Drying, Rewetting, and Storage Effects on the Radiocarbon Signature of Heterotrophic Respiration in Laboratory Soil Incubations

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Key Points:

* ∆14C of CO2 measured in incubations of archived soils provides an additional time point for constraining soil carbon models
* Air-drying and rewetting soils shifted the ∆14C of respired CO2 by 10 to 20‰, but storage duration did not have a significant effect
* The direction of the shift in ∆14C of CO2 depended on sampling year and the magnitude of change on ecosystem type (forest < grassland)

Abstract

Laboratory incubations are a useful technique for quantifying soil carbon pools are accessible and available to microorganisms. Measuring the radiocarbon signature of the CO2 released in incubations (14C-CO2) provides an integrative signal of the age of carbon leaving the soil (transit time), but in order to convert radiocarbon values to age a model is needed. Soil archives have the potential to provide multiple observations of respired CO2 at the same location over time, which could greatly reduce model uncertainty by providing additional constraints for model parameterization. However, air-drying, storage, and subsequent rewetting may lead to changes in the contribution of slower and faster cycling soil carbon pools to microbial respiration. We assessed the effects of air-drying, rewetting, and storage duration on 14C-CO2 observed in incubations of soil samples from forests and grasslands collected over the past two decades. We found that air-drying and rewetting led to significant (*p* < 0.05) changes in ∆14C-CO2, with changes smaller in forests (12.1‰) than in grasslands (21.4‰). These changes are equivalent to the difference in atmospheric radiocarbon over three to six years during the period 2000 to 2020. In contrast, the duration of storage did not appear to affect the difference between control samples and samples undergoing air-drying and rewetting. ∆14C-CO2 measured immediately following rewetting of soils was not significantly different than that measured during equilibrium respiration, suggesting that air-drying and rewetting induces lasting effects on substrate availability in laboratory incubations. While the shift in ∆14C-CO2 induced by air-drying and rewetting is significant, it is small enough that achived soil incubation deserves to be a valuable tool for improving soil carbon model uncertainty in the future.

**Plain Language Summary**

Soils play a key role in the global carbon cycle by storing it as organic matter for decades to millennia and thus acting as efficient carbon sink in the past, but it is unclear if they will continue to provide this ecosystem service as the climate changes. Microbial decomposition of soil organic matter returns CO2 back to the atmosphere, and radiocarbon dating of this returning CO2 can be used to quantify the potential for soil carbon storage. Archived soils can provide a unique insight as the radiocarbon signature of CO2 fixed from the atmosphere has changed markedly in the last decades. However, air-drying, time spent in archives, and subsequent rewetting of soils may affect the balance of younger versus older carbon leaving the soil, biasing the estimates of soil carbon storage potential. We compared the radiocarbon signature of CO2 from soils incubated with and without air-drying and archiving, and found that the air-dried soils appeared to release slightly older carbon than soils that had never been air-dried. The amount of time the soils were archived did not appear to have an effect. However, the effect of air-drying on the age of soil carbon decomposed by microbes was small: increasing the apparent storage time of carbon in soils by 5 years in forests and 10 years in grasslands. These results suggest that incubating archived soils is a promising technique for improving our ability to model near-term soil carbon storage and our understanding of global climate change.

1 Introduction

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

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

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

Calculating ages and transit times of soil carbon from measurements of Δ14C requires modeling soil carbon decomposition as a function of inputs, outputs, stocks, and if applicable, transfers between different soil carbon pools. However, model parameterization is challenging, due to uncertainty in defining soil carbon pools corresponding to relevant soil carbon stabilization mechanisms, as well as a lack of observational constraints. Radiocarbon observations at a single point in time are useful, but due to the curvature of the bomb-C peak there are two points in time with the same atmospheric radiocarbon value, which can lead to multiple model solutions (Trumbore, 2000). Observations of Δ14C-CO2 at more than one point in time can greatly reduce model uncertainty by serving as additional constraints (Baisden, Parfitt, Ross, Schipper, & Canessa, 2013).

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

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

Air-drying has been shown to result in the formation of new or stronger mineral-organic associations, increased aggregate stability, decreased microbial biomass, and a higher quantity of water-extractable organic matter (Kaiser, Kleber, & Berhe, 2015). Air-drying and rewetting effects appear to be soil-specific, with desorption of mineral-associated carbon upon rewetting observed for smectite-rich or highly charged soils, and differences in the quantity and rate of CO2 release following rewetting varying with soil texture and degree of aggregation (Kaiser et al., 2015).

In an incubation study of California grassland soils, exposure to multiple air-drying and rewetting cycles altered the 14C of respired CO2: leading to depletion between the first and last cycle in surface soils, but enrichment in subsoils (Schimel, Wetterstedt, Holden, & Trumbore, 2011).

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

However, if the relative contribution to respiration from soil organic matter pools with different intrinsic cycling rates changes in a short-term incubation following air-drying and rewetting, this should be detectable in 14C-CO2

For example, disruption of soil aggregates following drying and rewetting would likely lead to greater accessability of soil organic matter formerly protected from decomposition via physical occlusion. The effect on 14C-CO2 would be to increase the contribution to respiration from this relatively slower soil organic matter pool (Fig. 1b, open squares). 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.

The promise of improving soil carbon models by obtaining 14C-CO2 measurements from archived soils is tantalizing, but first the possible effects of air-drying and rewetting, as well as the effect of the duration of storage, must be quantified. The direction and magnitude of any change in 14C-CO2 induced by these disturbances should be indicative of the change in substrate, i.e. increased contribution of either faster or more slowly cycling carbon pools (Fig. 1b).

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

1. Air-drying and rewetting will lead to transient mobilization of a small pool of slower cycling carbon, shifting the 14C-CO2 of the CO2 pulse released immediately following rewetting;
2. 14C-CO2 released during the equilibrium respiration period from previously air-dried soils will not be significantly different from that of undried control samples;
3. Differences between control and treatment 14C-CO2 obsevered during the equilibrium respiration period will not be affected by the duration of storage of air-dried samples.

2 Materials and Methods

We devised three experiments to assess the feasibility of measuring 14C-CO2 in incubations of archived soils. In all three experiments the controls represent soils incubated without air-drying or storage. Experimental conditions for the first two experiments, looking at the effects of air-drying and rewetting in combination with storage (Experiment 1), and at the effect of air-drying and rewetting alone (i.e. without the storage effect, Experiment 2), are summarized in Table 1. We conducted a third experiment to assess the impact of storage duration on observed 14C-CO2 in which incubation conditions were more variable, as they had been conducted by different investigators as part of different experiments. Details of the experimental conditions for both the original (control) and the treatment incubations (following air-drying and storage) are given in full in supplemental table \_.

2.1 Experiment 1 and Experiment 2

2.1.1 Sample selection and field sampling

Soils analyzed in Experiment 1 were collected in 2011 as part of a larger study from the Biodiversity Exploratories project (Fischer et al., 2010). We choose a subset of these samples for the present study from two ecosystem types (forest and grassland) and from a range of soil textural classes, from the relatively sandy soils of the Schorfheide-Chorin geographic region to the more clay-rich soils from the Hainich-Dün. We omitted samples that showed the presence of inorganic C during the control incubations by using the δ13C signature of respired CO2, deeming any samples with δ13C-CO2 > -25‰ as a conservative indicator of samples potentially affected by the release of inorganic C. We then selected three grassland (50 m by 50 m) and three forest (100 m by 100 m) plots from each of the two geographic regions (n total = 12 sites), using the additional criterium that the 14C-CO2 observed in 2011 fell within the within the interquartile range observed for the ecosystem type and region.

For Experiment 2, we returned in July 2019 to the Hainich-Dün region to collect new samples from the same plots at the same sites 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 we collected three cores (0-10 cm depth, as in 2011), 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 sites.

Samples for Experiment 3 were obtained from the archives of S. Trumbore. Soils were originally collected from various locations around the United States and had been in storage for 4 to 14 years following the control sample incubations. All samples came from forest ecosystems, as no grassland samples were available. Owing to a lack of samples from deeper soil horizons, the samples included in this study were restricted to the A horizon only (see Supplemental Table 1 for more details on sample provenance).

2.1.2 Sample preparation

Following sample collection in both 2011 (Experiment 1) and 2019 (Experiment 2), soils were sieved to <2 mm at field-moisture, and water holding capacity was determined on a 10 g subsample. The remaining soil was then split, with one split air-dried at 40º C (treatment) and the other split left at field moisture (control). Control samples for both Experiment 1 (control-1) and Experiment 2 (control-2) were stored in recloseable plastic bags at 4º C until incubation. After air-drying, treatment samples for Experiment 1 (air-dry + storage) were placed in recloseable plastic bags, which were then stored inside large plastic boxes in a cool (ca. 15º C) dark room for seven years prior to incubation in 2018. Treatment samples for Experiment 2 (air-dry only) were briefly stored under the same conditions, but for less than three months prior to incubation in November 2019.

Carbon and nitrogen content was determined by dry combustion in a CN analyzer (Vario Max, Elementar Analysensysteme GmbH, Hanau, Germany) using material from the air-dried sample split. Inorganic carbon content was determined after combustion of organic carbon in a muffle furnace at 450º C for 16 h. Soil organic carbon content was then calculated as the difference between total carbon and inorganic carbon. Additional analyses performed on the air-dried soils 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).

2.1.3 Incubations

All incubations were conducted in duplicate. Soils were weighed out into 250 ml beakers and placed into 1000 ml mason jars with airtight lids fitted with two sampling ports. For Experiment 1, the mass of soil used for the control-1 samples varied between 70 and 250 g (air-dry equivalent), depending on the soil carbon content. Owing to limited sample quantity, only 20 g of soil was used for the Experiment 1 treatment samples (air-dry + storage); for Experiment 2 20 g of soil was used for both control (control-2) and treatment (air-dry only) incubations.

Prior to sealing the jars, soil moisture content was adjusted from either field-moisture status (control samples) or from air-dry status (treatment samples) to 60% of water holding capacity. Following moisture adjustment, jars were flushed with CO2-free air and left to incubate for a four-day pre-incubation period. Following pre-incubation jars were flushed again, and CO2 was then allowed to accumulate for a second period under equilibrium respiration conditions. All samples were incubated at 20º C.

Headspace gas sampling was conducted at slightly different times for Experiment 1 and Experiment 2. For Experiment 1, we measured headspace CO2 concentrations of control-1 samples at the end of the pre-incubation period, and then on days 1, 3, 7 and 14 of the equilibrium respiration period. We measured treatment sample headspace CO2 concentrations for Experiment 1 (air-dry + storage) daily during the pre-incubation period, and thrice weekly for the first two weeks of the equilibrium respiration period, and weekly thereafter. For Experiment 2, we measured the headspace CO2 concentrations of both control (control-2) and treatment (air-dry only) samples daily during the pre-incubation period (day 1 to day 4), while equilibirum respiration CO2 concentrations were measured three times per week for the first two weeks, and weekly thereafter.

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

Headspace 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 Experiment 1 control samples (control-1), for which only equilibrium respiration period 14C-CO2 was measured. However, only nine of the twelve air-dry + storage samples (Experiment 1) respired enough CO2 to measure 14C following the pre-incubation period.

Note that respiration rates had not yet reached equilibrium levels for the majority of samples by the end of the pre-incubation period, but as a four-day pre-incubation was used in the initial incubations conducted in 2011 (control-1 samples), we maintained the same duration for the air-dry + storage incubations in 2018 (Experiment 1) and for the Experiment 2 incubations (control-2 and air-dry only samples).

2.2 Experiment 3

2.2. Sample selection and incubation conditions

We obtained archived (air- or oven- (80C) dried) soil samples from a range of sites across the United States and Germany (n = 39) collected over the past two decades and incubated close to the time of collection and under field-moist conditions. For the purposes of the present study, these initial incubations were considered to be the controls for Experiment 3 (control-3 samples). Since the control-3 incubations were conducted in different laboratories by different investigators, incubation conditions such as temperature, quantity of soil incubated, and pre-incubation period duration varied. However, we adjusted the corresponding treatment incubations (storage duration) so that moisture content and equilibrium period respired carbon (mg CO2-C g soil C-1) were identical to the control-3 sample incubations. When possible storage duration treatment incubations were conducted in triplicate, but owing to limited quantities of soil, duplicate or single sample incubations were performed for some sites. Further details on incubation conditions, replicates, headspace gas sampling, and sample provenance for Experiment 3 are given in Supplementary Table 1.

For the control sample incubations, CO2 concentrations were only measured during the equilibrium respiration period (with the exception of two samples, see Supplementary Table 1). Owing to the lack of pre-incubation respiration data (see Fig./Table x), treatment incubation CO2 measurements were only made for a single period of the treatment sample incubations. Incubation vessels were sealed immediately following rewetting and samples and were allowed to respire until an equivalent amount of CO2 had been released (mg CO2 g soil C-1) as during the control sample equilibrium respiration period. Headspace CO2 concentrations were measured every three days for the first two weeks of incubation, and weekly as needed thereafter.

2.3 Isotopic analyses

We separated CO2 from the gas samples collected from incubation jar headspace using a vacuum line, and splits of the purified CO2 were analyzed for both δ 13C and ∆14C. Radiocarbon analyses for Experiments 1, 2, and treatment samples for Experiment 3 (storage duration samples) were conducted at the Max Planck Institute for Biogeochemistry (MPI-BGC) accelerator mass spectrometer facility using a MICADAS instrument (Ionplus, Dietikon, Switzerland), while the control samples for Experiment 3 (control-3 samples) were analyzed at the University of California Irvine (UCI) W.M. Keck Facility for Accelerator Mass Spectrometry. Radiocarbon values are reported in units of ∆14C, which is 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. To correct for potential mass-depenedent fractionation effects, the 14C/12C ratio of all samples is corrected to a common δ13C value of -25 per mil (Steinhof, 2013; Stuiver & Polach, 1977). As with the radiocarbon analyses, measurements of δ13C for Experiments 1, 2, and treatment samples from Experiment 3 (storage duration samples) were made at MPI-BGC (**instrument**), while control samples for Experiment 3 (control-3 samples) were measured at UCI (**instrument**).

2.5 Statistical analysis

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

3 Results

3.1 Respiration rates

3.1.1 Experiment 1 (air-dry + storage treatment)

Respiration rates increased strongly following rewetting for the air-dry + storage treatment in comparison to control samples, similar to what has been observed in other air-dry/rewetting studies [cite?].

The magnitude and timing of peak respiration rate diverged between grassland and forest sites (Fig. 2). Among the air-dry + storage samples, respiration rates were more than twice as high in grassland soils than in forest soils, reaching a maximum of 3.8 mg CO2 g soil C-1 d-1 after 92 h, followed by a sharp decline. Mean respiration rates in forest sites peaked at 1.5 mg CO2 g soil C-1 d-1after 166 h, followed by a much more gradual decline than in grassland sites. Control-1 samples responded more weakly and more gradually. As in the treatment samples, respiration was greater in control-1 grassland soils than in control-1 forest soils. Peak respiration rates for control-1 incubations were 1.9 and 0.6 mg CO2 g soil C-1 d-1 after 115 h for grassland and forest soils, respectively.

3.1.2 Experiment 2 (air-dry only treatment)

Respiration rates for the air-dry only treatment samples showed a similarly strong increase in comparison to the control-2 samples as was observed for the air-dry + storage treatment samples compared to the control-1 samples in Experiment 1. However, unlike the air-dry + storage treatment in Experiment 1, peak respiration rates for the air-dry only samples in Experiment 2 were not significantly different (p > 0.05) between forest and grassland soils: peaking at 3.0 and 3.3 mg CO2 g soil C-1 d-1 after 95 h for grassland and forest soils, respectively (Fig. 2).

3.2 Radiocarbon

3.2.1 Pre-incubation versus equilibrium respiration ∆14C-CO2 (Experiments 1 and 2)

Despite the significant differences in respiration rates, and in contrast to hypothesis 1, we did not observe significant differences between ∆14C-CO2 respired during the pre-incubation period and ∆14C-CO2 respired during the equilibrium respiration period: neither for the air-dry + storage treatment, nor for the air-dry only treatment, nor for the control-2 samples (Fig. 3). The interaction with land use was not significant nor was the interaction with experiment, so all data were pooled for statistical analysis.

Note the one outlier (forest, control-2) for which the pre-incubation CO2 was substantially depleted relative to equilibrium period respiration. However, even when this outlier was included in the statistical analysis, the difference between pre-incubation ∆14C-CO2 and equilibrium ∆14C-CO2 was not significant. Due to lower respiration rates during pre-incubation only three of the six forest air-dry + storage treatment samples in Experiment 1 generated enough CO2 to measure radiocarbon. 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.

3.2.3 Time series analysis of changes in ∆14C-CO2 (2011 to 2019)

A subset of the locations sampled for Experiment 1 in 2011 were resampled for Experiment 2 in 2019, allowing us to compare how ∆14C-CO2 changes over time in forest versus grassland soils. Mean ∆14C-CO2 values reported for the Experiment 1 incubations (control-1 and air-dry + storage treatment) are averaged across samples collected from both the Hainich-Dün region (n = 6 sites) and the Schorfheide-Chorin region (n = 6 sites), while Experiment 2 ∆14C-CO2 values are averaged across samples from the Hainich-Dün region only (n = 6 sites). The Δ14C of equilibrium period respired CO2 was enriched relative to atmospheric ∆14C (∆∆14C) for all samples at both time points (Fig. 4). However, mean ∆∆14C values observed in grassland samples were smaller than what was observed in forest samples (Fig. 4).

The mean ∆∆14C observed in control-1 forest soils (i.e. collected and incubated without air-drying in 2011) was similar to the mean ∆∆14C observed in control-2 forest soils (i.e. collected and incubated from without air-drying in 2019): 56.5±2.3‰ and 50.2±6.0, respectively. In contrast, we observed an increase in mean ∆∆14C between the values observed for the 2011 samples Experiment 1 forest treatment samples (air-dry + storage)

as 44.9±1.5‰ (air-dry + storage samples, Experiment 1) and increased to 62.9±8.3‰ for forest samples collected, air-dried, and incubated in 2019 (air-dry only samples, Experiment 2). Grassland samples showed an increase in ∆∆14C between 2011 and 2019 for both control (control-1: 17.2+5.7‰, control-2: 31.4±3.2‰) and treatment (air-dry+storage: 40.5±1.9‰, air-dry only: 50.9±6.6‰) samples.

The difference in ∆14C-CO2 over time within groups, i.e. control-1 or air-dry + storage samples collected in 2011 compared to control-2 or air-dry only samples collected in 2019, tended to be smaller (30 to 40‰) than the change observed in the atmosphere over the same period (48.4‰). Forest control samples were an exception: for these samples the difference between control-1 samples collected in 2011 and control-2 samples collected in 2011 was slightly greater than the difference in atmospheric ∆14C (54.7‰, with a standard deviation of 7.8‰).

3.2.4 Treatment effects on observed equilibrium period 14C-CO2 (Experiments 1 and 2)

Relative to the control-1 samples, the air-dry + storage treatment (Experiment 1, open squares in Fig. 4) led to enrichment in grassland soils, but depletion in forest soils. In contrast, the air-dry only treatment (Experiment 2, open circles, Fig. 4) led to enrichment for both forest and grassland soils collected and incubated in 2019. Treatment effects on ∆14C-CO2 were signifcant for both forests and grassland soils in Experiment 1 (2011 points, open squares in Fig 4), and significant for grassland samples but not forest samples in Experiment 2 (2019 points, open circles in Fig. 4). Compared across Experiments 1 and 2, the absolute value of the mean difference in ∆14C-CO2 between control and treatment samples was greater in grassland samples (21.4‰) than in forest samples (12.1‰). In contrast, while mean differences within laboratory replicates were lower than treatment differences, differences among replicates were slightly higher for forest samples (8.1‰, standard deviation ±3.8‰) than for grassland samples (5.0‰, standard deviation 3.8‰) across both Experiments 1 and 2.

3.2.4 Effect of cumulative respired carbon on ∆14C-CO2

[maybe expand with stats for other explanatory factors? e.g. texture, N content, change in moisture upon rewetting, etc…]

We looked at the possible effect of the difference in the amount of carbon respired (mg CO2-C g soil C-1) on the differences between control and treatment 14C-CO2 using a linear regression model, but it was not significant overall. When data from Experiment 1 and Experiment 2 were considered separately, we observed a slight positive trend (p = 0.063) between the difference in respired carbon and the difference in 14C-CO2 within Experiment 2, while there was no such effect in Experiment 1.

3.2.5 Air-dry + storage effects on ∆14C-CO2 (Experiments 1 and 3)

Difference between control and treatment samples from all experiments show that treatment effects, i.e. air-drying followed by rewetting or air-drying followed by storage and subsequent rewetting, typically result in changes in ∆14C-CO2 between ±20‰ to ±40‰ (dashed and dotted lines, respectively, in Fig. 5), with the majority within ±20‰. Put in context, differences in ∆14C of 20‰ to 40‰ are equivalent to the change in atmospheric radiocarbon over five and ten years, respectively, during the period of 2000 to 2020. The samples from Oak Ridge (magenta points) are an exception. However, these points do not show only bomb-C enrichment, but rather the results of exposure to a localized plume of 14C enriched CO2 from a nearby incinerator four years prior to sample collection (Cisneros-Dozal, Trumbore, & Hanson, 2006). Treatment 14C-CO2 for these highly enriched samples were more depleted relative to the controls than were the samples only labeled with bomb-C.

The air-drying + storage treatment tended to lead to enrichment in grassland samples (points above the 1:1 line, Fig. 5) while forest samples were depleted following treatment, regardless of origin. A notable exception to this trend are the three German forest samples collected in 2019, for which the air-drying only treatment (Experiment 2) led to enrichment (black triangles above 1:1 line in Fig. 5).

3.3 Storage duration

There does not seem to be evidence for a storage duration effect in the samples that only contain bomb-C (Fig. 6). In contrast, the highly enriched samples from Oak Ridge show an increasing trend in the differences in ∆14C-CO2 between control samples incubated from field-moist conditions and treatment samples (air-dry + storage) with increasing storage duration, suggesting losses of the most recently fixed carbon over time. These samples were included primarily because it was assumed that they would be more sensitive to potential losses of recently fixed carbon, as the experimental label should only be present in this pool of soil C.

4 Discussion

The increase in respiration rates seen in this study following air-drying and rewetting align with what many others have seen (cite?). However, the significant difference in the 14C of respired CO2 between the control and treatment samples in this study show that this increased respiration appears to be fueled at least in part by an extracellular substrate source that is only available to the microbial community following air-drying and rewetting.

However, in contrast to hypothesis 2, the ∆14C-CO2 respired immediately after rewetting (during the pre-incubation period) was not significantly different than what was observed later during the equilibrium respiration period. This suggests that the change in substrate availability initiated by air-drying and rewetting persists throughout the incubation. Previous studies have found mechanistic evidence for microbial osmolytes or lysed cells providing the fuel for the pulse of CO2 observed following rewetting of dried soils (N. Fierer & Schimel, 2003), as well as extracellular carbon (Xiang, Doyle, Holden, & Schimel, 2008). The results from this study provide support for a mechanism that makes extracellular carbon available to the microbial community with a distinct 14C signature and in sufficient quantity to fuel respiration beyond the initial rewetting pulse.

Air-drying and subsequent rewetting clearly has a significant effect on the 14C of respired CO2, but our results show that the direction and magnitude of the trend is dependent on two factors: when the sample was collected and the ecosystem type (forest versus grassland). Relative to un-dried control samples, respiration from forest soils analyzed in this study tend to show depletion in ∆14C-CO2 following air-drying and rewetting, while grassland soils show enrichment. The forest soils incubated in Experiment 2, collected in 2019, stand out as a counter example in that the air-dry and rewetting treatment lead to enrichment in ∆14C-CO2 relative to the controls. Yet the soils collected in 2011 from these same forest sites showed the same trend and magnitude of depletion in response to treatment as was observed in all other forest sites (Fig. 6).

In forest soils collected prior to 2019 (Fig. 5, all triangles except black ones above the 1:1 line), the depletion in ∆14C-CO2 observed in comparison to control sample incubations would suggests that the carbon respired in forest soils in response to treatment is older than that respired in grassland soils. This explanation is also consistent with what is seen in the highly enriched samples from Oak Ridge: increased depletion in 14C-CO2 relative to the controls due to a much greater difference in 14C between the most recently fixed carbon and the older carbon in the soil.

The switch from depletion to enrichment in ∆14C-CO2 following treatment that we observed in the forest soils from Central Germany between 2011 and 2019 could be explained by a corresponding shift in the relative enrichment of the more slowly cycling soil carbon pool in comparison to the fast cycling pool. This scenario is illustrated in Fig. 1b as a crossing of the slow and fast pool 14C curves between the (hypothetical) observation of the system in 1992 and 2019. Evidence for a possible crossing of slow and fast pool 14C curves between 2011 and 2019 at the Central Germany forest sites may also be inferred from the relatively smaller difference between control and treatment 14C-CO2 observed at both time points, in comparison to the grassland samples from the same regions. Alternatively, a different mechanism may be at play in these 2019 outlier samples, possibly due to the very dry growing season conditions experienced in 2019 as compared to 2011.

The relative increase in ∆14C-CO2 seen in the grassland soils may suggest that there is a slowly cycling carbon pool that is more enriched than the fastest cycling pool, and it is carbon from this more slowly cycling pool that is contributing more to respiration in treatment samples than in control samples (cf. square symbols in 2019, Fig. 1b). However, the hypothetical soil system depicted in Fig 1 (a, and b), is a simplification and may in fact contain more than two pools or transfers between pools. It rather could suggest the opposite, i.e. that carbon from a faster cycling pool has been mobilized (open circles, Fig. 1b) and the slow and fast ∆14C curves simply have yet to cross, as is the case for the 1992 sampling point in the hypothetical scenario in Fig. 1b.

Drying and rewetting is both more common and more extreme in grassland sites than in forest sites, potentially leading to increased storage of osmolytes in the soil over time (Warren, 2016), in grassland soils as compared to forest soils. Such a pool would likely be enriched with bomb-C and could be the substrate source observed in the grassland sample respiration following air-drying and rewetting. However, even within the same grassland soil, such a pool could have a different interpretation or be considered to be part of a completely different soil carbon pool in an air-dried and rewet soil than in a field-moist soil. A scenario like this is demonstrated for the water extractable organic carbon pool by Slesserov et al. (2020), which increases in size following air-drying and rewetting, fueling subsequent respiration. In the current study, without further information for model parameterization such as inputs, ∆14C of bulk soil, or pool sizes from mechanistic fractionation methods, confident determination of which pool is fueling the change in ∆14C-CO2 following treatment for grassland samples remains elusive.

If we assume that the respiration flux is dominated by the fast cycling soil carbon pool, then control sample ∆14C-CO2 should decline at nearly the same rate or just slightly slower than atmospheric ∆14C, as is observed for the forest and grassland soils sampled in both 2011 and 2019 for Experiments 1 and 2. While the difference from the atmosphere is greater for the 2019 grassland control samples than for the grassland controls in 2011, this may be simply because the bulk soil organic matter ∆14C content of the additional grassland sites included in the 2011 incubations is assumed to be lower. Bulk soil ∆14C was not measured for these samples, but measurements made in 2011 for nearby grassland sites in the Hainich-Dün region had a mean ∆14C content of 50.9‰ (n = 10), while mean 14C for the grassland sites in the Schorheide-Chorin region was 13.9‰ (n = 10). However, the consistent enrichment of ∆14C-CO2 in relation to the atmosphere observed in almost all samples is strong evidence that the dominant pool contributing to respiration is more enriched than the atmosphere, and therefore must be comprised of predominantly decadally cycling, bomb-C enriched, carbon.

Soils in this study spanned a relatively small range of storage duration, from 0 to 14 years, but within this range the duration of storage did not have a significant effect on the difference observed in ∆14C-CO2. To test this effect fully, it would be ideal to measure splits of the same sample at multiple points in time, but this was not possible within the confines of this study. The slight increase in the difference between control and treatment sample ∆14C-CO2 seen with increased duration of storage in the highly enriched samples from Oak Ridge, analyzed in Experiment 3, suggests that some of the most recently fixed carbon may be preferentially lost over time. 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 whether or not storage leads to losses. However, as the incubations conducted after 4 years of storage were done in a different laboratory under different conditions than the incubations after 14 years of storage, we caution that this may not represent a real trend.

Overall, the slight increase in the apparent age of respired CO2 seems to be consistent across the soils studied, but stronger in grassland soils than in forest soils. In the context of modeling applications, the differences in 14C-CO2 caused by air-drying and subsequent rewetting observed in this study would lead to shifts in apparent transit time of soil carbon by 5 to 10 years relative to estimates from incubations of soils that have not undergone air-drying. Depending on the needed resolution, this difference may be negligible, but future studies should consider the possible consequences of this shift. In conclusion, we believe the radiocarbon incubation technique for archived soils is promising approach for improving soil carbon models, and that the benefit of having observations of the system at multiple time points outweighs the slight shift in ∆14C-CO2 caused by the processes of air-drying, storage, and rewetting.

5 Conclusion

Air-drying and rewetting of soils leads to significant differences in the 14C of respired CO2 in laboratory incubations. These differences are apparently not affected by the duration of storage and are within 20‰ for the majority of forest soils and 20‰ for the more limited number of grassland samples studied. This is often comparable to the standard deviation among replicate incubated samples and can be considered relatively small (though systematic) error when calculating ages and transit times, i.e. equivalent to 5 to 10 years of change in atmospheric 14C over the first decade of the 20th century. Forest and grassland soils respond differently to air-drying and rewetting, with enrichment in grassland samples, and depletion in forest soils collected in and before 2011, but enrichment in the forest soils collected in 2019. The mechanism behind these differences is not clear, but the data from this study suggest that air-drying and rewetting increases the contribution of older carbon to respiration less in forests than in grasslands. 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. However, potential biases from air-drying and rewetting need to be considered, and may increase estimated mean transit times of soil carbon.

Acknowledgments, Samples, and Data

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**Figure 1**.

[see accompanying file for figures]

**Table 1.** Incubation conditions for Experiment 1 and Experiment 2

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  | period | | | |
|  |  |  |  |  |  | pre-incubation | | equilibrium respiration | |
| experiment | treatment | sample collection date | incubation date | initial moisture content\* | adjusted moisture content | time | ∆14C measured | time | ∆14C measured |
|  |  |  | *year* | *% water holding capacity* | *% water holding capacity* | *days* |  | *days* |  |
| 1 | control | 2011 | 2011 | 18 | 60 | 4 | no | 14 | yes |
| air-dry + storage | 2011 | 2018 | 0 | 60 | 4 | yes | 5 to 45 | yes |
| 2 | control | 2019 | 2019 | 19 | 60 | 4 | yes | 10 to 38 | yes |
| air-dry | 2019 | 2019 | 0 | 60 | 4 | yes | 7 | yes |

\* mean field moisture content for control samples (n = 12 for Experiment 1, n = 6 for Experiment 2); air-dry moisture content for treatment samples