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

**Jeffrey Beem-Miller1, Marion Schrumpf1, Alison Hoyt1,2, Georg Guggenberger3, and Susan Trumbore1,4**

1Max Planck Institute for Biogeochemistry, Jena, Germany, 2Lawrence Berkeley National Laboratory, Berkeley, CA, USA, 3Institute of Soil Science, Leibniz Universität Hannover, Hannover, Germany, 4Department of Earth System Sciences, University of California, Irvine, CA, USA

Corresponding author: Jeffrey Beem-Miller ([jbeem@bgc-jena.mpg.de](mailto:jbeem@bgc-jena.mpg.de))

**Key Points:**

· ∆14C of CO2 measured in incubations of archived soils provides additional constraints for 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, with stronger effects in grassland than in forest soils

Abstract

The radiocarbon signature of respired CO2 (∆14C-CO2) measured in laboratory soil incubations integrates the contribution of soil carbon pools with distinct intrinsic decomposition rates. ∆14C-CO2 can thus be used to infer transit times of carbon­—the time between entering and exiting the soil. Incubating archived soils enriched by “bomb-C” from mid-20th century nuclear weapons testing would enable us to trace this pulse over time, a potentially powerful soil carbon model constraint. However, air-drying, duration of storage, and subsequent rewetting of archived soils may each lead to changes in the relative contribution to respiration from faster or more slowly cycling soil carbon pools. We assessed these effects on ∆14C-CO2 measured in incubations of forest and grassland soils conducted before and after 7-14 years of storage. Air-drying and rewetting led to small but significant (*p* < 0.05) shifts in ∆14C-CO2 relative to undried controls,with mean differences smaller in forests (12.1‰) than in grasslands (21.4‰); storage duration had no significant effect on ∆14C-CO2. Relative to the change in atmospheric ∆14C over the study period, these shifts suggest a bias of 2 to 4 years for forest and grassland soil transit times, respectively. Our results indicate that air-drying and rewetting soils mobilizes a distinct pool of carbon that would otherwise be inaccessible to microbes, an effect that persists throughout the incubation. However, as the bias in ∆14C-CO2 from air-drying and rewetting is small, measuring ∆14C-CO2 in incubations of archived soils appears to be a promising technique for constraining soil carbon models.

**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 do so as the climate changes. Microbial decomposition of soil organic matter returns carbon back to the atmosphere, and radiocarbon dating of this returning CO2 (∆14C-CO2) can be used to quantify how long carbon is stored in soils. Incubating archived soils could provide unique insight into soil carbon sequestration potential by quantifying the change in ∆14C-CO2 over time. However, air-drying, duration of archiving, and subsequent rewetting of soils may bias estimates of sequestration potential by altering the balance of younger versus older carbon leaving the soil. We compared ∆14C-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 (by two years in forests and four years in grasslands). The amount of time the soils were archived did not have an effect. Since the bias from air-drying and rewetting was small, incubating archived soils appears to be a promising technique for improving our ability to model soil carbon cycling under global climate change.

**1. Introduction**

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 (Sierra et al., 2018).

Depletion of natural abundance radiocarbon due to radioactive decay 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. Thus 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 the soil (Sierra et al., 2017).

Extracting and measuring the radiocarbon content of specific soil carbon pools is hampered by spatial and temporal heterogeneity of the processes 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 size, density, or resistance to decomposition from acid, alkaline, or oxidative reagents can be useful. However, these methods result in mixtures of pools with different age distributions (Feng et al., 2016; Kleber et al., 2011; Lehmann & Kleber, 2015). In contrast, although they may introduce artifacts due to disturbance and potential alteration of the microbial community, laboratory incubations of bulk soil make use of the same fractionation agent as is found *in situ*: the microbial community (Schädel et al., 2020). Measuring the radiocarbon signal of CO2 (Δ14C-CO2) released in laboratory incubations of bulk soils provides an integrated measure of the weighted contribution to the soil efflux from carbon pools with distinct processing rates (Trumbore, 2000). Using this integrated signal provides key insights into the relative processing rate of carbon (or transit time) in soil, i.e. the average period of time between when an atom of carbon enters the soil and when it leaves.

Calculating ages and transit times of soil carbon from measurements of Δ14C requires a model. Soil carbon dynamics are typically modeled as a function of carbon inputs, outputs, stocks, and if applicable, transfers between different soil carbon pools. However, parameterizing soil carbon models is challenging due to the uncertainty in defining soil carbon pools that correspond to relevant soil carbon stabilization mechanisms. Additionally, due to the curvature of the bomb-C peak, there are two points in time at which the Δ14C signature of atmospheric CO2 was identical, which can lead to multiple model solutions if observations of the system are made at only one point in time (Trumbore, 2000). Observations of Δ14Cat multiple time points is therefore a powerful tool for reducing model uncertainty (Baisden et al., 2013).

Soil archives have proved to be a valuable resource when looking at changes in soil carbon over time (Jenkinson et al., 2008). Air-drying soils for storage in archives is a common practice, 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 yet to be documented.

Incubation 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 (Birch, 1958). This phenomenon has been extensively studied over the past several decades (Borken & Matzner, 2009; Schimel, 2018). Hypothesized 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 (Fierer & Schimel, 2003), or desorption of mineral-associated organic matter, and typically represent a combination of these sources (Kaiser et al., 2015; Slessarev et al., 2020).

Ecosystem type may play an important role in how a soil responds to drying and rewetting. For example, in a study from California, forest soils showed a stronger respiration response to drying and rewetting than grassland soils from an adjacent site, hypothesized to be due to a greater proportion of microbial biomass in the forest soils (Fierer & Schimel, 2003). 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 et al., 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 drying and rewetting.

Air-drying and rewetting of soil occurs *in situ* and is also common in laboratory incubation studies as a means of controling moisture content, underscoring the importance of this kind of disturbance in understanding soil carbon cycling (Borken & Matzner, 2009; Jarvis et al., 2007). 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 accessibility of soil organic matter formerly protected from decomposition via physical occlusion. Drying followed by 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 as a shift 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 assessed in order to exclude or quantify potential artifacts. 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:

H1) **Rewetting pulse**: the pulse of CO2 released immediately following rewetting will be older in air-dried soils than in non-air-dried soils, due to greater mobilization of protected carbon in the air-dried soils;

H2) **Equilibrium respiration**: CO2 released after the initial rewetting pulse will be younger than that released during the rewetting pulse, but will not differ between air-dried soils and non-air-dried soils;

H3) **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. All three experiments consider the effect of air-drying followed by subsequent rewetting, but with varying storage duration. Experiment 1 focuses on the effects of air-drying and 7 y of storage prior to rewetting (air-dry/rewet + storage), Experiment 2 on the effect of air-drying and rewetting alone, i.e. without storage (air-dry/rewet), and Experiment 3 on the air-dry/rewet + storage effect for samples stored between 7 and 14 y.

2.1. Sample selection and field sampling

Soils analyzed for Experiment 1, Experiment 2, and a subset of the samples from Experiment 3 were collected from plots established as part of the Biodiversity Exploratories project (Fischer et al., 2010). Basic soil properties for Experiment 1 and Experiment 2 samples are provided in Table 1, while data for Experiment 3 samples are provided in Supplemental Table 1 (along with additional data for Experiment 1 and Experiment 2 samples). Owing to a lack of samples from deeper soil horizons, the samples included in this study were restricted to the A horizon only.

Experiment 1 samples were collected in 2011, and comprised a subset of samples originally collected for a different study (Solly et al., 2014). Two ecosystem types (forest and grassland) were sampled from across a range of soil textures in two geographic regions, Hainich-Dün and Schorheide-Chorin (Table 1). 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 (which may be present in previously limed soils in this region). We then selected soils from three grassland plots (50 m by 50 m) and three forest plots (100 m by 100 m) in 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. Further details on the soil collection and sampling strategy can be found in Solly et al. (2014).

For Experiment 2, we returned to the Hainich-Dün region in July 2019 to collect new samples from the same plots 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.

In addition to the Biodiversity Exploratories samples, the remaining samples analyzed in Experiment 3 were collected from various locations around the United States over the past two decades as part of unrelated studies conducted by different investigators (n total sites = 39). In each of these studies the investigators measured ∆14C-CO2 respired in incubations of fresh (i.e. non-air-dried) soils and archived sample splits of the soils prior to incubation. These samples were all collected in forest ecosystems, as we were unable to obtain any grassland soils for which ∆14C-CO2 had previously been measured aside from the Biodiversity Exploratory samples. Supplemental Table 1 provides further 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, because Experiment 3 samples were collected as part of different experiments conducted by various investigators at different times, slight differences in processing among these samples was unavoidable.

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 aliquot air-dried at 40º C (air-dry/rewet + storage samples, Experiment 1, and air-dry/rewet samples, Experiment 2), while the other aliquot was left at field moisture (control-1, control-2). Control-1 and control-2 samples were stored in re-sealable plastic bags at 4º C until incubation. After air-drying, air-dry/rewet + storage samples (Experiment 1) were placed in re-sealable plastic bags, and stored inside large plastic boxes in a cool (ca. 15º C) dark room for seven years. Experiment 2 samples were stored for <3 mo.

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 1). As with control-1 and control-2 samples, control-3 samples were split while still field-moist, with one sample aliquot air-dried and placed in storage, and the other aliquot left at field moisture.

Carbon and nitrogen contents of both Experiment 1 and Experiment 2 samples were determined by dry combustion in a CN analyzer (Vario Max, Elementar Analysensysteme GmbH, Hanau, Germany) following fine grinding with a ball-mill (Retsch MM400, Hanau, Germany) using material from the air-dried sample splits. Carbon contents were then corrected for residual moisture content. 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. Soil incubations

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 2. As with sample preparation, incubation

conditions for Experiment 3 differed from Experiments 1 and 2, and are therefore described

**Table 1.**

*Soil Properties for Experiment 1 and Experiment 2 Samples*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Field Moisture | | | |  |  |  |  | Particle Size\*\* | | | | | |
|  |  | 2011 | | 2019 | | Organic C\*\* | | Total N\*\* | | Sand | | Silt | | Clay | |
| Region | Ecosystem\* | mean | sd | mean | sd | mean | sd | mean | sd | mean | sd | mean | sd | mean | sd |
|  |  | *% water holding capacity* | | | | *g kg-1* | | | | | | | | | |
| Schorfheide-Chorin | mixed forest | 10.8 | 3.6 | - | - | 22.1 | 8.1 | 1.1 | 0.3 | 861 | 44 | 91 | 26 | 47 | 19 |
| Schorfheide-Chorin | grassland | 7.5 | 4.3 | - | - | 22.8 | 1.5 | 2.2 | 0.1 | 730 | 98 | 158 | 75 | 111 | 30 |
| Hainich-Dün | deciduous forest | 30.6 | 1.3 | 17.4 | 1.3 | 23.7 | 0.5 | 1.7 | 0.1 | 54 | 17 | 753 | 6 | 192 | 15 |
| Hainich-Dün | grassland | 22.8 | 5.3 | 19.8 | 3.1 | 41.8 | 1.9 | 3.9 | 0.1 | 32 | 16 | 553 | 78 | 413 | 65 |

\* Grassland species are all cool-season grasses (C3 photosynthetic pathway); mixed forest is a mix of coniferous and deciduous species.

\*\* Analyses performed on 2011 samples only.

separately in section 2.3.2.

2.3.1. Experiment 1 and Experiment 2

All Experiment 2 incubations were conducted in duplicate, as were the air-dry/rewet + storage incubations for Experiment 1, but the control-1 incubations were performed on single samples (Supplemental Table 1). Soils for both experiments 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 we reduced the mass of soil incubated to 20 g for the Experiment 1 air-dry/rewet + storage treatmentincubations. We used 20 g of soil for both control-2 and air-dry/rewet incubations in Experiment 2 as well.

Soil moisture of Experiment 1 and Experiment 2 samples was adjusted to 60% of water holding capacity (WHC) prior to sealing the jars, either from field moisture (control-1 and control-2 samples) or from air-dried conditions (air-dry/rewet + storage and air-dry/rewetsamples). Following moisture adjustment, jars were flushed with CO2-free air, sealed, and left to incubate for a four-day pre-incubation period. After the pre-incubation 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. In contrast, the duration of the equilibrium respiration period for control-2 samples was determined by the total amount of CO2 respired. High CO2 concentrations can affect soil pH, thus a conservative upper limit of 1% CO2 in the jar headspace of control-2 samples was chosen to limit potential pH effects but still allow for sufficient CO2 production. The lower limit was set at 0.5 mg of carbon respired per sample, which is the minimum needed to measure the radiocarbon content.

The duration of the equilibrium respiration period for Experiment 1 and Experiment 2 treatment samples was determined by the amount of CO2 respired by the corresponding control-1 and control-2 samples: air-dry/rewet + storage (Experiment 1) and air-dry/rewet (Experiment 2) incubations were allowed to proceed until an equivalent amount of carbon had been respired per g of initial soil C as in the control incubations.

Headspace CO2 concentrations for control-1 incubations were measured once at the end of the pre-incubation period, but were measured daily during pre-incubation for air-dry/rewet + storage samples in Experiment 1, and both control-2 and air-dry/rewet samples in Experiment 2. We measured headspace CO2 concentrations one to three times per week during the equilibrium respiration period for both Experiment 1 and Experiment 2, with more frequent measurements made for samples with faster respiration rates. 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 equilibrium respiration period.

2.3.2. Experiment 3

We attempted to replicate the control-3 incubation conditions as closely as possible when conducting the corresponding air-dry/rewet + storage 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). Although moisture content varied among control-3 sample incubations due to different objectives of the initial investigators, we were able to maintain the same moisture content between paired control-3 and storage duration treatment incubations for all samples for which the control-3 moisture conditions were known (Supplementary Table 1).

Treatment samples for Experiment 3 were only incubated for a single enclosure period as neither data on the pre-incubation period duration nor CO2 release data were available for many of the control-3 samples. Treatment sample incubation vessels were immediately sealed following moisture adjustment 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. Both the duration and the CO2 measurement frequency varied for the control-3 incubations. ∆14C-CO2 and δ13C measurements were made at the end of the equilibrium

**Table 2.**

*Experimental Design*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  | Period | | | |
|  |  |  |  |  |  | Pre-incubation | | Equilibrium Respiration | |
| Experiment | Treatment | Sample Collection Year | Incubation Year | Initial Moisture Content\* | Incubation Moisture Content | Length | ∆14C measured | Length | ∆14C measured |
|  |  |  |  | *% water holding capacity* | *% water holding capacity* | *days* |  | *days* |  |
|  |  |  |  |  |  |  |  |  |  |
| 1 | control-1 | 2011 | 2011 | 18 (10) | 60 | 4 | no | 14 | yes |
| air-dry/rewet + storage | 2011 | 2018 | 0 | 60 | 4 | yes | 5 to 45 | yes |
| 2 | control-2 | 2019 | 2019 | 19 (2) | 60 | 4 | yes | 10 to 38 | yes |
| air-dry/rewet | 2019 | 2019 | 0 | 60 | 4 | yes | 7 | yes |
| 3 | control-3 | 1999 to 2011 | 1999 to 2011 | various | various | various | various | various | yes |
| air-dry/rewet + storage | 1999 to 2011 | 2009 or 2018 | 0 | various | NA | NA | 5 to 45 | yes |

\* Mean field moisture content is shown for control samples (n = 12 for Experiment 1, n = 6 for Experiment 2); moisture content following air-drying is shown for the treatment samples. Values given are means, standard deviations are given in parentheses. Note that the high standard deviation for control-1 samples is because samples came from two locations, Hainich-Dün and Schorheide-Chorin, while control-2 samples came from Hainich-Dün only. See Supplemental Table 1 for the moisture content of individual samples.

respiration period for control-3 samples and at the end of the single enclosure period for the air-dry/rewet + storage samples. We conducted the majority of the Experiment 3 air-dry/rewet + storage incubations in 2018 at the Max Planck Institute for Biogeochemistry (MPI-BGC) but a few of the treatment sample incubations were performed in 2009 at the University of California Irvine (UCI) (Supplementary Table 1).

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 the majority of the air-dry/rewet + storage samples from Experiment 3 were conducted at the MPI-BGC accelerator mass spectrometer facility (Steinhof, 2013). All other samples were analyzed at the UCI W.M. Keck Facility for Accelerator Mass Spectrometry (Xu et al., 2007). 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-dependent fractionation effects, the 14C/12C ratio of all samples is corrected to a common δ13C value of -25 per mil (Stuiver & Polach, 1977). Although the effect was small, ∆14C data from air-dry/rewet + storage samples (Experiments 1 and 3) were also corrected for depletion due to radioactive decay that occurred during storage.

The ∆14C notation is particularly useful for discussing decadal scale carbon dynamics, as positive values reflect the presence of bomb-C fixed from the atmosphere at some point over 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. At the present time (2020), systems dominated by decadally cycling C could be expected to have relatively large positive ∆∆14C values, due to the influence of bomb-C. 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 the majority of air-dry/rewet + storage samples from Experiment 3 were made at MPI-BGC (Delta+XL, Thermo Finnigan, Bremen, Germany), while control samples for Experiment 3 (control-3 samples) were measured at UCI (Thermo Delta Plus, Thermo Fisher Scientific). Data are reported using δ13C notation, which refers to the deviation in parts per thousand of the ratio of 13C/12C in the PDB standard.

2.5. Statistical analysis

We determined the statistical significance of differences between control and treatment samples using paired t-tests (alpha = 0.05). In order to identify potential influences on the observed treatment effects we also performed linear regression analysis using the difference between treatment and control ∆14C-CO2 as the response variable, and the difference in CO2 respired (treatment – control), soil carbon and nitrogen content, change in moisture content upon rewetting, and silt + clay content 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 for field-moist soils in both Experiment 1 (air-dry/rewet + storage treatment) and Experiment 2 (air-dry/rewet treatment) when compared within ecosystem types. However, the magnitude and timing of peak respiration rates diverged among experiments and between grassland and forest soils (Fig. 1).

Among the air-dry/rewet + storage samples in Experiment 1 (Fig. 1a, dashed lines), respiration rates were more than twice as high in grassland soils than in forest soils. Grassland air-dry/rewet + storage samples reached a maximum of 3.8 mg CO2 g soil C-1 d-1 after 3.8 d followed by a sharp decline (Fig 1a), while mean respiration rates in air-dry/rewet + storage forest sites peaked at 1.5 mg CO2 g soil C-1 d-1 after 6.9 d, followed by a much more gradual decline (Fig. 1a). In contrast, control-1 forest and grassland samples (solid lines, Fig. 1a) responded more weakly and more gradually than the air-dry/rewet + storage samples, but with similar trends with respect to



**Figure 1.** Respiration rates for Experiment 1 and Experiment 2 samples

**a)** Experiment 1 samples, collected in 2011; **b)** Experiment 2 samples, collected in 2019. Vertical gray line at day 4 demarcates the end of the pre-incubation period and the start of the equilibrium respiration period. Points show measurements and lines show trends in mean respiration rate. Shaded ribbons represent one standard error of the mean. The final measurement points for a few samples which took > 18 d to reach CO2 targets are excluded for display reasons; respiration rates for those samples remained constant. Note that headspace CO2 concentrations for control-1 samples (panel a) were only measured once during the pre-incubation period (day 4) in contrast to daily measurements for all other samples.

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 4.8 d.

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

Respiration data for Experiment 3 samples are shown separately (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 (Supplementary Table 1). 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 across all three experiments.

3.2. Rewetting pulse versus equilibrium respiration

We did not observe significant differences between ∆14C-CO2 respired during the pre-incubation period (the rewetting pulse) and ∆14C-CO2 respired during the equilibrium respiration period (Fig. 2). This finding was in contrast to our hypothesis (H1), and surprising in light of the significant differences in respiration rates between these two periods. The lack of significance was consistent across all samples for which ∆14C-CO2 was measured for both enclosure periods: air-dry/rewet + storage samples in Experiment 1 (open squares, Fig. 2), and both air-dry/rewet (open circles, Fig. 2) and control-2 (filled circles, Fig. 2) samples in Experiment 2. All data were pooled for statistical analysis as 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.

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 overall difference between pre-incubation ∆14C-CO2 and equilibrium respiration ∆14C-CO2 was not significant. Due to lower respiration rates during pre-incubation only three of the six air-dry/rewet + storage treatment samples from the forest soils incubated in Experiment 1 (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.



**Figure 2.** ∆14C-CO2 of the rewetting pulse versus the equilibrium respiration period

Points are means of laboratory duplicates and error bars are the min and max (except for Experiment 1 control samples, which were not replicated). Note that rewetting pulse Δ14C-CO2 was not measured for control-1 samples; additionally samples from three of the forest plots of the air-dry/rewet + storage samples from Experiment 1 failed to accumulate enough CO2 during the pre-incubation period to measure Δ14C. The outlier point with the substantially depleted pre-incubation Δ14C-CO2 is from Experiment 2 (control).

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/rewet + storage samples in Experiment 1 (Supplemental Fig. 2). Equilibrium respiration CO2 of the air-dry/rewet + storage forest soils was significantly enriched in δ13C compared to the rewetting pulse CO2 (-24.2‰ ± SD of 1.1‰ and -25.7‰ ± SD of 0.8‰, respectively), while we observed the opposite for the air-dry/rewet + 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 differences in δ13C between rewetting pulse CO2 and equilibrium period CO2 for any of the samples in Experiment 2. Note that as with ∆14C, δ13C-CO2 was note measured following the pre-incubation period for 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: for both the air-dry/rewet + storage treatment and the air-dry/rewet treatment. This was in contrast to our expectation that the air-drying and rewetting treatment would not affect equilibrium period ∆14C-CO2 (H2). We included data from all three experiments in the overall analysis of the effect of air-drying and rewetting (Fig. 3). Treatment sample incubations, including both the air-dry/rewet + storage treatment in Experiments 1 and 3, and the air-dry/rewet treatment in Experiment 2, typically resulted in differences between 20‰ and 40‰ relative to control sample incubations, although the majority of the differences were within ±20‰. The 20‰ to 40‰ range is shown for context (Fig. 3, dashed and dotted lines, respectively): these differences correspond to the change in ∆14C of the atmosphere over four and eight years, respectively, during the period of 2000 to 2020 (Graven et al., 2017).

The samples from Oak Ridge (Fig. 3) are an exception in that the majority of the air-dry/rewet + storage treatment samples and corresponding control-3 samples differ by ≥40‰. However, these points show the impact of not only bomb-C, but additional enrichment from exposure to a localized plume of 14C-enriched CO2. This localized 14C plume was released from a nearby incinerator four years prior to sample collection, and resulted in 14C enrichment of both surface litter and root inputs that persisted until the time of sample collection (Cisneros-Dozal et al., 2006). In these samples, the depletion observed in the ∆14C-CO2 of the air-dry/rewet + 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 (p < 0.05) 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/rewet treatment in Experiment



**Figure 3.** Overall treatment effect on Δ14C-CO2

Points show data from all three experiments and are the mean of laboratory replicates (for replicated samples); error bars are 2x standard error. Solid line is 1:1. For context, the dashed and dotted lines show differences of ±20‰ and ±40‰, equivalent to the decline in Δ14C in atmospheric CO2 over 4 and 8 y respectively, during the period of 2000 to 2020 (Graven et al., 2017).

2 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/rewet + 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.

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 (Supplementary Fig. 3). The mean difference in δ13C-CO2 observed in Experiment 1 air-dry/rewet + storage samples 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 in δ13C-CO2 observed in the air-dry/rewet samples from Experiment 2 were similar for both forest and grassland soils (-1.56‰ ± SD of 0.67‰ and -1.11‰ ± SD of 0.19‰, respectively) (Table 3).

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. We did not find it to be a significant predictor of the difference between control and treatment ∆14C-CO2 (Fig. 4). However, we did observe a slight increase in the difference between control and treatment ∆14C-CO2 with the increase in storage duration for the highly enriched samples from Oak Ridge, which were measured at two different timepoints: after 5 y and 14 y of storage. As the experimental label is concentrated in only the most recently fixed carbon, these samples are particularly senstitive to the potential loss of this carbon over time, which may explain this slight apparent storage duration effect.



**Figure 4.** Treatment effect on Δ14C-CO2 in relation to storage duration

Points show data from experiments 1 and 3. Data are averaged by site (some locations had multiple sites) and error bars show 2x standard error. Solid line is 1:1. For context, the dashed and dotted lines show differences of ±20‰ and ±40‰, equivalent to the decline in Δ14C in atmospheric CO2 over 4 and 8 y respectively, during the period of 2000 to 2020 (Graven et al., 2017).

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-CO2 can provide insight into the relative contribution of more slowly cycling carbon compared to faster cycling carbon at a 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 ∆14C-CO2 of respiration 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 both the air-dry/rewet + storage (Experiment 1) and the air-dry/rewet (Experiment 2) treatments had a significant effect on ∆14C-CO2 when assessed within ecosystem types using paired t-tests (p < 0.05) (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 (Fig. 5). Averaged across experiments, mean absolute differences in ∆14C-CO2 were 21.4‰ and 12.1‰ for grassland and forest soils respectively (Table 3). Mean differences of laboratory duplicates were lower than treatment differences for both Experiment 1 and Experiment 2; differences among duplicates were slightly higher for forest samples (8.1‰, ± SD of 3.8‰) than for grassland samples (5.0‰, ± SD of 3.8‰) (Table 3).

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



**Figure 5. Time series of control and treatment Δ14C-CO2 (Experiments 1 and 2)**

Points show mean Δ14C-CO2 within ecosystem and treatment groups; error bars show 2x standard error. Arrows show the direction of change in Δ14C-CO2 observed in treatment samples relative to the controls for a given time point. Atmospheric ∆14C-CO2 data (gray line) are from Graven et al. (2017) up to the year 2015, while data points from 2015 to 2019 are extrapolated (Sierra, 2018). All atmospheric radiocarbon data are for the northern hemisphere (zone 2).

**4. Discussion**

4.1. Implications for constraining soil carbon models

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 constructing time series of respired ∆14C-CO2 and constraining soil carbon models. The two treatments considered, air-drying alone or air-drying + storage, 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, atmospheric ∆14C has declined at about 5 per mille per year over the past two decades (Fig. 6) (Graven et al., 2017), meaning that the differences of roughly 10‰ to 20‰ that we observed Experiments 1 and 2 of this study 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 (Fig. 6); 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.

4.2. Detecting change over time in 14C-CO2 with incubations of archived soils

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 (Baisden et al., 2013; Gaudinski et al., 2000; Schrumpf & Kaiser, 2015). In most cases a slightly more complex system with two pools and no transfer of carbon between the two pools (a two-pool parallel model) is the simplest applicable model for fitting ∆14C-CO2 data observed over time (Sierra et al., 2014). Although model fitting is beyond the scope of this study, we developed a two-pool parallel model with simulated data to illustrate potential scenarios that could explain the results of this study (Fig. 6). Carbon stocks and pool sizes of the conceptural model depicted in Fig. 6 are based on density fraction data for the Hainich-Dün forest site (Schrumpf et al. 2013). Using single-pool steady-state models, Schrumpf et al. (2013) estimated a mean decomposition rate of between 3 and 5 years for the free light fraction, 90 years for the occluded light fraction, and 115 years for the heavy fraction of the 0-5 cm depth layer. We increased the intrinsic decomposition rate (*k*) of the fast cycling pool in our two-pool model system to 1/6 yr-1 to account for the increased contribution of fine roots relative to leaf litter in the 0-10 cm depth layer, and set the decomposition rate for the slow cycling pool to 100 years to account for the contribution of both occluded and heavy fraction carbon.

Two possible outcomes of the air-drying and rewetting treatment effect on respired 14C-CO2 are shown in Fig. 6b. The filled circles show hypothetical observations of ∆14C-CO2 respired from control incubations, while the open symbols show respiration from treatment incubations. In the first scenario (open squares), an increased contribution from the slow pool following air-drying and rewetting shifts ∆14C-CO2 toward the slow pool curve (blue line), while in the second scenario (open circles), an increased contribution from the fast pool shifts ∆14C-CO2 toward the fast pool curve (magenta line). Due to the crossing of the slow and fast pool curves in 2009, an increased contribution of the slow pool to respiration following treatment leads to relative depletion of ∆14C-CO2 in 1991, but relative enrichment of ∆14C-CO2 in 2019, while the opposite is true for the increased fast pool contribution scenario.

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**Figure 6**. **a)**Modeled trajectories of ∆14C over time for a hypothetical two-pool parallel soil carbon model in relation to atmospheric ∆14C-CO2, **b)** *(inset)* Depiction of two competing scenarios in which air-drying and rewetting shifts ∆14C of respired CO2 toward either the fast or slow soil carbon pool curve. Atmospheric ∆14C-CO2 data are from Graven et al. (2017) up to the year 2015, while data points from 2015 to 2019 are extrapolated (Sierra, 2018). All atmospheric radiocarbon data is for the northern hemisphere (zone 2).

The relative position and shape of the modeled ∆14C curves in Fig. 6a are determined by both *k* and the partitioning of inputs into the two model pools. The fast cycling carbon pool in Fig. 6a becomes enriched in ∆14C relative to the atmosphere in the mid-1970s, but as the carbon in this pool turns over relatively quickly, ∆14C then follows the decline in the atmosphere with a slight offset. In contrast, ∆14C of the slowly cycling pool does not cross the atmospheric curve (gray line, Fig. 6) until just after the year 2000, and then remains relatively enriched, buoyed by the presence of bomb carbon incorporated over the past several decades. Finally, as the fast cycling soil carbon pool dominates the respiration flux, the shape of the respiration curve is similar to the fast pool curve but slightly dampened by the contribution of the slow cycling pool.

The reversal of the air-dry/rewet + storage treatment effect between 2011 and 2019 in the Central German forest soils observed in this study (Fig. 5 and Table 3) may initially appear to suggest that the effect of air-drying/rewetting + storage differs from that of air-drying/rewetting without storage. However, the results of Experiment 3 show that storage duration does not have a significant effect on ∆14C-CO2. The increased slow pool contribution scenario depicted in Fig. 6b provides an alternative explanation for the reversal. For this scenario to apply, the relative 14C enrichment of the fast and slow cycling carbon pools at the Hainich-Dün forest sites must have switched between 2011 and 2019 as they did between 1991 and 2019 in the conceptual model (i.e. the crossing of the slow and fast model pool curves, Fig. 6).

In contrast to the forest soils, the grassland samples analyzed from the same region show enrichment of a similar magnitude in response to both the air-dry/rewet + storage treatment in 2011 and the air-dry/rewet treatment in 2019, providing more evidence that storage is not driving the reversal observed in the forest soils. If we apply the conceptual model in Fig. 6 to the results from the grassland soils, the data suggest that either carbon is being mobilized from a slowly cycling carbon pool that had already become more enriched than the faster cycling pool prior to 2011, or that the air-drying and rewetting treatment is mobilizing faster cycling carbon in grassland soils. The more parsimonius explanation is that the air-drying and rewetting treatment is mobilizing additional carbon from a more slowly cycling pool in both forest and grassland soils, but with different effects on ∆14C-CO2 due to differences in relative cycling rates of carbon in forest versus grassland ecosystems.

4.3. Potential role of pre-aged C inputs in forest versus grassland soils

A key difference in carbon cycling between forest and grassland ecosystems is the potential for carbon storage in woody tissues after it is fixed from the atmosphere (Gaudinski et al., 2000). Carbon entering the soil in forest ecosystems 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 the carbon in 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, for fine root inputs 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.

4.4. Significance of drying and rewetting, and storage duration, on ∆14C-CO2

We found that the common practice of air-drying and rewetting soils prior to incubation 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). However, 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/rewet + storage sample ∆14C-CO2 (Fig. 6). 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 loss of carbon over time or some other storage related mechanism.

4.4.1. ∆14C-CO2 of the rewetting pulse versus equilibrium respiration

Our first hypothesis was focused on the ∆14C of the rewetting pulse, the CO2 released immediately following moisture adjustment, which we expected to 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 for all of the samples in which we measured ∆14C-CO2 in both the pre-incubation and the equilibrium respiration period (Fig. 3). These results suggest that the change in substrate availability initiated by air-drying and rewetting may not be limited to the rewetting pulse, and may persist throughout the incubation. (For context, the mean amount of CO2 respired in the incubations in this study was 0.8 percent of the initial total soil organic carbon).

4.4.2. Persistence of drying and rewetting effects on ∆14C-CO2

The ∆14C-CO2 results from this study provide support for a mechanism that makes a new pool of carbon available to the microbial community following air-dring and rewetting: additional substrate with a distinct ∆14C signature that appears to be older than what is respired in soils that have not undergone air-drying. 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. However, the shift to older ∆14C-CO2 following treatment that we observed suggests extracellular carbon plays an important part in fueling this pulse, e.g. dissolved organic carbon from the soil solution or other non-microbial soil carbon pools such aggregate-protected C (Miller et al., 2005; Xiang et al., 2008).

4.4.3. Implication of δ13C-CO2 shifts following drying and rewetting

Alternatively, the consistent enrichment in δ13C-CO2 seen following both the air-dry/rewet + storage treatment and the air-dry/rewet treatment (Table 3, Supplemental Fig. 3), could indicate that the fuel for the rewetting pulse is microbial in origin, but derived from microbes that have been dormant for decades. Such a scenario would explain the concommitant shift in ∆14C-CO2, but to our knowledge such a response has not been previously documented. While we cannot rule out this interpretation, such an argument is complicated by the fact that older soil organic matter also tends to be enriched in δ13C, and thus mobilization of older, extracellular C would be in line with the observed shift in δ13C-CO2 seen in both the air-dry/rewet and the air-dry/rewet + storage samples (Wynn et al., 2005). Yet another complication in interpreting the shift in δ 13C-CO2 observed in the treatment sample incubations is microbial recycling, a process has also been

**Table 3.**

*∆14C, ∆∆14C, and δ13C of Respired CO2 in Experiment 1 and Experiment 2 Samples\**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  | Equilibrium Respiration | | | | | Difference (Treatment - Control) | | | |
|  |  |  |  |  | ∆14C-CO2 | | | δ13C-CO2 | | ∆14C-CO2 | | δ13C-CO2 | |
| Experiment | Ecosystem | Treatment | Year Sampled | n | *mean* | *mean ∆∆* | *sd* | *mean* | *sd* | *mean* | *CI95* | *mean* | *CI95* |
|  |  |  |  |  | ‰ | | | | | | | | |
| 1 | forest | air-dry/rewet + storage | 2011 | 6 | 82.2 | 44.9 | 7.3 | -24.2 | 1.1 |  |  |  |  |
| 1 | forest | control | 2011 | 6 | 93.8 | 56.5 | 6.9 | -26.8 | 0.2 | -11.6 | [-20.3, -2.8] | 2.4 | [1.5, 3.2] |
| 1 | grassland | air-dry/rewet + storage | 2011 | 6 | 77.8 | 40.5 | 11.7 | -27.2 | 0.5 |  |  |  |  |
| 1 | grassland | control | 2011 | 6 | 54.5 | 17.2 | 17.1 | -27.7 | 0.4 | 23.3 | [17.1, 29.5] | 0.5 | [0.1, 0.9] |
| 2 | forest | air-dry/rewet | 2019 | 3 | 51.8 | 62.9 | 27.4 | -24.5 | 1.4 |  |  |  |  |
| 2 | forest | control | 2019 | 3 | 39.1 | 50.2 | 19.6 | -26.1 | 0.8 | 12.7 | [5.3, 20.1] | 1.6 | [-1.9, 5.1] |
| 2 | grassland | air-dry/rewet | 2019 | 3 | 39.8 | 50.9 | 21.7 | -27.5 | 0.4 |  |  |  |  |
| 2 | grassland | control | 2019 | 3 | 20.2 | 31.4 | 10.3 | -28.6 | 0.3 | 19.6 | [12.2, 27.0] | 1.1 | [ 0.3, 1.9] |

\* Soils sampled in 2011 came from both the Schorheide-Chorin and the Hainich-Dün sites, whereas 2019 samples were from Hainich-Dün only. Each sample is a composite of three field replicates, and was analyzed in duplicate in the laboratory.

shown to lead to enrichment in δ13C (Wynn et al., 2005), and had been shown to be enhanced following air-drying and rewetting (Brödlin et al., 2019; Slessarev et al., 2020). As noted previously, the ∆14C unit accounts for mass-dependent fractionation effects, thus this phenomenon does not affect the radiocarbon results as reported.

We observed greater enrichment of δ13C-CO2 in forest soils than in grassland soils, which could indicate greater microbial recycling in forest soils or potentially more mobilization of mineral-associated carbon in forest soils than in grassland soils following treatment. Mineral-associated carbon has been shown to be more enriched in δ13C as well as older on average than bulk soil organic matter (Schrumpf et al., 2013), a combination of observations that suggest more mineral-associated carbon may have been released upon rewetting in the forest soils than in the grassland soils. However, the similarity in the direction of the δ13C-CO2 response across forest and grassland soils (Supplemental Fig. 3) suggests that a similar mechanism is at work in both ecosystems.

4.5. Air-drying and rewetting effects on forest soil ∆14C-CO2

Nearly all of the forest soils analyzed in this study exhibited depletion of ∆14C-CO2 following air-drying + storage (Fig. 3, Fig. 4). The depletion in the forest soils was greatest in the samples from Oak Ridge (magenta triangles, Fig. 3), 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 cycling 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 (Experiment 2) (Fig. 3). While the behavior of the anomalous Harvard Forest sample is hard to explain, the different behavior of the Central German forest soils from 2019 could be explained by invoking the increased slow pool contribution scenario depicted in Fig. 6b: by the year 2019, even in forest soils with lagged inputs and potentially slower overall carbon dynamics, 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 turnover of bomb carbon incorporated decades earlier.

4.6. Drying and rewetting effects on grassland soil ∆14C-CO2

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, our interpretation is that these data are consistent with the same general mechanism as in forests: 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 in our dataset, 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. 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 a smaller vegetative lag effect. The grassland soils that were observed in both 2011 (Experiment 1) and 2019 (Experiment 2) showed enrichment following air-drying and rewetting (Fig. 5), suggesting that the crossing point of the slow and fast cycling carbon curves occurred prior to 2011 in these soils.

4.7. Drying and rewetting mobilizes older carbon in both forest and grassland 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 susceptible 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. While we cannot say with certainty what mechanism is driving the response observed, 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. However, it is clear that overall treatment differences are not driven solely by the amount of CO2 released, but rather by changes in the carbon pools fueling respiration, specifically the mobilization of substrate from older soil carbon pools. This general response holds across both forest and grasslands, and is supported by the fact that we did not observe a significant difference between pre-incubation period ∆14C-CO2 and equilibrium period ∆14C-CO2 within incubations, nor did we observe a significant relationship between the amount of CO2 released and the difference observed between control and treatment ∆14C-CO2.

**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. The 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. Mean differences for the well-controlled Experiment 1 and Experiment 2 samples show less impact of methodological artifacts and thus likely represent the actual bias of the air-drying and rewetting effect more accurately. Mean differences between control and treatment incubations for these samples were only 12.1‰ and 20.4‰, for forest and grasslands respectively, which correspond to two years (10‰) to four years (20‰) of change in atmospheric ∆14C over the first decade of the 20th century. The implication of the bias introduced by air-drying and rewetting is that the technique may not be sensitive enough to detect sub-decadal changes in ∆14C-CO2, but remains valuable over longer time scales. However, when comparing ∆14C-CO2 of recently collected soils to those from an archive, we recommend that both samples undergo the same air-drying and rewetting procedure so as to make the results comparable.

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**

Code and data have been archived on Zenodo as a github release (v1.0) with the DOI 10.5281/zenodo.4106666. The file ‘arc-inc\_data-wrangling\_2020-07-30.Rmd’ within the zip file of the repository release contains the R script for running all analyses and generating all figures in the text. The authors would like to acknowledge the invaluable assistance of M. Rost in the laboratory and the field, and I. Schoening, M. Cisneros-Dozal, J. Koarashi, F. Hopkins, C. Lawrence, and S. Trumbore for sharing data and details on control-3 sample incubations. Funding was provided by the European Research Council (Horizon 2020 Research and Innovation Programme, grant agreement 695101; 14Constraint).

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