Impacts of Drying and Rewetting on the Radiocarbon Signature of Respired CO$_2$ and Implications for Incubating Archived Soils

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Abstract The radiocarbon signature of respired CO_2 ($\Delta^{14}C$-CO_2) measured in laboratory soil incubations integrates contributions from soil carbon pools with a wide range of ages, making it a powerful model constraint. Incubating archived soils enriched by "bomb-C" from mid-20th century nuclear weapons testing would be even more powerful as it would enable us to trace this pulse over time. However, air-drying and subsequent rewetting of archived soils, as well as storage duration, may alter the relative contribution to respiration from soil carbon pools with different cycling rates. We designed three experiments to assess air-drying and rewetting effects on $\Delta^{14}C$-CO_2 with constant storage duration (Experiment 1), without storage (Experiment 2), and with variable storage duration (Experiment 3). We found that air-drying and rewetting led to small but significant ($\alpha < 0.05$) shifts in $\Delta^{14}C$-CO_2 relative to undried controls in all experiments, with grassland soils responding more strongly than forest soils. Storage duration (4–14 y) did not have a substantial effect. Mean differences (95% CIs) for experiments 1, 2, and 3 were: 23.3‰ (±6.6), 19.6‰ (±10.3), and 29.3‰ (±29.1) for grassland soils, versus −11.6‰ (±4.1), 12.7‰ (±8.5), and −24.2‰ (±13.2) for forest soils. Our results indicate that air-drying and rewetting soils mobilizes a slightly older pool of carbon that would otherwise be inaccessible to microbes, an effect that persists throughout the incubation. However, as the bias in $\Delta^{14}C$-CO_2 from air-drying and rewetting is small, measuring $\Delta^{14}C$-CO_2 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 CO_2 ($\Delta^{14}C$-CO_2) can be used to quantify how long carbon is stored in ecosystems. Incubating archived soils could provide unique insight into soil carbon sequestration potential by quantifying the change in $\Delta^{14}C$-CO_2 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 $\Delta^{14}C$-CO_2 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 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

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 C ages (Sierra et al., 2018). Measuring the radiocarbon signature of heterotrophic respiration ($\Delta^{14}C$-CO_2) in laboratory incubations is a powerful constraint for modeling soil carbon dynamics because it provides an integrated measure of the carbon-weighted contribution to the soil efflux from carbon pools with distinct C sources and cycling rates

(Trumbore, 2000). Using archived soils to construct a time series of $\Delta^{14}C$-CO_2 has the potential to amplify the power of this model constraint, but it is unclear how air-drying, storage, and subsequent rewetting of archived soils may affect $\Delta^{14}C$-CO_2 observed in laboratory incubations.

The distribution of soil carbon among faster and more slowly cycling pools has important implications for predicting the response of the soil carbon reservoir to changes in inputs or decomposition rates resulting from climate change (Trumbore, 2000). Soils with large pools of slowly cycling carbon would be expected to sequester more carbon with increased inputs than soils dominated by fast cycling pools, while shifts in temperature or moisture regimes may affect decomposition rates differently depending on the stabilization mechanism. $\Delta^{14}C$-CO_2 reflects respiration fluxes dominated by the decomposition of fast cycling carbon in contrast to bulk soil $\Delta^{14}C$, which is dominated by large stocks of relatively slowly cycling carbon (Sierra et al., 2018). Together, these measurements can improve predictions of the response of soil C to global change.

Soil archives offer a window into the past, and incubating archived soils provides an opportunity to observe how $\Delta^{14}C$-CO_2 changes over time. The pulse of radiocarbon introduced into the biosphere from nuclear weapons testing ("bomb-C"), which peaked in the mid-20th century, (Trumbore, 2009) serves as an ideal tracer. New C inputs to the soil over the decades following the bomb-C peak carry distinct annual radiocarbon signatures due to the decrease in the concentration of atmospheric ^{14}C over this period. Following the bomb-C tracer in $\Delta^{14}C$-CO_2 respired from soils collected and archived over the latter half of the 20th century and first decades of the 21st could therefore provide unique insight into decadal scale soil C dynamics.

A critical challenge for the interpretation of $\Delta^{14}C$-CO_2 data is that, due to the curvature of the bomb-C peak, there were two points in time at which the $\Delta^{14}C$ signature of atmospheric CO_2 was identical. This means observations of $\Delta^{14}C$ from just a single point in time can be fit to models with different intrinsic decomposition rates. Trumbore (2000) gives the example of a two independent, homogenous pools of soil carbon, one with an intrinsic decomposition rate (k) of 6.6 years and the second with $k = 50$ years, both of which would have had a $\Delta^{14}C$ of 166‰ in 1996. Observations of $\Delta^{14}C$-CO_2 measured in incubations of archived soils could help resolve this ambiguity by enabling the construction of a time series of $\Delta^{14}C$-CO_2. The trajectory of $\Delta^{14}C$ in a soil carbon pool turning over every 6.6 years is quite different from one with an intrinsic decomposition rate of 50 years (Baisden et al., 2013), making a $\Delta^{14}C$-CO_2 time series a strong additional constraint for model parameterization.

Prior to long term storage soils are commonly air-dried. However, this process is known to affect biological, physical, and chemical properties of the soil (Bartlett & James, 1980; Jones et al., 2019). For example, incubation of soils following air-drying and rewetting typically leads to a rapid increase in CO_2 production, ranging from hours to several days (the Birch effect), before returning to equilibrium respiration rates (Birch, 1958). Hypothesized sources for the CO_2 released following soil rewetting include (and typically represent a combination of): 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), and desorption of mineral-associated organic matter (Kaiser et al., 2015; Slessarev et al., 2020). While the impact of air-drying and rewetting on soil respiration rates has been extensively studied (Borken & Matzner, 2009; Schimel, 2018), the potential effects of air-drying, long-term storage, and rewetting on $\Delta^{14}C$-CO_2 has yet to be documented.

If air-drying and rewetting affects the relative contribution to respiration of soil organic matter pools with different intrinsic cycling rates, this should be detectable in $\Delta^{14}C$-CO_2. 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 $\Delta^{14}C$-CO_2. However, if the rewetting pulse derives mainly from lysed microbial cells or the release of microbial osmolytes, little change in $\Delta^{14}C$-CO_2 would be expected.

Obtaining $\Delta^{14}C$-CO_2 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.

We designed three experiments to answer the following questions:

1. Is Δ^{14}C-CO$_2$ measured in incubations of soils prior to air-drying altered by the process of air-drying, storage, and subsequent rewetting?
2. What is the effect of air-drying and rewetting alone, that is without storage, on Δ^{14}C-CO$_2$?
3. Does the duration of storage affect Δ^{14}C-CO$_2$?

We present the results of these three experiments, along with an applied example of interpreting a time series of Δ^{14}C-CO$_2$ constructed by incubating archived soils. Our results provide support for the utility of incubating archived soils to understand rates of soil C cycling and provide constraints for C cycle models. They also provide insight into long-standing questions about the substrates fueling rewetting pulse respiration, as well as differences in soil C dynamics between forest and grassland ecosystems. We conclude with suggestions for how best to employ the radiocarbon incubation technique with archived soils beyond our sample set.

2. Materials and Methods

We devised three experiments to quantify potential shifts in Δ^{14}C-CO$_2$ measured in laboratory soil incubations following air-drying, storage, and rewetting. All three experiments consider the effect of air-drying followed by subsequent rewetting, but with varying storage duration, from less than 1 month (no storage) to 14 years. Experiment 1 focuses on the effects of air-drying and 7 years of storage prior to rewetting (air-dry/rewet + storage), Experiment 2 on the effect of air-drying and rewetting alone, that is without storage (air-dry/rewet), and Experiment 3 on the effect of varied storage duration (storage duration). All soils were split following sample collection, with one split air-dried, and the other refrigerated under field-moisture conditions until incubation. For each experiment we considered the undried split to be the control sample and the air-dried split to be the treatment sample.

2.1. Experiment 1: Air-Dry/Rewet With Long-Term Storage

2.1.1. Experiment 1 Sample Selection and Field Sampling

Soils analyzed for Experiment 1 were collected in 2011 from plots established as part of the Biodiversity Exploratories project (Fischer et al., 2010). The samples used in this study comprise a subset of samples originally collected for a study by Solly et al. (2014). Two ecosystem types (forest and grassland) were sampled from two regions of central Germany, Schorfheide-Chorin (Central Germany 1) and Hainich-Dün (Central Germany 2). The two regions have similar climates, but are characterized by different soil textures (Table 1). We selected carbonate-free 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 criterion that the Δ^{14}C-CO$_2$ observed in the 2011 incubations fell 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).

2.1.2. Experiment 1 Sample Preparation

Following sample collection, soils for Experiment 1 were sieved to <2 mm at field-moisture, and water holding capacity (WHC) was determined on a 10 g subsample. Briefly, we removed the tips from 50 ml centrifuge tubes and covered them with a fine mesh (<50 μm). We filled the tubes with soil and placed them upright with the mesh-side down in a glass dish filled with deionized water. Tubes were left overnight. The following day we moved them to a second glass dish filled with sand. We allowed the soils to drain for 30 min before weighing again to determine the amount of water absorbed. The remaining soil was then split, with one aliquot air-dried at 40°C (air-dry/rewet + storage treatment samples, $n = 12$), while the other aliquot was left at field moisture (control-1 samples, $n = 12$). Control-1 samples were stored in re-sealable plastic bags at 4°C until incubation. After air-drying, air-dry/rewet + storage samples were placed in re-sealable plastic bags, and stored in large plastic boxes in a cool (ca. 15°C) dark room for seven years.

2.1.3. Experiment 1 Incubations

Control-1 incubations were performed in 2011 on single samples due to time and space limitations within the original experiment. Soils were weighed out into 250 ml beakers and placed into 1,000 ml mason jars

Table 1
Mean Soil Properties by Sampling Region[a]

							Nutrients				Particle size					
							Organic C		Total N		Sand		Silt		Clay	
			MAT	MAP	n[c]		Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Experiment	Region	Ecosystem[b]	°C	mm yr^{-1}	sites		g kg^{-1}									
1	Central Germany 1	forest	8.3	550	3		22.1	8.1	1.1	0.3	861	44	92	27	47	20
1	Central Germany 1	grassland	8.3	550	3		22.8	1.5	2.2	0.1	731	99	158	75	111	31
1, 2, 3	Central Germany 2	forest	7.3	650	3		23.7	0.5	1.7	0.1	54	18	754	7	193	15
1, 2, 3	Central Germany 2	grassland	7.3	650	3		41.8	1.9	3.9	0.1	32	17	553	78	414	65
3	Oak Ridge, USA	forest	14.1	1360	2		24.9	0.0	1.1	0.1	-	-	-	-	-	-
3	Sierra Nevada, USA	forest	9.8	960	2		28.4	1.4	1.1	0.1	700	141	210	85	100	71
3	Harvard Forest,USA	forest	7.9	1075	1		60.0	-	-	-	-	-	-	-	-	-
3	Duke FACE, USA	forest	15.5	1140	1		16.6	-	0.8	-	-	-	-	-	-	-

[a]The Central Germany regions are from the Biodiversity Exploratory project: Schorheide-Chorin (region 1) and Hainich-Dün (region 2). Climate data for these sites are from Fischer et al. (2010). Harvard Forest nutrient data from Gaudinski et al. (2000); climate data are the ten-year averages from 1991 to 2000 (Boose & Gould, 2021); all Oak Ridge data are from Cisneros-Dozal et al. (2006); Duke FACE data are from Hopkins et al. (2012); Sierra Nevada data are from Koarashi et al. (2012). Note that not all data were available for all sites. [b]Central Germany 2, Harvard Forest, and Oak Ridge forest sites are mixed deciduous; Central Germany 2 forest sites include both coniferous and deciduous stands; Sierra Nevada and Duke FACE forest sites are exclusively coniferous. Grasslands were all cool-season grasses (C3 photosynthetic pathway). [c]See Table 2 for the total number of samples per experiment, and Table 3 for the number of samples per site per experiment.

with airtight lids fitted with two sampling ports. The mass of soil used for control-1 incubations ranged from 45 to 75 g (air-dry equivalent), based on estimated respiration rates from previous work at the sites. Soil masses were adjusted to ensure that enough CO_2 would be respired to measure $\Delta^{14}C$-CO_2 (>0.5 mg) while at the same time preventing excessive CO_2 build-up, as this has been shown to negatively impact heterotrophic respiration (MacFayden, 1973; Šantrůčková & Šimek, 1994).

Soil moisture content of control-1 samples was adjusted to 60% of WHC prior to sealing the jars. We moistened the soil from the top using a perforated luerlock cap attached to a 10 ml syringe that emitted water in small droplets for minimal disturbance. All control-1 samples were incubated for 4 days following moisture adjustment (the first enclosure period), after which the jars were flushed with CO_2-free air and allowed to accumulate CO_2 for a second enclosure period of 14 days.

We performed the air-dry/rewet + storage treatment incubations on the air-dried subsamples in 2018. We incubated the air-dry/rewet + storage samples in duplicate in order to quantify potential laboratory errors. Owing to a limited quantity of archived soil, we reduced the mass of soil incubated to 20 g. Using the same procedure as with control-1 samples, soil moisture content was adjusted to 60% WHC prior to flushing and sealing the jars. We maintained the same 4 day first enclosure period to capture the CO_2 released during the rewetting pulse. We determined the duration of the second enclosure period for the air-dry/rewet + storage treatment incubations according to the amount of CO_2 respired. We allowed the air-dry/rewet + storage treatment incubations to proceed until the same amount of CO_2 had been respired per g soil C as in the second enclosure period of corresponding control-1 sample incubations. Consequently, the incubation duration of the second enclosure period for the air-dry/rewet + storage treatment incubations varied (Table 2).

Headspace CO_2 concentrations for control-1 incubations were measured once at the end of the first enclosure period, but were measured daily during the first enclosure period for air-dry/rewet + storage incubations. We measured headspace CO_2 concentrations one to three times per week during the second enclosure period for both control-1 and air-dry/rewet + storage treatment incubations, with more frequent measurements made for samples with faster respiration rates. Headspace gas samples were collected and analyzed for $\Delta^{14}C$ and $\delta^{13}C$ content at the end of both the first enclosure period and the second enclosure period for the air-dry/rewet + storage treatment incubations. However, these measurements were only made following the second enclosure period for control-1 samples. All samples were incubated at 20°C.

Table 2
Experimental Design

				Sampling date	Incubation date	Moisture content[b] Initial	Adjusted	Enclosure period[c] 1st (rewetting pulse) Time	$\Delta^{14}C$	$\delta^{13}C$	2nd Time	$\Delta^{14}C$	$\delta^{13}C$
Experiment	n	Treatment	Reps[a]	*year*	*year*	*% grav*	*% grav*	*days*			*days*		
1	12	control-1	1	2011	2011	24-55 (11)	24-61 (13)	4	no	no	14	yes	yes
	12	air-dry/rewet + storage	2	2011	2018	<1	24-61 (13)	4	yes	yes	5–45	yes	yes
2	6	control-2	2	2019	2019	17-40 (10)	22-42 (9)	4	yes	yes	10–38	yes	yes
	6	air-dry/rewet	2	2019	2019	<1	22-42 (9)	4	yes	yes	7	yes	yes
3	29	control-3	1–3	1999–2011	1999–2011	6-95 (18)	18-95 (17)	1–10	no	no	5–14	yes	no
	29	storage duration	1–3	1999–2011	2009, 2018	<1	18-95 (17)	-	-	-	5–45	yes	no

[a]Laboratory incubation replicates. [b]Min. and max. values given for control samples, with standard deviations in parentheses. Initial moisture content for treatment samples was <1% following air-drying. Moisture content was adjusted to 60% of water holding capacity for all Experiment 1 and Experiment 2 samples (Methods), but as WHC was not determined for all of Experiment 3 samples the gravimetric (grav) data is provided instead. [c]First enclosure period duration range is only taken from a subset of the samples where it was explicitly reported (n = 4, Hopkins et al., 2012 and Koarashi et al., 2012). The duration was reported as an estimate for some samples (1 week, n = 20, Cisneros-Dozal et al., 2006) or not reported at all for other samples (n = 4, Gaudinski et al., 2000). $\Delta^{14}C$-CO_2 and respiration rates from the first enclosure period were only measured for 2 of the 29 control-3 samples (Koarashi et al., 2012). As we did not find significant differences between $\Delta^{14}C$-CO_2 of the 1st and 2nd enclosure periods (Results), we decided to incubate the storage duration samples in Experiment 3 for single enclosure period in order to better control the total amount of CO_2 respired.

2.2. Experiment 2: Air-Dry/Rewet Without Long-Term Storage

2.2.1. Experiment 2 Sample Selection and Field Sampling

We returned to the Central Germany 1 region (Hainich-Dün) in July 2019 to collect samples for Experiment 2 from the same plots originally sampled for Experiment 1 in 2011. We observed similar $\Delta^{14}C$-CO_2 across both Central Germany regions in Experiment 1, so we restricted the resampling to just Hainich-Dün 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. Following the protocol from the 2011 sampling, any aboveground vegetation was clipped, and organic horizons were scraped away prior to coring at the forest plots.

2.2.2. Experiment 2 Sample Preparation

Following sample collection, soils for Experiment 2 were sieved to <2 mm at field moisture, and WHC was determined on a 10 g subsample. The remaining soil was then split, with one aliquot air-dried at 40°C (air-dry/rewet treatment samples, n = 6), while the other aliquot was left at field moisture (control-2 samples, n = 6). Control-2 samples were stored in re-sealable plastic bags at 4°C until incubation. After air-drying, air-dry/rewet treatment samples were placed in re-sealable plastic bags, and stored in large plastic boxes in a cool (ca. 15°C) dark room for two months prior to incubation.

2.2.3. Experiment 2 Incubations

Incubation conditions for control-2 and air-dry/rewet treatment samples were identical. Incubations were performed in duplicate. We weighed out 20 g (air-dry equivalent) of soil into 250 ml beakers and placed them into the same incubation vessels as we used for Experiment 1. Prior to sealing the jars, we adjusted the soil moisture content to 60% WHC in the same manner as Experiment 1 samples (Section 2.1.3): either from field moisture (control-2 samples) or from the air-dried state (air-dry/rewet samples). Following moisture adjustment, jars were flushed with CO_2-free air, sealed, and left to incubate for the 4-day first enclosure period. After the first enclosure period the jars were flushed, and CO_2 was allowed to accumulate for a second enclosure period (Table 2).

Headspace CO_2 concentrations of both contol-2 and air-dry/rewet incubations were measured following the same protocol as the air-dry/rewet + storage incubations in Experiment 1: daily during the rewetting pulse period, and one to three times per week during the second enclosure period, depending on respiration rates.

Headspace gas samples were collected and analyzed for $\Delta^{14}C$ and $\delta^{13}C$ content at the end of both the rewetting pulse period and the second enclosure period. Control-2 samples were allowed to respire until >0.5 mg of CO_2-C was present in the jar headspace, which is the quantity needed to measure $\Delta^{14}C$. Incubations for the air-dry/rewet treatment samples were allowed to proceed until the same amount of CO_2 was respired per g of soil C as in the corresponding control-2 sample. All samples were incubated at 20°C.

2.3. Experiment 3: Storage Duration

Control-3 incubations were conducted by different investigators in different labs as part of six unrelated experiments. Due to the variation in experimental design among the control-3 incubations, we were forced to modify the incubation conditions for Experiment 3 samples slightly from the protocols followed in Experiments 1 and 2.

2.3.1. Experiment 3 Sample Selection

The main criteria for sample selection for Experiment 3 were: (a) samples were split prior to original incubation, with one portion air-dried and archived in amounts adequate for a repeated incubation; (b) $\Delta^{14}C$-CO_2 was measured from soils incubated close to the time of collection following a relatively short (one to three weeks) incubation period. We sought to cover a range of storage duration times (between 4 and 14 years, constrained by the availability of samples), and a range of soil types and climatic conditions (Table S5).

2.3.2. Experiment 3 Sample Preparation

Sieving protocols varied among control-3 samples, with some samples sieved to 2-mm while others remained unsieved (Table S5). All soils obtained for the storage duration incubations were air-dried splits made prior to control-3 incubations.

2.3.3. Experiment 3 Incubations

Soil mass and replication of corresponding storage duration treatment incubations varied (Table 2) according to the amount of soil material available. We kept the soil moisture the same between paired control-3 and storage duration treatment incubations. Incubation temperatures varied for control-3 incubations, but we conducted all storage duration treatment incubations at 20°C for simplicity. Although temperature has known effects on respiration rates, it has been shown that it does not affect $\Delta^{14}C$-CO_2 (Vaughn & Torn, 2019).

We did not have information on either the duration of the rewetting period or the corresponding amount of CO_2 respired during this period for all of the control-3 samples. Rather than impose a first enclosure period with an arbitrary duration, we decided to incubate the storage duration treatment samples for a single enclosure period beginning immediately after rewetting. We felt this was justified as we did not observe significant differences between first and second enclosure period $\Delta^{14}C$-CO_2 in the first two experiments (Results 3.2). We allowed respiration in the storage duration treatment samples to proceed until the same amount of CO_2 had been respired per g of soil C as in the second enclosure period of the corresponding control-3 sample incubations.

We measured headspace CO_2 concentrations every three days for the first two weeks of the storage duration treatment incubations, and weekly as needed thereafter; control-3 CO_2 measurement frequency varied. Aliquots of jar atmosphere were collected once the samples reached target CO_2 concentrations (7–48 mg CO_2 g C^{-1}), and then analyzed for $\Delta^{14}C$. We conducted the majority ($n = 16$) of the Experiment 3 storage duration treatment incubations in 2018 at the Max Planck Institute for Biogeochemistry (MPI-BGC) but the remainder ($n = 12$) of the treatment sample incubations were performed in 2009 at the University of California Irvine (UCI) (Table S5).

2.4. Soil Analyses

Total carbon and nitrogen contents of the Central Germany samples were determined by dry combustion in a CN analyzer (Vario Max, Elementar Analysensysteme GmbH) following fine grinding with a ball-mill (Retsch MM400). Soil texture of the Central Germany samples was determined using the pipette method, following removal of organic matter (Schlichting et al., 1995). Soil property data for the samples from all

other regions were obtained from the original studies (Cisneros-Dozal et al., 2006; Gaudinski et al., 2000; Hopkins et al., 2012; Koarashi et al., 2012; Solly et al., 2014) (Table 1).

2.5. Isotopic Analyses

For all three experiments, we separated CO_2 from the gas samples collected from incubation jar headspace using a vacuum line, with splits of the purified CO_2 analyzed for both $\delta^{13}C$ and $\Delta^{14}C$. Radiocarbon analyses were conducted at the MPI-BGC accelerator mass spectrometer facility (Steinhof, 2013) or the UCI W.M. Keck Facility for Accelerator Mass Spectrometry (Xu et al., 2007) (Table S5). Radiocarbon values are reported in units of $\Delta^{14}C$, defined as the deviation in parts per thousand of the ratio of ^{14}C–^{12}C from that of the oxalic acid standard measured in 1950. In order to account for potential mass-dependent fractionation effects, the $^{14}C/^{12}C$ ratio of all samples is corrected to a common $\delta^{13}C$ value of -25 per mil (Stuiver & Polach, 1977). Although the effect was small, $\Delta^{14}C$ data from air-dry/rewet + storage samples (Experiments 1 and 3) were also corrected for depletion of ^{14}C in the samples due to radioactive decay occurring during storage.

Measurements of $\delta^{13}C$ (Experiments 1 and 2 only) were made at MPI-BGC (Delta$^+$XL, Thermo Finnigan). Data are reported using $\delta^{13}C$ notation, which refers to the deviation in parts per thousand of the ratio of $^{13}C/^{12}C$ in the Pee Dee Belemnite standard.

2.6. Statistical Analysis

We compared the mean differences between treatment and control sample $\Delta^{14}C$-CO_2 and $\delta^{13}C$-CO_2 within ecosystem types for each experiment in order to assess the significance of the treatment effects. We quantified the analytical error associated with the radiocarbon incubation method by calculating the mean of the variance measured among replicates for all samples that were replicated. For samples that were not replicated we used the mean of the replicate variance measured across all samples. First we calculated mean differences between control and treatment samples, and the variance of this mean difference, and then we determined the mean and variance of the pooled sample. We calculated pooled statistics separately for forest and grassland soils in Experiments 1 and 2. Statistics were aggregated across ecosystem type for Experiment 3 as the direction of trend was the same for both forest and grassland soils (in this experiment), and we only had a limited number of grassland soils ($n = 3$).

The pooled mean is simply the average of the individual sample means weighted by the number of replicates. We determined the pooled variance (Equation 1) using the method of O'Neil (2014), which takes into account both sampled and unsampled variance for a finite population. We used this variance to determine 95% confidence intervals around the pooled mean difference, which we deemed significant if the confidence interval did not overlap zero.

$$S^2_N = \frac{\sum \left(n_i - 1\right) s_i^2 + \sum \left[n_i \cdot \left(\overline{x}_i - \overline{X}\right)\right]}{N - 1} \tag{1}$$

We conducted a parallel analysis using a linear mixed model approach, which we found supported our main findings with the paired difference approach. We decided to present only the results from the paired difference analysis in the interest of simplicity. However, details of the linear mixed model analysis and the results are provided in the suporting information (Text S1). We also conducted an exploratory analysis on the effect of the amount of C respired and the change in soil moisture content on the difference between control and treatment sample $\Delta^{14}C$-CO_2.

All statistical analyses were performed in R (R Core Team, 2020).

2.7. Conceptual Model

We developed a conceptual model for the forested sites from a single region, Hainich-Dün (Central Germany 2), to illustrate potential sources for the carbon respired following the air-drying and rewetting treatments imposed in this study. We did not use the $\Delta^{14}C$-CO_2 data observed in our study to constrain the model, but rather used a model developed for forested sites in the same region to validate our findings (Schrumpf & Kaiser, 2015). We implemented a two-pool parallel model, with inputs partitioned between slow and
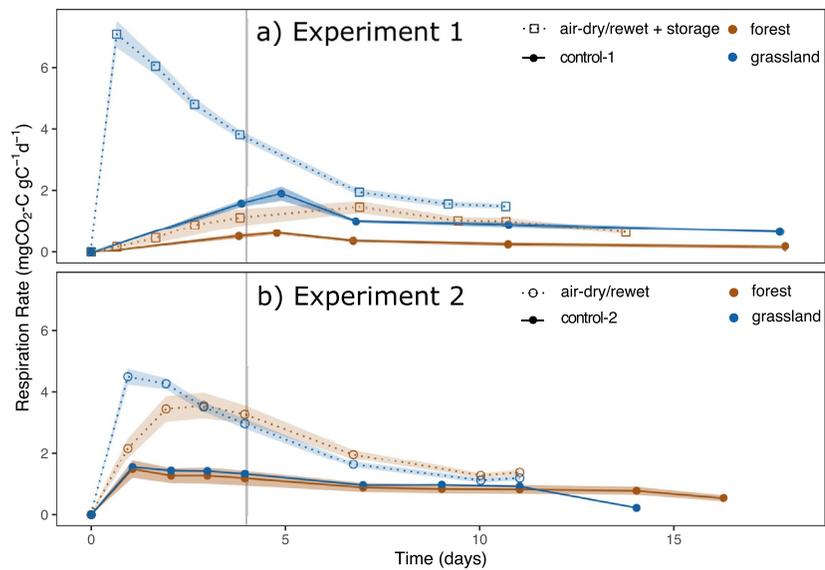
Figure 1. Respiration rates for Experiment 1 (Air-dry/rewet + storage) and Experiment 2 (Air-dry/rewet only) samples. (a) Experiment 1 samples; (b) Experiment 2 samples. Vertical gray line at day 4 demarcates the end of the first enclosure period (rewetting pulse). Points show measurements and lines 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 days to reach CO_2 targets are excluded for display reasons; respiration rates for those samples remained constant. Note that headspace CO_2 concentrations for control-1 samples (panel a) were only measured once during the first enclosure period (day 4) in contrast to daily measurements for all other samples.

fast cycling soil C pools and no transfers between pools, using the Soil R package (Sierra et al., 2014). In an earlier study, Schrumpf and Kaiser (2015) estimated first order C cycling rates and pool sizes for empirically defined soil C pools using a density fractionation procedure. We approximated the inverse of the first order cycling rates (turnover times) for the fast and slow pools of our model using Schrumpf and Kaiser (2015)'s empirical estimates for the free light fraction and the heavy fraction from the 0–5 cm depth increment: 4 and 115 years for the fast and slow pools, respectively. Schrumpf and Kaiser (2015) found that 10% of the carbon in the 0–5 cm depth layer was in the free light fraction. We used this proportion to partition soil C between the fast and slow pools, under the assumption that the free light fraction corresponds to the fast pool. Following the earlier study, we assumed a lag time of 8 years for inputs.

3. Results

3.1. Respiration Rates

We observed consistent differences between control and treatment sample respiration rates in Experiments 1 and 2, with control sample respiration rates lower than treatment sample respiration rates in both experiments (Figure 1). However, the magnitude and timing of maximum respiration rates diverged among experiments and between grassland and forest soils (Figure 1). Maximum respiration rates were more than twice as high in grassland soils than in forest soils for air-dry/rewet + storage treatment samples in Experiment 1 (Figure 1a), but were similar across ecosystem types for the air-dry/rewet treatment samples in Experiment 2 (Figure 1b). Respiration rates for Experiment 3 samples are shown in Figure S4. However, CO_2 flux rates cannot be meaningfully interpreted for these samples given the differences in incubation temperature, the degree to which rewetting pulse CO_2 was included in the control-3 incubations, and the wide variation in CO_2 measurement frequency among samples.

3.2. First and Second Enclosure Period $\Delta^{14}C$-CO_2 and $\delta^{13}C$-CO_2

We did not see significant differences when we compared $\Delta^{14}C$-CO_2 from the first enclosure period to that of the second enclosure period (Figure 2). This was true for all comparisons made within experiment,
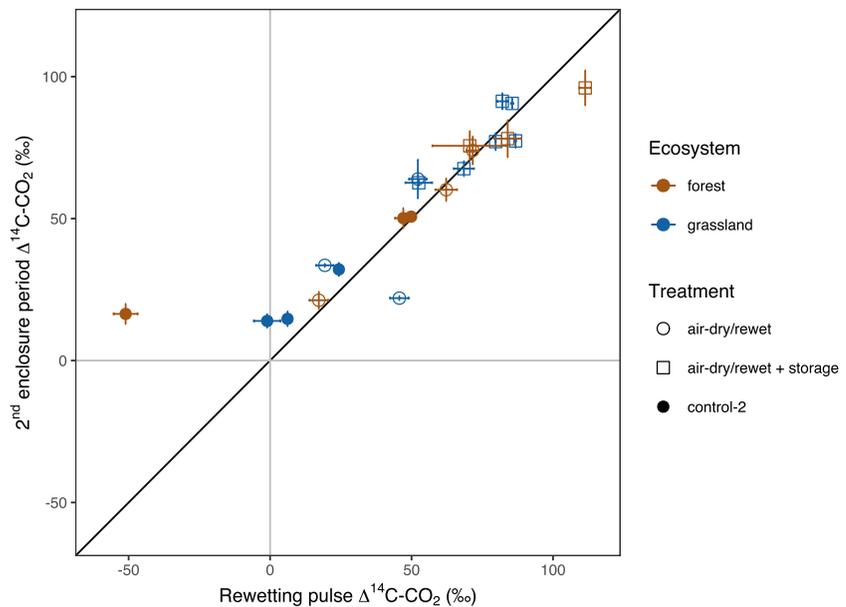
Figure 2. $\Delta^{14}C$-CO_2 of the rewetting pulse (first enclosure period) versus the second enclosure period. Points are means of laboratory duplicates and error bars are the minimum and maximum. Note that $\Delta^{14}C$-CO_2 was not measured for the first enclosure period (rewetting pulse) in control-1 samples; additionally samples from three of the forest plots of the air-dry/rewet + storage samples from Experiment 1 failed to accumulate enough CO_2 during this period to measure $\Delta^{14}C$-CO_2.

treatment, and ecosystem groups, with one exception: grassland control-2 samples had slightly higher $\Delta^{14}C$-CO_2 in the second enclosure period compared to the first (mean difference = 10.4‰, 95% CI = [6.0‰, 14.8‰]). When we combined data across experiments, ecosystem types, and treatments, the mean difference in $\Delta^{14}C$-CO_2 between enclosure periods was only 2.0‰ (95% CI = [−1.0‰, 5.0‰]), which is similar to the reported precision for ^{14}C measurements (1.7‰–2.7‰). (We excluded the forest control-2 sample that was clearly an outlier (Figure 2) from this combined analysis).

We note that, due to lower respiration rates during the first enclosure period, only three of the six forest soils in the air-dry/rewet + storage treatment group from Experiment 1 (Figure 2) generated enough CO_2 to measure radiocarbon content. In addition, it was not possible to compare $\Delta^{14}C$-CO_2 across enclosure periods for the control-1 samples as $\Delta^{14}C$-CO_2 of the first enclosure period was not measured in 2011.

In contrast to $\Delta^{14}C$-CO_2, we did observe significant differences between the $\delta^{13}C$-CO_2 of the first enclosure period and that of the second enclosure period for the forest soils in the air-dry/rewet + storage treatment group in Experiment 1 (mean difference = −1.16‰, 95% CI = [−1.69‰, −0.63‰]) and the control-2 grassland soils (Experiment 2) (mean difference = 0.85‰, 95% CI = [0.64‰, 1.07‰]) (Figure S5). Note that as with $\Delta^{14}C$, $\delta^{13}C$-CO_2 was not measured for the first enclosure period of control-1 incubations.

3.3. Overall Treatment Effects on $\Delta^{14}C$-CO_2 and $\delta^{13}C$-CO_2

We observed consistent differences between control and treatment sample $\Delta^{14}C$-CO_2 in the second enclosure period in all three experiments (Table 3). Treatment sample incubations typically resulted in differences between 20‰ and 40‰ relative to control sample incubations, although the majority of the differences were within ±20‰ (dashed lines, Figure 3). The samples from Oak Ridge are an exception in that mean difference in $\Delta^{14}C$-CO_2 between storage treatment samples and corresponding control-3 samples was −44.0‰ (Table 3).

Forest and grassland soil $\Delta^{14}C$-CO_2 shifted in opposite directions following treatment in Experiment 1: the air-dry/rewet + storage treatment led to depletion in forest soils, but enrichment in grassland soils (Table 3). In contrast, both forest and grassland soils in Experiment 2 responded to the air-dry/rewet treatment with

Table 3
$\Delta^{14}C$ and $\delta^{13}C$ of Respired CO_2 in the Second Enclosure Period[a]

Experiment[b]	Ecosystem	Treatment	n	$\Delta^{14}C\text{-}CO_2$ Mean	$\Delta^{14}C\text{-}CO_2$ Mean $\Delta\Delta$[c]	$\Delta^{14}C\text{-}CO_2$ sd	$\delta^{13}C\text{-}CO_2$ Mean	$\delta^{13}C\text{-}CO_2$ sd	Difference (treatment - control) $\Delta^{14}C\text{-}CO_2$ Mean	$\Delta^{14}C\text{-}CO_2$ CI95	$\delta^{13}C\text{-}CO_2$ Mean	$\delta^{13}C\text{-}CO_2$ CI95
								‰				
1	forest	air-dry/rewet + storage	6	82.2	44.9	8.8	−24.2	1.1				
1	forest	control-1	6	93.8	56.5	7.7	−26.8	0.2	−11.6	[−15.7, −7.5]	2.38	[1.83, 2.92]
1	grassland	air-dry/rewet + storage	6	77.8	40.5	11.4	−27.2	0.5				
1	grassland	control-1	6	54.5	17.2	16.8	−27.7	0.4	23.3	[16.7, 29.9]	0.51	[0.18, 0.83]
2	forest	air-dry/rewet	3	51.8	62.9	24.9	−24.5	1.4				
2	forest	control-2	3	39.1	50.2	17.9	−26.1	0.8	12.7	[4.2, 21.2]	1.56	[0.41, 2.72]
2	grassland	air-dry/rewet	3	39.8	50.9	19.9	−27.5	0.4				
2	grassland	control-2	3	20.2	31.4	9.6	−28.6	0.3	19.6	[9.3, 29.9]	1.11	[0.57, 1.66]
3a	forest	storage duration	9	-	-	-	-	-				
3a	forest	control-3	9	-	-	-	-	-	−24.2	[−37.4, −11.0]	-	-
3a	grassland	storage duration	3	-	-	-	-	-				
3a	grassland	control-3	3	-	-	-	-	-	−29.3	[−58.4, −0.2]	-	-
3b	forest	storage duration	17	-	-	-	-	-				
3b	forest	control-3	17	-	-	-	-	-	−44.0	[−52.0, −35.9]	-	-

[a]Experiment 3 storage duration treatment samples were only incubated for a single enclosure period and so data were measured following this period. [b]Results from Experiment 3 reported separately for the enriched samples from Oak Ridge (3b) and the nonenriched samples (3a). Mean control and treatment $\Delta^{14}C\text{-}CO_2$ are only reported for Experiments 1 and 2 where the aggregated data are representative of one site at one point in time. [c]The $\Delta\Delta$ notation denotes the difference from the atmosphere at the time of sampling.

enrichment in $\Delta^{14}C\text{-}CO_2$. Experiment 3 treatment sample $\Delta^{14}C\text{-}CO_2$ tended to be depleted relative to the controls (points below the 1:1 line in Figure 3) for the majority of forest and grassland soils.

We did not find evidence of a substantial effect of the amount of C respired on $\Delta^{14}C\text{-}CO_2$, nor consistent effects due to the change in soil moisture (Figures S7 and S8).

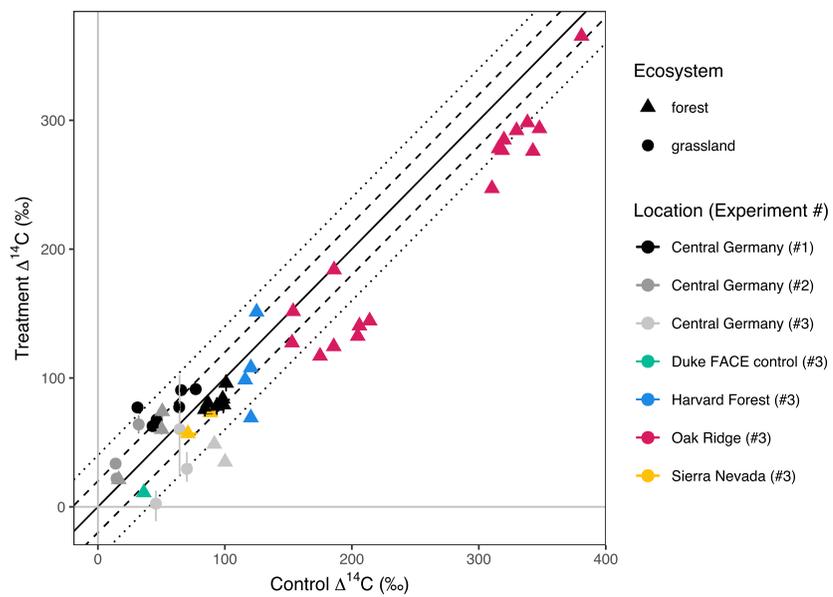Treatment samples in Experiment 1 and Experiment 2 showed significant differences ($\alpha = 0.05$) in $\delta^{13}C\text{-}CO_2$ relative to the controls for both forest and grassland soils (Figure S6). Overall differences in $\delta^{13}C\text{-}CO_2$ were slightly larger for forest soils than in grassland soils (Table 3). Note that comparisons of $\delta^{13}C\text{-}CO_2$ were not made in Experiment 3 owing to a lack of data for the control-3 samples.

3.4. Storage Duration Effect on $\Delta^{14}C\text{-}CO_2$

We used data from both Experiment 1 and Experiment 3 to assess the effect of storage duration. The longest duration of storage was 14 years, while the shortest was 5 years. Over this range of time we did not observe a trend in the difference between control and treatment $\Delta^{14}C\text{-}CO_2$ with increasing duration of storage (Figure 4).

3.5. Time Series Analysis of $\Delta^{14}C\text{-}CO_2$ (Experiments 1 and 2)

For the sites sampled in both 2011 (Experiment 1) and 2019 (Experiment 2), the absolute value of the mean difference in $\Delta^{14}C\text{-}CO_2$ between control and treatment samples was greater in grassland samples than in forest samples at both time points (Table 3). In addition to the absolute values of $\Delta^{14}C\text{-}CO_2$, the difference between respired $\Delta^{14}C\text{-}CO_2$ and the atmosphere in the year of sampling ($\Delta\Delta^{14}C$) is a useful indicator of soil C transit times, that is the duration of time from when CO_2 was fixed from the atmosphere to when it leaves the soil via respiration. We observed that sample $\Delta^{14}C\text{-}CO_2$ was enriched relative to the atmosphere across

Figure 3. Overall treatment effect on $\Delta^{14}C$-CO_2. Points show data from all three experiments and are the mean of laboratory replicates (for replicated samples); error bars are standard deviation of replicates. Solid line is 1:1. For context, the dashed and dotted lines show differences of $\pm20‰$ and $\pm40‰$, respectively. Location names are followed by the corresponding experiment number in parentheses. The samples from both Central Germany sites (Hainich-Dün and Schorfheide-Chorin) behaved similarly in Experiment 1, so samples analyzed in the same experiment are coded with the same colors in the above figure. Oak Ridge soils were part of a whole ecosystem ^{14}C label experiment (Cisneros-Dozal et al., 2006), where the label occurred within four years of original sample collection.

ecosystem types for all both control and treatment samples at all timepoints, that is $\Delta\Delta^{14}C$ values were all positive (Figure 5, Table 3). We measured lower $\Delta\Delta^{14}C$ values for grassland samples than forest samples in both 2011 and 2019, meaning grassland sample $\Delta^{14}C$-CO_2 was closer to the atmosphere than forest sample
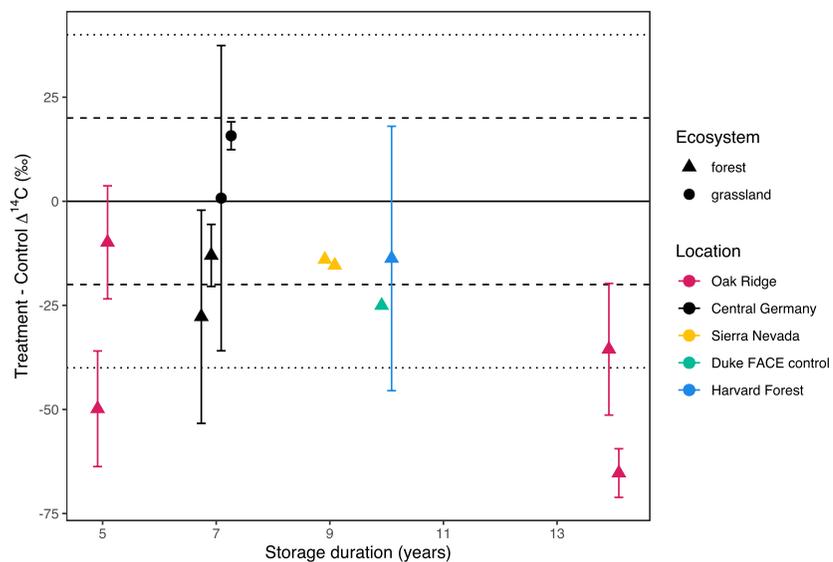
Figure 4. Treatment effect on $\Delta^{14}C$-CO_2 in relation to storage duration. Points show data from Experiments 1 and 3. Data are averaged by site (some regions had multiple sites, Table 3) and error bars show the standard deviation for the site mean. Note that Central Germany samples from Experiments 1 and 3 are averaged together here. For context, the dashed and dotted lines show differences of $\pm20‰$ and $\pm40‰$, respectively. The Oak Ridge sample points with the greater treatment-control difference at both 5 and 14 years of storage are from the Tennessee Valley site, which received more ^{14}C label than did the other Oak Ridge site, Walker Ridge.
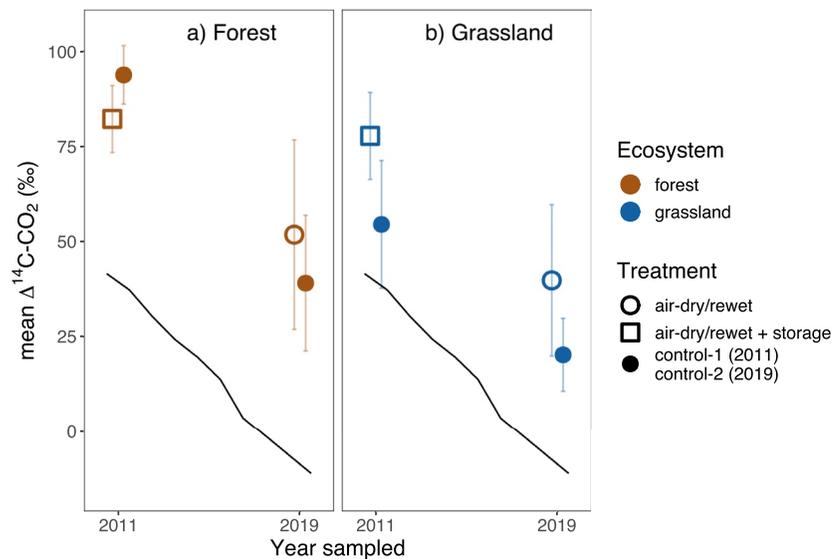
Figure 5. Time series of control and treatment $\Delta^{14}C\text{-}CO_2$ in Experiments 1 and 2. (a) forest sites, (b) grassland sites. Points show mean $\Delta^{14}C\text{-}CO_2$ within ecosystem and treatment groups; error bars show the standard deviation. Atmospheric $\Delta^{14}C\text{-}CO_2$ data (black 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).

$\Delta^{14}C\text{-}CO_2$ (Table 3). Within ecosystem types, control sample $\Delta\Delta^{14}C\text{-}CO_2$ values were lower than treatment samples for both 2011 and 2019 grassland soils, as well as the 2019 forest soils. However, we observed the opposite trend for the 2011 forest soils: for these soils the treatment sample $\Delta^{14}C\text{-}CO_2$ was closer to the atmosphere than control sample $\Delta^{14}C\text{-}CO_2$.

4. Discussion

4.1. How Closely do Incubations of Archived, Air-Dried and Rewetted Soils Match Results From Fresh Soil Incubations?

The results from all three experiments in this study show that measuring $\Delta^{14}C\text{-}CO_2$ in incubations of air-dried and archived soils is a promising technique for constructing time series of respired $\Delta^{14}C\text{-}CO_2$ and constraining soil carbon models. We observed that air-drying and rewetting shifted observed $\Delta^{14}C\text{-}CO_2$ relative to control incubations of soils that had never been air-dried, but these differences were relatively small: on the order of 10‰–25‰ (excluding the samples from the Oak Ridge labeling experiment, Table 3). However, differences between control and treatment $\Delta^{14}C\text{-}CO_2$ were significant for all three experiments (Table 3), suggesting that the process of drying and rewetting leads to utilization of substrates with distinct $\Delta^{14}C$ signatures.

4.2. Effects of Air-Drying and Rewetting on the Age of Respired CO_2

We suggest that air-drying and rewetting mobilizes carbon from more slowly cycling pools than would be available to the microbial community in soils that did not undergo air-drying and rewetting. Given the trajectories of $\Delta^{14}C$ in slow and fast cycling soil carbon pools over time, we can expect different responses to air-drying and rewetting in $\Delta^{14}C\text{-}CO_2$ in soils sampled at different times. The time series data from the Hainich-Dün sites sampled in both 2011 and 2019 provide a case-study for this behavior. At these sites we observed enrichment in $\Delta^{14}C\text{-}CO_2$ following air-drying and rewetting for the forest soils collected in 2019 (Experiment 2) and the grassland soils collected in both 2011 (Experiment 1) and 2019 (Experiment 2), but depletion in the forest soils collected in 2011 (Experiment 1). We present an empirical model of soil C dynamics developed at the Hainich-Dün forest site in a previous study (Schrumpf & Kaiser, 2015) in order to illustrate the importance of the year of sampling and system-specific carbon dynamics in interpreting $\Delta^{14}C\text{-}CO_2$ following air-drying and rewetting (Figure 6).
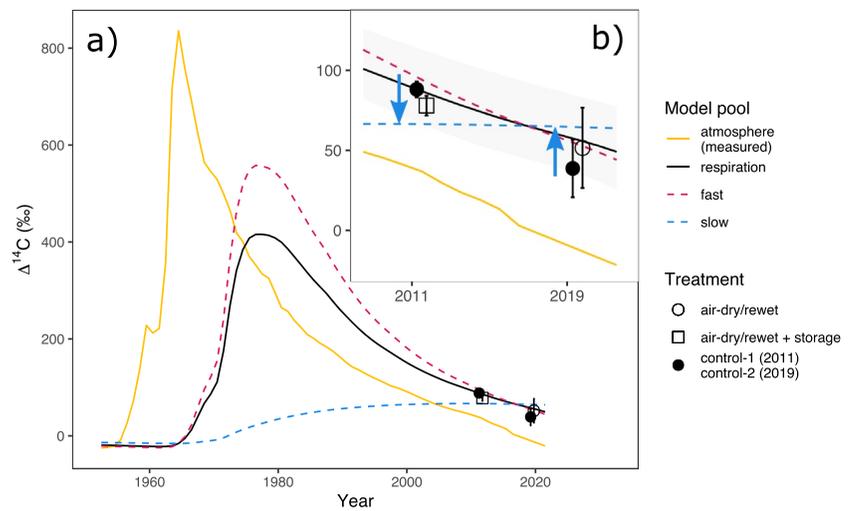
Figure 6. Conceptual model fit and observed $\Delta^{14}C$-CO_2 for the Hainich-Dün forest sites (Central Germany 2). (a) $\Delta^{14}C$ of soil pools and heterotrophically respired CO_2 predicted by the model adapted from Schrumpf and Kaiser (2015) alongside atmospheric $\Delta^{14}C$ for the bomb-C period (1950–2020) and $\Delta^{14}C$-CO_2 observed in this study (black points) (b) *(inset)* Zoom to study period. Blue arrows show the shift in $\Delta^{14}C$-CO_2 following air-drying and rewetting. Note that $\Delta^{14}C$-CO_2 shifts toward the slow pool in both 2011 and 2019, but the direction of the shift depends on sampling year. Points are jittered to prevent over plotting; error bars show standard deviations. Note that the model was not fit to the $\Delta^{14}C$-CO_2 observed in this study. Atmospheric $\Delta^{14}C$-CO_2 data are from Graven et al. (2017) up to the year 2015, while atmospheric points from 2015 to 2019 are extrapolated (Sierra, 2018). All atmospheric radiocarbon data is for the northern hemisphere (zone 2).

Comparing model projections of the trajectories for fast, slow and respired $\Delta^{14}C$ with the $\Delta^{14}C$-CO_2 measured in this study (Figure 6) indicates that our data are consistent with the mobilization of carbon from the slow C pool following drying and rewetting. Following treatment, $\Delta^{14}C$-CO_2 (black points) shifts toward the slow pool $\Delta^{14}C$ curve (dashed blue line), indicating an increased contribution to respiration from this pool. Due to the crossing of the slow and fast (magenta) pool curves in 2015, increased contribution of the slow pool to respiration following air-drying and rewetting leads to relative depletion of $\Delta^{14}C$-CO_2 in 2011, but relative enrichment of $\Delta^{14}C$-CO_2 in 2019. Thus, the bias in $\Delta^{14}C$-CO_2 introduced by air-drying and rewetting could be either higher or lower relative to a sample incubated without air-drying depending on the year of sampling.

4.3. Explaining Differences in Forest Versus Grassland Soil $\Delta^{14}C$-CO_2 in Experiments 1 and 2

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 Central Germany 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 years, in comparison to 1–2 years for fine roots in the grassland ecosystems. This pre-aging, or lag effect, for fine root inputs may explain the greater $\Delta\Delta^{14}C$ values seen for the respiration from forest ecosystems as compared to the grassland ecosystems in this study (Table 3).

In contrast to forests, the grassland soils responded to the air-drying and rewetting treatment with relative enrichment in $\Delta^{14}C$-CO_2 in both 2011 (Experiment 1) and in 2019 (Experiment 2) (Figure 5). However, we believe that the grassland soil response is due to the same mechanism as in the forest soils: a greater contribution of more slowly cycling carbon to respiration following air-drying and rewetting. The smaller positive $\Delta\Delta^{14}C$-CO_2 values we observed in grassland soils, in addition to the known shorter 'lag' effect, suggest that overall C cycling rates are faster in grasslands than in forests, which would lead to an earlier crossing of the ^{14}C curves for the fast and slow cycling soil carbon pools (see Figure 6). Our results indicate that for the

Central Germany sites we sampled in this study, this crossing occurred prior to 2011 for the grassland soils, but between 2011 and 2019 for the forest soils. If this is correct, even though the net change in $\Delta^{14}C$-CO_2 due to air-drying and rewetting differed between forests and grasslands in Experiment 1, both outcomes are still be consistent with the explanation that air-drying and rewetting mobilizes additional carbon from a more slowly cycling pool.

4.4. Is Rewetting Pulse CO_2 Derived From Different C Sources?

There are competing hypotheses for the source of CO_2 released immediately following rewetting, which seek to explain the immediate increase in respiration as well as the subsequent return to basal respiration rates (Fierer & Schimel, 2003; Kaiser et al., 2015; Slessarev et al., 2020; Warren, 2016; Williams & Xia, 2009). Due to the often dramatic differences in respiration rates between the rewetting period and subsequent respiration (e.g., Figure 1), previous authors posit differences in the substrates fueling rewetting versus subsequent respiration. However, we did not find a significant difference in $\Delta^{14}C$-CO_2 between these two respiration periods. This finding was true for all of the samples in which we measured $\Delta^{14}C$-CO_2 in both the rewetting pulse period and a second enclosure period (Figure 3). These results suggest that the change in substrate availability initiated by air-drying and rewetting may not be limited to the rewetting pulse.

There is a large body of literature that provides evidence for different chemistry of the substrates fueling the rewetting pulse compared to that of the substrates fueling basal respiration (Franzluebbers et al., 2000; Williams & Xia, 2009; Wu & Brooks, 2005; Xiang et al., 2008). However, as other recent work has shown, persistence of soil organic matter is not solely due to chemistry (Dungait et al., 2012; Lützow et al., 2006; Marschner et al., 2008; Schmidt et al., 2011). The similarity in $\Delta^{14}C$ across substrates utilized in the rewetting pulse and the second enclosure period, despite likely diverging in chemistry (cf. change in $\delta^{13}C$-CO_2, Table 3 and Figure S6), is therefore in line with the modern paradigm (Lehmann & Kleber, 2015; Lehmann et al., 2020). Alternatively, microbial recycling over the relatively short duration of the incubations in this study (mean = 9 d) could also explain the lack of change in $\Delta^{14}C$-CO_2 between enclosure periods. For context, we note that the mean amount of CO_2 respired in the incubations in this study was 0.8% of the initial total soil organic carbon. This microbial recycling hypothesis is also supported by the shifts in $\delta^{13}C$ observed between the rewetting pulse and the second enclosure period, which we did find to be significant.

While it is beyond the scope of this study, the age of C released has potential to help refine hypotheses about underlying mechanisms. For example, the decomposition of older, physically protected organic matter via disruption of aggregates (Kaiser et al., 2015) could be consistent with our observations, which could be tested by comparing measurements of C isotopes in physically separated aggregates to those in respired CO_2. Similarly, future experiments could be designed to investigate more specific hypotheses regarding extracellular versus microbially derived sources for fuelling the rewetting pulse. For example, comparing C isotopes in microbial phospholipid fatty acids (PLFA) versus those in water extractable organic C to what is respired before and after air-drying and rewetting would be one approach for assessing the relative importance of microbial recycling versus mineral-associated C (Slessarev et al., 2020).

4.5. Implication of $\delta^{13}C$-CO_2 Shifts Following Drying and Rewetting

The consistent enrichment in $\delta^{13}C$-CO_2 seen following both the air-dry/rewet + storage treatment and the air-dry/rewet treatment (Table 3, Figure S5) could have multiple possible causes. Microbial recycling has been shown to lead to $\delta^{13}C$ enrichment (Wynn et al., 2005), and to be enhanced following air-drying and rewetting (Brödlin et al., 2019; Slessarev et al., 2020). If the carbon substrate responsible for the rewetting pulse is derived from mobilization of older, microbially processed, and/or mineral-associated C, increases in $\Delta^{14}C$-CO_2 and $\delta^{13}C$-CO_2 such as those observed in both the air-dry/rewet and the air-dry/rewet + storage samples could be expected (Wynn et al., 2005). As noted previously, the $\Delta^{14}C$ unit accounts for mass-dependent fractionation effects, thus this phenomenon does not affect the radiocarbon results as reported.

We observed greater enrichment of $\delta^{13}C$-CO_2 in forest soils than in grassland soils, which could indicate greater microbial recycling in forest soils or potentially more mobilization of mineral-associated organic matter in forest soils than in grassland soils following treatment. Mineral-associated organic matter has been shown to be more enriched in $\delta^{13}C$ as well as older on average than bulk soil organic matter (Schrumpf

et al., 2013). This combination of observations indicates that more mineral-associated organic carbon may have been released upon rewetting in the forest soils than in the grassland soils. However, the similarity in the direction of the δ^{13}C-CO$_2$ response across forest and grassland soils (Figure S5) suggests that a similar mechanism is at work in both ecosystems.

4.6. Assessing Potential Storage Effects on Δ^{14}C-CO$_2$

Data from Experiment 1 and Experiment 3 showed that storage duration does not have a strong effect on Δ^{14}C-CO$_2$, at least within a period of 5–14 years (Figure 4). Nearly all of the soils incubated were from forests soils collected before 2019, and these all exhibited depletion of Δ^{14}C-CO$_2$ following air-drying/re-wet + storage treatment (Figures 3 and 4). However, the depletion in the forest soils was greatest in the samples from Oak Ridge (magenta triangles, Figure 3), which had been substantially enriched in Δ^{14}C above background levels through release of enriched ^{14}C from a nearby incinerator four years prior to sample collection. This and a subsequent manipulation experiment resulted in ^{14}C enrichment of both surface litter and root inputs (at levels between +400‰ and + 1,000‰) that persisted until the time of sample collection (Cisneros-Dozal et al., 2006).

One explanation for the greater shift observed for the Oak Ridge soils as compared to the nonlabeled forest soils is that for these labeled soils there is a greater difference in Δ^{14}C between the carbon fixed in the two decades prior to sampling (∼80–200‰) and the labeled carbon (+400‰−1,000‰) introduced to the soil in the four years prior to sampling. The consistently lower Δ^{14}C-CO$_2$ for samples incubated after air-drying and rewetting adds further support to the idea that C being mobilized comes primarily from CO$_2$ made available for decomposition from C fixed from the atmosphere >4 years previously. Alternatively, the greater difference observed in the Oak Ridge samples could indicate that the most recently fixed carbon in archived soils is lost over the storage period. However, given that storage of air-dried samples has not been linked to substantial loss of soil C in previous studies (Blake et al., 2000), this seems unlikely.

Thus, our major finding is that incubation of archived soils can provide useful information on the dynamics of soil carbon and, in particular, be helpful for constraining models of soil carbon. As it is clear that the process of air-drying and rewetting likely mobilizes and increases the contribution of older soil C to respiration in incubations, we recommend that modern soil comparisons use the same treatment (air-drying and rewetting) when creating a time series using newly collected soils from the same location.

5. Conclusion

Measuring Δ^{14}C-CO$_2$ in incubations of air-dried and archived soils is a promising technique for constructing time series of respired Δ^{14}C-CO$_2$ and constraining soil carbon models. Air-drying and rewetting of soils led to small but significant differences in the Δ^{14}C of respired CO$_2$ in laboratory incubations when compared to incubations of the same soils without air-drying. The magnitudes of these differences do not appear to be affected by the duration of storage and are within 25‰ for the majority of forest soils and 40‰ for the more limited number of grassland samples studied. Samples collected and analyzed in the same laboratory had smaller differences of 12.1‰, and 20.4‰, for forest and grasslands, respectively. (For context, Δ^{14}C of atmospheric CO$_2$ has declined by ca. 5‰ per year between 2000 and 2015 (Graven et al., 2017)).

Overall, our results demonstrate that differences in Δ^{14}C-CO$_2$ between archived soils and what might have been observed in samples incubated prior to air-drying and rewetting depend on two key variables: the year of sample collection and the carbon dynamics of the system being studied. Determining the exact mechanism driving the differences in Δ^{14}C-CO$_2$ is beyond the scope of this study, but our results suggest that the CO$_2$ released upon rewetting air-dried soils is fueled predominantly by older carbon, specifically through the mobilization of substrate from soil organic matter pools dominated by carbon fixed years to decades previously. Furthermore, this shift in Δ^{14}C-CO$_2$ persists beyond the rewetting pulse, suggesting that simply excluding the rewetting pulse CO$_2$ when measuring Δ^{14}C-CO$_2$ does not eliminate the bias introduced by air-drying and rewetting. Finally, we recommend that when comparing Δ^{14}C-CO$_2$ between recently collected soils and archived soils, both samples should undergo the same air-drying and rewetting procedure to minimize bias.

Data Availability Statement

Code and data have been archived on Zenodo as a github release (v2.0) with the DOI https://doi.org/10.5281/zenodo.4959705. The file 'arc-inc_ms-code_2021-06-15.Rmd' within the zip file of the repository release contains the R script for running all analyses, and the file "arc-inc_figs_2021-06-15.Rmd" will generate all figures in the main text. Supporting Information S1 can be reproduced with the file "SI_ArcInc_2021-06-15.Rmd".

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