



## Radiocarbon incubations of archived soils: insights into drying/rewetting effects and constraining soil C models

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Radiocarbon measurements of heterotrophically respired C ( $\Delta^{14}\text{C-CO}_2$ ) in laboratory soil incubations provide information about the age and source of microbially-available soil organic matter. However, due to the influence of “bomb” radiocarbon (from nuclear weapons testing in the mid-20<sup>th</sup> century), measurements of  $^{14}\text{C}$  at a single time point can yield multiple solutions when modeling soil C cycling rates. Measuring  $\Delta^{14}\text{C-CO}_2$  on archived soils would provide additional time points to assess which solution is appropriate. We had two hypotheses regarding the effect of archiving on  $\Delta^{14}\text{C-CO}_2$ : 1) long-term storage does not affect  $\Delta^{14}\text{C-CO}_2$ , and 2) drying and rewetting effects on  $\Delta^{14}\text{C-CO}_2$  are limited to  $\text{CO}_2$  released immediately following rewetting, without significant effects on  $\text{CO}_2$  released after respiration rates equilibrate.

To address the first hypothesis, sample splits of soils collected at nine grassland and 21 forest sites ( $n=30$ ) between 2004 and 2011 (for which  $\Delta^{14}\text{C-CO}_2$  had been previously measured) were incubated again in 2018 after undergoing air-drying and storage. The difference in  $\Delta^{14}\text{C-CO}_2$  measured before and after archiving was significant ( $p < 0.05$ ); however, in line with our hypothesis, the number of years archived was not a significant predictor of the difference in a regression analysis.

To test the second hypothesis we first collected and analyzed  $\Delta^{14}\text{C-CO}_2$  following the “pre-incubation” period, i.e. the period immediately following rewetting, as well as after the equilibrium respiration period for the subset of samples (six grassland, six forest) for which we had data on the original pre-incubation period. In this subset we observed different responses in forest versus grassland soils in the equilibrium respiration period:  $\Delta^{14}\text{C-CO}_2$  decreased from the original value by  $12.7 (\pm 4.5)$  per mille in forests ( $p = 0.08$ ), but increased by  $22.2 (\pm 6.7)$  per mille in grasslands ( $p < 0.05$ ) (errors are twice the standard error of the mean difference). In contrast to our second hypothesis the  $\Delta^{14}\text{C}$  of the  $\text{CO}_2$  released immediately following rewetting was not significantly different from the  $\Delta^{14}\text{C}$  of the  $\text{CO}_2$  respired under equilibrium respiration conditions, despite the much higher rate of respiration following rewetting. A final incubation experiment comparing freshly collected soils that were dried but not archived was conducted to distinguish conclusively between rewetting and storage effects, but we are still awaiting the data.

In conclusion, the drying/rewetting effect appears to drive the differences between

$\Delta^{14}\text{C-CO}_2$  measured in incubations before and after archiving, rather than duration of storage. The radiocarbon incubation technique for archived samples is promising: the 12 to 22 per mille differences observed are not insignificant, but in many cases should be within the range of acceptable error in a modeling context. The wider implication of our results is that drying and rewetting soils appears to mobilize a different pool of soil organic matter than would otherwise be available to microbes, an effect that persists throughout an incubation and affects grassland and forest soils differently. This effect applies to radiocarbon incubations in general and warrants further investigation.