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This pre-print manuscript “*Drying/rewetting cycles mobilize old C from deep soils from a California annual grassland*” was subsequently accepted by *Soil biology & biochemistry*.

This version of the manuscript has not been peer-reviewed.

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The published final version of this paper can be found here:  
<https://doi.org/10.1016/j.soilbio.2011.01.008>

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# Drying/rewetting cycles mobilize old C from deep soils from a California annual grassland

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## **Abstract**

We measured the natural abundance of  $^{14}\text{C}$  in  $\text{CO}_2$  respired from surface and deep soils released through multiple dry/rewetting cycles in laboratory incubations. While the C respired from surface soils was 'young', that respired from deep soils came from C pools with an average turnover time of 650–850 years. This reinforces previous research suggesting that a substantial amount of deep soil C is chemically labile but physically inaccessible to microorganisms, but also suggests that substantial amounts of that C may not be so strongly bound to minerals as to be effectively inert, raising the question of why it hasn't already been metabolized.

*Keywords:* soil carbon, deep soil, physical protection, radiocarbon, priming.

## Short Communication

Deep soils (below 20 cm) are the largest reservoir of organic C on earth, containing more organic C than vegetation or the ocean, and more total C than is in the atmosphere (Jobbágy and Jackson, 2000). The nature of that C pool, however, remains something of a mystery, with questions remaining about its chemical nature, turnover time, and the processes that regulate it (Baisden and Parfitt, 2007). Because deep soil C typically has  $^{14}\text{C}$  dates in the thousands of years (Trumbore, 2000), it has been largely assumed that this C is either chemically recalcitrant or so strongly sorbed to mineral surfaces as to be functionally inert (Basiden and Parfitt, 2007). That the  $\text{CO}_2$  found in the soil profile dates roughly modern (Fierer *et al.*, 2005) reinforces that conclusion—the old C deep in the soil is not substantially metabolized *in situ*.

However, several recent studies have challenged the assumption that deep soil C is inert. For example, Fontaine *et al.* (2007) found that by adding labile C, they could stimulate breakdown of deep soil C, and concluded that it was of poor enough quality that microbes could not grow on it without exogenous energy sources. In contrast, Xiang *et al.* (2008) found that in grassland soils from 1 m depth, multiple dry/wet cycles increased microbial total respiration and biomass, up to 6-fold. They concluded that native C could support growth if microbes could just access the OM and that it was not so tightly sorbed as to be unavailable. Such studies emphasize the need to better understand the nature of deep soil C and of its biogeochemical nature.

We wanted to follow up on the results of Xiang *et al.* (2008) and determine how old the C released by repeated dry/wet cycles was. Was this recent material from deep roots or leachates or was it old C that had been preserved in the soil? We therefore repeated the Xiang *et al.* (2008) experiment, but measured the  $^{14}\text{C}$  enrichment of the C respired to estimate its “age” or the turnover time of the soil C pool it came from, comparing surface and deep soils.

The soil is a pachic argixeroll at the Sedgwick Reserve in Central California (34°41'29.4"N; 120°02'42.7"W), and is more fully described in Xiang *et al.* (2008). For surface soils (0-10 cm; 2.2% C), we collected triplicate soil cores and composited them. To collect deep soil (B horizon soil from 1m depth; 1.16% C), we dug a soil pit, collected soil from each side wall, and composited the samples. Soils (100 g and 200 g dry weight

equivalents for the surface and deep soils respectively) were weighed into incubation jars (1l) and run through five wetting/drying cycles. Carbon dioxide was collected during the first and last rewetting pulses. Gas samples were collected in stainless steel gas cans, converted into graphite targets, and analyzed for  $^{14}\text{C}$  enrichment at the W.M Keck Carbon Cycle accelerator mass spectrometry facility at the University of California Irvine (Southon *et al.*, 2004). We report radiocarbon results as fraction Modern—the ratio of  $^{14}\text{C}/^{12}\text{C}$  in the sample to that of a standard representing the  $^{14}\text{C}/^{12}\text{C}$  ratio in preindustrial air. The standard  $^{14}\text{C}/^{12}\text{C}$  ratio is corrected for mass dependent isotope fractionation so all data are reported as if they had a common  $\delta^{13}\text{C}$  signature of  $-25\text{‰}$  (Stuiver & Polach, 1977).

We inferred turnover times of the carbon pool contributing to  $\text{CO}_2$  in two ways. Both assume that carbon is constantly added to and removed (by decomposition) from soil, with decomposition represented by a first-order decay constant ( $k$ ). This carbon pool is assumed to be homogeneous (i.e. all one age) and at steady state. The first method, which can only be used for samples with fraction Modern  $<1$ , calculates the turnover time assuming there has been no bomb  $^{14}\text{C}$  incorporated into the sample. The turnover time ( $1/k$ ) is estimated using a model that accounts for differences in the loss rates of C ( $k$ ) versus radiocarbon ( $k + \lambda$ , where  $\lambda$  is the rate constant for radioactive decay of  $^{14}\text{C}$ ). Because turnover times are relatively short compared to the mean life of radiocarbon, they are close to the values we would calculate assuming all the C respired had been fixed at the same time and isolated from exchange that might add younger carbon.

The second method may be used for samples with fraction Modern greater or less than 1.0. This simple model accounts for the changing natural abundance of  $^{14}\text{C}$  since the start of atmospheric nuclear testing (in the late 1950's) as well as tracking  $^{14}\text{C}$  loss to decomposition and radioactive decay (see Gaudinski *et al.*, 2000; Torn *et al.*, in press). Turnover times ( $1/k$ ) are varied until the observed  $\delta^{14}\text{C}$  signature in the year of measurement (2007) is observed. Turnover times estimated using this method are longer than those from the first method (Table 1) because we assume some of the  $^{14}\text{C}$  in the measured sample comes from bomb  $^{14}\text{C}$ .

Table 1.  $^{14}\text{C}$  natural abundance of C respired during the first and last rewetting cycles in soils from the surface and 1 m depth in a California annual grassland. Values in parentheses are standard deviations.

| Soil        | $^{14}\text{C}$ fraction Modern | Std. Dev n=3 | $\Delta^{14}\text{C}^*$<br>‰ | Turnover time estimated<br>from FM (yr) |
|-------------|---------------------------------|--------------|------------------------------|---|
| First cycle |                                 |              |                              |   |
| Surface     | 1.017                           | 0.0048       | 10.5                         | -                                       |
| 1 m deep    | 0.926                           | 0.0051       | -80.4                        | 660 (50)                                |
| Final cycle |                                 |              |                              |   |
| Surface     | 1.0059                          | 0.0015       | -1.1                         | -                                       |
| 1 m deep    | 0.9738                          | 0.0182       | -56.7                        | 220 (160)                               |

\* measured 2007

The C respired by surface soils had fraction Modern values greater than 1.0, indicating the presence of  $^{14}\text{C}$  produced by atmospheric nuclear weapons testing. In the initial pulse, the average enrichment was +10.5 ‰ decreasing to -1.5 ‰ (older) over the incubation (Table 1). Estimated turnover times increased over the course of the incubation from 280 to 320 years. This decline may result from rapid loss of carbon that was fixed more recently (i.e. had higher  $^{14}\text{C}$  content) than the bulk of the material.

Carbon respired from deep soils was considerably less enriched in  $^{14}\text{C}$  (-56 to -80 ‰; Table 1) indicating that this C was from a pool dominated by much older material than that in the surface soil. In contrast to the surface horizons, radiocarbon signatures from the deep soils increased slightly from the first to final wetting cycle; estimated turnover times decreased by several hundred years (Table 1), although the C remained quite old. The  $^{14}\text{C}$  enrichment of the deep-soil respired C was still substantially less depleted (i.e. younger) than that of the bulk soil C, which averages around -400 ‰, but was substantially more  $^{14}\text{C}$  depleted than the  $\text{CO}_2$  present in the soil profile, which averages around +100 ‰ (Fierer *et al.*, 2005). Xiang *et al.* (2008) suggested that in deep soils, drying/rewetting cycles mobilize otherwise stable carbon; this study established that conclusively. The C released by dry/wet cycles is old, and is not merely fresh root or leachate material.

These data and other recent studies (Xiang *et al.*, 2008, Fontaine *et al.*, 2007) show that a surprisingly large amount of the deep C is not be stable but rather “metastable”—unused but not unusable. This work suggests that in deep soils, a significant amount of deep soil C is chemically labile and

does not require additional substrate to allow the microorganisms to make new enzymes to degrade these compounds. The conclusion that some old soil C is bio-available to microbes is supported by finding old C in microbial phospholipids from 30 cm deep in an agricultural soil (Kramer and Gleixner, 2008).

The old  $^{14}\text{C}$  dates for C mobilized and respired from deep soils as a result of dry/wet cycles  $\text{CO}_2$  reinforces the hypothesis that substantial amounts of C are simply physically inaccessible to microbes in deep soils, but challenges the assumption that the material is old by virtue of being strongly sorbed by minerals. It reinforces the question: if the material is chemically labile, and mobilizable by a modest physical/chemical disruption, why is it so old? Why haven't microbes found and metabolized this C already?

## Acknowledgements

We thank the UCSB Sedgwick Reserve for providing research support and Dr. Chris Still for laboratory space to do radiocarbon work. We thank the SLU fund for internationalisation of postgraduate studies for supporting Wetterstedt's travel to the U.S. and the U.S. National Science Foundation's Ecology and Ecosystem Studies Programs, and the Kearney Foundation of Soil Science for financial support.



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