**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‰, while the duration of storage had no significant impact

· The direction and magnitude of the shift in ∆14C of CO2 depended on sampling year, with stronger effects in grassland than in forest soils

Abstract

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

**Plain Language Summary**

Soils play a key role in the global carbon cycle by sequestering carbon from the atmosphere for decades to millennia. However, it is unclear if they will continue to do so as the climate changes. Microbial decomposition of soil organic matter returns carbon back to the atmosphere, and radiocarbon dating of this returning CO2 (∆14C-CO2) can be used to quantify how long carbon is stored in soils. Incubating archived soils could provide unique insight into soil carbon sequestration potential by quantifying the change in ∆14C-CO2 over time. However, air-drying, duration of archiving, and subsequent rewetting of soils may bias estimates of sequestration potential by altering the balance of younger versus older carbon leaving the soil. We compared ∆14C-CO2 from soils incubated with and without air-drying and archiving, and found that the air-dried soils appeared to release slightly older carbon than soils that had never been air-dried (by two years in forests and four years in grasslands). The amount of time the soils were archived did not have an effect. Since the bias from air-drying and rewetting was small, incubating archived soils appears to be a promising technique for improving our ability to model soil carbon cycling under global climate change.

**1. Introduction**

Soil carbon is a heterogeneous mixture of organic matter, some components of which persist in the soil for months or years, while others persist for centuries or millennia. The persistence of soil carbon can be understood through the concept of different “pools” of carbon, each defined by the mechanism by which carbon is stabilized in the soil and characterized by a distinct probability distribution of ages (Sierra et al., 2018). 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 large fast cycling pools, while shifts in temperature or moisture regimes may affect decomposition rates differently depending on the stabilization mechanism.

Empirical approaches for quantifying soil carbon pools that cycle at different rates include fractionation by size, density, or resistance to decomposition from acid, alkaline, or oxidative reagents. However, these methods result in mixtures of pools with different age distributions (Feng et al., 2016; Kleber et al., 2011; Lehmann & Kleber, 2015). In contrast, although they may introduce artifacts due to disturbance and potential alteration of the microbial community, laboratory incubations of bulk soil make use of the same fractionation agent as is found *in situ*: the microbial community (Schädel et al., 2020).

Laboratory soil incubation is a commonly used technique for understanding soil carbon dynamics, but measuring the radiocarbon signature of heterotrophically respired CO2 (∆14C-CO2) is not. However, Δ14C-CO2 is a powerful constrait for modeling soil carbon dynamics because it provides an integrated measure of the weighted contribution to the soil efflux from carbon pools with distinct processing rates (Trumbore, 2000). ∆14C-CO2 can thus be used to infer the relative processing rate of carbon (or transit time) for the whole soil, i.e. the average period of time between when an atom of carbon enters the soil and when it leaves (Sierra et al., 2017). In contrast to bulk soil ∆14C, which is dominated by relatively slowly cycling carbon, ∆14C-CO2 reflects mainly fast cycling carbon (Sierra et al., 2018).

Depletion of natural abundance radiocarbon due to radioactive decay provides information about the age of carbon in soil organic matter on centennial or millennial scales, while insight into decadal scale dynamics can be gained from tracing the pulse of radiocarbon introduced into the biosphere from nuclear weapons testing (“bomb-C”) in the mid-20th century (Trumbore, 2009). The bomb-C pulse peaked in the atmosphere in the 1960s, but due to differential rates of biological processing, the peak is lagged in time and dampened in soils.

A critical issue with interpreting bomb-C radiocarbon is that there are two points in time at which the Δ14C signature of atmospheric CO2 is identical, due to the curvature of the bomb-C peak. This creates a challenge when using Δ14C to infer intrinsic decomposition rates when observations are only available from a single point in time, as the same Δ14C value could be fit to models with different rates. Trumbore (2000) gives the example of a two independent, homogenous pools of soil carbon, one with an intrinsic decomposition rate (*k*) of 6.6 years and the second with *k* = 50 years, both of which would have a Δ14C of 166‰ in 1996. However, this multiple solution problem can be mitigated with multiple observations of ∆14C over time, as 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).

Soil archives have proven to be a valuable resource when constructing time series of bulk soil radiocarbon (Jenkinson et al., 2008). However, measuring Δ14C-CO2 in incubations of archived soils presents additional challenges. 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). 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.

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).

Air-drying and rewetting of soil occurs *in situ* and is also common in laboratory incubation studies as a means of controling moisture content, underscoring the importance of this kind of disturbance in understanding soil carbon cycling (Borken & Matzner, 2009; Jarvis et al., 2007). If air-drying and rewetting affects the relative contribution of soil organic matter pools with different intrinsic cycling rates to respiration, this should be detectable in ∆14C-CO2. For example, disruption of soil aggregates following drying and rewetting would likely lead to greater accessibility of soil organic matter formerly protected from decomposition via physical occlusion. Drying followed by rewetting could also lead to desorption of organic matter sorbed to minerals, increasing the accessibility of this formerly protected substrate. If drying and rewetting mobilizes carbon from these relatively slowly cycling soil organic matter pools the effect should be detectable as a shift in ∆14C-CO2. However, if the rewetting pulse derives mainly from lysed microbial cells or the release of microbial osmolytes little change in ∆14C-CO2 would be expected.

Obtaining ∆14C-CO2 measurements from incubations of archived soils would be a valuable tool for further constraining and improving soil carbon models, but first the possible effects of air-drying and rewetting, as well as the effect of storage duration, must be assessed in order to exclude or quantify potential artifacts. The direction and magnitude of any change in ∆14C-CO2 induced by these treatments should be indicative of the change in substrate, i.e. increased contribution of either faster or more slowly cycling carbon pools to heterotrophic respiration.

We 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 hypothesized that the pulse of CO2 released immediately following rewetting would be older in stored, air-dried soils than was measured initially on undried, freshly collected sample splits, due to greater mobilization of protected, relatively slowly cycling carbon through the process of drying and rewetting. However, we expected any differences in ∆14C-CO2 would be limited to the rewetting pulse and would not persist through a second enclosure period. Furthermore, we expected that any differences between ∆14C-CO2 measured in incubations of archived soils would be due to air-drying and rewetting effects alone. Therefore we expected soils that had been air-dried and rewet without storage would exhibit the same differences in ∆14C-CO2 as in archived soils, i.e. we did not expect a significant storage duration effect.

**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).

2.1 Experiment 1

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 different study by Solly et al. (2014). Two ecosystem types (forest and grassland) were sampled from two regions of central Germany, Schorheide-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 used the δ13C signature of respired CO2 (δ13C-CO2) from the second enclosure period of the incubations conducted in 2011 to omit samples containing inorganic carbon, conservatively deeming any samples with δ13C-CO2 > -25‰ as potentially affected by the release of inorganic C (which may be present in previously limed soils in this region). We then selected soils from three grassland plots (50 m by 50 m) and three forest plots (100 m by 100 m) in each of the two geographic regions (n total = 12 sites), using the additional criterium that the ∆14C-CO2 observed in the 2011 incubations fell within the 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. 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.

An additional 10g aliquot was removed from the air-dried sample and further dried at 105ºC in order to determine water holding capacity (WHC). Briefly, the tip was removed from a 50 ml centrifuge tube and covered with a fine mesh (<50 µm). The tube was filled with soil and placed mesh-side down overnight in a glass dish filled with a mass of water equal to twice that of the mass of soil. The following day the tube was moved to a second glass dish filled with sand and allowed to drain for 30 minutes before weighing again (wet mass). WHC is defined as the difference between the wet mass and the oven-dry mass over the oven-dry mass.

2.1.3 Experiment 1 incubations

Control-1 incubations were performed 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, either from field moisture (control-1 samples) or from air-dried conditions (air-dry/rewet + storage samples). We moistened the soil from the top by means of 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 in duplicate in order to quantify potential laboratory errors. However, owing to a limited quantity of archived soil we had to reduce the mass of soil incubated to 20 g for all air-dry/rewet + storage treatmentsamples. 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. However, to compensate for the difference in the mass of soil incubated and to account for the fact that the amount of CO2 respired is thought to affect ∆14C-CO2, 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

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. As we did not observe significant treatment differences between the two geographic regions in the results of Experiment 1, we restricted the resampling to just one region to save on cost and time. At each plot (n = 6) we collected three cores from the same depth interval as 2011 (0-10 cm), which were then homogenized to yield one composite sample. 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, and so will be described jointly. Incubations were performed in duplicate to quantify potential laboratory errors. As with the air-dry/rewet + storage treatment samples in Experiment 1, 20 g (air-dry equivalent weight) of soil were weighed out into 250 ml beakers and placed into 1000 ml mason jars with airtight lids fitted with two sampling. Soil moisture content was adjusted to 60% WHC prior to sealing the jars 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.

The duration of the second enclosure period differed between control-2 and air-dry/rewet samples. 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. In contrast, 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 paired control-2 sample. All samples were incubated at 20º C.

2.3 Experiment 3

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

2.3.1 Experiment 3 sample selection

The main criteria for sample selection for Experiment 3 were: 1) samples were split prior to incubation, 2) ∆14C-CO2 was available from the second enclosure period of the initial incubations, and 3) adequate soil material from the unincubated sample split was available for the storage duration treatment incubation. We sought to cover a range of storage duration, but this was ultimately constrained to be between 4 and 14 y due to the difficulty of obtaining samples. We were able to obtain samples from a range of soil types and climatic conditions, and as with the first two experiments, we limited the samples studied to the A horizon (SI Table 1).

2.3.2 Experiment 3 sample preparation

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

**Table 1.**

*Mean soil properties by sampling region\**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | Nutrient | | | | Particle size | | | | | |
|  |  |  |  | Organic C | | Total N | | Sand | | Silt | | Clay | |
| Experiment | Region | Ecosystem\*\* | n\*\*\* | Mean | sd | Mean | sd | Mean | sd | Mean | sd | Mean | sd |
|  |  |  | *sites* | *g kg-1* | | | | | | | | | |
| 1 | Central Germany 1 | forest | 3 | 22.1 | 8.1 | 1.1 | 0.3 | 861 | 44 | 92 | 27 | 47 | 20 |
| 1 | Central Germany 1 | grassland | 3 | 22.8 | 1.5 | 2.2 | 0.1 | 731 | 99 | 158 | 75 | 111 | 31 |
| 1, 2, 3 | Central Germany 2 | forest | 3 | 23.7 | 0.5 | 1.7 | 0.1 | 54 | 18 | 754 | 7 | 193 | 15 |
| 1, 2, 3 | Central Germany 2 | grassland | 3 | 41.8 | 1.9 | 3.9 | 0.1 | 32 | 17 | 553 | 78 | 414 | 65 |
| 3 | Oak Ridge | forest | 2 | 24.9 | 0 | 1.1 | 0.1 | - | - | - | - | - | - |
| 3 | Sierra Nevada, CA | forest | 2 | 28.4 | 1.4 | 1.1 | 0.1 | 700 | 141 | 210 | 85 | 100 | 71 |
| 3 | Harvard Forest | forest | 1 | 60 | - | - | - | - | - | - | - | - | - |
| 3 | Duke FACE | forest | 1 | 16.6 | - | 0.8 | - | - | - | - | - | - | - |

\* The Central Germany regions are two sites from the Biodiversity Exploratories project: 1) Schorfheide-Chorin, 2) Hainich-Dün. Data from Harvard Forest provided by Gaudinki et al. (2000), Oak Ridge data from Cisneros-Dozel et al. (2006), Duke FACE data from Hopkins et al. (2012), Sierra Nevada data from Koarashi et al. (2009). Central Germany region data are from the database of the Biodiversty Exploratories project (BEXIS). 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 are a mix of coniferous and deciduous species; 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.

2.3.3 Experiment 3 Incubations

Replication and the mass of soil incubated in control-3 incubations varied according to the objectives of the original investigators. Soil mass and replication for the corresponding paired storage duration treatment incubations were kept the same as control-3 incubations unless this was not possible due to the amount of archived soil available. Due to the different objectives of the initial investigators soil moisture and incubation temperature also varied among control-3 samples. The soil moisture content of paired storage duration and control-3 samples was the same. However, all storage duration 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 were unable to obtain data on the first enclosure period duration or the amount of CO2 released during this period for all of the control-3 samples. Given this lack of data, and since we did not observe significant differences between rewetting pulse ∆14C-CO2 and ∆14C-CO2 collected following the second enclosure period in Experiment 1 air-dry/rewet + storage samples or in Experiment 2 samples (Results 3.2), we decided to conduct the storage duration treatment incubations for Experiment 3 with only a single enclosure period.

The duration of the second enclosure period and the CO2 measurement frequency varied for the control-3 incubations. However, we were able to obtain data on the cumulative CO2 respired during the second enclosure period and ∆14C of this CO2 for all of the control-3 samples. For the storage duration treatment samples we sealed the incubation jars following moisture adjustment (section 2.1.3) and flushing with CO2-free air. Incubations of storage duration treatment samples were allowed to proceed until an equivalent amount of CO2 had been released (mg CO2 g soil C-1) as during the second enclosure period of the corresponding control-3 samples. We measured headspace CO2 concentrations every three days for the first two weeks of the storage duration treatment sample incubations, and weekly as needed thereafter. Aliquots of jar atmosphere were collected once the samples reached target CO2 concentrations and subsequently analyzed for ∆14C. We conducted the majority (n = 16) of the Experiment 3 air-dry/rewet + storage 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) (Supplementary Table 1).

2.4 Soil analyses

Carbon and nitrogen contents of Experiment 1 and Experiment 2 samples were determined by dry combustion in a CN analyzer (Vario Max, Elementar Analysensysteme GmbH, Hanau, Germany) following fine grinding with a ball-mill (Retsch MM400, Hanau, Germany) using material from the air-dried sample splits. Nutrient contents were then corrected for residual moisture content. Additional analyses performed on the air-dried soils for Experiment 1 samples included texture (pipette method following removal of organic matter, Schlichting et al., 1995) and pH (1:2.5 mixture of soil and 0.01 M CaCl2). Data on the soil properties for Experiment 3 samples were obtained from the original investigators (Gaudinski et al. 2000; Cisneros et al., 2005; 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 for Experiments 1, 2, and the storage duration samples from Experiment 3 incubated in 2018 were conducted at the MPI-BGC accelerator mass spectrometer facility (Steinhof, 2013). All other samples were analyzed at the UCI W.M. Keck Facility for Accelerator Mass Spectrometry (Xu et al., 2007). Radiocarbon values are reported in units of ∆14C, defined as the deviation in parts per thousand of the ratio of 14C to 12C from that of the oxalic acid standard measured in 1950. In order to account for potential mass-dependent fractionation effects, the 14C/12C ratio of all samples is corrected to a common δ13C value of -25 per mil (Stuiver & Polach, 1977). Although the effect was small, ∆14C data from air-dry/rewet + storage samples (Experiments 1 and 3) were also corrected for depletion due to radioactive decay that occurred during storage. An additional unit used in this study is ∆∆14C, which is defined as the difference between the ∆14C measured in a sample and that of the atmosphere in the year of sampling.

As with the radiocarbon analyses, measurements of δ13C for Experiments 1, 2, and the storage duration samples from Experiment 3 incubated in 2018 were made at MPI-BGC (Delta+XL, Thermo Finnigan, Bremen, Germany), while control-3 samples were measured at UCI (Thermo Delta Plus, Thermo Fisher Scientific). Data are reported using δ13C notation, which refers to the deviation in parts per thousand of the ratio of 13C/12C in the PDB standard.

**Table 2.**

*Experimental design*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  | Enclosure period\*\*\* | | | | | |
|  |  |  |  |  |  | Moisture content\*\* | | 1st (rewetting pulse) | | | 2nd | | |
| Experiment | n | Treatment | Reps\* | Sampling date | Incubation date | Intial | 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 | 28 | control-3 | 1-3 | 1999-2011 | 1999-2011 | 6-95 (18) | 18-95 (17) | 1-10 | no | no | 5-14 | yes | no |
| 28 | storage duration | 1-3 | 2018 | 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. 2005) 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.

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 first 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. We assumed this mean variance for those samples that were not run in replicate so we could more accurately calculate the error of the difference between treatment and control means for each sample. We then determined the pooled mean and variance of the treatment-control differences within ecosystem types separately for Experiment 1 and Experiment 2 samples, and pooled across ecosystem type for Experiment 3. We did not consider ecosystem type for Exeperiment 3 as the direction of trend was the same for both forest and grassland soils and additionally 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 tested our hypothesis that changes in ∆14C-CO2 due to treatment would be limited to the rewetting pulse alone by comparing the paired differences in ∆14C-CO2 between the first and second enclosure periods. This was only possible for those samples where ∆14C-CO2 was measured in both periods: Experiment 1 air-dry/rewet + storage samples, and Experiment 2 samples. All samples were replicated, so we used the measured laboratory replicate variance when calculating the error on mean differences by sample. We calculated pooled means, variances, and 95% confidence intervals within each experiment, treatment, and ecosystem type using the same method described above. Confidence intervals on the mean difference overlapped zero for all but one group, so we conducted the same analysis on the whole sample set as well, pooling across experiment, ecosystem type, and treatment, in order to assess the overall effect of enclosure period on ∆14C-CO2.

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

**3. Results**

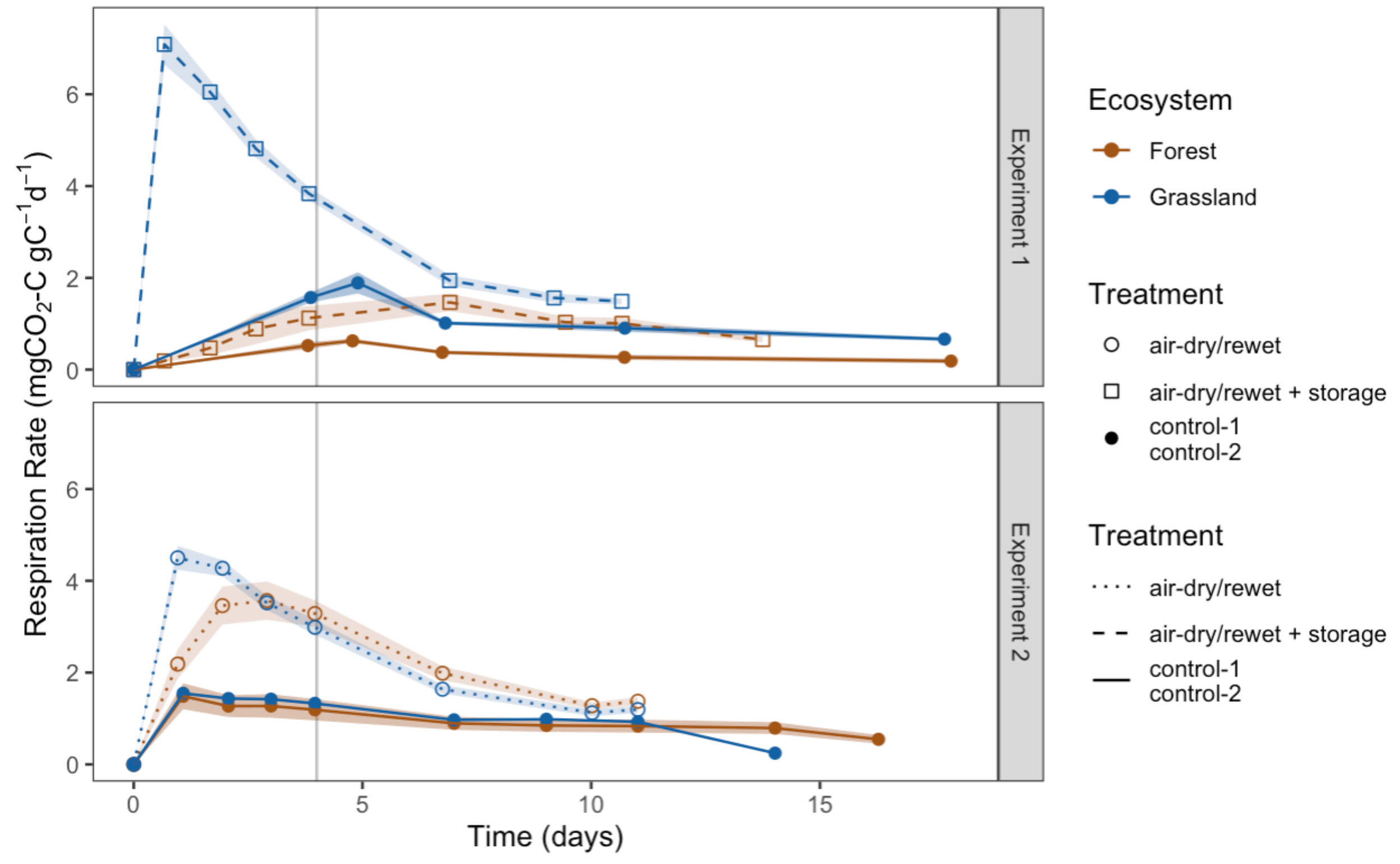
3.1. Respiration rates

The increase in respiration rates following moisture adjustment was greater for treatment soils than for the control soils in both Experiment 1 (air-dry/rewet + storage treatment) and Experiment 2 (air-dry/rewet treatment) when compared within ecosystem types. However, the magnitude and timing of peak respiration rates diverged among experiments and between grassland and forest soils (Fig. 1).

Among the air-dry/rewet + storage samples in Experiment 1 (Fig. 1a, dashed lines), the maximum respiration rates were more than twice as high in grassland soils than in forest soils. Grassland air-dry/rewet + storage samples reached a maximum of 7.1 mg CO2 g soil C-1 d-1 after 0.7 d followed by a sharp decline (Fig 1a), while mean respiration rates in air-dry/rewet + storage forest sites reached a maximum of 1.5 mg CO2 g soil C-1 d-1 after 6.9 d, followed by a much more gradual decline (Fig. 1a). In contrast, control-1 forest and grassland samples (solid lines, Fig. 1a) responded more weakly and more gradually than the air-dry/rewet + storage samples, but with similar trends with respect to ecosystem type. Respiration rates were higher in control-1 grassland soils than in control-1 forest soils: reaching maximal rates of 1.9 and 0.6 mg CO2 g soil C-1 d-1 for grassland and forest soils respectively after 4.8 d.

We observed similar patterns in the respiration rates of Experiment 2 samples (Fig. 1b) as in Experiment 1, with the treatment samples (air-dry/rewet) respiring at a significantly higher rate than control-2 samples (p < 0.05). However, unlike the air-dry/rewet + storage treatment samples in Experiment 1, maximal respiration rates were similarly low for both grassland and forest air-dry/rewet treatment samples in Experiment 2. We obseved maximal respiration rates of 3.0 and 3.3 mg CO2 g soil C-1 d-1 after 95 h for grassland and forest air-dry/rewet samples, respectively.

Given the differences in incubation temperature, duration of the pre-incubation and second enclosure periods, and the wide variation in CO2 measurement frequency both among control-3 samples from different studies and between paired control-3 and storage duration treatment incubations in this study, CO2 flux rates cannot be meaningfully interpreted (Supplementary Fig. 1).

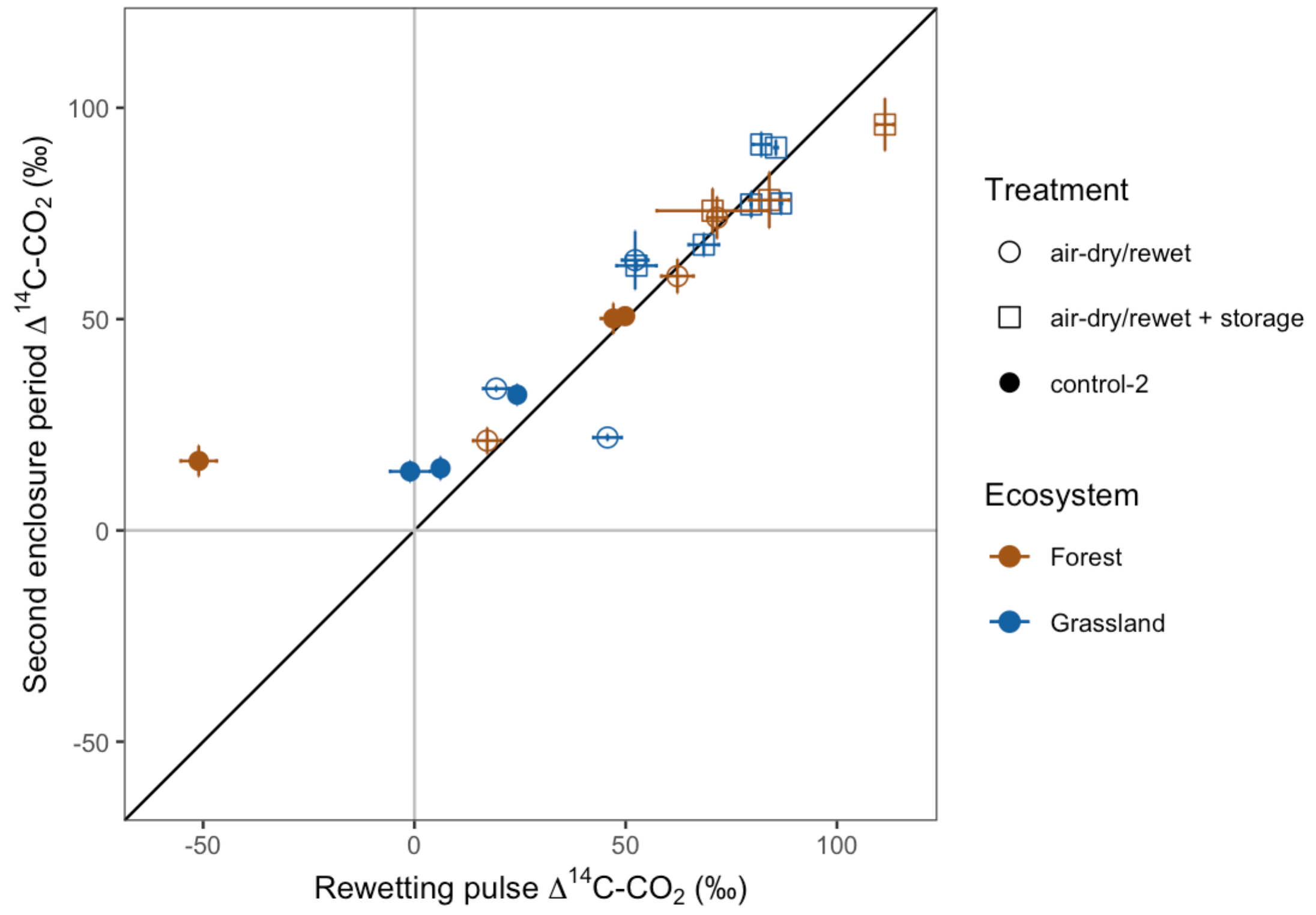


**Figure 1.** Respiration rates for Experiment 1 and Experiment 2 samples

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

3.2. Differences in first and second enclosure period ∆14C-CO2 and δ13C-CO2

Contrary to our hypothesis, ∆14C-CO2 measured shortly after rewetting (during the first enclosure period) did not differ substantially from ∆14C-CO2 measured during the second enclosure period. Ninety-five percent confidence intervals around the mean difference in ∆14C-CO2 included zero for all comparisons made within experiment, treatment, and ecosystem groups, with the exception of the grassland control samples in Experiment 2. For these samples ∆14C-CO2 was significantly higher in the second enclosure period than in the first (mean difference = -10.4‰, 95% CI = [-14.8‰, -6.0‰]). However, when we combined data across experiments, ecosystem types, and treatments the difference was not signifcant (mean difference = -2.0, 95% CI = [-5.0‰, 1.0‰]). There was one exceptional sample in the Experiment 2 forest control group for which the ∆14C-CO2 of the first enclosure period was strongly depleted relative to the second enclosure period (Fig. 2). While this difference was consistent in both laboratory replicates and thus may well represent a real phenomenon, we excluded it from the overall analysis as it does not appear to be representative of the trend observed in the sample population as a whole.



**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 min. and max.. 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.

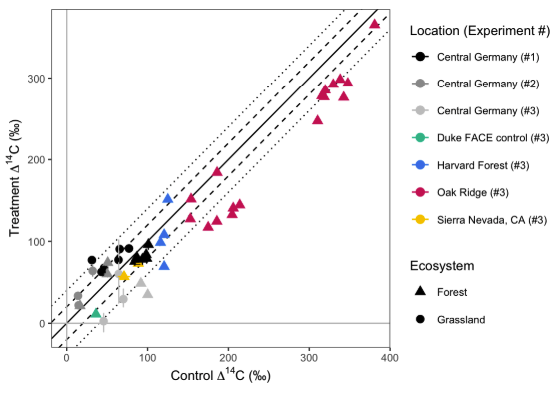
We note that due to lower respiration rates during the first enclosure period only three of the six forest soils in the air-dry/rewet + storage treatment group from Experiment 1 (Fig. 2) generated enough CO2 to measure the radiocarbon content. In addition, it was not possible to compare ∆14C-CO2 across enclosure periods for the control-1 samples as ∆14C-CO2 of the first enclosure period was not measurred in 2011.

We observed 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. 2). 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 observed in the second enclosure period in all three experiments (Table 3). This was in contrast to our expectation that treatment effects would be limited to the first enclosure period. Treatment sample incubations, including both the air-dry/rewet + storage treatment in Experiments 1 and 3, and the air-dry/rewet treatment in Experiment 2, typically resulted in differences between 20‰ and 40‰ relative to control sample incubations, although the majority of the differences were within ±20‰. The 20‰ to 40‰ range is shown for context (Fig. 3, dashed and dotted lines, respectively): these differences correspond to the change in ∆14C of the atmosphere over four and eight years, respectively, during the period of 2000 to 2020 (Graven et al., 2017). The samples from Oak Ridge are an exception in that the majority of the air-dry/rewet + storage treatment samples and corresponding control-3 samples differ by ≥40‰ (Table 3).

We observed opposite trends in the treatment ∆14C-CO2 response for forest and grassland soil in Experiment 1, with the air-dry/rewet + storage treatment leading to depletion for forests, but enrichment for grsslands (Table 3). In contrast, both forest and grassland soils in Experiment 2 responded to the air-dry/rewet treatment with enrichment in ∆14C-CO2, while the Experiment 3 treatment sample ∆14C-CO2 tended to be depleted relative to the controls for both forest and grassland soils.



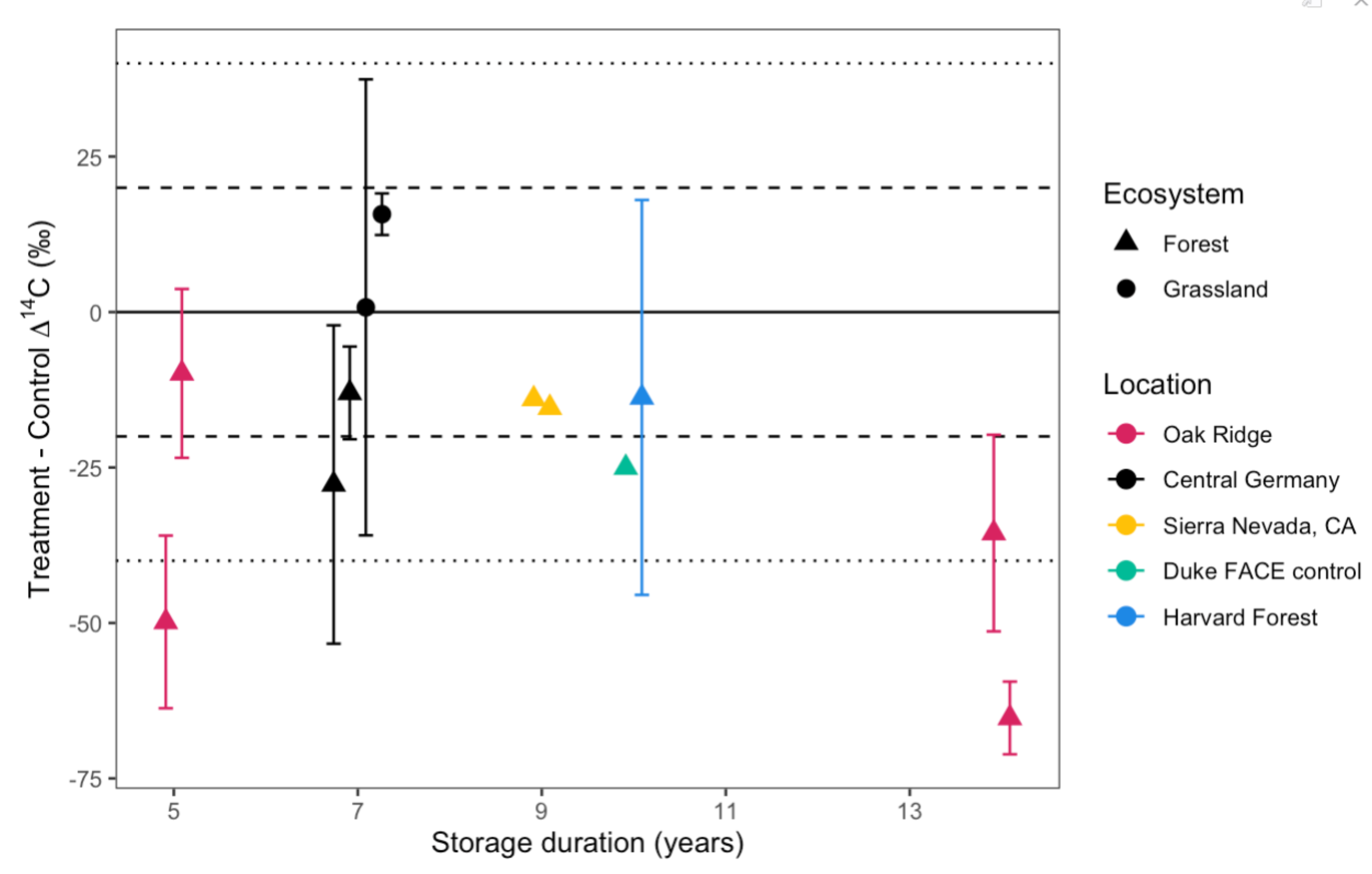
**Figure 3.** Overall treatment effect on Δ14C-CO2

Points show data from all three experiments and are the mean of laboratory replicates (for replicated samples); error bars are standard deviation of replicates. Solid line is 1:1. For context, the dashed and dotted lines show differences of ±20‰ and ±40‰, equivalent to the decline in Δ14C in atmospheric CO2 over 4 and 8 y respectively, during the period of 2000 to 2020 (Graven et al., 2017). The samples from both Central Germany sites (Hainich-Dün and Schorfheide-Chorin) behaved similarly in Experiment one, so samples analyzed in the same experiment are are coded with the same colors in the above figure.

Treatment samples in Experiment 1 and Experiment 2 consistently showed significant differences (alpha = 0.05) in δ13C-CO2 relative to the controls for both forest and grassland soils (Supplementary Fig. 3). 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.

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).

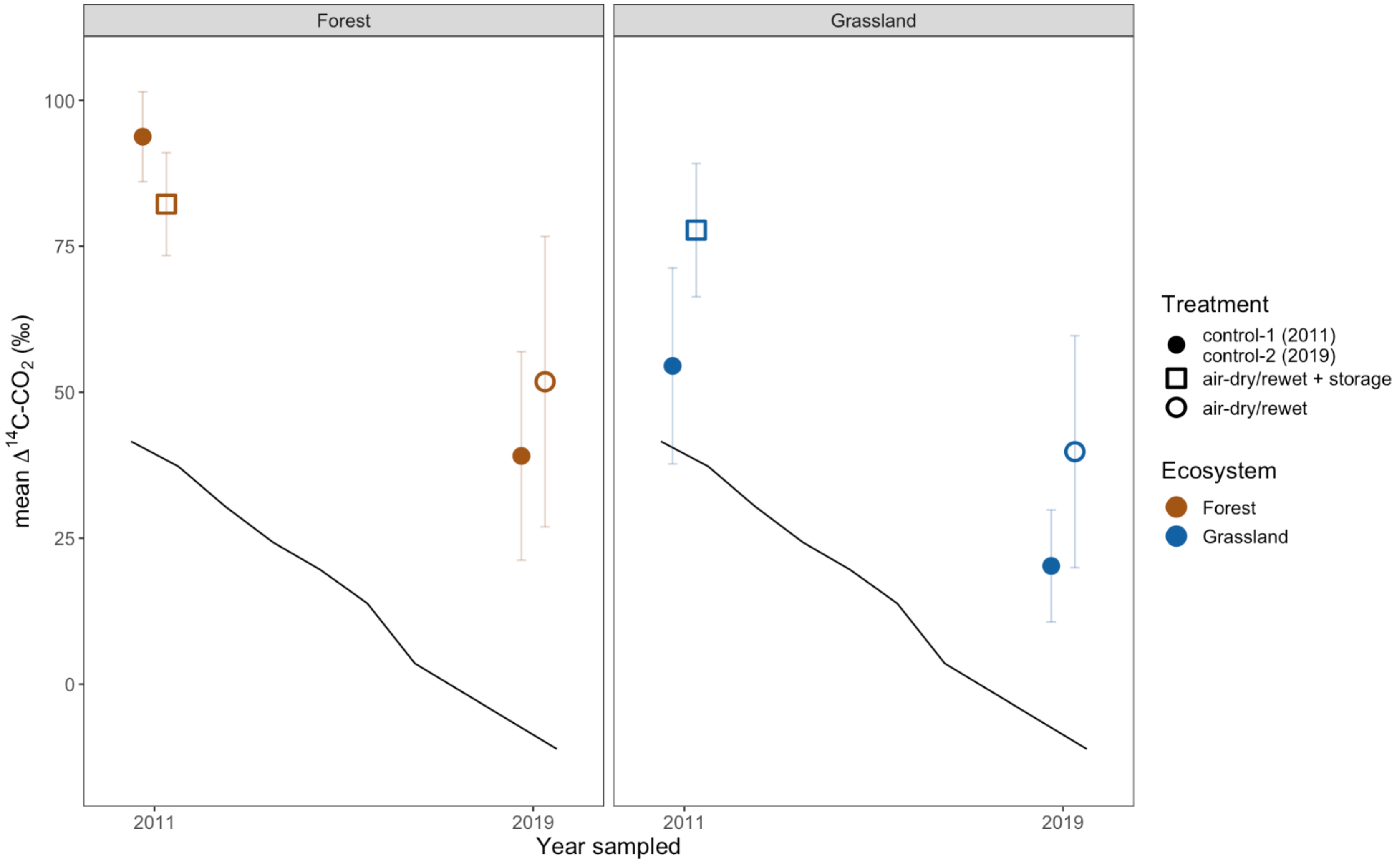


**Figure 4.** Treatment effect on Δ14C-CO2 in relation to storage duration

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‰, equivalent to the decline in Δ14C in atmospheric CO2 over 4 and 8 y respectively, during the period of 2000 to 2020 (Graven et al., 2017).

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

A subset of the sites sampled for Experiment 1 in 2011 were resampled for Experiment 2 in 2019, allowing us to assess both the change in ∆14C-CO2 of respiration over time in control-1 versus control-2 samples, as well as the potential impact of air-drying and rewetting on any observed changes. The absolute value of the mean difference in ∆14C-CO2 between control and treatment samples was greater in grassland samples than in forest samples for both Experiment 1 and Experiment 2 (Table 3). We observed enrichment in grassland soil ∆14C-CO2 in response to the air-drying/rewetting + storage treatment in Experiment 1 and the air-drying/rewetting treatment in Experiment 2. In contrast, the forest soils collected in 2011 exhibited depletion in ∆14C-CO2 following treatment in Experiment 1, but enrichement in Experiment 2.



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

Points show mean Δ14C-CO2 within ecosystem and treatment groups; error bars show the pooled standard deviation. Atmospheric ∆14C-CO2 data (black line) are from Graven et al. (2017) up to the year 2015, while data points from 2015 to 2019 are extrapolated (Sierra, 2018). All atmospheric radiocarbon data are for the northern hemisphere (zone 2).

Mean ∆Δ14C-CO2 was positive, i.e. sample Δ14C-CO2 was enriched relative to the atmosphere, across ecosystem types for both treatment and control samples collected in 2011 (Experiment 1) and in 2019 (Experiment 2) (Fig. 5, Table 3). However, we observed that grassland soil ∆Δ14C-CO2 smaller than forest soil ∆Δ14C-CO2 at both time points (Table 3). Comparing ∆∆14C-CO2 between control and treatment samples within ecosystem types, we observed lower values in grassland control samples than in grassland treatment samples at both time points. We also observed lower ∆∆14C-CO2 values in forest control samples than in forest treament samples for the soils collected in 2019, but for the forest soils collected in 2011 treatment sample ∆∆14C-CO2 was lower than in the control samples. Between 2011 and 2019 ∆∆14C-CO2 increased in both control and treatment grassland samples and in forest treatment soils, while ∆∆14C-CO2 for forest control soils remained nearly constant over this time period.

**4. Discussion**

4.1. Implications for constraining soil carbon models

The results from all three experiments in this study show that measuring ∆14C-CO2 in incubations of air-dried and archived soils is a promising technique for constructing time series of respired ∆14C-CO2 and constraining soil carbon models. 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 per mille, if we exclude the samples from the Oak Ridge labeling experiment (Table 3). However, 95% confidence intervals around the mean differences between control and treatment ∆14C- CO2 did not include zero for any of the three experiments (Table 3), suggesting that the process of drying and rewetting does lead to utilization of substrates with distinct ∆14C signatures.

Atmospheric ∆14C has declined at about 5 per mille per year over the past two decades (Fig. 6) (Graven et al., 2017), meaning that the differences we observed following the air-drying and rewetting treatments are equivalent to the difference expected between photosynthate fixed between two and five years apart. Soil is an open reservoir: recently fixed carbon entering the soil is mixed with carbon in soil organic matter that has persisted in the soil for many years. This makes identification of the different substrates fueling respiration in control versus treatment incubations difficult, and is beyond the scope of this study. However, analysis of the relative magnitude of the treatment effect on forest versus grassland soils, ∆∆14C-CO2 (the difference between the radiocarbon signature of respired CO2 and that of the atmosphere in the year of sample collection), and whether treatment led to enrichment or depletion of ∆14C-CO2 can be used to infer qualitative differences in the mean age of the respired carbon among treatments and ecosystem types.

4.2 Interpretation of treatment effects on the age of respired CO2

Data from samples collected at the same location (Hainich-Dün) at two different time points (for Experiment 1 in 2011 and Experiment 2 in 2019) illustrate both the potential of the archive

**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. \*\*\* Calculated using pooled variance (Methods).

incubation approach and the challenges inherent in interpreting radiocarbon data. Consider that we observed enrichment following 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). However, we do not believe that the difference in trend between forest control and treatment samples in Experiment 1 and Experiment 2 is due to the fact that the treatment in Experiment 1 included the effect of storage, while the treatment in Experiment 2 looked at the effect of air-drying and rewetting alone. If the difference in trend were due to storage, this would indicate that storage affects forest and grassland samples differently. While this is possible, it is the simplest explanation.

We developed a simple hypothetical model to illustrate a potential scenario that we believe provides a simpler explanation for the reversal in the treatment effect trend. Modeling soil radiocarbon with a single pool for a given soil layer has been found repeatedly to be inadequate for explaining soil carbon dynamics (Baisden et al., 2013; Gaudinski et al., 2000; Schrumpf & Kaiser, 2015). In most cases a slightly more complex system with two pools and no transfer of carbon between the two pools (a two-pool parallel model) is the simplest applicable model for fitting ∆14C-CO2 data observed over time (Sierra et al., 2014).

We implemented a two-pool parallel model structure and parameterized the carbon stocks and pool sizes using density fraction data collected from forest sites in the Hainich-Dün region by Schrumpf et al. (2013) for a previous study. Using single-pool steady-state models fit separately to each density fraction, Schrumpf et al. (2015) estimated a mean decomposition rate of between 3 and 5 years for the free light fraction, 90 years for the occluded light fraction, and 115 years for the heavy fraction of the 0-5 cm depth layer. We increased the intrinsic decomposition rate (*k*) of the fast cycling pool in our two-pool model system to 1/6 yr-1 to account for the increased contribution of fine roots relative to leaf litter in the 0-10 cm depth layer, and set the decomposition rate for the slow cycling pool to 100 years to account for the contribution of both occluded and heavy fraction carbon (Fig. 6).

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

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

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

Another line of evidence suggesting that the reversal of the air-dry/rewet + storage treatment effect between 2011 and 2019 in the Central German forest soils observed in this study (Fig. 5 and Table 3) is not due to the effect of storage comes from the results of Experiment 3. 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. below that of a fast cycling pool only slightly lagged from the atmospheric ∆14C curve. Additionally, the forest soils in Experiment 3 also showed the same pattern of depletion following treatment. This result is also in line with increased contribution to respiration from a slowly cycling soil carbon pool with ∆14C

We suggest that the increased slow pool contribution scenario depicted in Fig. 6b provides an alternative explanation for the reversal, and explains the effect of air-drying and rewetting on the ∆14C of respired CO2 in incubations of air-dried soils. For this scenario to apply in the context of this study, the mean relative ∆14C enrichment of the fast and slow cycling carbon pools at the Hainich-Dün forest sites must have switched between 2011 and 2019 as they did between 1991 and 2019 in the conceptual model (i.e. the crossing of the slow and fast model pool curves, Fig. 6). Given that we paratmeterized the model using data collected from this site, this seems plausible.

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

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

A key difference in carbon cycling between forest and grassland ecosystems is the potential for carbon storage in woody tissues after it is fixed from the atmosphere (Gaudinski et al., 2000). Carbon entering the soil in forest ecosystems may be “pre-aged” compared to inputs in grassland ecosystems. Earlier work in some of the same 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). Additionally, a greater lag in forest systems would also mean that the crossing point for the ∆14C curves of the slow and fast cycling soil carbon pools (e.g. the blue and magenta lines in Fig. 6), would be later in time relative to grassland soils. Again, if the fast and slow soil carbon pool curves crossed between 2011 and 2019 for the forest soils, but had already crossed by 2011 in the grassland soils, this would explain the differences in the treatment responses observed in this study between the two ecosystem types. This could be tested by comparing the ∆14C of empirically defined “fast” and “slow” carbon pools over this time period relative to observed ∆14C-CO2, e.g. free light particulate organic matter and mineral associated organic matter. However, this is beyond the scope of this study.

4.4. ∆14C-CO2 of the rewetting pulse versus the second enclosure period

We hypothesized that the CO2 released immediately following moisture adjustment, i.e. the rewetting pulse, would be older than the CO2 released during the second enclosure period. However, in contrast to our expectations, we did not find a significant difference in ∆14C-CO2 between these two respiration periods. This finding was true for all of the samples in which we measured ∆14C-CO2 in both the 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, and may persist throughout the incubation.

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 soley 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 that likely diverge in chemistry 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 percent 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. 3) could be due to many possible scenarios, not just microbial recycling. Microbial recycling has been shown to lead to enrichment in δ13C (Wynn et al., 2005), and has also been shown to be enhanced following air-drying and rewetting (Brödlin et al., 2019; Slessarev et al., 2020). Another scenario is that the fuel for the rewetting pulse is microbial in origin, but derived from microbes that have been dormant for decades. Such a scenario would explain the concommitant shift in ∆14C-CO2, but to our knowledge such a response has not been previously documented. While we cannot rule out this interpretation, such an argument is complicated by the fact that older soil organic matter also tends to be enriched in δ13C due to the ongoing process of decomposition, and thus mobilization of older, extracellular C would be in line with the observed shift in δ13C-CO2 seen in both the air-dry/rewet and the air-dry/rewet + storage samples (Wynn et al., 2005). 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), a combination of observations that suggest 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. 3) suggests that a similar mechanism is at work in both ecosystems.

4.6. Quantifying the treatment effect on ∆14C-CO2

Nearly all of the forest soils analyzed in this study 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 artificial means. This localized 14C plume was released from a nearby incinerator four years prior to sample collection, and resulted in 14C enrichment of both surface litter and root inputs that persisted until the time of sample collection (Cisneros-Dozal et al., 2006). These samples were included precisely because the highly enriched label was concentrated in the most recently fixed carbon, and therefore should be a sensitive indicator of mobilization of older (> 4 y) versus younger soil carbon.

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 past two decades as compared to the labeled carbon introduced to the soil in the past four years than the difference between ∆14C of the atmosphere two decades previously and the ∆14C of atmospheric C fixed in the past decade for the non-labeled forest soils. This could indicate that C mobilized by air-drying and rewetting comes primarily from CO2 fixed from the atmosphere one to two decades previously in forest soils. Alternatively, the greater difference observed in the Oak Ridge samples may suggest 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, this seems unlikely.

In contrast to the forest sites, almost all of the soils from grassland sites exhibited enrichment in ∆14C-CO2 following air-drying + storage (Fig. 3). However, our interpretation is that these data are consistent with the same general mechanism as in forests: air-drying and rewetting of grassland soils increases the contribution of more slowly cycling carbon to respiration. There are two compelling explanations for why this increased contribution from the slow pool leads to enrichment in grasslands in our dataset, rather than depletion as in the majority of the forest soils we analyzed. First, the oldest grassland samples analyzed were not collected until 2011, on average several years later than the forest soils. Second, as discussed previously, the crossing point of the fastest cycling soil carbon pool and the more slowly cycling pools should occur earlier in grassland soils due to a smaller vegetative lag effect. The grassland soils that were observed in both 2011 (Experiment 1) and 2019 (Experiment 2) showed enrichment following air-drying and rewetting (Fig. 5), suggesting that the crossing point of the slow and fast cycling carbon curves occurred prior to 2011 in these soils.

While we cannot say with certainty what mechanism is driving the response observed, the mobilization of a larger pool of soil C following rewetting of grassland soils is supported by the significantly greater increase in respiration rates that we observed in grassland soils as compared to forest soils. However, it is clear that overall treatment differences are not driven solely by the amount of CO2 released, but rather by changes in the carbon pools fueling respiration. This general response holds across both forest and grasslands soils, and is supported by 1) we did not observe a significant difference between the rewetting pulse ∆14C-CO2 and ∆14C-CO2 release in the second enclosure period within incubations, 2) nor did we observe a significant relationship between the amount of CO2 released and the difference observed between control and treatment ∆14C-CO2.

**5. Conclusion**

Air-drying and rewetting of soils leads to significant differences in the ∆14C of respired CO2 in laboratory incubations when compared to incubations of the same soils without air-drying. The magnitude of these differences do not appear to be affected by the duration of storage and are within 25‰ for the majority of forest soils and 40‰ for the more limited number of grassland samples studied. Mean differences between control and treatment incubations for Experiment 1 and Experiment 2 samples, for which the samples were analyzed in the same laboratory, were only 12.1‰ and 20.4‰, for forest and grasslands respectively. Our results suggest that when analyzing ∆14C-CO2 of recently collected soils along with those from an archive, both samples should undergo the same air-drying and rewetting procedure to minimze bias and make the results more comparable.

Determining the exact mechanism driving the differences in ∆14C-CO2 is beyond the scope of this study, but our results suggest that the pulse of 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 between two and three 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. The shift in ∆14C-CO2 is greater for grassland than for forest soils, indicating that the pool of carbon that is mobilized by this process is either more susceptible or larger in grassland ecosystems than in forests. However, we advise caution in extending these results to soils from very different climates or with very different mineral assemblages, as such soils may respond differently to air-drying and rewetting. 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, provided that the minor bias introduced by air-drying and rewetting is accounted for, and can provide a strong constraint for soil C models in the future.

**Acknowledgments, Samples, and Data**

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

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