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

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Deep soils (below 20 cm) are one of the major reservoirs of organic C on earth, containing more C than is in either vegetation or 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 14C 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 effectively inert, raising the question of why it hasn’t already been metabolized. It also demonstrates the contribution of C fixed before the 1960s to CO2 metabolized in the surface soils at this site.

These observations emphasize our lack of understanding of the processes that allow C to persist for thousands of years in deep soils, and conversely, the processes that might make deep, old soil C amenable to decomposition. For example, incubation of deep soils from a California grassland environment (Ewing et al., 2006) demonstrated that the breaking of soil structures can result in respiration of old C, suggesting that physical structures can be important in making C unavailable for decomposition. Fontaine et al. (2007) found that adding labile C 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. When these soils were kept constantly moist, respiration collapsed to near zero, while drying and rewetting them a new pulse of respiration with each dry/wet cycle. They concluded that native C could support growth if microbes could just access it and that it was not so tightly sorbed as to be unavailable. Such studies emphasize the need to better understand the biogeochemical nature of deep soil C.

To follow up the results of Xiang et al. (2008), we asked whether the C released by repeated dry/wet cycles was recent material from deep roots or leachates or old C that had been preserved in the soil. We repeated their experiment using the same soil, but measured...
the $^{13}$C and $^{14}$C enrichment of the C respired to estimate the turnover time of the soil C pool it came from, comparing surface and deep soils.

The site is at the Sedgwick Reserve in Central California (34°41'29.4"N; 120°02'42.7"W). It has been dominated by invasive C-3 annual grasses for the last several decades, but had a history of agriculture before that. The soil is a pachic argixeroll and is more fully described in Xiang et al. (2008). For surface soils (0–10 cm; 2.2% 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 (1 L) and run through five wet/dry 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 using a sealed-tube zinc reduction method (Xu et al., 2007), and analyzed for $^{14}$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 $\Delta^{14}$C:

$$\Delta^{14}\text{C} = \left[ \frac{^{14}\text{C}}{^{12}\text{C}} \right]_{\text{sample}} - 25 \times 0.95^{14}\text{C}_{\text{PDB}} \times \frac{1}{(9\times1950)/8267} - 1$$

where the $^{14}\text{C}/^{12}\text{C}$ ratio of the sample is corrected for mass dependent isotope fractionation so all data are reported as if they had a common $^{13}\text{C}$ signature of $-25\%_{\text{o}}$ (Stuiver and Polach, 1977). The $\Delta^{14}\text{C}$ notation also accounts for decay of the oxalic acid standard since 1950, and is thus an expression of the absolute amount of $^{14}\text{C}$ in the sample in the year (y) it was measured. $\Delta^{14}\text{C}$ values equal to zero indicate that the $^{14}\text{C}/^{12}\text{C}$ ratio of the sample equals that of preindustrial wood. Positive $\Delta^{14}\text{C}$ values indicate the presence of ‘bomb’ $^{14}\text{C}$ produced by weapons testing in the late 1950s and early 1960s, while negative $\Delta^{14}\text{C}$ values indicate that the C has been isolated from exchange with the atmosphere and has experienced significant radioactive decay.

We also report $\delta^{13}\text{C}$ signatures measured by continuous flow isotope ratio mass spectrometry (Thermo/Fisher Delta plus equipped with a Gasbench II interface) on an aliquot of the purified CO$_2$ we used for graphitization and radiocarbon analysis (Xu et al., 2007). The data are reported in standard notation as the parts per thousand deviation from the $^{13}\text{C}/^{12}\text{C}$ ratio of the PDB standard.

We estimated the turnover time of the carbon pool contributing to CO$_2$ by modeling its radiocarbon signature, assuming that it is a single, homogeneous pool at steady state. We assume carbon is constantly added to and removed (by decomposition) from the pool, with decomposition represented by a first-order decay constant ($k$). The amount of carbon in the pool contributing to decomposition is given by the rate of input ($I$) minus $k$ (the decomposition rate constant) times the size of the pool ($C$). At steady state, $I = k \times C$. The rate of change of radiocarbon differs in that the radiocarbon signature of the inputs vary with the years since 1900 (values from Levin et al., 2010), and the rate of loss is $k + \lambda$, where $\lambda$ is the rate constant for radioactive decay of $^{14}\text{C}$; 1/8267 yr. The $\Delta^{14}\text{C}$ signature for the soil organic matter pool in any given year ($t$) is thus:

$$\Delta^{14}\text{C}_{\text{RS}}(t) = \left( \Delta^{14}\text{C}_{\text{RS}}(t - 1) + \left( \Delta^{14}\text{C}_{\text{atmosphere}} \times I \right) - (k+\lambda) \times \left( \Delta^{14}\text{C}_{\text{RS}}(t - 1) \right) \right)$$

where: $\Delta^{14}\text{C}_{\text{RS}}(t)$ and $\Delta^{14}\text{C}_{\text{RS}}(t - 1) = 14\text{C}$ content of respiration source pool in the year ($t$) or ($t - 1$) respectively; and

$$\Delta^{14}\text{C}_{\text{atmosphere}}(t) = 14\text{C} \text{ content of atmospheric CO}_2 \text{ in the year } t \text{ from Levin et al., 2010. Turnover times } (1/k) \text{ are varied until the observed } \Delta^{14}\text{C} \text{ signature in the year of measurement (2007) is matched} \text{ (see Gaudinski et al., 2000; Torn et al., 2009 for more details).}$$

The initial pulse of CO$_2$ released after wetting dry surface soils had $\Delta^{14}\text{C}$ values greater than 0 $\%_{\text{o}}$ (Table 1), indicating the presence of $^{14}\text{C}$ produced by atmospheric nuclear weapons testing. However, this value (+10.5 $\%_{\text{o}}$) was also less than that of the 2007 atmosphere (+50 $\pm$ 5 $\%_{\text{o}}$), indicating a substantial contribution of C fixed prior to the 1960s to the respired CO$_2$. The CO$_2$ respired after the 5th rewetting pulse had decreased, to $-1.5\%_{\text{o}}$ (Table 1). Estimated turnover times corresponding to these radiocarbon signature increased over the course of the incubation from 280 to 320 years. This decline is consistent with decreased contributions of a ‘younger’ C source with $^{13}\text{C}$ closer to that of the 2007 atmosphere with repeated rewetting.

Carbon respired from deep soils was considerably less enriched in $^{14}\text{C}$ ($-56 \text{ to } -80\%_{\text{o}}$; Table 1) indicating that this C was from a pool dominated by older material than that in the surface soil. In contrast to the surface horizons, radiocarbon signatures from the deep soils increased from the first to final wetting cycle; estimated turnover times therefore 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 $-200 \text{ to } -400\%_{\text{o}}$ for 1 m depth at this site, but was substantially more $^{14}\text{C}$ depleted than the CO$_2$ present in the soil profile, which averaged 15–30$\%_{\text{o}}$ higher in $\Delta^{14}\text{C}$ than the atmospheric $^{14}\text{CO}_2$ (Fierer et al., 2005). These results suggest that dry/wet cycles release C that is not normally accessible (i.e. is not contributing a major fraction of metabolized C in the field.)

Our results are consistent with trends in the $^{14}\text{C}$ ages of DOC with depth in another California grassland soil (Sanderman et al., 2008), and with the values reported by Ewing et al. (2006). However, they are more enriched in $^{14}\text{C}$ compared to the CO$_2$ respired from California grassland soil B horizons immediately after physical disruption that mechanically broke apart soil peds (Ewing et al., 2006).

The $\delta^{13}\text{C}$ signatures of CO$_2$ respired from surface and deep soils differed considerably (Table 1). Surface soils respired C with $\delta^{13}\text{C}$ values closer to those of C4 grasses, which suggests that a legacy remains in the SOC pools of cropping with C4 crops such as corn or sorghum. The $\delta^{13}\text{C}$ signatures did not change between the first and fifth rewetting of the surface soil. In contrast, the CO$_2$ respired from the deep soil had $\delta^{13}\text{C}$ values showing no inputs of C4 carbon, reflecting either historical C3 vegetation or recent invasives. The $\delta^{13}\text{C}$ signatures increased by about 1$\%_{\text{o}}$ over the course of the incubation, which could reflect a change in the quality of substrate being respired between the first and fifth rewetting.

| Soil     | $\Delta^{14}\text{C}$ | Std. dev. | $\delta^{13}\text{C}$ | Std. dev. | Turnover time estimated from bomb $^{14}\text{C}$ |
|----------|------------------------|-----------|------------------------|-----------|---------------------------------------------|
| Surface  | $10.5 \pm 4.8$         | 0.3       | $-15.9 \pm 0.3$        | 0.3       | 280 (30)                                    |
| 1 m deep | $-80.4 \pm 5.1$        | 0.1       | $-23.5 \pm 0.1$        | 0.1       | 850 (40)                                    |
| Final cycle | $-1.1 \pm 1.5$    | 0.3       | $-15.8 \pm 0.3$        | 0.3       | 325 (15)                                    |
| 1 m deep | $-56.7 \pm 2.8$        | 0.1       | $-22.4 \pm 0.1$        | 0.1       | 660 (40)                                    |

* Measured 2007.
There is a large pool of old, deep soil C (Jobbágy and Jackson, 2000). The mechanisms for its stability, however, have not been clear. The most common explanations have been chemical sorption and physical encapsulation, processes which make C unavailable for microbial attack (e.g. Ewing et al., 2006). However, the ability to mobilize C by either priming (Fontaine et al., 2007) or dry/wet cycles (Xiang et al., 2008), and the occurrence of old C in microbial phospholipids from subsoil (Kramer and Gleixner, 2008) all suggest that some fraction of this deep C is biologically available and relatively easily accessible to microorganisms: not stable but “meta-stable”—unused but not unusable.

This is the first study to use radiocarbon to evaluate the nature of deep soil SOM that can be mobilized by simple environmental perturbation such as dry/wet cycles in a poorly structured deep soil. We found that the C respired as a result of dry/retting cycles had 14C dates that were centuries old, reinforcing the hypothesis that some old C is vulnerable to attack without requiring strong physical or chemical forcing to make it available.

If that is true, it begs the question: what additional mechanisms could help explain the meta-stability of deep soil C? One possibility is limited diffusion of C to active microbes. In deep soils, where microbes and resources are sparse, C must potentially diffuse along a chromatography column, slowing C movement to microbes. If C supply is low, microbes would lapse into dormancy, reducing the strength of diffusion gradients, and so further reducing C movement. Such a “diffusion collapse” could work in concert with other mechanisms to explain the presence of old, labile C in deep soil. The mass flow of water associated with rewetting, however, would overcome it and promote a release of old C, as we observed.

Such a mechanism would fit into developing conceptual models of soil dynamics, which increasingly focus on spatial interactions as a control over C and N mineralization in soils. For example, Schimel and Bennett (2004) suggested that changes in N processing within, and movement between, N-rich and N-poor microsites may explain why soils appear to shift from amino acid, through NH4+, to NO3- domination along an N-availability gradient. These models suggest that microsite interactions produce more than mere heterogeneity, they produce overall patterns of soil process. The centuries old 14C dates of C respired in this study could reflect such a pattern.

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