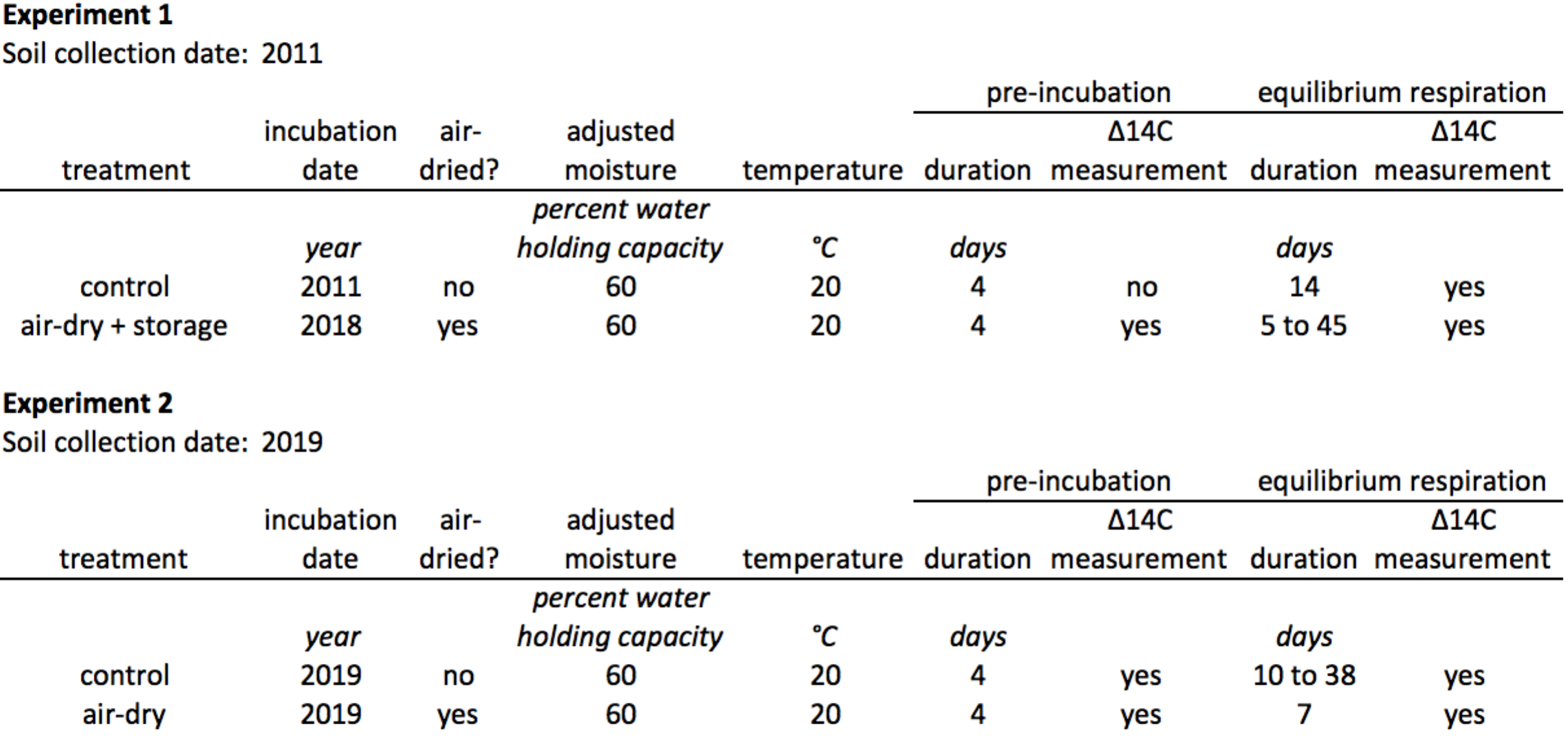
Drying and rewetting effects and the effect of storage on the radiocarbon signature of heterotrophic respiration in laboratory soil incubations

1. **Introduction**
   * The spike and subsequent decline in atmospheric radiocarbon due to nuclear weapons testing in the mid-20th century enables the detection of decadally cycling carbon in soils. 14C enrichment of soil organic matter from “bomb-C” pulse indicates the presence of recently fixed carbon, while 14C depletion, due to the natural process of radioactive decay, is indicative of carbon that has persisted in the soil on centennial to millennial scales.
   * Natural abundance radiocarbon incubations can provide a powerful soil C model constraint, as they provide an integrated signal of the organic matter actively respired by soil microbes.
   * More rapidly cycling pools of soil organic matter dominate the flux of carbon exiting the soil system via heterotrophic respiration, which can be inferred from the observation that respiration is almost always more enriched in 14C than bulk soil.
   * [something about transit time, multiple pools]
   * However, the decline in atmospheric 14C following the mid-20th century peak and the mixing of carbon from soil organic matter pools with different cycling rates (and therefore different mean 14C signatures) complicates the interpretation of both bulk soil 14C and 14C-CO2. Even for a single-pool system, observations of the soil system at a single point in time typically yield multiple solutions for the mean age of carbon: a younger age if the carbon is mostly derived from the right side of the bomb peak, or an older age if it is from the left side. This problem is compounded in a complex, multiple pool system.
   * A simple method for reducing uncertainty when modeling mean soil carbon age is to make radiocarbon observations of the soil system at multiple time points: tracking the increase or decrease in 14C-CO2 relative to the atmosphere indicates the relative contribution of decadal versus more slowly cycling soil organic matter to respiration, providing key insight into soil carbon dynamics.
   * Soil archives have the potential to provide these additional time points, with the added benefit of greater 14C enrichment from the decades closer to the bomb peak increasing the difference in 14C between the youngest and oldest carbon.
   * Standard soil archiving procedure is to air-dry at temperatures <60˚C, followed by storage in an air-tight containers with temperature maintained at <20˚C. During storage soils should be protected from light and fluctuations in temperature should be avoided. Soil archives have proved to be a valuable resource for looking at the change in soil carbon over time, with the most extreme example being the >150 year archives from the Rothamsted long-term experiments, used for parameterizing the well-known soil carbon model Roth-C. However, the effects of air-drying, storage, and subsequent rewetting on 14C-CO2 observed in soil incubations have not been documented.
   * Following air-drying and rewetting, most soils exhibit a characteristic rapid increase in CO2 production, before returning to equilibrium respiration rates. The mechanism or mechanisms driving this pulse of CO2 have been extensively studied over the past several decades. The source of the CO2 released in the rewetting pulse has been hypothesized to come from the lysis of microbial cells subjected to osmotic shock (Williams and Xia, 2009; Warren et al 2014), disruption of soil aggregates, osmolytes released from microbes emerging from aridity induced dormancy (Fierer and Schimel, 2002/2003), desorption of mineral-associated organic matter, or a combination of these sources.
   * [cf, Jones et al., 2019: CO2 release as a function of storage duration; only a shift in the timing of rewetting pulse]
   * Air-drying has been shown to result in the formation of new or stronger mineral-organic associations, increased aggregate stability, decreased microbial biomass, and a higher quantity of water-extractable organic matter. Air-drying and rewetting effects appear to be soil-specific, with desorption of minC upon rewetting observed for smectite-rich or highly charged soils, and differences in the quantity and rate of CO2 release following rewetting varying with soil texture and degree of aggregation (Kaiser et al., 2014).
   * During short-term incubations, the majority of CO2 is assumed to derive from the substrates consumed by the microbial community *in situ*. In longer duration incubations, the lack of new inputs to the system is assumed to lead to shifts in substrate utilization, from easily accessible, shorter-cycling pools to less accessible pools, i.e. protected from decomposition in some manner (Schädel et al. 2020). If the relative contribution to respiration from soil organic matter pools with different intrinsic cycling rates changes in a short-term incubation following air-drying and rewetting, this should be detectable in the 14C-CO2.
   * For example, disruption of soil aggregates following drying and rewetting would likely lead to greater availability of soil organic matter formerly protected from decomposition via physical occlusion. The effect on 14C-CO2 would be to increase the contribution to respiration from this relatively slower soil organic matter pool. 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.
   * Hypotheses: 1) air-drying and rewetting will lead to transient mobilization of a small pool of slower cycling carbon, shifting the 14C-CO2 of the CO2 pulse released immediately following rewetting; 2) 14C-CO2 released during the equilibrium respiration period will not be significantly different from control samples rewet from field-moist conditions; 3) storage will not affect 14C-CO2 of equilibrium period respiration.
2. **Methods**
   * We devised two experiments to assess the feasibility of measuring 14C-CO2 in incubations of archived soils (Table 1).

**Table 1.** Experimental design



* + In the first experiment we looked at the effect on 14C-CO2 from air-drying and rewetting in combination with storage (treatment: air-dry + storage). The control samples for this experiment were collected and incubated in 2011. 14C-CO2 from 2011 incubations of the control samples were then compared to a second set of incubations performed seven years later (in 2018) on splits of the same samples following air-drying and storage. Note that the samples were homogenized and split prior to the initial (2011) incubations.
  + A second experiment was designed to assess the effect of air-drying and rewetting directly, without the potentially confounding effect of storage. For this experiment additional soils were collected in 2019 from a subset of the sites sampled in 2011. After collection, soils were homogenized and split into two subsamples, one of which was air-dried (treatment: air-dry) prior to incubation, the other of which was incubated without air-drying (control).
  + Incubation conditions were the same for both experiments. Soils were first sieved to <2mm at field-moisture, and water holding capacity was determined on a subsample. Soils were weighed out as duplicates into 200 ml beakers and placed into 1L mason jars with airtight lids fitted with two sampling ports. Prior to sealing the jars moisture content was adjusted to 60% of soil water holding capacity, either from field-moist conditions for control samples or from an air-dried state for treatment samples. Jars were then flushed with CO2-free air and left to incubate for a four-day pre-incubation period. Following pre-incubation jars were flushed again, and CO2 was allowed to accumulate for a second period under equilibrium respiration conditions. Note that respiration rates had not yet reached equilibrium levels for the majority of samples by the end of the pre-incubation period, but as a four-day pre-incubation was used in the initial 2011 incubations for the control samples in experiment 1, we maintained the same duration for the treatment incubations in 2018 and for the air-drying and rewetting experiment conducted in 2019 (experiment 2).
  + Finally, we conducted a third experiment to assess the impact of storage duration on observed 14C-CO2. We obtained soil samples from studies conducted in various laboratories over the past two decades covering a range of storage duration from 7 to 14 years (n total sites = 39). Since the control incubations were conducted in different laboratories by different investigators, incubation conditions such as temperature, quantity of soil incubated, and pre-incubation period duration varied. However, the moisture content for the treatment incubations, i.e. those conducted after air-drying and storage, were adjusted to the same level as in the control incubations. When possible treatment incubations were conducted in duplicate or triplicate, but owing to limited quantities of soil, single sample incubations were performed for some sites. As these incubations were not as rigorously controlled as in experiments 1 and 2, we analyzed the data from this third experiment separately. Further details on incubation conditions, headspace gas sampling, and sample provenance are given in Supplementary Table 1.
  + *Headspace gas sampling, experiment 1 (2011 samples)*
  + For control incubations, headspace CO2 concentrations were measured at the end of the pre-incubation period and then on days 1, 3, 7 and 14 during the equilibrium respiration period. For the air-dry + storage treatment incubations, headspace CO2 concentrations were measured daily during the pre-incubation, and on days 3, 5, and 7, then additionally on days 10, 38, and 45 for those samples that had not yet reached target CO2 concentrations.
  + CO2 targets for the air-dry + storage treatment incubations were set to the amount of CO2 respired by the corresponding control sample during the equilibrium respiration period.
  + Headspace samples were collected and analyzed for 14C and 13C content at the end of the equilibrium respiration period for control incubations, but were collected after both the pre-incubation and equilibrium respiration periods for the air-dry + storage treatment incubations. However only nine of the twelve samples reached CO2 concentrations adequate for measuring 14C following the pre-incubation period for the air-dry + storage treatment incubations.
  + *Headspace gas sampling, experiment 2 (2019 samples)*
  + Headspace CO2 concentrations were measured daily during the pre-incubation period for both control incubations and the air-dry treatment incubations. During the equilibrium respiration period for the control incubations, CO2 concentrations were measured on days 3, 5, and 7, and then weekly until CO2 concentrations were adequate for measuring 14C. Similar to experiment 1, CO2 targets for the air-dry treatment incubations were determined by the amount of CO2 respired by the corresponding control samples during the equilibrium respiration period. Due to higher respiration rates, all air-dry treatment samples reached the CO2 targets after seven days of incubation, with CO2 concentrations measured on days 3, 5, and 7.
  + Headspace samples were collected and analyzed for both 14C and 13C content after both the pre-incubation and equilibrium respiration periods for both control and air-dry treatment incubations.
  + *Sample selection and field sampling*
  + Incubations conducted in 2011 were part of a larger study from the Biodiversity Exploratories project. We choose a subset of the samples for the present study to cover two ecosystem types (forest and grassland) and to span a range of soil textural classes, from the relatively sandy soils of the Schorfheide-Chorin geographic region to the more clay-rich soils from the Hainich-Dunn (Table 1 [sites, depths, texture, c/n, 13C, moisture]). We omitted samples that showed the presence of inorganic C during the 2011 incubations using the δ13C signature: any samples with δ13C > -25‰ were considered as potentially affected by the release of inorganic C. We then selected three grassland and three forest samples from the interquartile range of 14C-CO2 observed in 2011 for each geographic region (n total = 12 sites).
  + For the second experiment we returned in July 2019 to the Hainich-Dunn region to collect new samples from the same sites that were originally sampled in 2011. As we did not observe significant treatment differences between the two geographic regions in the results of experiment 1, we restricted the resampling to just one region to save on cost and time. As in 2011, for each plot three 10cm cores (0-10 cm depth) were collected and homogenized to yield one composite sample per plot. Any aboveground vegetation was clipped, and organic horizons were scraped away prior to coring at the forest sites.
  + *Additional measurements*
  + We measured both organic and inorganic carbon content of all soils, as well as total nitrogen content and particle size distribution. We also measured the delta 13C signature of CO2 released during the incubation for each time point when 14C was measured. Radiocarbon analyses were conducted at the Max Planck Institute for Biogeochemistry accelerator mass spectrometer facility. All radiocarbon measurements are reported with respect to the international standards (Stuiver and Pollack 1977; Steinhoff...?).
  + *Statistics*
  + We determined the statistical significance of treatment effects using paired t-tests (alpha = 0.05). In order to identify potential influences on the observed treatment effects we performed a linear regression analysis using the difference between control and treatment CO2 fluxes and observed 14C-CO2 as the response variable

1. **Results**

*Respiration rates: Experiment 1 (air-dry + storage treatment, 2011 samples)*

* + Respiration rates increased dramatically following rewetting for the air-dry + storage treatment, similar to what has been observed in other air-dry/rewetting studies [cite]. However, the magnitude and trend of the respiration rates were significantly different between grassland and forest sites [statistics; other forest/grassland studies for comparison?].
  + For the grassland sites, respiration was four times greater than in forest sites, reaching a maximum of 28 mg CO2 gC-1 d-1 after 36 h, followed by a sharp decline. Mean respiration rates in forest sites peaked at 7 mgCO2 gC-1 d-1 after 96 h, followed by a much more gradual decline than in grassland sites.
  + Control samples responded more weakly and more gradually to rewetting, although as in the treatment samples respiration was greater in grassland soils than in forest soils. Peak respiration rates for control incubations were 11 mg CO2 gC-1 d-1 and 3 mg CO2 gC-1 d-1 after 114 h for grasslands and forests, respectively [Fig. resp rates, A].

*Respiration rates: Experiment 2 (air-dry treatment, 2019 samples)*

* + Compared to control samples, respiration rates increased dramatically following rewetting in the air-dry treatment samples, just as the air-dry + storage treatment samples in experiment 1. [add statistics comparing exp 1 w/ exp 2]. However, unlike the air-dry + storage treatment, forest and grassland soils responded similarly to the air-dry treatment in experiment 2 and total CO2 release during the pre-incubation period was not significantly different between forests and grasslands (p = 0.56) [Fig. resp rates, B].

*Radiocarbon results: pre-incubation vs. equilibrium respiration (experiment 1 and experiment 2)*

* + Despite the significant differences in respiration, and in contrast to our hypothesis, we did not observe significant differences between 14C-CO2 respired during the pre-incubation period and 14C-CO2 respired during the equilibrium respiration period: neither for the air-dry + storage treatment (2011 samples) nor for the air-dry treatment alone (2019 samples) [Fig. pre vs. main inc 14C, 1:1].
  + Differences between forest samples were not significantly different than differences between grassland samples (paired t-test mean difference 0.52 ± 5.67, p = 0.86 on 8 degrees of freedom), so forests and grasslands were pooled for statistical analysis. Note that due to lower respiration rates during pre-incubation, only three of the six forest samples in experiment 1 generated enough CO2 to measure radiocarbon. Additionally, it was not possible to compare pre-incubation and equilibrium respiration 14C-CO2 for the control samples in experiment 1 since pre-incubation 14C-CO2 was not measured for these samples in 2011.
  + There was one outlier for which the pre-incubation CO2 was substantially depleted relative to equilibrium period respiration, however even when included in the statistical analysis the overall differences between pre-incubation 14C-CO2 and equilibrium 14C-CO2 were not significant (Supplementary figure 2).

*Radiocarbon results: equilibrium respiration*

* For forest soils, the air-dry + storage treatment (2011 samples, experiment 1) led to significant depletion in equilibrium respiration relative to the controls (statistics), whereas grassland soils showed the opposite trend (statistics).
* *Plotting 14C-CO2 from the 2011 samples against the amount of CO2-C respired per gram of soil C shows that forest soils tend to become more depleted in 14C with increasing C respired, but grassland soils do not [fig. mean 14C vs. C respired].*
* [perhaps this suggests that the “fast” pool in forests is smaller than in grasslands?]
* In contrast to the results from experiment 1, both forest and grassland soils responded to the air-dry treatment (2019 samples) with relative enrichment in 14C-CO2 respired during the equilibrium respiration period relative to control samples, and both forest and grassland samples followed a trend of enrichment in 14C-CO2 with increasing amounts of CO2-C respired per gram of soil C [fig. 14C vs. C respired].

*Radiocarbon results: air-drying vs. air-drying + storage*

* The direction of change in 14C-CO2 in response to either the air-drying or the air-drying + storage treatment is difficult to predict due to uncertainty about the trajectory of 14C-CO2 over time and its position relative to the slower and faster cycling soil organic matter pools that contribute to heterotrophic respiration [conceptual figure 2]. Site-specific soil organic matter dynamics make these responses particularly challenging to interpret. For example, one of the grassland sites analyzed has
* Separating the effects of air-drying from the combined effect of air-drying + storage is challenging due to the non-linearity of 14C trends over time (due to the influence of the bomb curve) as well as site-specific soil organic matter dynamics.
* We sought to compensate for these confounding factors by comparing trends in the differences in 14C-CO2 over time (2011 versus 2019) in control and treatment samples for the subset of sites that were sampled in both years [fig. control/treatment 14C over time].
* On average, 14C-CO2 declined for both control and treatment samples between 2011 and 2019, although at a slower rate than atmospheric 14C. The same general trends in the change of 14C-CO2 over time are seen in the treatment samples as in the control samples.
* both forest and grassland soils responded to the air-dry treatment with relative enrichment in 14C-CO2 respired during the equilibrium respiration period relative