

# Diverse organic carbon dynamics captured by radiocarbon analysis of distinct compound classes in a grassland soil

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## Supplementary Material

### *1 Density Separation*

To fully characterize the soil samples from the Hopland site, we compared the compound class measurements and the physical fractionations to a separate SPT density separation experiment as well as a “bulk” <sup>13</sup>C nuclear magnetic resonance (NMR) experiment to compare the change in chemical structure in <2mm soils.

#### *1.1 Methods*

Bulk soil samples (~20 g) were density fractionated using a low C and N sodium polytungstate (SPT-O, Geoliquids) into three density fractions: the free light fraction (FLF), the occluded light fraction (OLF), and the dense fraction (DF) according to the method in [McFarlane *et al.*, 2013]. The density separation was done in triplicate for each depth sample. The separation density was 1.65 g mL<sup>-1</sup>. For the experiment all glassware was pre-combusted to reduce C contamination. For our heavy liquid we used 850 g of SPT powder in 825 g of 18.2 ΩM water in a 1 L beaker and was constantly stirred using a stir bar. Repeat measurements of the SPT solution ensured the correct density of 1.65 g mL<sup>-1</sup>.

30 For each replicate, 20 grams of 2 mm sieved soil from each depth (0-10 cm, 10-20 cm,  
31 20-50 cm, and 50-100 cm) was transferred into an acid-washed centrifuge tube and 100ml of  
32 SPT-0, the tube was inverted by hand ensuring the entire sample was in contact with the SPT.  
33 The samples were centrifuged at 3500 rpm for 1 hour and the floating material was aspirated and  
34 collected via vacuum filtration. Samples were washed repeatedly through a Pall Supor 0.45 mm  
35 47 mm PES filter using 18.2 MΩ water. The FLF was dried in a 65°C oven overnight, weighed,  
36 and then transferred into a 105°C oven overnight. Once cooled, samples were ground with a  
37 mortar and pestle.

38 To recover the OLF fraction the centrifuge tubes were filled with 75ml SPT. The sample  
39 was mixed using a benchtop mixer for 1 minute at 1400 rpm. The sample vial was then  
40 transferred into an ice filled Styrofoam box where it cooled for 5 minutes and then sonicated for  
41 1 minute at 80% amplitude. The samples were centrifuged at 3500 rpm for 1 hour to recover the  
42 OLF. The floating material (OLF) was then aspirated into a side-arm flask and rinsed five times  
43 with about 150 mL of 18.2 MΩ water. The samples were dried at a 65°C overnight, weighed, and  
44 then transferred to a 105°C oven overnight. The sample was then ground using a mortar and  
45 pestle and transferred into vials for later analysis.

46 The remaining SPT was then aspirated from the centrifuge bottles, leaving just the  
47 residual DF. The DF was rinsed until the density of the supernatant reached 1g/ml. The samples  
48 were dried in a 65°C oven overnight, weighed, and then transferred to a 105°C oven overnight.  
49 The DF was grinded using a ball mill and transferred into vials for later analysis.

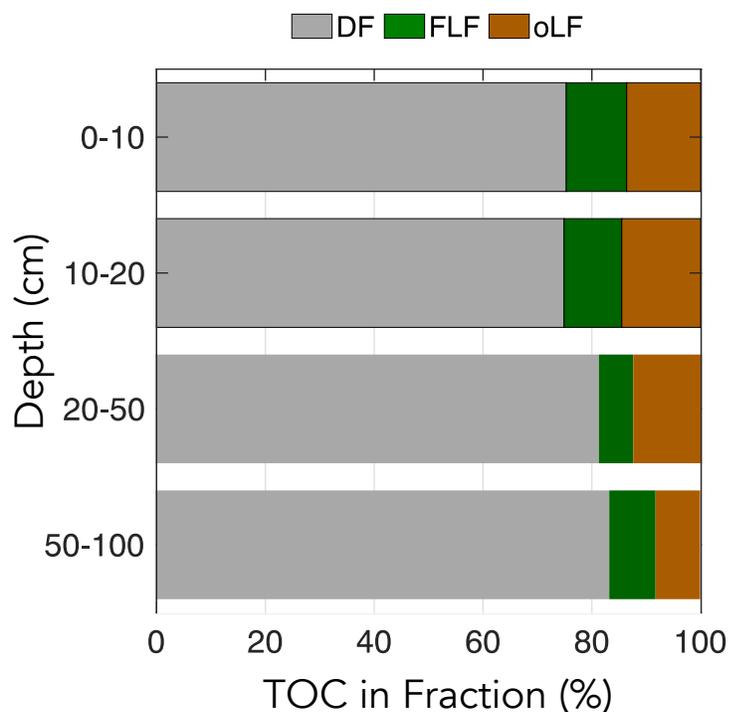
50 This entire process was performed in triplicate for each depth; four depths, twelve samples.

51 As described above, each of the fractions (FLF, OLF, and DF) was loaded into quartz tubes  
52 for <sup>14</sup>C analysis, and foil balled for δ<sup>13</sup>C analysis.

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54 ***1.2 Results:***

55 To compare our size fractionated samples to a traditional density fractionation, we carried out the  
56 separated our <2mm “bulk soil” into three fractionations with a heavy liquid. The three fractions  
57 were the free light fraction (FLF) or mineral free, the occluded light fraction (OLF) is also  
58 mineral free, but is found in aggregates and requires disruptive (sonication) energy input, and the  
59 dense fraction (DF) is the mineral associated fraction (Plaza et al. 2019). At each depth the FLF  
60 was the youngest fraction and had roughly 26 %OC, the OLF by comparison generally is very  
61 condensed with %OC ranging from 32 to 43%. The FLF ranged from  $+3 \pm 5\%$  in the surface to -  
62  $350 \pm 110\%$  at depth. The OLF ranged from  $18 \pm 7$  to  $-633 \pm 21\%$ . The DF ranged from  $14 \pm 5$   
63 to  $-563 \pm 9\%$ . In general, the DF was older than both the sand and <63  $\mu\text{m}$  fractions at each  
64 depth, suggesting some modern, mineral-free carbon is present in both the sand and silt/clay  
65 fractions. While these separations are not perfect, they help us understand where most carbon is  
66 concentrated within a sample. Generally, these HREC soils have very little FLF and OLF by  
67 mass, the DF consists of 80% of the total carbon within the sample (SI Table). The HF includes  
68 both the sand and silt/clay fractions. Because our >63 $\mu\text{m}$  fraction has younger  $\Delta^{14}\text{C}$  values than  
69 the <2mm to >63 $\mu\text{m}$  or DF fractions we can assume the <63 $\mu\text{m}$  fraction includes free particulate  
70 carbon which cycles faster than the truly mineral-associated DF. The OLF is older than both the  
71 DF and the FLF at the deepest depth, which could mean aggregation is a mechanism for greater  
72 stability in these soils.



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75 Figure S1. A. Density separation radiocarbon results for the FLF, OLF, and DF.B. fraction of OC  
 76 contained in each density fraction. Error bars represent standard error on triplicate experiment  
 77 measurements.

78 **2 Nuclear Magnetic Resonance (NMR) Spectroscopy**

79 **2.1 Methods**

80 Semi-quantitative solid-state cross polarization magic angle spinning (CPMAS) <sup>13</sup>C NMR was  
 81 performed on a depth profile of <2mm soil horizon samples to identify target compound classes.  
 82 In order to reduce paramagnetic iron interferences, soil samples were first de-mineralized using  
 83 2% hydrofluoric acid (HF) following the protocols of [Sanderman *et al.*, 2017]. Approximately  
 84 0.5 g of de-mineralized soil was crushed into a fine powder with an agate mortar and pestle and  
 85 loaded into a 7.5mm rotor for NMR analysis. <sup>13</sup>C-NMR spectra were collected on a Bruker Neo

86 console operating at a Larmor frequency of 75.71 MHz and externally referenced to adamantane  
87 ( $\delta=38.48$  ppm).  $^1\text{H}$ - $^{13}\text{C}$  cross polarization measurements were collected while spinning at 6 khz  
88 with a contact time of 1ms and a recycle delay of 2s. Spectra were acquired with between  
89 115518 and 346996 scans depending on sample organic carbon concentration. Spectral intensity  
90 was normalized by mass and number of scans in Matlab. This method allows for direct  
91 comparison between the spectra measured with the same parameters. Spectra were processed  
92 with Bruker Topspin software and peak integrations were done with Matlab (vR2022b). Data  
93 was categorized by the following functional group shifts: 210-165 ppm, 165-145 ppm, 145-95  
94 ppm, 95-52 ppm, 52-0 ppm, which correspond to C=O groups, aromatic C-O groups, other  
95 aromatics and olefinics, O- and N-alkyl groups, and alkyl carbon groups respectively [*Baldock et*  
96 *al.*, 2004; *Mao et al.*, 2017].

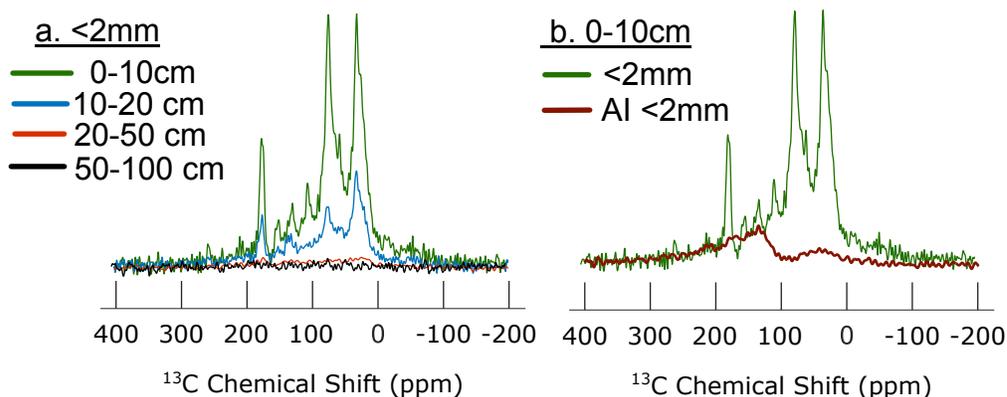
## 97 **2.2 Results:**

98 NMR spectra and total soil organic carbon content were collected from < 2 mm  
99 hydrofluoric acid-rinsed soil samples (Figure 2). Total carbon declined from 3.4% at the surface  
100 to 0.2% in the 50-100cm interval, with the steepest decline between the 0-10 and 10-20cm  
101 intervals. The normalized relative abundances of the five molecular classes we identified by  $^{13}\text{C}$ -  
102 NMR are listed in Table 1. Generally, the magnitude of all peaks decreased between the surface  
103 and subsurface soils, and the 50-100 cm depth had no detectable peaks over baseline. The  
104 aromatic peak had the least decline within the sample set, with over 60% of the initial peak  
105 intensity retained throughout the depth profile. By comparison, the 20-50 cm depth had >75%  
106 less alkyl C relative to the surface soil. We note that the radiocarbon values between the de-  
107 mineralized (hydrofluoric acid-rinsed) soils and bulk soils indicate a slightly older value in the  
108 de-mineralized soil (SI Table 1), which suggests that some younger, highly labile carbon was

109 removed during this procedure.

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113 Figure S2: (A) Soil state  $^{13}\text{C}$ -NMR and characterization of bulk (< 2 mm) hydrofluoric acid-  
114 treated soil collected from four depths of an annual grassland in Hopland, CA. Total organic  
115 carbon was calculated separately on three physical size fractions at each depth. (B). Direct  
116 comparison of NMR from AI fraction and bulk soil.

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### 118 **3.1 Radiocarbon Blank Assessment Method**

119 To calculate the amount of exogenous C added during WEOC, TLE, and AA extractions,  
120 process blanks were carried out independently. Blank analysis for compound specific  
121 radiocarbon analysis (CSRA) becomes increasingly important when the extraction preparations  
122 are complex and the sample size of the target compound is small [Sun *et al.*, 2020]. We used  $^{14}\text{C}$   
123 modern and dead standard materials to quantify the excess C acquired through each extraction  
124 procedure. For the acid insoluble fraction and untreated soil samples background corrections, we  
125 ran  $^{14}\text{C}$ -free coals as is standard at CAMS.

126 For the TLE extraction, an empty ASE cell was extracted by the same method as the soil

127 samples. A known amount of modern or dead  $^{14}\text{C}$  materials was added to the dried down “blank  
128 sample” in the quartz tube. This method uses the “indirect blank” calculation, using a  
129 comparison of fraction modern ( $F^{14}\text{C}$ ) to an accepted value, because there is not enough excess  
130 carbon to measure the blank directly [Santos *et al.*, 2010]. For the AA blank analysis, a  $^{14}\text{C}$ -dead  
131 alanine powder and a  $^{14}\text{C}$ -modern commercial protein powder were digested and processed  
132 through the resin column following AA procedures.

133 In total, three  $^{14}\text{C}$ -modern and nine  $^{14}\text{C}$ -dead samples were analyzed to quantify the AA  
134 blank (SI Table). For the TLE blank quantification, four  $^{14}\text{C}$ -modern and four  $^{14}\text{C}$ -dead samples  
135 were analyzed. The size and  $F^{14}\text{C}$  of the blank were then determined using the methods and  
136 published R script from Sun *et al.* (2020). The R script was run in R Studio version 4.1.2 (R Core  
137 Team, 2021). Briefly, a Bayesian model was used to fit thousands of linear regression lines  
138 between the  $F^{14}\text{C}$  and inverse of the sample size ( $1/\mu\text{g C}$ ), allowing for the calculation of the  
139  $F^{14}\text{C}$  and size of the blank, as well as their associated uncertainties.

### 140 ***3.2 Radiocarbon blank assessment Results***

141 Extraneous C was quantified for the TLE and AA extractions (SI Table 4). The TLE blank  
142 is  $8.16 \pm 2.54 \mu\text{g}$  of C with an  $F^{14}\text{C}$  value of  $0.671 \pm 0.252$ . The exogenous C added from the AA  
143 extraction procedure is  $11.934 \pm 6.205 \mu\text{g}$  of C with  $F^{14}\text{C}$  value of  $0.807 \pm 0.389$ . The WEOC  
144 blank contribution is  $2.818 \pm 0.753$  with a  $F^{14}\text{C}$  value of  $0.298 \pm 1.22$  (details reported in Finstad  
145 *et al.*, 2023). Generally, the extracted  $^{14}\text{C}$  samples were large enough ( $> 250 \mu\text{g C}$ ) that the  
146 contribution of the blank did not significantly shift the  $^{14}\text{C}$  values outside the variability of

147 sample replicates. Future efforts will identify the source of extraneous C to lower this blank  
148 contribution from materials or a methodology step and increase the applicability of this method  
149 for smaller and more <sup>14</sup>C-depleted samples.

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