intrinsic decomposition rate (Sierra et al., 2017).

Extracting and measuring the radiocarbon content of specific soil carbon pools is hampered by spatial and temporal heterogeneity of the mechanisms that lead to soil carbon persistence, such as physical occlusion in aggregates, association with minerals, or chemical recalcitrance. Defining soil carbon pools empirically with techniques such as density, size, or resistance to chemical attack can be useful, but these methods also introduce artifacts and likely result in mixtures of pools with different age distributions (Feng et al., 2016; Kleber et al., 2011; Lehmann & Kleber, 2015). In contrast, although they also introduce artifacts due to disturbance and potential alteration of the microbial community, laboratory soil incubations of bulk soil make use of the same fractionation agent as is found in situ: the microbial community (Schaedel et al., 2020). Measuring the radiocarbon signal of CO2 (Δ14C-CO2) released in laboratory incubations of bulk soils is thus a powerful tool for understanding the relative processing rate of carbon in soil (or transit time) as it provides an integrated measure of the weighted contribution to the release flux from pools of soil carbon with distinct processing rates (Trumbore, 2000).

Calculating ages and transit times of soil carbon from measurements of Δ14C requires modeling soil carbon decomposition as a function of inputs, outputs, stocks, and if applicable, transfers between different soil carbon pools. Parameterizing these models is challenging, however, due to uncertainty in defining soil carbon pools corresponding to relevant soil carbon stabilization mechanisms, as well as a lack of observational constraints. In particular, due to the curvature of the bomb-C peak there are two points in time at which the atmospheric radiocarbon carbon value was identical, which can lead to multiple model solutions when observations are only made at one point in time (Trumbore, 2000). Observations of Δ14Cat multiple time points can therefore greatly reduce model uncertainty (Baisden, Parfitt, Ross, Schipper, & Canessa, 2013).

Soil archives have proved to be a valuable resource for looking at the change in soil carbon over time, with perhaps the most well-known example being the >150 year archives from the Rothamsted long-term experiments, which were used for parameterizing the soil carbon model Roth-C (Jenkinson, Poulton, & Bryant, 2008). Air-drying soils for storage in archives is a common practice of convenience, but causes long-recognized effects on biological, physical, and chemical properties (Bartlett & James, 1980; Jones et al., 2019). However, the effect of air-drying, storage, and subsequent rewetting on ∆14C-CO2 observed in soil incubations has not been documented.

Incubations of soils following air-drying and rewetting typically leads to a rapid increase in CO2 production, ranging from hours to several days (the Birch effect), before returning to equilibrium respiration rates. This phenomenon has been extensively studied over the past several decades (Birch, 1958; Borken & Matzner, 2009; Schimel, 2018). Hypopthesized sources for the CO2 released following soil rewetting include the lysis of microbial cells subjected to osmotic shock (Warren, 2016; Williams & Xia, 2009), disruption of soil aggregates, osmolytes released from microbes emerging from aridity induced dormancy (N. Fierer, Schimel, & Holden, 2003; Noah Fierer & Schimel, 2002), desorption of mineral-associated organic matter, or a combination of these sources (Slessarev et al., 2020).

Different soils may differ in the susceptibility to drying and rewetting effects due to the relative importance of soil carbon stabilization mechanisms in a particular soil, potentially leading to differences in ∆14C-CO2 following disturbance. Desorption of mineral-associated carbon upon rewetting has been observed for smectite-rich or highly charged soils, while differences in the quantity and rate of CO2 release following rewetting has been shown to vary with soil texture and degree of aggregation (Kaiser, Kleber, & Berhe, 2015). More generally, air-drying appears to result in the formation of new or stronger mineral-organic associations, increased aggregate stability, decreased microbial biomass, and a higher quantity of water-extractable organic matter across a range of different soils (Kaiser et al., 2015).

Ecosystem type may also play an important role in how a soil responds to drying and rewetting. In a study from California, forest soils showed a stronger respiration response to drying and rewetting than grassland soils from an adjacent site (N. Fierer & Schimel, 2003), hypothesized to be due to a greater proportion of microbial biomass in the forest soils. A study comparing forests, grasslands, and croplands in Germany also found differences in the proportion of soil carbon protected in aggregates (Wiesmeier et al., 2014), which could lead to ecosystem level differences in the respiration response if aggregate disruption plays an important role in fueling respiration following drying and rewetting. In another incubation study of California grassland soils, exposure to multiple air-drying and rewetting cycles altered ∆14C-CO2: leading to depletion between the first and last cycle in surface soils, but enrichment in subsoils with more slowly cycling carbon (Schimel, Wetterstedt, Holden, & Trumbore, 2011). This change in the respiration response following exposure to the drying and rewetting stress suggests that the climatic regime of an ecosystem may also play an important role in determining the relative contribution of faster or more slowly cycling soil carbon to heterotrophic respiration following these kind of disturbances.

In longer duration incubations the lack of new inputs to the system is assumed to lead to shifts in substrate utilization from readily available, faster cycling pools to carbon from less available or less accessible pools (Townsend, Vitousek, Desmarais, & Tharpe, 1997; Schädel et al., 2020). The preferential depletion of the most readily available carbon can be detected through the shift in the ∆14C-CO2 measured at the beginning of an incubation compared to ∆14C-CO2 measured at the end of an incubation (Cusack, Torn, Mcdowell, & Silver, 2010; Ewing, Sanderman, Baisden, Wang, & Amundson, 2006; Mueller et al., 2014). In contrast, the microbial community and the substrates accessible to microbes in short-duration incubations can be assumed to be unaffected by substrate limitation, suggesting that ∆14C-CO2 should be close to what is respired *in situ*.

However, if air-drying and rewetting affects the relative contribution of soil organic matter pools with different intrinsic cycling rates to respiration, this should be detectable in ∆14C-CO2. For example, disruption of soil aggregates following drying and rewetting would likely lead to greater accessability of soil organic matter formerly protected from decomposition via physical occlusion. Drying followed be rewetting could also lead to desorption of organic matter sorbed to minerals, increasing the accessibility of this formerly protected substrate. If drying and rewetting mobilizes carbon from these relatively slowly cycling soil organic matter pools the effect should be detectable in ∆14C-CO2. However, if the rewetting pulse derives mainly from lysed microbial cells or the release of microbial osmolytes little change in ∆14C-CO2 would be expected.

Obtaining ∆14C-CO2 measurements from incubations of archived soils would be a valuable tool for further constraining and improving soil carbon models, but first the possible effects of air-drying and rewetting, as well as the effect of storage duration, must be quantified. The direction and magnitude of any change in ∆14C-CO2 induced by these treatments should be indicative of the change in substrate, i.e. increased contribution of either faster or more slowly cycling carbon pools to heterotrophic respiration.

We developed the following hypotheses regarding the potential effects of air-drying, moisture adjustment, and storage duration on ∆14C-CO2 observed in laboratory soil incubations:

1. **Rewetting pulse**: ∆14C of the pulse of CO2 released immediately following rewetting will be older in air-dried soils than non air-dried soils, due to greater mobilization of protected carbon in the air-dried soils;
2. **Equilibrium respiration**: CO2 released after the initial rewetting pulse will be younger than that released during the rewetting pulse, and will not differ between air-dried soils and non air-dried soils;
3. **Storage duration**: duration of storage will not affect ∆14C-CO2 observed in incubations of archived soils.

2 Materials and Methods

We devised three experiments to assess the feasibility of measuring ∆14C-CO2 in incubations of archived soils. Experiment 1 is focused on the effects of air-drying + storage, Experiment 2 on the effect of air-drying alone, without storage, and Experiment 3 on the effect of air-drying + storage with varying storage duration times.

2.1 Sample selection and field sampling

Soils analyzed for Experiment 1 and Experiment 2, as well as a subset of the samples from Experiment 3, were collected at sites from the Biodiversity Exploratories project (Fischer et al., 2010). Experiment 1 samples were collected in 2011 as part of a larger study (Solly et al., 2014). We choose a subset of these samples for the present study from two ecosystem types (forest and grassland) and from a range of soil textural classes: from the relatively sandy soils of the Schorfheide-Chorin geographic region to the more clay-rich soils from the Hainich-Dün. We used the δ13C signature of respired CO2 from the incubations conducted in 2011 to omit samples containing inorganic carbon, conservatively deeming any samples with δ13C-CO2 > -25‰ as potentially affected by the release of inorganic C. We then selected soils from three grassland plots (50 m by 50 m) and three forest plots (100 m by 100 m) from each of the two geographic regions (n total = 12 sites), using the additional criterium that the ∆14C-CO2 observed in the 2011 incubations fell within the within the interquartile range observed for the ecosystem type and region. See Solly et al. (2014) for futher details on soil collection and sampling strategy for Experiment 1 samples.

For Experiment 2, we returned in July 2019 to the Hainich-Dün region to collect new samples from the same plots (both forest and grassland) that were originally sampled in 2011. As we did not observe significant treatment differences between the two geographic regions in the results of Experiment 1, we restricted the resampling to just one region to save on cost and time. At each plot (n = 6) we collected three cores from the same depth interval as 2011 (0-10 cm), which were then homogenized to yield one composite sample. Any aboveground vegetation was clipped, and organic horizons were scraped away prior to coring at the forest plots.

The remaining samples analyzed in Experiment 3 were collected from various locations around the United States over the past two decades (n total sites = 39). All of the remaining samples came from forest ecosystems, as we were unable to obtain any grassland samples for which ∆14C-CO2 had previously been measured. Owing to a lack of samples from deeper soil horizons, the samples included in this study were restricted to the A horizon only (see Supplemental Table 1 for more details on sample provenance and references to the original studies).

2.2 Sample preparation

Experiment 1 and Experiment 2 samples were processed in the same way. However, as Experiment 3 samples were collected as part of different experiments conducted by various investigators, there were slight differences in processing among these samples.

Following sample collection, soils for Experiment 1 and Experiment 2 were sieved to <2 mm at field-moisture and water holding capacity was determined on a 10 g subsample. The remaining soil was then split, with one split air-dried at 40º C (air-dry + storage samples, Experiment 1, and air-dry only samples, Experiment 2), while the other split was left at field moisture (control-1, control-2). Control-1 and control-2 samples were stored in recloseable plastic bags at 4º C until incubation. After air-drying, air-dry + storage samples (Experiment 1) were placed in recloseable plastic bags, and stored inside large plastic boxes in a cool (ca. 15º C) dark room for seven years. Air-dry onlysamples (Experiment 2) were briefly stored under the same conditions as the air-dry + storage samples, but only for three months, which we considered negligible in terms of storage duration.

The majority of Experiment 3 control samples (control-3) were also sieved to <2 mm, but samples from one site (Oak Ridge) were incubated unsieved (Supplementary Table 3). As with control-1 and control-2 samples, control-3 samples were split while still field moist, with one sample split air-dried and placed in storage, while the other was left at field moisture.

Carbon and nitrogen content for both Experiment 1 and Experiment 2 samples was determined by dry combustion in a CN analyzer (Vario Max, Elementar Analysensysteme GmbH, Hanau, Germany) following fine grinding with a ball-mill (Retch MM400, Hanau, Germant) using material from the air-dried sample splits, after correcting for residual moisture content. Inorganic carbon content was determined after combustion of organic carbon in a muffle furnace at 450º C for 16 h. Soil organic carbon content was then calculated as the difference between total carbon and inorganic carbon. Additional analyses performed on the air-dried soils for Experiment 1 and Experiment 2 included texture (pipette method following removal of organic matter, Schlichting et al., 1995) and pH (1:2.5 mixture of soil and 0.01 M CaCl2). Details of analyses performed on Experiment 3 samples are given in Supplementary Table 1.

2.3 Incubation conditions

Incubation conditions for Experiment 1 and Experiment 2 were similar and are described jointly in section 2.3.1, as well as summarized in Table 1. As with sample preparation, incubation conditions for Experiment 3 differed from Experiments 1 and 2, and are therefore described separately in section 2.3.2, and summarized along with sample preparation details in Supplementary Table 1.

2.3.1 Experiment 1 and Experiment 2

Experiment 1 air-dry + storage incubations and all Experiment 2 incubations were conducted in duplicate, while control-1 incubations were performed on single samples (Table 1). Soils were weighed out into 250 ml beakers and placed into 1000 ml mason jars with airtight lids fitted with two sampling ports. The mass of soil used for control-1 samples ranged from 70 g to 250 g (air-dry equivalent), depending on the soil carbon content. Owing to limited sample quantity remaining for the air-dry + storagesamples, only 20 g of soil was used for these incubations and we opted to use this same mass of soil for all Experiment 2 incubations. Soil moisture content of all Experiment 1 and Experiment 2 samples was adjusted to 60% of water holding capacity (WHC) prior to sealing the jars, either from field moisture status (control-1 and control-2 samples) or from air-dry status (air-dry + storage and air-dryonly samples). Following moisture adjustment, jars were flushed with CO2-free air and left to incubate for a four-day pre-incubation period. After the pre-incubation period the jars were flushed again, and CO2 was allowed to accumulate for a second enclosure period (equilibrium respiration period). All samples were incubated at 20º C.

The total incubation duration varied among samples in both Experiment 1 and Experiment 2. The equilibrium respiration period for the control-1 incubations was set at 14 d in order to allow for adequate time to observe CO2 fluxes, as this was a key goal of the original study (Schöning et al. unpublished). In contrast, the duration of the equilibrium respiration period for control-2 samples was not determined by a set time, but rather by the amount of CO2 respired, as the primary goal for these incubations was to measure ∆14C-CO2. Accordingly, control-2 incubations were allowed to proceed until CO2 concentrations were adequate for measuring the radiocarbon content of respired CO2. Since high CO2 concentrations can affect soil pH, a conservative target of 1% CO2 in the jar headspace was chosen to limit potential pH effects but still allow for sufficient CO2 production. The duration of the equilibrium respiration period in treatment sample incubations in both Experiment 1 and Experiment 2 (air-dry + storage and air-dryonly samples, respectively) was determined by the amount of CO2 respired by the corresponding control-1 and control-2 samples: treatment sample incubations were allowed to proceed until the same amount of carbon had been respired per g of initial soil C as in the control incubations.

Headspace gas sampling was conducted at slightly different times for control-1 samples than for air-dry + storage samples in Experiment 1 and for both control-2 and air-dry only samples in Experiment 2. Headspace CO2 concentrations for control-1 incubations were measured once at the end of the pre-incubation period, and then on days 1, 3, 7 and 14 of the equilibrium respiration period. For air-dry + storage samples, and both control-2 and air-dryonly samples, we measured sample headspace CO2 concentrations daily during the pre-incubation period, thrice weekly for the first two weeks of the equilibrium respiration period, and once per week thereafter as needed. Headspace gas samples were collected and analyzed for ∆14C and δ13C content at the end of both the pre-incubation period and the equilibrium respiration period for all incubations except for the control-1 samples, for which ∆14C-CO2 was only measured for the second period. Due to low rates of respiration, only nine of the twelve air-dry + storage samples from Experiment 1 respired enough CO2 to measure ∆14C following the pre-incubation period; however all of the Experiment 2 samples respired adequate CO2 for measuring ∆14C following pre-incubation.

2.3.2 Experiment 3

We attempted to replicate the control-3 incubation conditions as closely as possible when conducting the corresponding air-dry + storage treatment incubations, but owing to missing data from the original incubations and limited sample quantity this was not entirely possible. Replication and the mass of soil incubated in control-3 incubations varied according to the experimental design of the original experiment (Supplementary Table 1). Due to limited sample mass, replication and soil mass for the storage duration treatment incubations diverged in some cases from the corresponding control-3 incubations (Supplementary Table 1). Although moisture content varied among control-3 sample incubations, we kept it consistent between paired control-3 and storage duration treatment incubations (Supplementary Table 1).

Treatment samples for Experiment 3 were only incubated for a single enclosure period as data on the pre-incubation period duration and CO2 release were not available for many of the control-3 samples. Incubation vessels were immediately sealed following moisture adjustment of the air-dry + storage treatment samples and soils were allowed to respire until an equivalent amount of CO2 had been released (mg CO2 g soil C-1) as during the equilibrium respiration period of the corresponding control-3 samples. Headspace CO2 concentrations were measured every three days for the first two weeks of the treatment sample incubations, and weekly as needed thereafter. The duration of the equilibrium respiration period and CO2 measurement frequency for control-3 sample incubations varied (Supplementary Table 1). Radiocarbon and δ13C measurements were measured at the end of the equilibrium respiration period for control-3 samples and at the end of the single enclosure period for the air-dry + storage treatment samples.

2.4 Isotopic analyses

For all three experiments, we separated CO2 from the gas samples collected from incubation jar headspace using a vacuum line, with splits of the purified CO2 analyzed for both δ13C and ∆14C. Radiocarbon analyses for Experiments 1, 2, and air-dry + storage samples from Experiment 3 were conducted at the Max Planck Institute for Biogeochemistry (MPI-BGC) accelerator mass spectrometer facility, while the control-3 samples from Experiment 3 were analyzed at the University of California Irvine (UCI) W.M. Keck Facility for Accelerator Mass Spectrometry. Radiocarbon values are reported in units of ∆14C, defined as the deviation in parts per thousand of the ratio of 14C to 12C from that of the oxalic acid standard measured in 1950. In order to account for potential mass-depenedent fractionation effects, the 14C/12C ratio of all samples was corrected to a common δ13C value of -25 per mil (Steinhof, 2013; Stuiver & Polach, 1977).

The ∆14C notation is particularly useful for discussing decadal scale carbon dynamics, as positive values reflect the presence of bomb-produced carbon that was fixed from the atmosphere at some point over the the past several decades, while negative values indicate the dominance of carbon fixed at some point prior to 1950. An additional unit used in this study is ∆∆14C, which is defined as the difference between the ∆14C measured in a sample and that of the atmosphere in the year of sampling. Highly negative ∆∆14C values would be expected to be found in systems with slower carbon dynamics, due to the process of radioactive decay, while systems dominated by annual inputs should have small positive ∆∆14C values, and at the present time (2020) systems dominated by decadally cycling C could be expected to have relatively large positive ∆∆14C values. However, due to the mixing of fast and slow cycling carbon in soils, ∆∆14C cannot be used in isolation for inferring soil carbon processing rates.

As with the radiocarbon analyses, measurements of δ13C for Experiments 1, 2, and treatment samples from Experiment 3 (storage duration samples) were made at MPI-BGC (**instrument**), while control samples for Experiment 3 (control-3 samples) were measured at UCI (**instrument**). Data are reported using δ13C notation, which refers to the deviation in parts per thousand of the ratio of 12C to 13C in the PDB standard.

2.5 Statistical analysis

We determined the statistical significance of differences between treatment effects using paired t-tests (alpha = 0.05). In order to identify potential influences on the observed treatment effects we performed a linear regression analysis using the difference between treatment and control ∆14C-CO2 as the response variable, and the difference in CO2 respired (control – treatment), soil carbon and nitrogen content, change in moisture content upon rewetting, and particle size as explanatory variables. All statistical analyses were performed in R (R Core Team 2019).

3 Results

3.1 Respiration rates

The increase in respiration rates following moisture adjustment was significantly greater for air-dried soils than field-moist soils for both Experiment 1 (air-dry + storage treatment) and Experiment 2 (air-dry only treatment) when compared within ecosystem types. However, the magnitude and timing of the peak respiration rate response diverged among experiments and between grassland and forest soils (Fig. 1). Respiration data for Experiment 3 samples are shown separately in the supplementary materials (Supplementary Fig. 1) as the samples in Experiment 3 were only incubated for a single enclosure period and were adjusted to a different incubation moisture content than Experiment 1 and Experiment 2 samples,. Additionally, CO2 concentration was only measured at a single time point for some of the Experiment 3 samples, limiting our ability to compare respiration rates.

Among the air-dry + storage samples in Experiment 1, respiration rates were more than twice as high in grassland soils than in forest soils. Grassland air-dry + storage samples reached a maximum of 3.8 mg CO2 g soil C-1 d-1 after 92 h followed by a sharp decline (blue dashed lines, Fig 1a), while mean respiration rates in air-dry + storage forest sites peaked at 1.5 mg CO2 g soil C-1 d-1after 166 h, followed by a much more gradual decline (brown dashed lines, Fig. 1a). In contrast, control-1 forest and grassland samples (solid brown and blue lines, Fig. 1a) responded more weakly and more gradually than the air-dry + storage samples, but with similar trends with respect to ecosystem type. Respiration rates were higher in control-1 grassland soils than in control-1 forest soils: peaking at 1.9 and 0.6 mg CO2 g soil C-1 d-1 for grassland and forest soils respectively, after the first day of the second enclosure period (115 h).

We observed similar patterns in the respiration rates of Experiment 2 samples as seen in Experiment 1, with the air-dry only treatment samples respiring at a significantly higher rate than control-2 samples (p < 0.05) (Fig. 1b). However, unlike the air-dry + storage treatment in Experiment 1, peak respiration rates in forest and grassland soils were not significantly different (p > 0.05) for the air-dry only samples in Experiment 2, peaking at 3.0 and 3.3 mg CO2 g soil C-1 d-1 after 95 h for grassland and forest soils, respectively.

Soils incubated in Experiment 1 were collected in the spring of 2011, while soils incubated in Experiment 2 were collected in summer 2019, leading to differences in the field-moisture content, as well as differences in prior exposure to drying and rewetting cycles between the two experiments. Mean field moisture contents of soils collected for Experiment 1 were 25.7% WHC (±1.0% SD) for forest soils and 23.9% WHC (±2.0% SD). In comparison, mean field moisture contents of soils collected for Experiment 2 were slightly higher, with the increase greater for grassland soils than for forests: 27.9% WHC (±1.4% SD) for forests and 31.4% WHC (±3.5%) for grasslands. Due to these differences in field-moisture content, we added a greater amount of water to the control-1 treatment samples in order to reach the target incubation moisture content of 60% WHC than we added to control-2 samples to reach the same target WHC.

3.2 Rewetting pulse versus equilibrium respiration

Despite the significant differences in respiration rates, and in contrast to the rewetting pulse hypothesis (H1), we did not observe significant differences between ∆14C-CO2 respired during the pre-incubation period and ∆14C-CO2 respired during the equilibrium respiration period (Fig. 2). The lack of significance was consistent across all samples for which ∆14C-CO2 was measured for both enclosure periods: air-dry + storage samples in Experiment 1 (open squares, Fig. 2), and both air-dry only (open circles, Fig. 2) and control-2 (filled circles, Fig. 2) samples in Experiment 2. We did not find a significant interaction with ecosystem type or experiment in a linear regression model using the difference between pre-incubation and second enclosure period ∆14C-CO2 as the response variable, so all data were pooled for statistical analysis.

There was one outlier forest site among the control-2 samples for which the pre-incubation CO2 was substantially depleted relative to equilibrium period respiration (Fig. 2). However, even when this outlier was included in the statistical analysis, the difference between pre-incubation ∆14C-CO2 and equilibrium ∆14C-CO2 was not significant. Due to lower respiration rates during pre-incubation only three of the six air-dry + storage treatment samples from the forest soils incubated in Experiment 1 (open brown squares, Fig. 2) generated enough CO2 to measure the radiocarbon content. In addition, it was not possible to compare pre-incubation and equilibrium respiration ∆14C-CO2 for the control-1 samples as pre-incubation ∆14C-CO2 was not measured for these samples in 2011.

Unlike the ∆14C-CO2 response, we did observe significant differences between the δ13C-CO2 of the rewetting pulse and that of the equilibrium period for one of the treatment groups, the air-dry + storage samples in Experiment 1. Equilibrium respiration CO2 of the air-dry + storage forest soils was significantly enriched in δ13C compared to the rewetting pulse CO2 of the same samples (-24.2‰ ± SD of 1.1‰ and -25.7‰ ± SD of 0.8‰, respectively), while we observed the opposite for the air-dry + storage grassland samples: δ13C-CO2 of the equilibrium respiration period was significantly depleted relative to the rewetting pulse CO2 (-27.2‰ ± SD of 0.5‰ and 26.9‰ ± SD of 0.4‰, respectively). We did not observe significant difference in δ13C between the rewetting pulse CO2 and the equilibrium period CO2 for any of the samples in Experiment 2, and as with ∆14C, we did not measure the δ13C-CO2 during the pre-incubation period of the control-1 samples.

3.3 Overall treatment effects on equilibrium respiration

We observed consistent differences between control and treatment sample ∆14C-CO2 during the equilibrium respiration period across all experiments, in contrast to the expectations of H2. Due to the lack of a signicant difference between ∆14C-CO2 observed in the pre-incubation period and ∆14C-CO2 observed in the equilibrium respiration period in Experiments 1 and 2, we have included the data from Experiment 3 with just a single enclosure period in the overall analysis of the treatment effects (Fig. 3).Treatment sample incubations, including both the air-dry + storage treatment (Experiments 1 and 3), and the air-dry only treatment (Experiment 2), typically resulted in differences between 20‰ and 40‰ (Fig. 3, dashed and dotted lines, respectively) relative to control sample incubations, with the majority within ±20‰. The range of 20‰ to 40‰ is shown for context: these differences correspond to the change in ∆14C of the atmosphere over four and eight years, respectively, during the period of 2000 to 2020.

The samples from Oak Ridge (magenta points, Fig. 3) are an exception in that the differences between the air-dry + storage treatment and corresponding control-3 samples exceeds 40‰ for some samples. However, these points show the impact not only of bomb-C, but also additional enrichment from exposure to a localized plume of 14C-enriched CO2, which was released from a nearby incinerator four years prior to sample collection (Cisneros-Dozal, Trumbore, & Hanson, 2006). In these samples, the depletion observed in the ∆14C-CO2 of the air-dry + storage samples relative to the controls provides strong evidence that the treatment increased the respiration contribution of carbon fixed prior to this incinerator plume enrichment event (> 4 y).

Ecosystem type had a significant effect on the shift in ∆14C-CO2 following treatment in all three experiments. Grassland samples tended to show enrichment following treatment (circles above the 1:1 line, Fig. 3) while forest samples showed depletion (triangles below the 1:1 line, Fig. 3), regardless of origin. Forest samples from the air-dry only treatment in Experiment 2 (black triangles above 1:1 line in Fig. 3) are a notable exception in that equilibrium respiration was more enriched in ∆14C-CO2 relative to the corresponding control-2 samples. Interestingly, this response is in contrast to what we observed for the air-dry + storage treatment samples collected seven years earlier (in 2011) at the same sites (Hainich-Dün, Central Germany), which were depleted in ∆14C-CO2 relative to the control-1 samples.

3.4 Storage duration effect on ∆14C-CO2

We used data from both Experiment 1 and Experiment 3 to test the effect of storage duration (H3), but in line with our initial hypothsis, we did not find it to be a significant predictor of the difference between control and treatment ∆14C-CO2 (Fig. 4). When considered in isolation, the highly enriched samples from Oak Ridge, which were measured at two different points over the duration storage (5 and 14 y), we did observe a slight increase in the difference between control and treatment ∆14C-CO2 with the increase in storage duration. These samples should be more sensitive to potential losses of the most recently fixed carbon, as the experimental label should only be present in this pool of soil C, and these data suggest that this may be the case for these samples.

In contrast to ∆14C trends, treatment samples in Experiment 1 and Experiment 2 consistently showed significant enrichment in δ13C-CO2 relative to the controls for both forest and grassland soils. For the air-dry + storage samples in Experiment 1, the mean difference in δ13C-CO2 was greater for forest soils (-2.58‰ ± SD of 0.32‰) than for grassland soils (-0.51 ± SD of 0.21), while the mean differences for the air-dry only samples in Experiment 2 were similar for both forest and grassland soils (-1.56‰ ± SD of 0.67‰ and -1.11‰ ± SD of 0.19‰, respectively) (Supplementary Fig. 3).

3.5 Time series analysis of ∆∆14C-CO2 (Experiments 1 and 2)

The change over time in ∆14C-CO2 of respiration compared to the change over time in atmospheric ∆14C can provide insight into the relative contribution of more slowly cycling carbon versus faster cycling carbon pools for given site. A subset of the locations sampled for Experiment 1 in 2011 were resampled for Experiment 2 in 2019, allowing us to assess both the change in the ∆14C of respired carbon over time in control-1 versus control-2 samples, as well as the potential impact of air-drying and rewetting on any observed changes.

We observed that the air-dry + storage treatment had a significant effect on ∆14C-CO2 in both Experiment 1 and Experiment 2, across ecosystem types, when assessed using paired t-tests (Table 3). The absolute value of the mean difference in ∆14C-CO2 between control and treatment samples was greater in grassland samples than in forest samples for both Experiment 1 and Experiment 2. Averaged across experiments these differences were 21.4‰ and 12.1‰ in grassland and forest soils respectively. Mean differences within laboratory duplicates were lower than treatment differences for both Experiment 1 and Experiment 2, but differences among duplicates were slightly higher for forest samples (8.1‰, standard deviation ±3.8‰) than for grassland samples (5.0‰, standard deviation 3.8‰).

Relative to the atmosphere, mean Δ14C-CO2 was enriched in both treatment and control samples collected in 2011 (Experiment 1) and 2019 (Experiment 2) (Fig. 5). However, ∆∆14C was smaller for grassland samples than for forest samples at both time points, indicating that respiration in the grassland soils contained a greater proportion of more recently fixed carbon (Table 3). When comparing the change in ∆∆14C-CO2 over time in control versus treatment samples we also observed slightly different trends in grassland soils as compared to forest soils. For grassland samples we saw an increase in ∆∆14C between 2011 and 2019 for both control and treatment samples. In contrast, control samples from the forested sites had similar mean ∆∆14C values in both 2011 and 2019, while mean ∆∆14C observed in forest treatment samples increased over this period (Table 3).

4 Discussion

The results from all three experiments in this study show that measuring ∆14C-CO2 in incubations of air-dried and archived soils is a promising technique for improving our understanding of soil carbon dynamics. The two treatments considered (air-drying alone or air-drying + storage prior to moisture adjustment) both resulted in significant differences in ∆14C-CO2 relative to control incubations of soils that had never been air-dried. However, while significant, these differences were relatively small (Table 3). For context, the average rate of change in atmospheric ∆14C over the past two decades has been 5‰ (Graven et al., 2017), meaning that the differences of roughly 10‰ to 20‰ that we observed in the first two experiments of this study (for which the control and treatment incubations were conducted in the same laboratory), would equate to an offset of two to four years in the apparent mean age of respired carbon if the system were interpreted with a simple one pool model. Taking into account variability within ecosystems and treatment groups, these differences suggest that samples would have to be collected at least seven to eight years apart to detect a significant change over time when comparing ∆14C-CO2 observed in fresh samples, i.e. incubated without air-drying, and ∆14C-CO2 observed in incubations of air-dried and subsequently rewet soils obtained from an archive. However, this is only true for the past two decades, since between the 1960s and 1990s the annual rate of change in atmospheric ∆14C was much greater, which would lead to correspondingly greater differences between samples collected over a smaller time period.

Data from samples collected at the same location (Hainich-Dün) at two different time points (for Experiment 1 in 2011 and Experiment 2 in 2019) illustrate both the potential of the archive incubation technique and the challenges inherent in interpreting radiocarbon data. The one pool model interpretation mentioned above, for example, has been found repeatedly to be inadequate for explaining soil carbon dynamics [cite]. A slightly more complex system with two pools and no transfer of carbon between the two pools (a two-pool parallel model), is a more helpful tool for interpreting the results of this study (C. A. Sierra, Müller, & Trumbore, 2014). Such a model system is depicted in Fig. 6, with one pool of soil carbon cycling faster (magenta lines), one pool cycling more slowly (blue lines), and the respiration flux, as it must by definition, tracing a path between the two curves.

The ∆14C of the fast cycling carbon pool in Fig. 6, with an intrinsic decomposition rate (*k*) of 1/6, becomes enriched relative to the atmosphere in the mid-1970s, but as the carbon in this pool turns over relatively quickly, the ∆14C then follows the decline in the atmosphere with a slight offset. The ∆14C of the slowly cycling pool (k = 1/100) does not cross the atmospheric curve (gray line, Fig. 6) until just after the year 2000, and then after crossing it stays relatively enriched, buoyed by the slow turn-over of bomb carbon incorporated over the past several decades. Finally, the shape of the respiration flux curve is similar to that of the fast cycling soil carbon pool, since fast-cycling carbon dominates the respiration flux, but due to the contribution of the slow cycling pool the respiration curve appears slightly compressed by comparison.

In a two pool model system an increase in the relative contribution of the one of the pools to the respiration flux due to a disturbance, such as air-drying and rewetting, could lead to depletion of the respiration flux at one point in time yet lead to enrichment at a later point in time. This scenario is illustrated in Fig. 6b. Increased contribution from the slow cycling soil carbon pool (open gold squares) pulls the ∆14C of respiration closer to the slow pool curve, leading to depletion of ∆14C-CO2 relative to the control (filled gold circles) in 1991, but enrichment of ∆14C-CO2 in 2019 (Fig. 6b). If the treatment were to increase the contribution from the fast pool (open gold circles, Fig. 6b), the same reversal would occur, but in the opposite direction, i.e. enrichment in 1991, but depletion in 2019. The apparent reversal of the air-dry + storage treatment effect between 2011 and 2019 in the Central German forest soils observed in this study (Fig. 5, Table 3) could be explained by this phenomenom. Alternatively, the effect of air-drying + storage may be different than that of air-drying only. However, the grassland samples analyzed in 2011 and 2019 from the same region as the forest soils responded with enrichment to both air-drying + storage and air-drying alone, lending support to the interpretation that the same mechanism is operating in both soils, but with different effects due to differences in the relative cycling rate of carbon in forest versus grassland ecosystems.

A key difference in carbon cycling between forest and grassland ecosystems is the potential for storage of carbon in woody tissues after it is fixed from the atmosphere (Gaudinski, Trumbore, & Davidson, 2000). Carbon entering the soil in forest ecosystems, then, may be “pre-aged” compared to inputs in grassland ecosystems. Earlier work in some of the same forest and grassland ecosystems analyzed in this study (the Hainich-Dün and Schorfheide-Chorin regions) provides support for the pre-ageing of carbon in forest ecosystems: Solly et al. (2013) found the mean age of fine roots in the forest ecosystems to be approximately 10 y, in comparison to 1 to 2 y for fine roots in the grassland ecosystems. This pre-aging, or lag effect, may explain the greater ∆∆14C values seen for the respiration from forest ecosystems as compared to the grassland ecosystems in this study (Table 3). Additionally, a greater lag in forest systems would also mean that the crossing point for the ∆14C curves of the slow and fast cycling soil carbon pools (e.g. the blue and magenta lines in Fig. 6), would be later in time relative to grassland soils. Again, if the fast and slow soil carbon pool curves crossed between 2011 and 2019 for the forest soils, but had already crossed by 2011 in the grassland soils, this would explain the differences in the treatment responses observed in this study between the two ecosystem types.

In contrast to our second hypothesis, we found that air-drying and rewetting soils prior to incubation, a common practice in many studies, significantly changed the ∆14C of respired CO2 as compared to control sample incubations in which the soils had never been air-dried. The shift in ∆14C-CO2 following air-drying and rewetting was consistent for both soils that had been stored in archives following air-drying (Experiment 1 and Experiment 3), as well as soils that were air-dried and then rewetted soon after, without long-term storage (Experiment 2). In line with our third hypothesis, we did not find that storage duration (Experiment 3) had a significant effect on the difference between control sample ∆14C-CO2 and air-dry + storage sample ∆14C-CO2. Together, these results suggest that the shifts in ∆14C-CO2 observed in this study are driven primarily by air-drying and rewetting, rather than from ageing of soil C or some other storage related mechanism.

Our first hypothesis was focused on the ∆14C of the rewetting pulse, the CO2 released immediately following moisture adjustment, which we thought would be older than the CO2 released during equilibrium respiration. However, in contrast to our expectations, we did not find a significant difference in ∆14C-CO2 between these two respiration periods. This finding was true all of the samples in which we measured ∆14C-CO2 in both the pre-incubation and the equilibrium respiration period (Fig. 3). These results suggests that the change in substrate availability initiated by air-drying and rewetting may not be limited to the rewetting pulse, potentially persisting throughout the incubation. (For context, the mean of amount of CO2 respired in the incubations in this study was 0.8 percent of the initial carbon).

Previous studies have found mechanistic evidence for microbial osmolytes or lysed cells providing the fuel for the pulse of CO2 observed following rewetting of dried soils (N. Fierer & Schimel, 2003), as well as extracellular carbon (Xiang, Doyle, Holden, & Schimel, 2008). The ∆14C-CO2 results from this study provide support for a mechanism that makes a new pool of extracellular carbon available to the microbial community: additional substrate with a distinct ∆14C signature. This pool of additional carbon is likely a primary driver of the increase in respiration rates seen following moisture adjustment in air-dried soils (Fig. 1), but it is not clear from our data whether a sufficient quantity of additional substrate is made available to fuel respiration into the equilibrium period, or whether we are observing the same ∆14C-CO2 during both the pre-incubation period and the equilibrium respiration period due to microbial recycling.

The consistent enrichment in δ 13C-CO2 seen following both the air-dry + storage treatment and the air-dry only treatment could indicate that an increase in microbially derived carbon is also contributing to the increase in respiration rates following rewetting. The accompanying change in ∆14C could indicate that the air-drying and rewetting treatment mobilized carbon from microbes that have been dormant for decades. However, older soil organic matter also tends to be enriched in 13C (Wynn, Bird, & Wong, 2005), meaning that the enrichment in δ13C-CO2 could be simply a function of older substrate fueling both the rewetting pulse and the subsequent respiration. Alternatively, the enrichment in δ 13C following treatment could also potentially indicate that we are seeing more microbial recycling in the treatment incubations, as this process has also been shown to lead to enrichment in δ13C (Wynn et al., 2005).

The greater enrichment in δ13C-CO2 seen in forest soils as compared to grassland soils could be an indication of different carbon sources mobilized by the air-drying and rewetting treatment. For example, mineral-associated carbon has been shown to be more enriched in δ13C, and therefore it is possible that more mineral associated carbon was released upon rewetting in the forest soils than in the grassland soils. Additionally, specific compounds found in plant tissues exhibit a range of δ 13C from ±5‰ relative to bulk leaf δ 13C (Bowling, Pataki, & Randerson, 2008), suggesting a possible role of different plant derived metabolites being made preferentially available in forests compared to grasslands following air-drying and rewetting. However, the similarity in the direction of the δ13C-CO2 response across forest and grassland soils suggests that a similar mechanism is at work in both ecosystems.

Nearly all of the forest soils analyzed in this study exhibited depletion of ∆14C-CO2 following air-drying + storage (Fig. 5). The depletion in the forest soils was greatest in the samples from Oak Ridge (magenta triangles, Fig. 5), which had been substantially enriched in ∆14C above background levels through artificial means. These samples were included precisely because the highly enriched label was concentrated in the most recently fixed carbon, and therefore should be a sensitive indicator of mobilization of older (> 5 y) versus younger soil carbon. If we assume that the same mechanism is operating in the forest soils from all of these sites, the Oak Ridge data provides strong evidence that the shift in ∆14C-CO2 following air-drying is due to an increase in the contribution of more slowly cyling carbon to respiration, i.e. carbon that was fixed from the atmosphere in over the past few decades, rather than the most recently fixed carbon.

The exceptions to the general trend of depletion in forest soils following air-drying are one sample from Harvard Forest, and the three forest samples from Central Germany that were collected in 2019 and did not undergo storage. The behavior of the anomalous Harvard Forest sample is hard to explain. However, the different behavior of the Central German forest soils from 2019 could be explained with the same logic as above: by the year 2019, even in forest soils with lagged inputs, the more slowly cycling soil carbon pool in these surface soils would be enriched relative to both the fastest cycling pool and the atmosphere due to the slow rate of turn-over of bomb carbon incorporated decades earlier.

In contrast to the forest sites, almost all of the soils from grassland sites exhibited enrichment in ∆14C-CO2 following air-drying + storage (Fig. 3). However, we suggest that the data are consistent with the same general mechanism as in forests: that air-drying and rewetting of grassland soils increases the contribution of more slowly cycling carbon to respiration. There are two compelling explanations for why this increased contribution from the slow pool leads to enrichment in grasslands, rather than depletion as in the majority of the forest soils we analyzed. First, the oldest grassland samples analyzed were not collected until 2011, on average several years later than the forest soils, and second, as discussed previously, the crossing point of the fastest cycling soil carbon pool and the more slowly cycling pools should occur earlier in grassland soils due to the much smaller lag effect. The grassland soils that were observed in both 2011 (Experiment 1) and 2019 (Experiment 2) both showed enrichment following air-drying and rewetting, suggesting that the crossing point of the slow and fast cycling carbon curves occurred prior to 2011 in these soils.

Differences between control and treatment ∆14C-CO2were significantly greater in grassland soils than in forest soils for all three experiments, suggesting either that protected carbon may be more suceptibe to air-drying and rewetting in grasslands than in forests, or that the pool of carbon mobilized by these disturbances is larger in grassland soils than in forests. The mobilization of a larger pool of soil C following rewetting of grassland soils is supported by the significantly greater increase in respiration rates that we observed in grassland soils as compared to forest soils. Slightly more carbon was respired in the air-dry + storage (Experiment 1) and air-dry only (Experiment 2) treatment incubations than in the corresponding control incubations due to the larger amount of CO2 released following rewetting. However, as there was no change in between pre-incubation period ∆14C-CO2 and equilibrium period ∆14C-CO2 within incubations, this indicates that the treatment differences are not driven solely by the amount of CO2 released, but rather by changes in the specific pools of carbon fueling respiration.

5 Conclusion

Air-drying and rewetting of soils leads to significant differences in the ∆14C of respired CO2 in laboratory incubations when compared to incubations of the same soils without air-drying. These magnitude of these differences do not appear to be affected by the duration of storage and are within 20‰ for the majority of forest soils and 40‰ for the more limited number of grassland samples studied. Differences of this magnitude correspond to 4 to 8 y of change in atmospheric ∆14C over the first decade of the 20th century, meaning this technique may not be sensistive enough to detect sub-decadal changes in ∆14C-CO2. Determining the exact mechanism driving the differences in ∆14C-CO2 is beyond the scope of this study, but our results indicate that the pulse of CO2 released upon rewetting air-dried soils is fueled predominantly by older carbon, and furthermore, that this shift in ∆14C-CO2 persists into the equilibrium respiration period. The shift in ∆14C-CO2 is greater for grassland than for forest soils, suggesting that the pool of carbon that is mobilized by this process is either more susceptible or larger in grassland ecosystems than in forests. Overall, the results of this study suggest that measuring the ∆14C of respired CO2 in laboratory incubations of archived soils is a promising technique for improving quantitative interpretation of soil C dynamics and can provide a strong constraint for soil C models in the future.

Acknowledgments, Samples, and Data

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