

# Archived Soil Incubations Project

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## Notes

- This workbook is intended to load and prepare the key data for analysis for the archive incubation project.
- in general, this is an updated version of script “./src/arc\_inc\_master.R”
- all code chunk options are set to “echo = FALSE”; see raw .Rmd file for data wrangling code.

## Introduction

The laboratory soil incubation is a commonly used technique for understanding soil carbon dynamics. Soil carbon is a heterogeneous mixture of organic matter, some of which persists in the soil for months or years, some for centuries or millenia. The persistence of soil carbon can be understood through the concept of different “pools” of carbon, each defined by the mechanism by which they persist in the soil, and characterized by an age distribution.

Natural abundance radiocarbon provides information about carbon dynamics on the scale of centuries to millenia, while insight into decadal scale dynamics can be gained from tracing the pulse of radiocarbon introduced into the biosphere from nuclear weapons testing in the mid-20<sup>th</sup> century. This “bomb-C” pulse peaked in the atmosphere in the 1950s (Fig. 1), but due to differential rates of abiotic incorporation and biological processing, the delay in the timing of this peak in different pools of soil carbon can be used to infer the relative rate at which carbon enters and leaves these pools, which is functionally equivalent to the intrinsic decomposition rate.

Extracting and measuring the radiocarbon content of specific soil carbon pools is hampered by spatial and temporal heterogeneity of the mechanisms that lead to soil carbon persistence, such as physical occlusion in aggregates, association with minerals, or thermodynamics. Defining soil carbon pools empirically with techniques such as density, size, or resistance to chemical attack can be useful, but these methods also introduce artifacts and likely result in mixtures of pools with different mean ages. In contrast, although they also introduce artifacts due to disturbance and potential alteration of the microbial community, laboratory soil incubations make use of the same fractionation agent as *in situ*: the microbial community. Measuring the radiocarbon signal of CO<sub>2</sub> ( $\Delta^{14}\text{C-CO}_2$ ) released in laboratory soil incubations is a powerful tool for understanding the relative processing rate of carbon in soil (or transit time) as it provides an integrated measure of the weighted contribution to the release flux from pools of soil carbon with distinct processing rates.

Interpreting  $\Delta^{14}\text{C-CO}_2$  from laboratory soil incubations requires the use of a model. However, parameterizing these models is challenging, both due to the uncertainty of the persistence mechanisms themselves as well as a lack of observational constraints. Radiocarbon observations at a single point in time are very useful, but due to the curvature of the bomb-C peak there are two points in time with the same atmospheric radiocarbon value, leading to multiple model solutions. Adding additional observations of  $\Delta^{14}\text{C-CO}_2$  at multiple points in time can greatly reduce model uncertainty.

Soil archives have already proven to be a valuable source of data for constraining soil carbon models by providing time series of the change in soil carbon <sup>14</sup>C content. The promise of improving models further by obtaining <sup>14</sup>C-CO<sub>2</sub> measurements from archived soils is tantalizing, but first the possible effects of air-drying and rewetting, as well as the effect of the duration of storage, must be quantified.

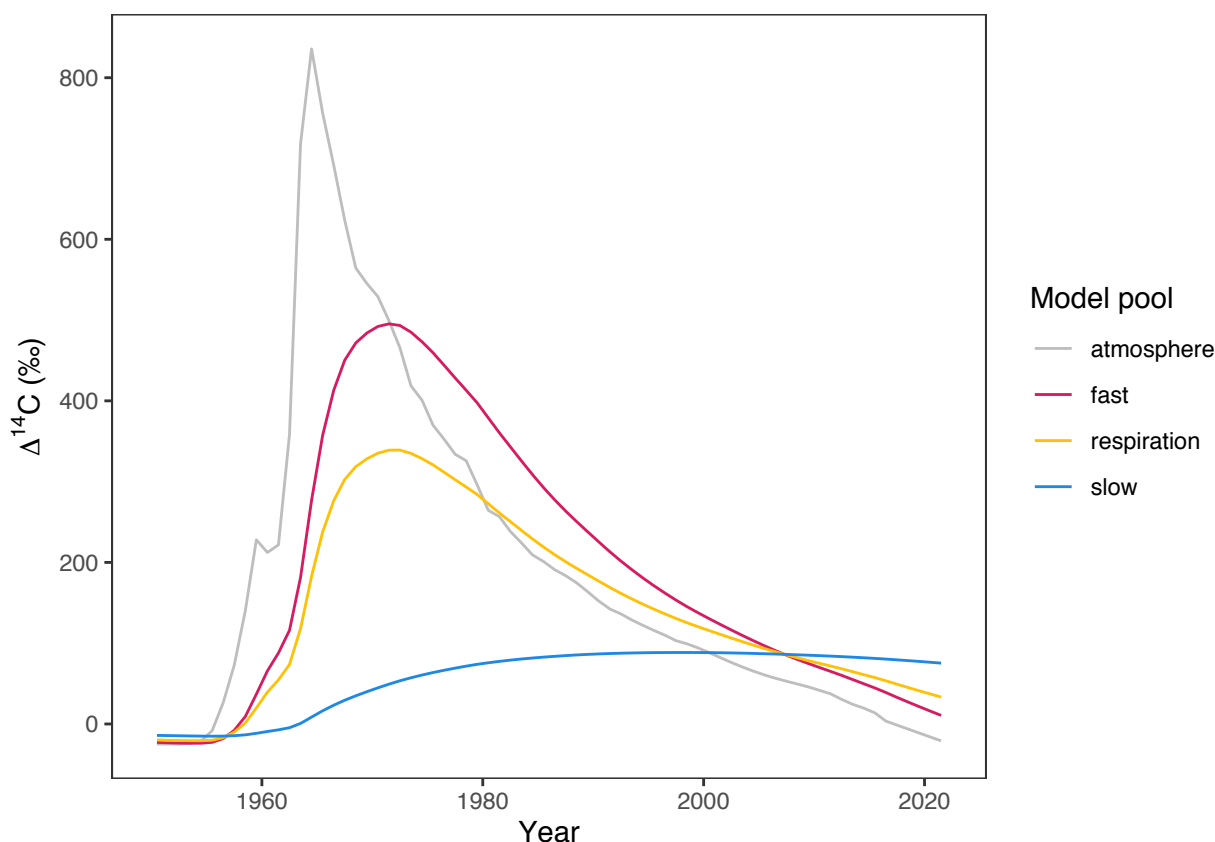
We designed an experiment to assess these effects, preliminary results of which are presented in the following report.

## Conceptual understanding

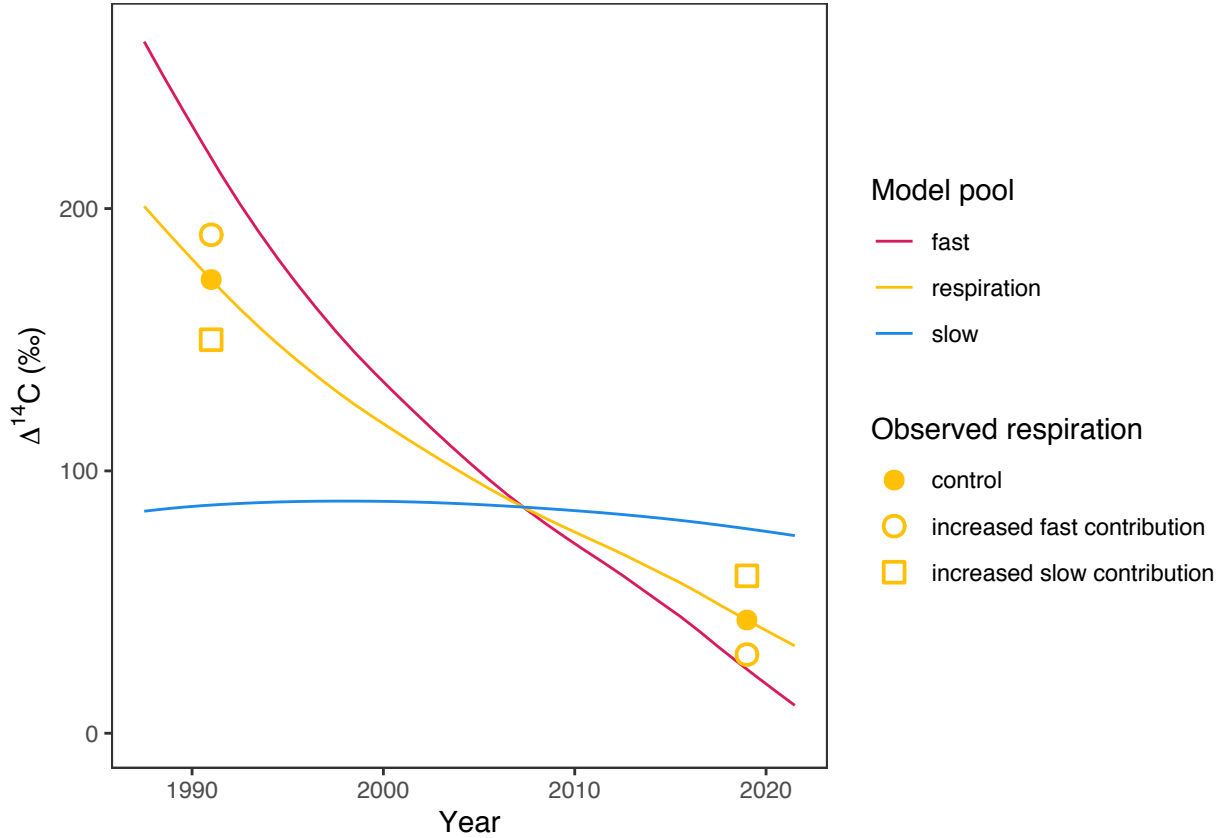
If the null hypothesis is disproved, i.e. air-drying and rewetting (air-dry) or air-drying, rewetting, and storage (air-dry + storage) have a significant effect on  $\Delta^{14}\text{C}$ - $\text{CO}_2$  relative to control samples, there are two possible outcomes: enrichment of the respiration flux or depletion.

In a simple two-pool soil carbon model, the treatment effect can be conceptualized as a relative change in the contribution of “slower” or “faster” cycling carbon to the respiration flux observed in control samples.

Trajectories of the change in  $\Delta^{14}\text{C}$  over time for “slow” and “fast” soil carbon pools are shown below (Fig. 1) in relationship to the  $\Delta^{14}\text{C}$  of the atmosphere and respired  $\text{CO}_2$  for a theoretical model system.



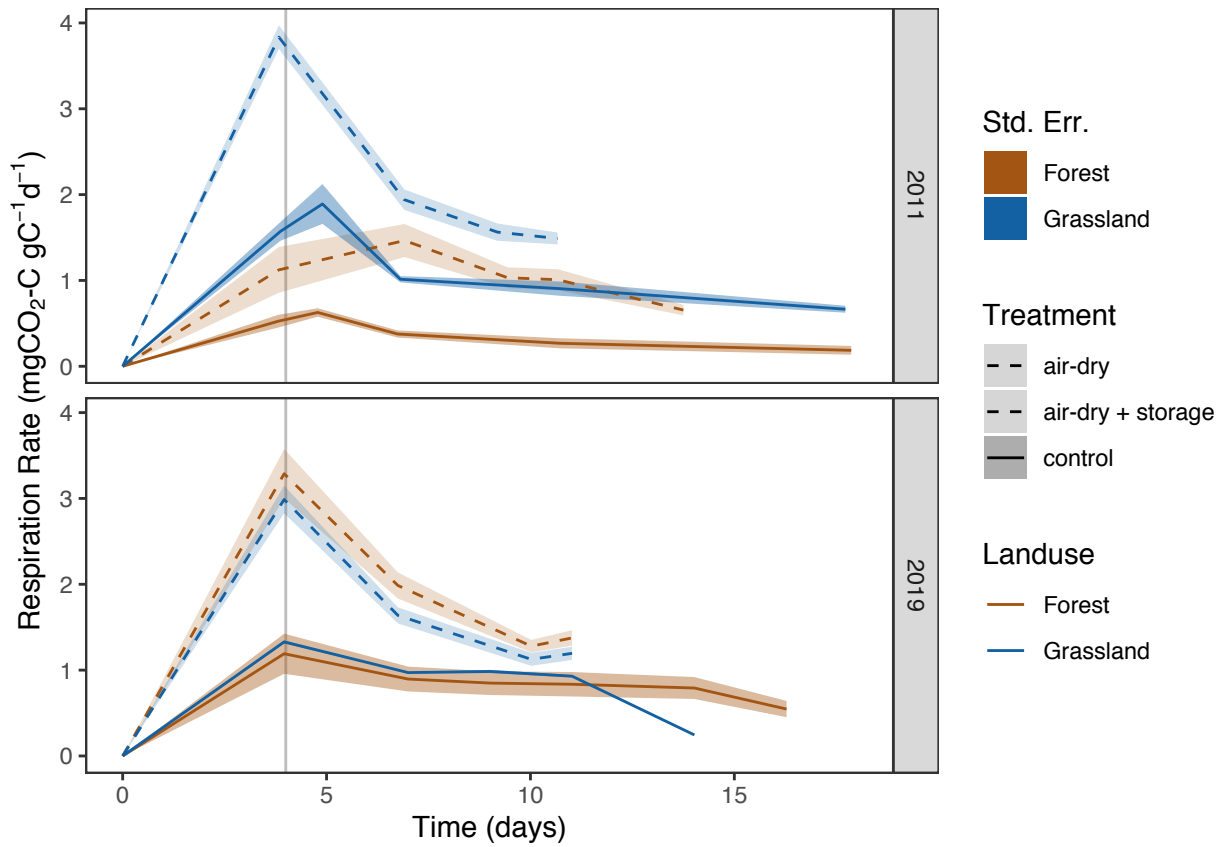
The simplest two-pool model is a parallel system: one in which carbon entering the soil is partitioned between the two pools without any transfers from one pool to the other. However, even in this simple system, the same treatment effect can result in either enrichment or depletion depending on the year the sample was collected. The direction of change will be dependent on system dynamics, specifically the intrinsic decomposition rates of the slow and fast pools, while in more complicated systems the amount of mixing between the pools will play a role as well. Potential treatment effects for the same parallel two-pool model system shown in Fig. 1 are shown below at two points in time (Fig. 2).



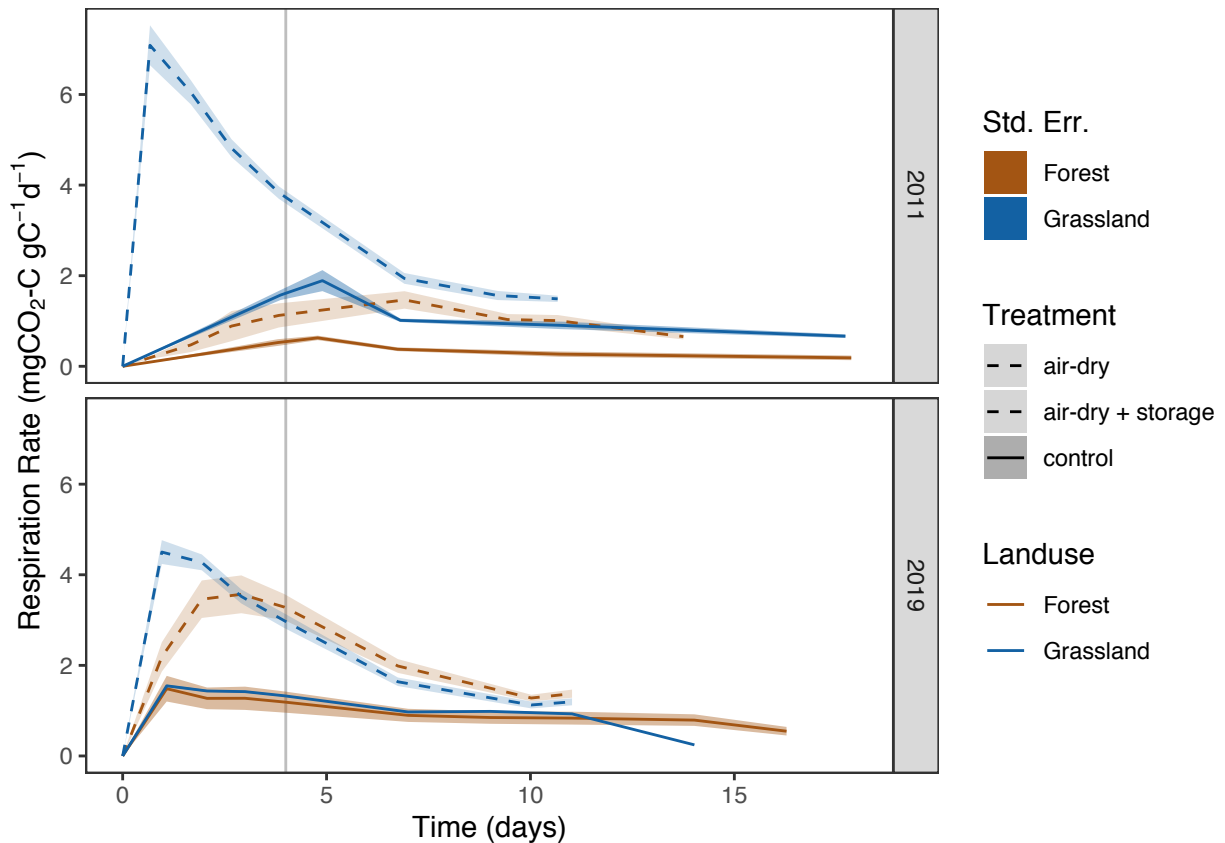
In the above plot (Fig. 2), increased contribution from the slow pool leads to depletion in  $\Delta^{14}\text{C}$  of respiration relative to the control observation in 1991 but relative enrichment in 2019.

## CO<sub>2</sub> fluxes and soil data

1. Load flux data from air-dry + storage (experiment 1) and storage duration (experiment 3) control samples, and convert from “wide” to “long” format so as to match other data.
2. Load flux data from air-dry + storage samples and from air-dry experiment (ctl & treatment), C & N data for all the Exploratories samples (measured in 2011), and soil mass and moisture data for all experiments.
3. Combine and summarize data in long format to calculate respiration rates and plot over time.
4. Plots of CO<sub>2</sub> fluxes over time. The final measurement points for a few samples which took >18 days to reach CO<sub>2</sub> targets are excluded for display reasons. Respiration rates for those samples remained flat.
  - While daily measurements were made during the pre-incubation period for the majority of samples, only the cumulative pre-incubation flux was measured for the 2011 control samples
  - Consequently, the respiration rate for the pre-incubation period was calculated cumulatively for all samples (Fig. 1)



- However, daily measurements made for the remaining samples shows that the initial rewetting pulse was much substantially greater for the treatment samples in both 2011 and 2019 (Fig. 2)



## Isotope data

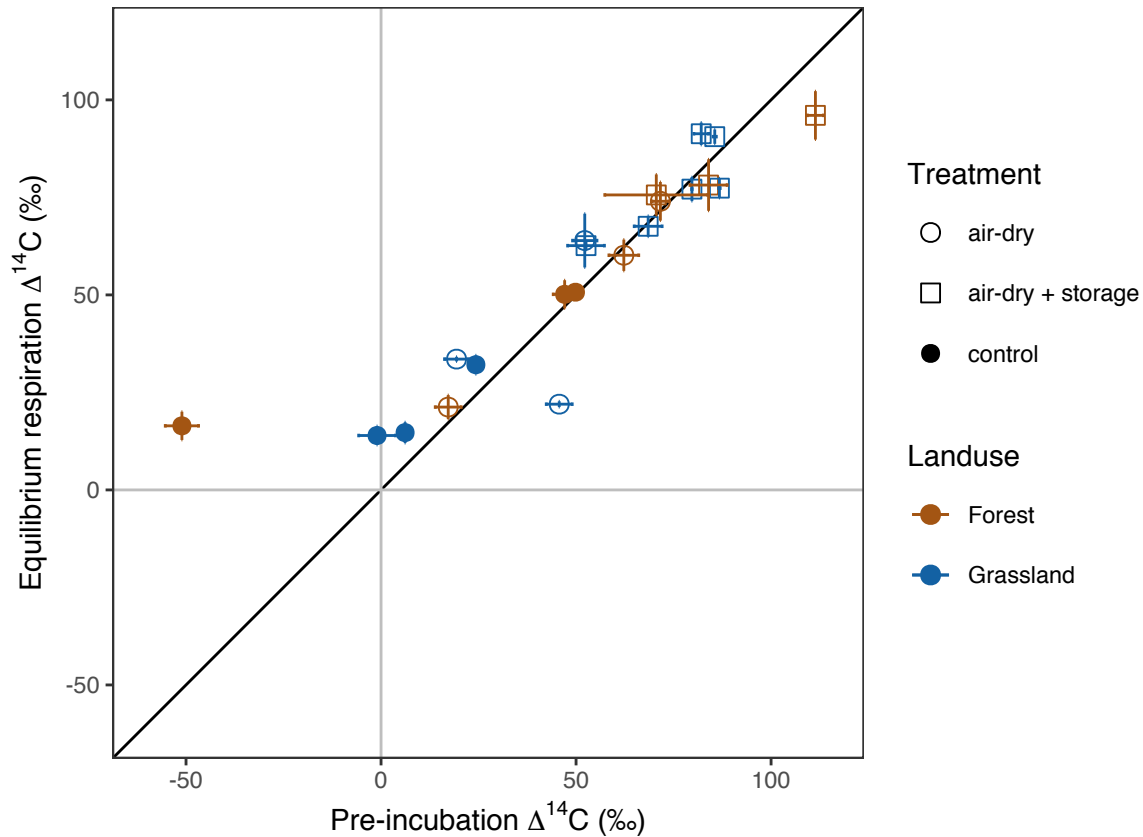
1. Read in isotope data from various sources. First load helper function 'read\_jena\_ams\_results.R'
2. Next read in data from the appropriate directories in 'data/raw'.
3. Create a "tidy" style template for the data, i.e. variables in columns.
  - Key variables are as follows:
    - SampleName (incorporates lab rep and treatment, e.g. "HEG10-1\_dry")
    - ID (plot IDs, e.g. for "HEG10" for Exploratory samples)
    - Treatment (3 treatments: air-dry, air-dry + storage, storage duration; + controls)
    - Type (2 levels: F = forest, G = grassland)
    - Period (incubation period, 2 levels: pre = preincubation, inc = equilibrium incubation)
    - Experiment (3 levels: arc = air-dry + storage, rewet = air-dry/rewet, time = storage duration)
  - Observational columns include:
    - d14c ( $\Delta^{14}\text{C-CO}_2$ )
    - d13c ( $\delta^{13}\text{C-CO}_2$ )
    - C\_g\_kg (C content)
    - dw\_g (dry weight)
    - mgCO2.C\_gS (mg CO<sub>2</sub>-C respired g<sup>-1</sup> soil Period<sup>-1</sup>)
    - time\_d (days in incubation period prior to measurement)
    - dH2O\_grav (percent change in gravimetric water content due to laboratory moisture adjustment)
    - dH2O\_whc (percent change in water holding capacity due to laboratory moisture adjustment)
4. Summarize observational data from timeseries data by unique IDs (SampleName).

5. Create helper functions for decay correction, converting  $\Delta^{14}\text{C}$  to fraction modern, and cleaning up extraneous values in raw  $^{14}\text{C}$  data. Archived sample  $\Delta^{14}\text{C}$  data should to be corrected for decay since the year of collection. (Although the correction is very small and likely insignificant, I will do it anyway).

- decay correction formula is:

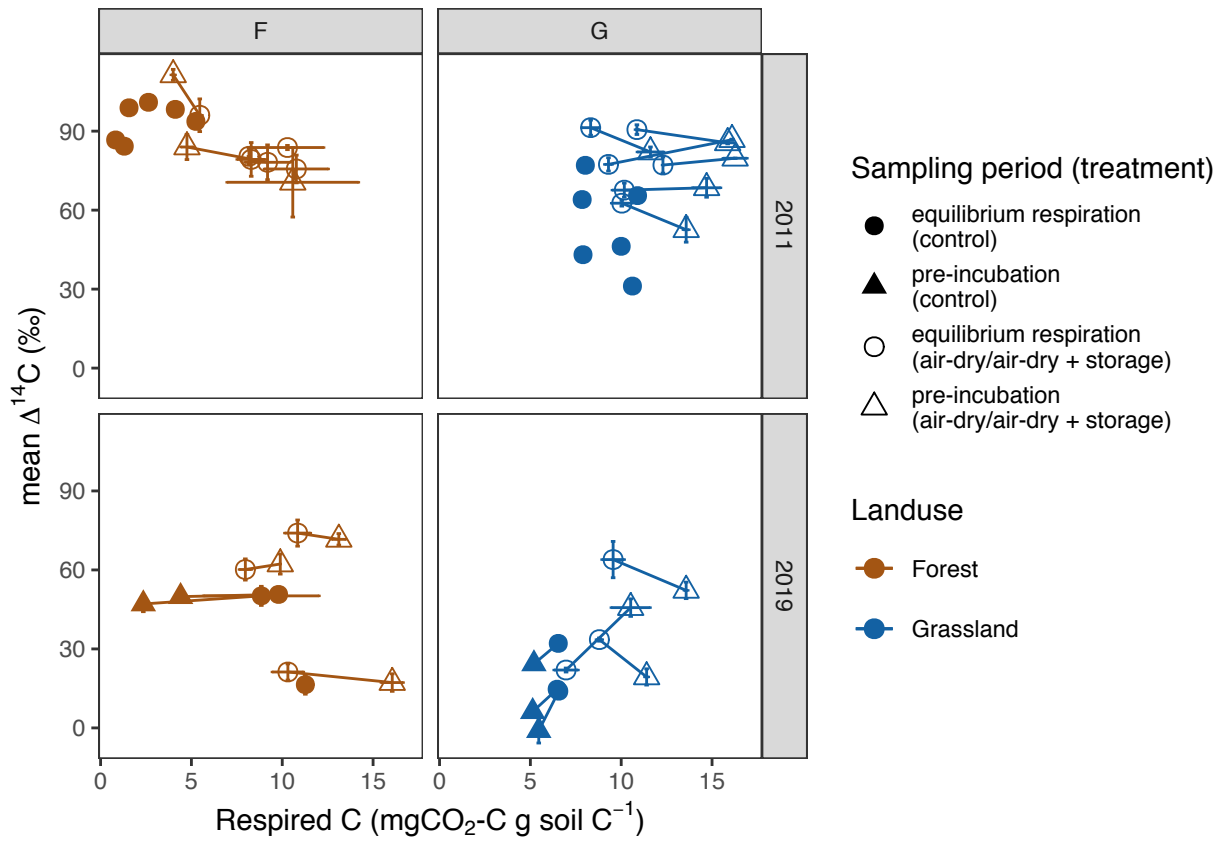
$$1000 \cdot \left( (FM \cdot e^{\frac{-\text{year}_{sampled} + 1950}{8267}}) - 1 \right)$$

6. Clean up  $^{14}\text{C}$  data and add external data points (tme experiment, Xplr control samples)
7. Combine data.
8. Count number of  $^{14}\text{C}$  observations for checking plots.
9. Plot pre-incubation period  $\Delta^{14}\text{C}$  against equilibrium respiration period  $\Delta^{14}\text{C}$ .
  - Points are means of duplicate lab reps and error bars are min and max (except for the 2011 control samples, which were not replicated)
  - Pre-incubation  $\Delta^{14}\text{C}$  was not measured for the 2011 control samples.
  - Relative outlier point is the very negative (mean =  $-51.1\text{‰}$ ) HEW22 pre-incubation control samples from the 2019 air-dry experiment.
  - Samples from three of the forest plots of the 2011 treatment samples failed to accumulate enough  $\text{CO}_2$  to measure  $^{14}\text{C}$ .

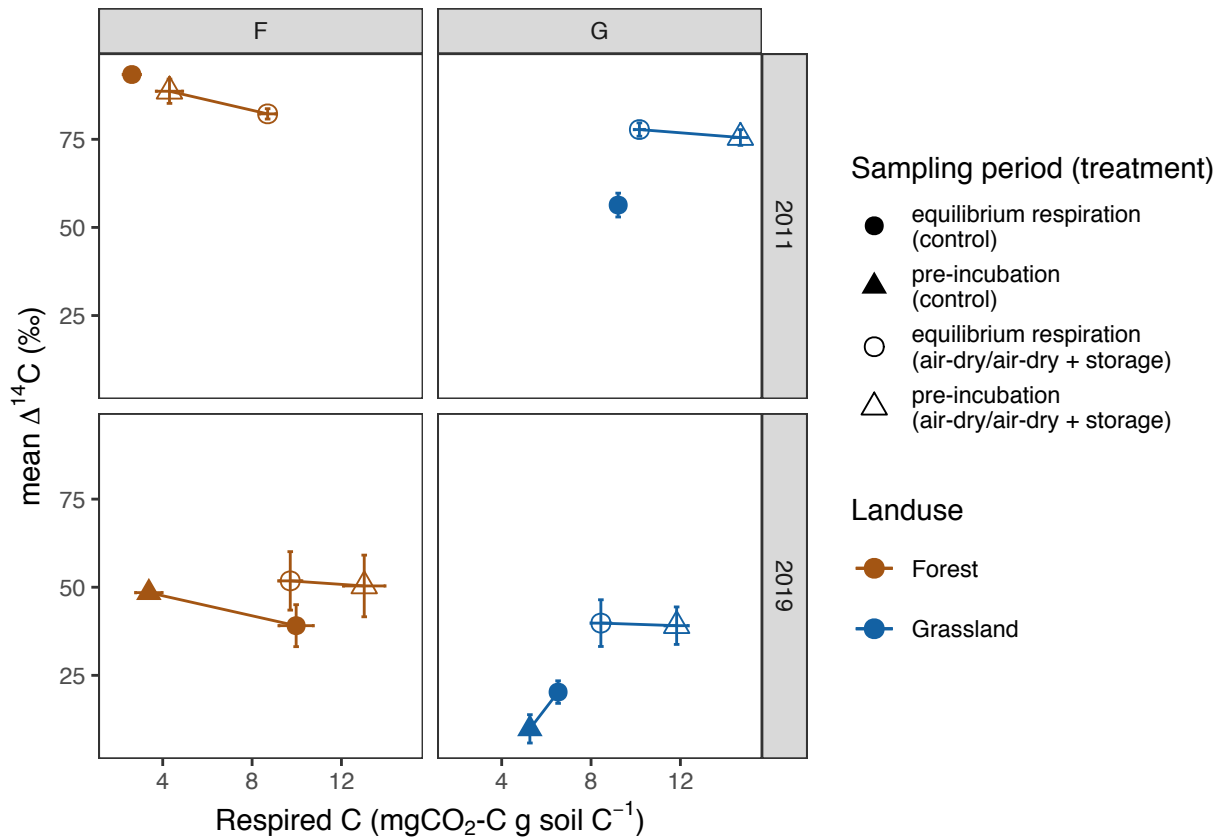


10. Plot  $\Delta^{14}\text{C}$  against proportion of soil C respired by experiment, land cover, and sampling period.

- First figure shows data averaged by plot
- (Note that pre-incubation  $\Delta^{14}\text{C}$  was not measured for the 2011 control samples)
- Limits exclude outlier point (HEW22 control pre-incubation) for improved legibility

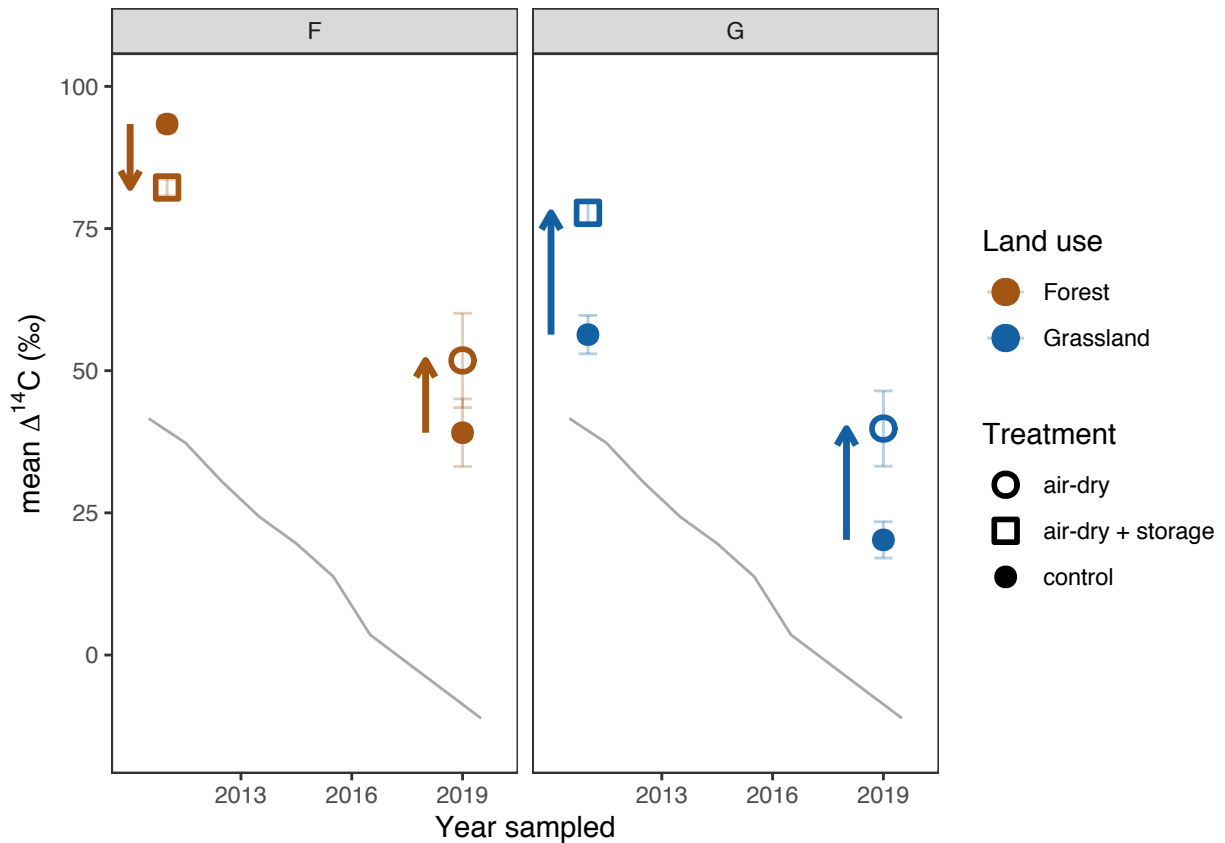


- Second figure shows data averaged by land use and treatment within sampling periods



- Code for a third plot with outlier data is in code chunk below, but not is currently included in report
12. Show treatment effects on observed equilibrium period  $^{14}\text{C}$ .
- Fig. 1 shows the direction of mean treatment effects over time in reference to the atmosphere (gray line). Points are means ( $n = 12$  for 2011 treatment points,  $n = 9$  for 2011 control points;  $n = 6$  for all 2019 points); error bars =  $2 \times$  std. err. of the mean.

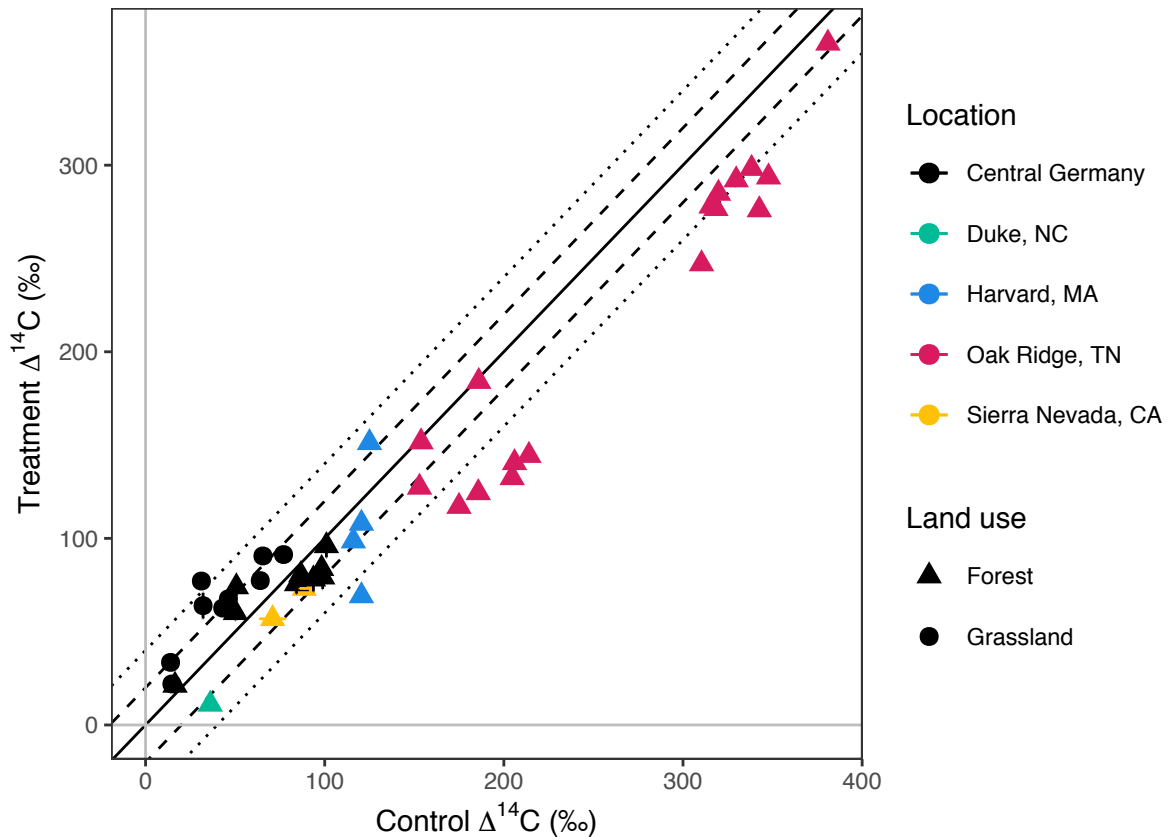




- Code below plots treatment effects as means with inferential error bars (2x SE), but is not included in the report.

15. Show overall effect of treatment on whole data set.

- Notes:
  - Control data shown on x-axis, treatment data shown on y-axis
  - Solid line is 1:1, dashed line is a 20 ‰ offset, and dotted line is a 40 ‰ offset (roughly equivalent to the atmospheric decline over five and 10 years respectively for the period 2000 to 2020)
  - Data from all three experiments conducted in this study are shown, as well as a handful of additional data points for which both control and treatment (i.e. after air-drying + storage) incubations were conducted in another laboratory (Harvard points)
  - Only A horizon data are shown here, as owing to sample availability only three samples were analyzed for organic and B horizons (respectively)
  - Points are means of replicates and error bars are min and max of replicates (number of replicates varies from 1 to 3); note that error bars are not visible at this scale for most points
- Key messages:
  - Difference between control and treatment samples is within a 5 year range for the majority of points
  - Grassland samples tend to be above 1:1 line, forest samples below, regardless of site
  - The three German forest samples above 1:1 line were analyzed in 2019 (air-dry only treatment, this study), lending support to the interpretation that slow and fast soil C pool curves for northern hemisphere forest samples have only recently crossed or may have yet to cross.



16. Show the effect of storage duration by plotting the difference between control and treatment  $^{14}\text{C}$  as a function of storage duration. However, the expected Ctl-Trt difference would also likely have a trend over time. Perhaps plot an expected trend...? Not sure how much this matters.
- As previously, dashed and dotted lines are plotted to give a sense of error introduced by the treatment by showing a difference of 20‰ and 40‰ (roughly equivalent to the atmospheric decline over five and 10 years respectively for the period 2000 to 2020)
  - Position of points jittered to avoid overplotting; storage duration has been rounded down to the nearest whole year
  - In general, the trend in the differences due to treatment for the highly enriched samples from Oak Ridge, TN suggest losses of the most recently fixed carbon over the duration of storage
    - These samples were included primarily because it was assumed that they would be more sensitive to potential losses of recently fixed carbon, as the label should only be present in this pool of soil C
    - However, in contrast, there does not seem to be evidence for a storage duration effect in the samples that only contain bomb-C

