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

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**Key Points:**

· ∆14C of CO2 measured in incubations of archived soils provides additional constraints for soil carbon models

· Air-drying and rewetting soils shifted the ∆14C of respired CO2 by 10 to 20‰ independent of the duration of storage

· Differences in direction and magnitude of ∆14C-CO2 shifts between forests and grasslands depended on sampling year and system C dynamics

Abstract

The radiocarbon signature of respired CO2 (∆14C-CO2) 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, duration of storage, and subsequent rewetting of archived soils may each lead to changes in the relative contribution to respiration from faster or more slowly cycling soil carbon pools. We assessed these effects on ∆14C-CO2 measured in incubations of forest and grassland soils conducted before and after storage. Air-drying and rewetting led to small but significant (α < 0.05) shifts in ∆14C-CO2 relative to undried controlsin all three of our experiments, and grassland soils responded more strongly than forest soils. Mean absolute differences (95% CIs) by experiment were: 23.3‰ (±6.6), 19.6‰ (±10.3), and -29.3‰ (±29.1) for grasslands, versus -11.6‰ (±4.1), 12.7‰ (±8.5), and -24.2‰ (±13.2) for forests. Storage durations of 4-14 y did not have a substantial effect on ∆14C-CO2. 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 ∆14C-CO2 from air-drying and rewetting is small, measuring ∆14C-CO2 in incubations of archived soils appears to be a promising technique for constraining soil carbon models.

**Plain Language Summary**

Soils play a key role in the global carbon cycle by sequestering carbon from the atmosphere for decades to millennia. However, it is unclear if they will continue to do so as the climate changes. Microbial decomposition of soil organic matter returns carbon back to the atmosphere, and radiocarbon dating of this returning CO2 (∆14C-CO2) can be used to quantify how long carbon is stored in ecosystems. Incubating archived soils could provide unique insight into soil carbon sequestration potential by quantifying the change in ∆14C-CO2 over time. However, air-drying, duration of archiving, and subsequent rewetting of soils may bias estimates of sequestration potential by altering the balance of younger versus older carbon leaving the soil. We compared ∆14C-CO2 from soils incubated with and without air-drying and archiving, and found that the air-dried soils appeared to release slightly older carbon than soils that had never been air-dried. 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 (∆14C-CO2) in laboratory incubations is a powerful constraint for modeling soil carbon dynamics because it provides an integrated measure of the weighted contribution to the soil efflux from carbon pools with distinct ages that reflect their C sources and cycling rates (Trumbore, 2000). Using archived soils to construct a time series of ∆14C-CO2 would be an even more powerful model constraint, but it is unclear how air-drying, storage, and subsequent rewetting of archived soils may affect ∆14C-CO2 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. ∆14C-CO2 reflects respiration fluxes dominated by the decomposition of fast cycling carbon in contrast to bulk soil ∆14C, 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 Δ14C-CO2 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 decline in the concentration of atmospheric 14C over this period. Following the bomb-C trace in Δ14C-CO2 respired from soils collected and archived over this period could therefore provide unique insight into decadal scale soil C dynamics.

A critical challenge for the interpretation of Δ14C-CO2 data is that there were two points in time at which the Δ14C signature of atmospheric CO2 was identical, due to the curvature of the bomb-C peak. This means observations of Δ14C from a single point in time can be fit to models with different rates of intrinsic decomposition. 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 a Δ14C of 166‰ in 1996. Observations of Δ14C-CO2 measured in incubations of archived soils could help resolve this ambiguity by enabling the construction of a Δ14C-CO2 time series. The trajectory of ∆14C 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), a strong additional constraint for model parameterization.

Incubations of archived soils present challenges, however. Prior to long term storage soils are commonly air-dried, but this process is known to affect biological, physical, and chemical properties of the soil (Bartlett & James, 1980; Jones et al., 2019). Incubation of soils following air-drying and rewetting typically leads to a rapid increase in CO2 production, ranging from hours to several days (the Birch effect), before returning to equilibrium respiration rates (Birch, 1958). Hypothesized sources for the CO2 released following soil rewetting include the lysis of microbial cells subjected to osmotic shock (Warren, 2016; Williams & Xia, 2009), disruption of soil aggregates, osmolytes released from microbes emerging from aridity-induced dormancy (Fierer & Schimel, 2003), or desorption of mineral-associated organic matter, and typically represent a combination of these sources (Kaiser et al., 2015; Slessarev et al., 2020). 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 Δ14C-CO2 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 ∆14C-CO2. For example, disruption of soil aggregates following drying and rewetting would likely lead to greater accessibility of soil organic matter formerly protected from decomposition via physical occlusion. Drying followed by rewetting could also lead to desorption of organic matter sorbed to minerals, increasing the accessibility of this formerly protected substrate. If drying and rewetting mobilizes carbon from these relatively slowly cycling soil organic matter pools the effect should be detectable as a shift in ∆14C-CO2. However, if the rewetting pulse derives mainly from lysed microbial cells or the release of microbial osmolytes little change in ∆14C-CO2 would be expected.

Obtaining ∆14C-CO2 measurements from incubations of archived soils would be a valuable tool for further constraining and improving soil carbon models, but first the possible effects of air-drying and rewetting, as well as the effect of storage duration, must be assessed.

We designed three experiments to answer the following questions:

1. Is ∆14C-CO2 observed 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, i.e. without storage, on ∆14C-CO2?
3. Does the duration of storage affect ∆14C-CO2?

We present the results of these three experiments, along with an applied example of interpreting a time series of ∆14C-CO2 with archived soils. Our results support 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 ∆14C-CO2 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 y of storage prior to rewetting (air-dry/rewet + storage), Experiment 2 on the effect of air-drying and rewetting alone, i.e. without storage (air-dry/rewet), and Experiment 3 on the effect of varied storage duration (storage duration). All soils were split following sample collection, with one split air-dried, and the other refrigerated at field-moisture 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 comprised 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 ∆14C-CO2 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 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 minutes before weighing again to determine the amount of water absorbed. The remaining soil was then split, with one aliquot air-dried at 40º 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 4º C until incubation. After air-drying, air-dry/rewet + storage samples were placed in re-sealable plastic bags, and stored inside large plastic boxes in a cool (ca. 15º 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 1000 ml mason jars with airtight lids fitted with two sampling ports. The mass of soil used for control-1 incubations ranged from 45 g 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 CO2 would be respired to measure ∆14C-CO2 (> 0.5 mg) following the second enclosure period while at the same time preventing excessive CO2 build-up as this has been shown to negatively impact heterotrophic respiration (MacFayden 1973; Santruckova and Simek 1994).

Soil moisture contents of control-1 samples were adjusted to 60% of water holding capacity (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 d following moisture adjustment (the first enclosure period), after which the jars were flushed with CO2-free air and allowed to accumulate CO2 for a second enclosure period of 14 d.

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. However, 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% water holding capacity prior to flushing and sealing the jars. We maintained the same 4 d first enclosure period to capture the CO2 released during the rewetting pulse. We determined the duration of the second enclosure period for the air-dry/rewet + storage treatment incubations by the amount of CO2 respired. We allowed the air-dry/rewet + storage treatment incubations to proceed until the same amount of CO2 had been respired per g soil C as in the second enclosure period of corresponding control-1 sample incubations. Accordingly, the incubation duration of the second enclosure period for the air-dry/rewet + storage treatmentincubations varied (Table 2).

Headspace CO2 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 CO2 concentrations one to three times per week during the second enclosure period for both control-1 and air-dry/rewet + storage treatmentincubations, with more frequent measurements made for samples with faster respiration rates. Headspace gas samples were collected and analyzed for ∆14C and δ13C content at the end of both the first enclosure period and the second enclosure period for the air-dry/rewet + storage treatmentincubations, but these measurements were only made following the second enclosure period for control-1 samples. All samples were incubated at 20º C.

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 ∆14C-CO2 across both Central Germany regions in Experiment 1, so we restricted the resampling to just Hainich-Dün to save on cost and time. 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 40º 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 4º C until incubation. After air-drying, air-dry/rewet treatment samples were placed in re-sealable plastic bags, and stored inside large plastic boxes in a cool (ca. 15º 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 style incubation vessels as used for the Experiment 1 incubations. 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 air-dried conditions (air-dry/rewet samples). Following moisture adjustment, jars were flushed with CO2-free air, sealed, and left to incubate for a 4 d first enclosure period. After the first enclosure period the jars were flushed again, and CO2 was allowed to accumulate for a second enclosure period (Table 2).

Headspace CO2 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 ∆14C and δ13C content at the end of both the rewetting pulse period and the second enclosure period. Control-2 samples were allowed to incubate until > 0.5 mg of CO2-C was present in the jar headspace, which is the quantity needed to measure ∆14C. Incubations for the air-dry/rewet treatment samples were allowed to proceed until the same amount of CO2 was respired per g of soil C as in the corresponding control-2 sample. All samples were incubated at 20º C.

2.3 Experiment 3: storage duration

We were forced to modify the incubation conditions for Experiment 3 samples slightly (both control and treatment) from the protocols followed for Experiments 1 and 2 in order to accommodate the variation in experimental design of the initial incubations, which were conducted by different investigators in different labs as part of six unrelated experiments. We designated these initial incubations as the control samples for Experiment 3 (hereafter “control-3”). While control-3 sample incubations varied according to the goals of the original experiments, we aimed to maintain the same amount of CO2 respired and the same soil moisture content between paired control-3 and storage duration treatment sample incubations.

2.3.1 Experiment 3 sample selection

The main criteria for sample selection for Experiment 3 were: 1) samples were split prior to original incubation, with one portion air-dried and archived in amounts adequate for a repeated incubation; 2) ∆14C-CO2 was measured from soils incubated close to the time of collection and air dried after a relatively short (one to three weeks) incubation period. We sought to cover a range of storage duration times (between 4 and 14 y, constrained by the availability of samples), and a range of soil types and climatic conditions, (SI Table 1).

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 (SI Table 1). All soils obtained for the storage duration incubations were air-dried splits made prior to control-3 incubations.

2.3.3 Experiment 3 Incubations

Control-3 incubation soil mass, replication, temperature, and adjusted moisture varied according to the objectives of the original investigators (Table 2). Soil mass and replication of corresponding storage duration treatment incubations varied according to the amount of soil material available. We kept the soil moisture the same between paired control-3 and storage duration treatment incubations. However, all storage duration treatment incubations were conducted at 20ºC for simplicity, as while temperature has known effects on respiration rates, it has been shown that it does not affect ∆14C-CO2 (Vaughn and Torn, 2019).

We did not have information on either the duration of the rewetting period or the corresponding amount of CO2 respired 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 ∆14C-CO2 in the first two experiments (Results 3.2). We allowed respiration in the storage duration treatment samples to proceed until the same amount of CO2 had been respired per g of soil C as in the second enclosure period of the corresponding control-3 sample incubations.

We measured headspace CO2 concentrations every three days for the first two weeks of the storage duration treatment incubations, and weekly as needed thereafter; control-3 CO2 measurement frequency varied



. Aliquots of jar atmosphere were collected once the samples reached target CO2 concentrations (7 to 48 mg CO2 g C-1) and were analyzed for ∆14C. 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) (SI Table 1).

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, Hanau, Germany) following fine grinding with a ball-mill (Retsch MM400, Hanau, Germany). 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 investigators (Gaudinski et al. 2000; Cisneros et al., 2006; Hopkins et al. 2012; Koarashi et al. 2012; Solly et al. 2014) (Table 1).

2.5 Isotopic analyses

For all three experiments, we separated CO2 from the gas samples collected from incubation jar headspace using a vacuum line, with splits of the purified CO2 analyzed for both δ13C and ∆14C. Radiocarbon analyses 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) (SI Table 1). Radiocarbon values are reported in units of ∆14C, defined as the deviation in parts per thousand of the ratio of 14C to 12C from that of the oxalic acid standard measured in 1950. In order to account for potential mass-dependent fractionation effects, the 14C/12C ratio of all samples is corrected to a common δ13C value of -25 per mil (Stuiver & Polach, 1977). Although the effect was small, ∆14C data from air-dry/rewet + storage samples (Experiments 1 and 3) were also corrected for depletion of 14C in the samples due to radioactive decay that occurred during storage.

Measurements of δ13C (Experiments 1 and 2 only) were made at MPI-BGC (Delta+XL, Thermo Finnigan, Bremen, Germany). Data are reported using δ13C notation, which refers to the deviation in parts per thousand of the ratio of 13C/12C in the PDB standard.

2.6 Statistical analysis

We compared the mean differences between treatment and control sample ∆14C-CO2 and δ13C-CO2 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. We calculated mean differences between control and treatment samples and the variance of this mean difference, and then determined the pooled mean and variances. We calculated pooled statistics separately for forest and grassland soils in Experiments 1 and 2, but pooled across ecosystem type for Experiment 3 as the direction of trend was the same for both forest and grassland soils in Experiment 3 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 (**Eq. 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.

**Eq. 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 supplemental information. 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 ∆14C-CO2.

All statistical analyses were performed in R (R Core Team 2019).

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 ∆14C-CO2 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 fast cycling soil C pools, and no transfers between pools, using the Soil R package (Sierra et al., 2014). In an earlier study, Schrumpf & Kaiser (2015) estimated intrinsic decomposition rates and pool sizes for empirically defined soil C pools using a density fractionation procedure. We approximated the intrinsic decomposition rates for the fast and slow pools of our model using Schrumpf & Kaiser (2015)’s mean estimates for the free light fraction and the heavy fraction from the 0-5 cm depth increment: 4-1 y and 115-1 y for the fast and slow pools, respectively. Schrumpf & Kaiser (2015) found that 10% of the carbon in the 0-5 cm depth layer was in the free light fraction. We used this fraction for the partitioning 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 y 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 (Fig. 1). However, the magnitude and timing of maximum respiration rates diverged among experiments and between grassland and forest soils (Fig. 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 (Fig. 1a), but were similar across ecosystem types for the air-dry/rewet treatment samples in Experiment 2 (Fig. 1b). Respiration rates for Experiment 3 samples are shown in Supplementary Figure 4, but CO2 flux rates cannot be meaningfully interpreted given the differences in incubation temperature, degree to which rewetting pulse CO2 was included in the control-3 incubations, and the wide variation in CO2 measurement frequency among samples.

3.2. First and second enclosure period ∆14C-CO2 and δ13C-CO2

We did not see significant differences when we compared ∆14C-CO2 from the first enclosure period to that of the second enclosure period. This was true for all comparisons made within experiment, treatment, and ecosystem groups, with one exception: grassland control-1 samples had slightly higher ∆14C-CO2 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 ∆14C-CO2 between enclosure periods was only 2.0‰ (95% CI = [-1.0‰, 5.0‰]), which is similar to the reported precision for 14C measurements (1.7‰ to 2.7‰). (We excluded the forest control-2 sample that was clearly an outlier (Fig. 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 (Fig. 2) generated enough CO2 to measure the radiocarbon content. In addition, it was not possible to compare ∆14C-CO2 across enclosure periods for the control-1 samples as ∆14C-CO2 of the first enclosure period was not measured in 2011.

However

, we did observe significant differences between the δ13C-CO2 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 grassland soils in the control group in Experiment 2 (mean difference = 0.85‰, 95% CI = [0.64‰, 1.07‰]) (Supplemental Fig. 5). Note that as with ∆14C, δ13C-CO2 was not measured for the first enclosure period of control-1 incubations.

3.3. Overall treatment effects on ∆14C-CO2 and δ13C-CO2

We observed consistent differences between control and treatment sample ∆14C-CO2 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, Fig. 3). The samples from Oak Ridge are an exception in that mean difference in ∆14C-CO2 between storage treatment samples and corresponding control-3 samples was -44.0‰ (Table 3).

Forest and grassland soil ∆14C-CO2  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 enrichment in ∆14C-CO2. Experiment 3 treatment sample ∆14C-CO2 tended to be depleted relative to the controls (points below the 1:1 line in Fig. 3) for the majority of forest and grassland soils.

Treatment samples in Experiment 1 and Experiment 2 consistently showed significant differences (α= 0.05) in δ13C-CO2 relative to the controls for both forest and grassland soils (Supplementary Fig. Differences in δ13C-CO2 were slightly larger in forest soils than grassland soils across experiments (Table 3). Comparisons of δ13C-CO2 were not made for Experiment 3 samples owing to a lack of data for the control-3 samples.

We did not find evidence of a substantial effect of the amount of C respired on ∆14C-CO2, nor consistent effects due to the change in soil moisture (Supplemental Figures 7 and 8).

3.4. Storage duration effect on ∆14C-CO2

We used data from both Experiment 1 and Experiment 3 to assess the effect of storage duration. The longest duration of storage was 14 y, while the shortest was 5 y. Over this range of time we did not observe a trend in the difference between control and treatment ∆14C-CO2 with increasing duration of storage (Fig. 4).

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

A subset of the sites sampled for Experiment 1 in 2011 was resampled for Experiment 2 in 2019. This time series allows us to assess both the change in ∆14C-CO2 of respiration over time in control-1 versus control-2 samples, as well as the potential impact of air-drying and rewetting on any observed changes. The absolute value of the mean difference in ∆14C-CO2 between control and treatment samples was greater in grassland samples than in forest samples at both time points: in 2011 (Experiment 1) and 2019 (Experiment 2) (Table 3).

The difference between respired ∆14C-CO2 and the atmosphere in the year of sampling (∆∆14C) is an important indicator of soil C cycling rates. Sample Δ14C-CO2 was enriched relative to the atmosphere across ecosystem types for all samples, i.e. ∆∆14C values were positive (Fig. 5, Table 3). However, we observed smaller ∆Δ14C-CO2 values in grassland soils than in forest soils at both time points (Table 3). Comparing ∆∆14C-CO2 between control and treatment samples within ecosystem types, we observed lower values in control samples than in treatment samples at both time points for the grassland soils. We saw the same trend for forest samples collected in 2019. In contrast, treatment samples from the forest soils collected 2011 had lower ∆∆14C-CO2 values than did control samples.

**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 ∆14C-CO2 in incubations of air-dried and archived soils is a promising technique for constructing time series of respired ∆14C-CO2 and constraining soil carbon models. We observed that air-drying and rewetting shifted observed ∆14C-CO2 relative to control incubations of soils that had never been air-dried, but these differences were relatively small: on the order of 10‰ to 25‰, if we exclude the samples from the Oak Ridge labeling experiment (Table 3). Differences between control and treatment ∆14C-CO2 were significant for all three experiments (Table 3), however, suggesting that the process of drying and rewetting leads to utilization of substrates with distinct ∆14C signatures.

4.2 Effects of air-drying and rewetting on the age of respired CO2

While respired ∆14C-CO2 was clearly affected by air-drying and rewetting treatment for samples collected at the same location (Hainich-Dün) at two different time points (for Experiment 1 in 2011 and Experiment 2 in 2019), the magnitude and sign of the difference compared to control incubations differed. For example, we observed enrichment following the air-drying and rewetting treatment in 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 forest soils collected in 2011 (Experiment 1).

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 ∆14C in slow and fast cycling soil carbon pools over time, we can expect different responses to air-drying and rewetting in ∆14C-CO2 in soils sampled at different times. The importance of the year of sampling and system-specific carbon dynamics are illustrated using a conceptual model developed by Schrumpf & Kaiser (2015) for forest sites in the Hainich-Dün (Fig. 6).

Comparing the data from the Hainich-Dün forest sites with model projections of the trajectories for fast, slow and respired ∆14C (Fig. 6) indicates they are consistent with mobilization of carbon from the slow C pool after drying and rewetting. Following treatment, ∆14C-CO2 of respired CO2 (black points) shifts towards the slow pool ∆14C 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, an increased contribution of the slow pool to respiration following treatment leads to relative depletion of ∆14C-CO2 in 2011, but relative enrichment of ∆14C-CO2 in 2019. Thus, depending on the sampling year, the bias in ∆14C-CO2 introduced by air-drying and rewetting could be either higher or lower relative to a sample incubated without air-drying.

4.3. Explaining differences in forest versus grassland soils 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 German forest and grassland ecosystems analyzed in this study (the Hainich-Dün and Schorfheide-Chorin regions) provides support for the pre-ageing of carbon in forest ecosystems: Solly et al. (2013) found the mean age of the carbon in fine roots in the forest ecosystems to be approximately 10 y, in comparison to 1 to 2 y for fine roots in the grassland ecosystems. This pre-aging, or lag effect, for fine root inputs may explain the greater ∆∆14C values seen for the respiration from forest ecosystems as compared to the grassland ecosystems in this study (Table 3).

In contrast to forests, the grassland soils that were collected in both 2011 (Experiment 1) and 2019 (Experiment 2) respired more 14C-enriched CO2 following air-drying and rewetting (Fig. 5) and differed from observations in forests (Fig. 3). In this case, we also expect that air-drying and rewetting increased the contribution of more slowly cycling carbon to respiration. There are two explanations for why this increased contribution from the slow pool leads to enrichment in respired ∆14C after treatment in grasslands in our dataset, rather than matching results from the forest soils. First, the oldest grassland samples analyzed were not collected until 2011, on average several years later than the forest soils. Second, faster overall C cycling in grasslands, including a shorter ‘lag’ effect, means that the year when the trajectories of 14C for fast and slow cycling soil carbon pools cross (see Fig. 6) would be expected to occur earlier in grassland soils. Thus, even though the effects of air-drying and rewetting on ∆14C-CO2 differ between forests and grasslands, both results are consistent with the explanation that the air-drying and rewetting treatment is mobilizing additional carbon from a more slowly cycling pool.

4.4. Is rewetting pulse CO2 derived from different C sources?

There are competing hypotheses for the source of CO2 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; Williams & Xia, 2009; Kaiser et al., 2015; Warren, 2016; Slessarev et al., 2020). Due to the often dramatic differences in respiration rates between the rewetting period and subsequent respiration (e.g. 1) these authors posit differences in the substrates fueling rewetting versus subsequent respiration. However, we did not find a significant difference in ∆14C-CO2 between these two respiration periods. This finding was true for all of the samples in which we measured ∆14C-CO2 in both the rewetting pulse period and a second enclosure period (Fig. 3). These results suggest that the change in substrate availability initiated by air-drying and rewetting may not be limited to the rewetting pulse.

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; Wu and Brooks, 2005; Xiang et al., 2008; Williams and Xia, 2009). However, as other recent work has shown, persistence of soil organic matter is not solely due to chemistry (Lützow et al., 2006; Marschner et al. 2008; Schmidt et al.; 2011; Dungait et al., 2012). The similarity in ∆14C across substrates utilized in the rewetting pulse and the second enclosure period, despite likely diverging in chemistry (cf. change in δ13C-CO2, Table 3 and Supplemental Fig. 6), is therefore in line with the modern paradigm (Lehmann and 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 ∆14C-CO2 between enclosure periods. For context, we note that the mean amount of CO2 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 δ13C observed between the rewetting pulse and the second enclosure period, which we did find to be significant.

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



The consistent enrichment in δ13C-CO2 seen following both the air-dry/rewet + storage treatment and the air-dry/rewet treatment (Table 3, Supplemental Fig. 5) could have multiple possible causes. Microbial recycling has been shown to lead to δ13C 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 ∆14C-CO2 and δ13C-CO2 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 ∆14C unit accounts for mass-dependent fractionation effects, thus this phenomenon does not affect the radiocarbon results as reported.

We observed greater enrichment of δ13C-CO2 in forest soils than in grassland soils, which could indicate greater microbial recycling in forest soils or potentially more mobilization of mineral-associated organic matter in forest soils than in grassland soils following treatment. Mineral-associated organic matter has been shown to be more enriched in δ13C 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 δ13C-CO2 response across forest and grassland soils (Supplemental Fig. 5) suggests that a similar mechanism is at work in both ecosystems.

Assessing potential storage effects on ∆14C-CO2

Data from Experiment 1 and Experiment 3 showed that storage duration does not have a strong effect on ∆14C-CO2, at least within a period of 5 to 14 y (Fig. 4). Nearly all of the soils incubated were from forests soils collected before 2019, and these all exhibited depletion of ∆14C-CO2 following air-drying/rewet + storage treatment (Fig. 3, Fig. 4). However, the depletion in the forest soils was greatest in the samples from Oak Ridge (magenta triangles, Fig. 3), which had been substantially enriched in ∆14C above background levels through release of enriched 14C from a nearby incinerator four years prior to sample collection. This and a subsequent manipulation experiment resulted in 14C enrichment of both surface litter and root inputs (at levels between +400 and +1000 ‰)

One explanation for the greater shift observed for the Oak Ridge soils as compared to the non-labeled forest soils is that for these labeled soils there is a greater difference between the carbon fixed in the two decades prior to sampling (~ 80-200‰) and the labeled carbon (+400-1000 ‰) introduced to the soil in the four years prior to sampling. The consistently lower ∆14C-CO2 for samples incubated after air-drying and rewetting adds further support to the idea that C being mobilized comes primarily from CO2 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, for example with newly collected soils from the same location.

**5.**

Measuring ∆14C-CO2 in incubations of air-dried and archived soils is a promising technique for constructing time series of respired ∆14C-CO2 and constraining soil carbon

models. Air-drying and rewetting of soils led to small but significant differences in the ∆14C of respired CO2 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, ∆14C of atmospheric CO2 has declined by ca. 5‰ per year between 2000 and 2015 (Graven et al., 2017).

Overall, our results demonstrate that differences in ∆14C 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 ∆14C-CO2 is beyond the scope of this study, but our results suggest that the CO2 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 ∆14C-CO2 persists beyond the rewetting pulse, suggesting that simply excluding the rewetting pulse CO2 when measuring ∆14C-CO2 does not eliminate the bias introduced by air-drying and rewetting. Finally, we recommend that when comparing ∆14C-CO2 between recently collected soils and archived soils, both samples should undergo the same air-drying and rewetting procedure to minimize bias.

**Acknowledgments, Samples, and Data**

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. Supplementary materials can be reproduced with the file “SI\_ArcInc\_2021-06-15.Rmd”. The authors would like to acknowledge the invaluable assistance of M. Rost in the laboratory and the field, and I. Schoening, M. Cisneros-Dozal, J. Koarashi, F. Hopkins, C. Lawrence, and S. Trumbore for sharing data and details on control-3 sample incubations. Funding was provided by the European Research Council (Horizon 2020 Research and Innovation Programme, grant agreement 695101; 14Constraint).

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**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).The final measurement points for a few samples which took > 18 d to reach CO2 targets are excluded for display reasons; respiration rates for those samples remained constant. Note that headspace CO2 concentrations for control-1 samples (panel a) were only measured once during the first enclosure period (day 4) in contrast to daily measurements for all other samples.

**Figure 2.** ∆14C-CO2 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 Δ14C-CO2 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 CO2 during this period to measure Δ14C-CO2.

**Figure 3.** Overall treatment effect on Δ14C-CO2. Points show data from all three experiments and are the mean of laboratory replicates (for replicated samples); error bars are standard deviation of replicatesFor context, the dashed and dotted lines show differences of ±20‰ and ±40‰. 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 14C label experiment (Cisneros-Dozal et al. 2006), where the label occurred within four years of original sample collection.

**Figure 4.** Treatment effect on Δ14C-CO2 in relation to storage duration**.** Points show data from Experiments 1 and 3. Data are averaged by site (some 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 ±20‰ and ±40‰. The Oak Ridge sample points with the greater treatment-control difference at both 5 y and 14 y of storage are from the Tennessee Valley site, which received more 14C label than did the other Oak Ridge site, Walker Ridge.

**Figure 5.** Time series of control and treatment Δ14C-CO2 in Experiments 1 and 2. **a)** forest sites, **b)** grassland sites.Points show mean Δ14C-CO2 within ecosystem and treatment groups; error bars show the standard deviation. Atmospheric ∆14C-CO2 data (black line) are from Graven et al.All atmospheric radiocarbon data are for the northern hemisphere (zone 2).

**Figure 6.** Conceptual model fit and observed ∆14C-CO2 for the Hainich-Dün forest sites (Central Germany 2). **a)**∆14C of soil pools and heterotrophically respired CO2 predicted by the model adapted from Schrumpf & Kaiser (2015) alongside atmospheric ∆14C for the bomb-C period (1950 to 2020) and ∆14C-CO2 observed in this study (black points) **b)** *(inset)* Zoom to study period. Blue arrows show the shift in ∆14C-CO2 following air-drying and rewetting. Note that ∆14C-CO2 shifts towards 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 ∆14C-CO2 observed in this study(2017) up to the year 2015, while atmospheric

**Table 1.**

*Mean soil properties by sampling region\**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  | |  | | Nutrients | | | | | | Particle size | | | | | | | | |
|  |  |  |  |  | |  | | Organic C | | | Total N | | | Sand | | | Silt | | | Clay | | |
| Experiment | Region | Ecosystem\*\* | MAT | | MAP | | n\*\*\* | | Mean | sd | | Mean | sd | | Mean | sd | | Mean | sd | | Mean | sd | |
|  |  |  | *°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 | 7.25 | | 650 | | 3 | | 23.7 | 0.5 | | 1.7 | 0.1 | | 54 | 18 | | 754 | 7 | | 193 | 15 | |
| 1, 2, 3 | Central Germany 2 | grassland | 7.25 | | 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 | | 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 | - | | - | - | | - | - | | - | - | | - | - | |
| 3 | Duke FACE, USA | forest | 15.5 | | 1140 | | 1 | | 16.6 | - | | 0.8 | - | | - | - | | - | - | | - | - | |

\* 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 Gaudinki et al. (2000); climate data are the ten-year averages from 1991 to 2000 (Boose et al., 2012); all Oak Ridge data are from Cisneros-Dozel et al. (2006); Duke FACE data are from Hopkins et al. (2012); Sierra Nevada data are from Koarashi et al. (2009). Note that not all data were available for all sites. \*\* 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). \*\*\* See Table 2 for the total number of samples per experiment, and Table 3 for the number of samples per site per experiment.

*Experimental design*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  | Enclosure period\*\*\* | | | | | |
|  |  |  |  |  |  | Moisture content\*\* | | 1st (rewetting pulse) | | | 2nd | | |
| Experiment | n | Treatment | Reps\* | Sampling date | Incubation date | Initial | Adjusted | Time | ∆14C? | δ13C? | Time | ∆14C? | δ13C? |
|  |  |  |  | *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 |

\*Laboratory incubation replicates. \*\* 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. \*\*\* 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 et al., 2006) or not reported at all for other samples (n = 4, Gaudinski et al., 2000). ∆14C-CO2 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 ∆14C-CO2 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 CO2 respired.

**Table 3.**

*∆14C and δ13C of respired CO2 in the 2nd enclosure period\**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  | | | | | Difference (treatment - control) | | | |
|  |  |  |  | ∆14C-CO2 | | | δ13C-CO2 | | ∆14C-CO2 | | δ13C-CO2 | |
| Experiment\*\* | Ecosystem | Treatment | n | Mean | Mean ∆∆\*\*\* | sd | Mean | sd | Mean | CI95 | Mean | 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] | - | - |

\* Experiment 3 storage duration treatment samples were only incubated for a single enclosure period and so data were measured following this period. \*\*Results from Experiment 3 reported separately for the enriched samples from Oak Ridge (3b) and the non-enriched samples (3a). Mean control and treatment ∆14C-CO2 are only reported for Experiments 1 and 2 where the aggregated data are representative of one site at one point in time. \*\*\* The ∆∆ notation denotes the difference from the atmosphere at the time of sampling.