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

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

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

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

Key Points:

* Air-drying and rewetting significantly alters 14C of respired CO2
* Magnitude and direction of change affected by year of sampling and ecosystem
* 14C of rewetting pulse CO2 is not significantly different from 14C of subsequent respiration

Abstract

Laboratory incubations are a useful technique for identifying soil organic matter that is accessible to microbes. Measuring the radiocarbon of the CO2 released in laboratory incubations (14C-CO2) provides an integrative signal of the age of organic matter leaving the soil (transit time), but in order to convert radiocarbon values to ages a model is needed. Soil archives have the potential to provide multiple observations of respired CO2 over time at the same location, which could greatly reduce model uncertainty by providing additional constraints for model parameterization. However, air-drying, rewetting, and storage 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 the duration of storage in archives on 14C-CO2 for 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 greater in grasslands (21.4‰) than in forests (12.1‰). 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. The shift in ∆14C-CO2 induced by air-drying and rewetting was significant, but still 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 absorbing excess carbon dioxide from the atmosphere and storing it for decades to millennia, but it is unclear if they will continue to provide this ecosystem service as the climate changes. Decomposition of soil organic matter returns carbon dioxide back to the atmosphere, and radiocarbon dating of this returning carbon dioxide can reveal how long carbon persists in the soil. Measuring the radiocarbon content of this returning carbon dioxide in incubations of archived soils could greatly reduce the uncertainty of carbon models by providing additional time points as model constraints, but air-drying, rewetting, and storage of soils may affect the balance of younger versus older carbon leaving the soil. We compared the radiocarbon signature of CO2 from soils incubated with and without air-drying and storage and found that the air-dried soils appeared to release slightly older carbon than soils that had never been air-dried. The duration of storage did not appear to have an effect. However, the difference in radiocarbon due to air-drying was small, equivalent of an apparent increase in the average amount of time carbon persists in the soil of 5 years in forests and 10 years in grasslands. These results suggest that incubating archived soils is a promising technique for improving carbon models 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 of which persists in the soil for months or years, while some persists for centuries or millennia. The persistence of soil carbon can be understood through the concept of different “pools” of carbon that are defined by the mechanism by which they persist in the soil and are characterized by distinct age distributions (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 (Fig. 1a), 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 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 Δ14C requires the use of a model. However, parameterizing these models is challenging, both due to the uncertainty of the persistence mechanisms themselves as well as a lack of observational constraints. Radiocarbon observations at a single point in time are very useful, but due to the curvature of the bomb-C peak there are two points in time with the same atmospheric radiocarbon value, leading to multiple model solutions (Fig. 1a). Observations of Δ14C-CO2 at multiple points 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 with 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 the most extreme example perhaps being the >150 year archives from the Rothamsted long-term experiments, used for parameterizing the well-known 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, before returning to equilibrium respiration rates. The mechanism or mechanisms driving this pulse of CO2 have been extensively studied over the past several decades (Birch, 1958; Borken & Matzner, 2009; Schimel, 2018). The source of the CO2 released in the rewetting pulse has been hypothesized to come from 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. 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., 2014).

During short-term incubations, the majority of CO2 can be assumed to derive from the substrates consumed by the microbial community *in situ*. In longer duration incubations, the lack of new inputs to the system is assumed to lead to shifts in substrate utilization, from easily accessible, shorter-cycling pools to less accessible pools, i.e. protected from decomposition in some manner (Schädel et al., 2020). 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 (Fig. 1b).

For example, disruption of soil aggregates following drying and rewetting would likely lead to greater availability 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 will not be significantly different from that of control sample incubations with moisture adjusted from field-moist conditions;
3. Differences between control and treatment 14C-CO2 will not be affected by the duration of storage.

2 Materials and Methods

We devised three experiments to assess the feasibility of measuring 14C-CO2 in incubations of archived soils. Experimental conditions for the first two experiments, looking at 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 described in Table 1. We conducted a third experiment to assess the impact of storage duration on observed 14C-CO2, that had variable control sample incubation conditions as they had been conducted by different investigators as part of different experiments. We rewetted and measured the 14C-CO2 of evolved CO2 during the equilibrium phase, as informed by the results of Experiments 1 and 2.

2.1 Sample selection and field sampling

Control incubations conducted in 2011 for Experiment 1 were part of a larger study from the Biodiversity Exploratories project (Solly et al. 2014). We choose a subset of these samples for the present study from two ecosystem types (forest and grassland) and from a range of soil textural classes, from the relatively sandy soils of the Schorfheide-Chorin geographic region to the more clay-rich soils from the Hainich-Dunn. We omitted samples that showed the presence of inorganic C during the control incubations by using the δ13C signature, deeming any samples with δ13C > -25‰ as potentially affected by the release of inorganic C. We then selected three grassland and three forest samples from the interquartile range of 14C-CO2 observed in 2011 for the two geographic regions (n total = 12 sites).

For Experiment 2, we returned in July 2019 to the Hainich-Dunn region to collect new samples from 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. As in 2011, at each plot three cores (0-10 cm depth) were collected and 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.

2.2 Experimental conditions

2.2.1 Experiment 1 (air-drying and rewetting + storage) and Experiment 2 (air-drying and rewetting only)

In Experiment 1 we looked at the effect on ∆14C-CO2 from air-drying and rewetting in combination with storage (treatment: air-dry + storage). The control samples for this experiment were collected and incubated in 2011. 14C-CO2 from control sample incubations were then compared to a second set of incubations performed seven years later (in 2018) on splits of the original samples. Following sample splitting, treatment sample splits were air-dried and stored in sealed plastic bags.

Experiment 2 was designed to assess the effect of air-drying and rewetting directly, without the potentially confounding effect of storage. For this experiment additional soils were collected in 2019 from a subset of the sites sampled in 2011. After collection soils were homogenized and split into two subsamples, one of which was air-dried (treatment: air-dry) prior to incubation, the other of which was incubated without air-drying (control).

Incubation conditions were the same for both Experiment 1 and Experiment 2. Soils were sieved to <2 mm at field-moisture, and water holding capacity was determined on a subsample. Soils were weighed out as duplicates into 200 ml beakers and placed into 1 L mason jars with airtight lids fitted with two sampling ports. Prior to sealing the jars moisture content was adjusted to 60% of soil water holding capacity (either from field-moist conditions for control samples or from an air-dried state for treatment samples, see Table 1). 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.

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 2011 incubations for the control samples in Experiment 1, we maintained the same duration for the treatment incubations in 2018 and for the air-drying and rewetting experiment conducted in 2019 (Experiment 2).

2.2.2 Experiment 3

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. These ‘control’ incubations were conducted in different laboratories by different investigators, so incubation conditions such as temperature, quantity of soil incubated, and pre-incubation period duration varied. We controlled the treatment incubations (i.e. after air-drying, storage, and rewetting) so that moisture content and equilibrium period respired carbon (mg CO2-C g soil C-1) were identical to control incubations. When possible treatment incubations were conducted in duplicate or triplicate, but owing to limited quantities of soil, 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.

2.3 Headspace gas sampling

2.3.1 Experiment 1 (air-dry + storage treatment)

For control incubations, headspace CO2 concentrations were measured at the end of the pre-incubation period and then on days 1, 3, 7 and 14 during the equilibrium respiration period. For the air-dry + storage treatment incubations, headspace CO2 concentrations were measured daily during the pre-incubation, and on days 3, 5, and 7, then additionally on days 10, 38, and 45 for those samples that had not yet reached target CO2 concentrations.

CO2 concentration targets for the air-dry + storage treatment incubations were set to the amount of CO2 respired by the corresponding control sample during the equilibrium respiration period.

Headspace samples were collected and analyzed for 14C and 13C content at the end of the equilibrium respiration period for control incubations, but were collected after both the pre-incubation and equilibrium respiration periods for the air-dry + storage treatment incubations. However, only nine of the twelve treatment samples respired enough CO2 to measure 14C following the pre-incubation period.

2.3.2 Experiment 2

Headspace CO2 concentrations were measured daily during the pre-incubation period for both control incubations and the air-dry treatment incubations. During the equilibrium respiration period for the control incubations, CO2 concentrations were measured on days 3, 5, and 7, and then weekly until adequate CO2 had been respired for measuring 14C. Similar to Experiment 1, CO2 concentration targets for the treatment incubations were determined by the amount of CO2 respired by the corresponding control samples during the equilibrium respiration period. Due to higher respiration rates, all air-dry treatment samples reached the CO2 targets after seven days of incubation, with CO2 concentrations measured on days 3, 5, and 7.

Headspace samples were collected and analyzed for 14C and 13C content after both the pre-incubation and equilibrium respiration periods for both control and air-dry treatment incubations.

2.3.3 Experiment 3

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, 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 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.

2.4 Additional measurements

We measured both organic and inorganic carbon content of all soils, as well as total nitrogen content and particle size distribution. Radiocarbon analyses were conducted at the Max Planck Institute for Biogeochemistry accelerator mass spectrometer facility (Experiments 1 and 2) or the University of California Irvine Keck Facility for Accelerator Mass Spectrometry (control samples, Experiment 3). All radiocarbon measurements are reported with respect to the international standards (Steinhof, 2013; Stuiver & Polach, 1977).

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 dramatically 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?]. However, 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 samples responded more weakly and more gradually to rewetting, although as in the treatment samples respiration was greater in grassland soils than in forest soils. Peak respiration rates for control 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 dramatic increase in comparison to the controls as was observed for the air-dry + storage treatment samples in Experiment 1. However, unlike the air-dry + storage treatment, peak respiration rates were not significantly different (p > 0.05) between forest and grassland soils in Experiment 2, peaking at 3.0 and 3.3 mg CO2 g soil C-1 d-1 after 95 h for grassland and forest soils, respectively (Fig. 2).

3.2 Radiocarbon

3.2.1 Pre-incubation versus equilibrium respiration ∆14C-CO2

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: either for the air-dry + storage treatment or for the air-dry treatment alone (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) 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 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 samples in Experiment 1 as pre-incubation ∆14C-CO2 was not measured for these samples in 2011.

3.2.2 Treatment effects on observed equilibrium period 14C-CO2

Relative to the controls the air-dry + storage treatment (Experiment 1, open squares in Fig. 4) led to enrichment in grassland samples, but depletion in forest samples. In contrast, the air-dry only treatment (Experiment 2, open circles, Fig. 4) led to enrichment for both forest and grassland samples (2019 points). Treatment effects on ∆14C-CO2 were signifcant for both forests and grassland soils in Experiment 1 (2011 points, Fig 4), and significant for grassland samples but not forest samples in Experiment 2 (2019 points, Fig. 4). The absolute mean difference in ∆14C-CO2 between control and treatment samples was greater in grassland samples (21.4‰) than in forest samples (12.1‰) for both experiments.

Δ14C of respired CO2 was enriched relative to the atmosphere for all samples in both experiments. Looking across experiments, the decline in 14C-CO2 between 2011 and 2019 paralleled that of atmospheric 14C for forest control samples and both control and treatment grassland samples, but was much smaller for the forest treatment samples.

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 between the difference in respired carbon and the difference in 14C-CO2 within Experiment 2, but it was only marginally significant (p = 0.063).

3.2.5 Treatment effect on ∆14C-CO2 for all samples (Experiments 1, 2, 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‰, with the majority within ±20‰ (Fig. 5). These difference are equivalent to the decline in atmospheric radiocarbon over 5 and 10 years, respectively, during the period of 2000 to 2020. The samples from Tennessee (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 (Trumbore et al., 2002). Treatment 14C-CO2 for these highly enriched samples were more depleted relative to the controls than were the samples only labeled with bomb-C.

Grassland samples tend to be above the 1:1 line, while forest samples are generally below, regardless of origin. A notable exception to this trend are the three German forest samples that are above 1:1 line, which were analyzed in 2019 (air-dry only treatment) (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 increasing trend in the differences due to treatment for the highly enriched samples from Oak Ridge, TN suggest losses of the most recently fixed carbon over the duration of storage. 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 (Cisneros-Dozal, Trumbore, & Hanson, 2006).

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 our initial hypothesis, the 14C of respired 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 TN: 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). Or it could suggest the opposite: 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. However, the hypothetical soil system depicted in Fig 1 (a, and b), is simplified and reality is likely more complex.

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-Dunn 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, TN, 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. As many published incubation studies dry then rewet samples, it should also be considered that this can influence not only respiration rates but also the 14C signature of respired CO2. 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 in when calculating ages and transit times: 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

Data are available in the International Soil Radiocarbon Database (ISRaD) and on Zenodo. The authors would like to acknowledge the invaluable assistance of M. Rost in the laboratory and the field, I. Schoening, M. Cisneros-Dozal, J. Koarashi, F. Hopkins, C. Lawrence, and S. Trumbore for sharing data and details on control sample incubations. Funding was provided by ERC grant # ...

References

Baisden, W. T., Parfitt, R. L., Ross, C., Schipper, L. A., & Canessa, S. (2013). Evaluating 50 years of time-series soil radiocarbon data : towards routine calculation of robust C residence times, 129–137. https://doi.org/10.1007/s10533-011-9675-y

Bartlett, R., & James, B. (1980). Studying Dried , Stored Soil Samples — Some Pitfalls 1. *Soil Sci. Soc. Am. J*, *44*, 721–724.

Birch, H. F. (1958). The effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil*, *10*(1), 9–31. https://doi.org/10.1007/BF01343734

Borken, W., & Matzner, E. (2009). Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Global Change Biology*, *15*(4), 808–824. https://doi.org/10.1111/j.1365-2486.2008.01681.x

Cisneros-Dozal, L. M., Trumbore, S., & Hanson, P. J. (2006). Partitioning sources of soil-respired CO2 and their seasonal variation using a unique radiocarbon tracer. *Global Change Biology*, *12*(2), 194–204. https://doi.org/10.1111/j.1365-2486.2005.001061.x

Feng, W., Shi, Z., Jiang, J., Xia, J., Liang, J., Zhou, J., & Luo, Y. (2016). Methodological uncertainty in estimating carbon turnover times of soil fractions. *Soil Biology and Biochemistry*, *100*, 118–124. https://doi.org/10.1016/j.soilbio.2016.06.003

Fierer, N., & Schimel, J. P. (2003). A Proposed Mechanism for the Pulse in Carbon Dioxide Production Commonly Observed Following the Rapid Rewetting of a Dry Soil. *Soil Science Society of America Journal*, *67*(3), 798–805. https://doi.org/10.2136/sssaj2003.0798

Fierer, N., Schimel, J. P., & Holden, P. A. (2003). Variations in microbial community composition through two soil depth profiles. *Soil Biology and Biochemistry*, *35*(1), 167–176. https://doi.org/10.1016/S0038-0717(02)00251-1

Fierer, Noah, & Schimel, J. P. (2002). Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry*, *34*(6), 777–787. https://doi.org/10.1016/S0038-0717(02)00007-X

Jenkinson, D. S., Poulton, P. R., & Bryant, C. (2008). The turnover of organic carbon in subsoils. Part 1. Natural and bomb radiocarbon in soil profiles from the Rothamsted long-term field experiments. *European Journal of Soil Science*, *59*(2), 391–399. https://doi.org/10.1111/j.1365-2389.2008.01025.x

Jones, A. R., Gupta, V. V. S. R., Buckley, S., Brackin, R., Schmidt, S., & Dalal, R. C. (2019). Geoderma Drying and rewetting e ff ects on organic matter mineralisation of contrasting soils after 36 years of storage. *Geoderma*, *342*(January), 12–19. https://doi.org/10.1016/j.geoderma.2019.01.053

Kleber, M., Nico, P. S., Plante, A., Filley, T., Kramer, M., Swanston, C., & Sollins, P. (2011). Old and stable soil organic matter is not necessarily chemically recalcitrant: Implications for modeling concepts and temperature sensitivity. *Global Change Biology*, *17*(2), 1097–1107. https://doi.org/10.1111/j.1365-2486.2010.02278.x

Lehmann, J., & Kleber, M. (2015). Perspective The contentious nature of soil organic matter. *Nature*, 1–9. https://doi.org/10.1038/nature16069

Schimel, J. P. (2018). Life in Dry Soils: Effects of Drought on Soil Microbial Communities and Processes. *Annual Review of Ecology, Evolution, and Systematics*, *49*(1), 409–432. https://doi.org/10.1146/annurev-ecolsys-110617-062614

Sierra, C. A., Hoyt, A. M., He, Y., & Trumbore, S. E. (2018). Soil Organic Matter Persistence as a Stochastic Process: Age and Transit Time Distributions of Carbon in Soils. *Global Biogeochemical Cycles*, *32*(10), 1574–1588. https://doi.org/10.1029/2018GB005950

Sierra, C. A., Müller, M., Metzler, H., Manzoni, S., & Trumbore, S. E. (2017). The muddle of ages, turnover, transit, and residence times in the carbon cycle. *Global Change Biology*, *23*(5), 1763–1773. https://doi.org/10.1111/gcb.13556

Slessarev, E. W., Lin, Y., Jiménez, B. Y., Homyak, P. M., Chadwick, O. A., D’Antonio, C. M., & Schimel, J. P. (2020). Cellular and extracellular C contributions to respiration after wetting dry soil. *Biogeochemistry*, *147*(3), 307–324. https://doi.org/10.1007/s10533-020-00645-y

Steinhof, A. (2013). Data Analysis at the Jena 14C Laboratory. *Radiocarbon*, *55*(3–4), 282–293. https://doi.org/10.2458/azu\_js\_rc.55.16350

Stuiver, M., & Polach, H. A. (1977). Discussion: Reporting of 14C Data. *Radiocarbon*, *19*(3), 355–363. https://doi.org/10.1017/S0033822200003672

Trumbore, S. (2000). Age of soil organic matter and soil respiration: Radiocarbon constraints on belowground C dynamics. *Ecological Applications*, *10*(2), 399–411. https://doi.org/10.1890/1051-0761(2000)010[0399:AOSOMA]2.0.CO;2

Trumbore, S. (2009). Radiocarbon and Soil Carbon Dynamics. *Annual Review of Earth and Planetary Sciences*, *37*(1), 47–66. https://doi.org/10.1146/annurev.earth.36.031207.124300

Warren, C. R. (2016). Soil Biology & Biochemistry Do microbial osmolytes or extracellular depolymerisation products accumulate as soil dries ? *Soil Biology and Biochemistry*, *98*, 54–63. https://doi.org/10.1016/j.soilbio.2016.03.021

Williams, M. A., & Xia, K. (2009). Characterization of the water soluble soil organic pool following the rewetting of dry soil in a drought-prone tallgrass prairie. *Soil Biology and Biochemistry*, *41*(1), 21–28. https://doi.org/10.1016/j.soilbio.2008.08.013

Xiang, S., Doyle, A., Holden, P. A., & Schimel, J. P. (2008). Soil Biology & Biochemistry Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils, *40*, 2281–2289. https://doi.org/10.1016/j.soilbio.2008.05.004

**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