

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

Katherine E. Grant^{1*}, Marisa N. Repasch^{1,2,3}, Kari M. Finstad¹, Julia D. Kerr¹, Maxwell Marple¹, Christopher J. Larson^{1,4}, Taylor A. B. Broek^{1,5}, Jennifer Pett-Ridge^{1,6}, and Karis J. McFarlane¹

¹Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA

²Institute of Arctic and Alpine Research, University of Colorado, Boulder, CO, USA

³Earth and Planetary Sciences, University of New Mexico, Albuquerque, NM, USA

⁴Department of Earth and Environmental Science, University of Pennsylvania, Philadelphia, PA, USA

⁵National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) Facility, Woods Hole Oceanographic Institution Woods Hole, MA, USA

⁶Life and Environmental Sciences Department, University of California-Merced, Merced, CA, USA

Correspondence to: Katherine E. Grant (grant39@llnl.gov)

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” ¹³C 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⁻¹. 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

30 and was constantly stirred using a stir bar. Repeat measurements of the SPT solution ensured the
31 correct density of 1.65 g mL⁻¹.

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

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

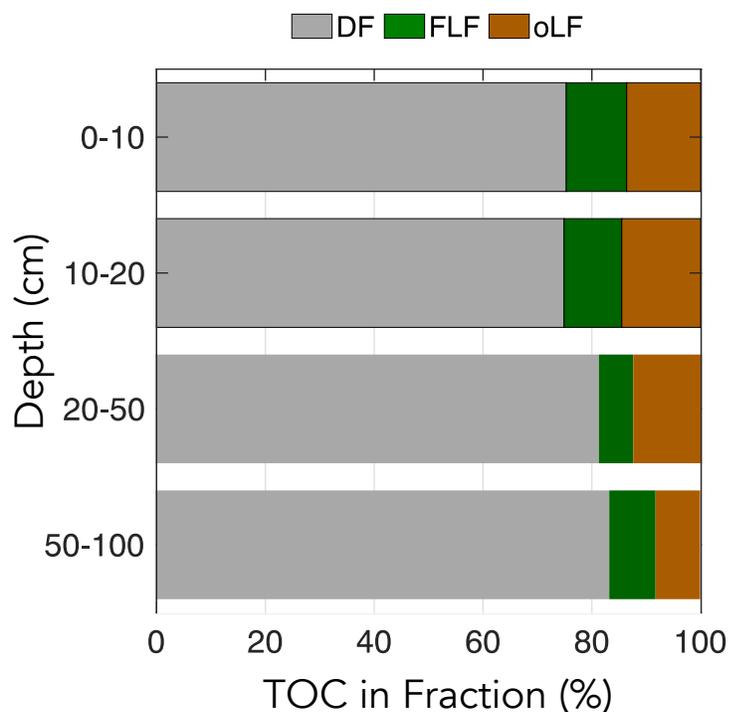
48 The remaining SPT was then aspirated from the centrifuge bottles, leaving just the
49 residual DF. The DF was rinsed until the density of the supernatant reached 1g/ml. The samples
50 were dried in a 65°C oven overnight, weighed, and then transferred to a 105°C oven overnight.
51 The DF was grinded using a ball mill and transferred into vials for later analysis.
52 This entire process was performed in triplicate for each depth; four depths, twelve samples.

53 As described above, each of the fractions (FLF, OLF, and DF) was loaded into quartz tubes
54 for ^{14}C analysis, and foil balled for $\delta^{13}\text{C}$ analysis.

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56 ***1.2 Results:***

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



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77 Figure S2. A. Density separation radiocarbon results for the FLF, OLF, and DF.B. fraction of OC

78 contained in each density fraction. Error bars represent standard error on triplicate experiment

79 measurements.

80 **2 Nuclear Magnetic Resonance (NMR) Spectroscopy**

81 **2.1 Methods**

82 Semi-quantitative solid-state cross polarization magic angle spinning (CPMAS) ^{13}C NMR was

83 performed on a depth profile of <2mm soil horizon samples to identify target compound classes.

84 In order to reduce paramagnetic iron interferences, soil samples were first de-mineralized using

85 2% hydrofluoric acid (HF) following the protocols of [Sanderman *et al.*, 2017]. Approximately

86 0.5 g of de-mineralized soil was crushed into a fine powder with an agate mortar and pestle and

87 loaded into a 7.5mm rotor for NMR analysis. ^{13}C -NMR spectra were collected on a Bruker Neo

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

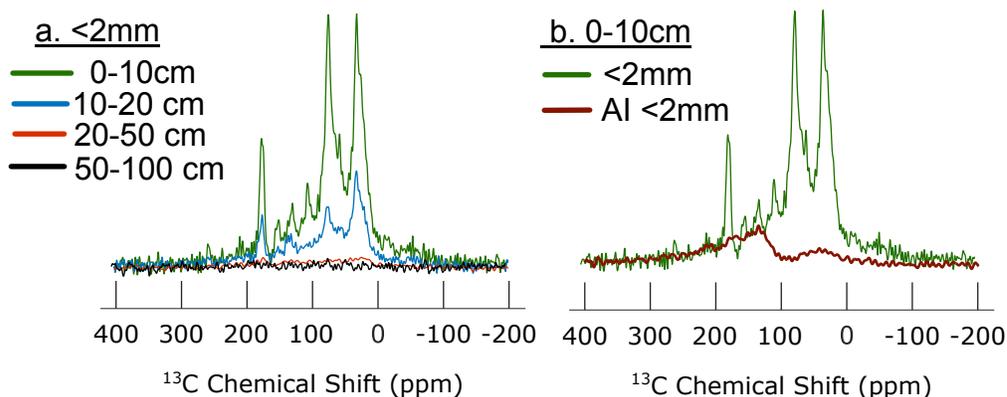
99 **2.2 Results:**

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

111 removed during this procedure.

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115 Figure S4: (A) Soil state ^{13}C -NMR and characterization of bulk (< 2 mm) hydrofluoric acid-
116 treated soil collected from four depths of an annual grassland in Hopland, CA. Total organic
117 carbon was calculated separately on three physical size fractions at each depth. (B). Direct
118 comparison of NMR from AI fraction and bulk soil.

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120 **3.1 Radiocarbon Blank Assessment Method**

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

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

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

135 In total, three ^{14}C -modern and nine ^{14}C -dead samples were analyzed to quantify the AA
136 blank (SI Table). For the TLE blank quantification, four ^{14}C -modern and four ^{14}C -dead samples
137 were analyzed. The size and $F^{14}\text{C}$ of the blank were then determined using the methods and
138 published R script from Sun *et al.* (2020). The R script was run in R Studio version 4.1.2 (R Core
139 Team, 2021). Briefly, a Bayesian model was used to fit thousands of linear regression lines
140 between the $F^{14}\text{C}$ and inverse of the sample size ($1/\mu\text{g C}$), allowing for the calculation of the
141 $F^{14}\text{C}$ and size of the blank, as well as their associated uncertainties.

142 ***3.2 Radiocarbon blank assessment Results***

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

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

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