

1 **Belowground allocation and dynamics of recently fixed plant carbon in a California annual**
2 **grassland soil**

3 Christina Fossum^{1*}, Katerina Estera-Molina¹, Mengting Yuan¹, Don Herman¹, Ilexis Chu-
4 Jacoby¹, Peter Nico², Keith Morrison³, Jennifer Pett-Ridge^{3,4}, Mary Firestone^{1,2}

5 **Author Affiliations**

6 ¹Department of Environmental Science, Policy, and Management, University of California,
7 Berkeley, CA; ² Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley CA;
8 ³Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore,
9 CA; ⁴Life and Environmental Sciences Department, University of California, Merced, CA

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13 **Corresponding author:** Mary Firestone, Department of Environmental Science, Policy, and
14 Management, 140 Mulford Hall, University of California, Berkeley CA 94720
15 (mkfstone@berkeley.edu)

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17 **Summary**

18 Plant roots and the organisms that surround them are a primary source for stabilized
19 organic C, particularly in grassland soils, which have a large capacity to store organic carbon
20 belowground. To quantify the flow and fate of plant fixed carbon (C) in a Northern California
21 annual grassland, we tracked plant carbon from a five-day ¹³CO₂ pulse field labeling for the
22 following two years. Soil and plant samples were collected immediately after the pulse labeling,
23 and again at three days, four weeks, six months, one year, and two years. Soil organic matter was

24 fractionated using a sodium polytungstate density gradient to separate the free-light fraction
25 (FLF), occluded-light fraction (OLF), and heavy fraction (HF). Using isotope ratio mass
26 spectrometry, we measured ^{13}C enrichment and total C content for plant shoots, roots, soil, soil
27 dissolved organic carbon (DOC), and the FLF, OLF, and HF. The HF was further analyzed by
28 solid state ^{13}C NMR spectroscopy.

29 At the end of the labeling period, the largest amount of ^{13}C was recovered in plant shoots
30 (60%), but a substantial amount (40%) was already found belowground in roots, soil, and soil
31 DOC. Density fractionation of 4-week soil samples (from which living roots were removed)
32 indicated that the highest isotope enrichment was in the mineral-rich heavy fraction, with similar
33 enrichment of the FLF and OLF. At the 6-month sampling, after the dry summer period during
34 which plants senesced and died, the amount of label in the FLF increased such that it was equal
35 to that in the HF. By the 1-year sampling, ^{13}C in the FLF had declined substantially and
36 continued to decline by the 2-year sampling. ^{13}C recovery in the OLF and HF, however, was
37 qualitatively stable between sampling times. By the end of the 2-year experiment, 69% of
38 remaining label was in the HF, 18% in the FLF and 13% in the OLF.

39 While the total ^{13}C content of the HF did not change significantly from the 4-week to the
40 2-year sample time, ^{13}C NMR spectroscopic analysis of spring HF samples from 2018, 2019, and
41 2020 suggests that the relative proportion of aliphatic/alkyl functional groups declined in the
42 newly formed SOC over the 2-year period. Simultaneously, aromatic and carbonyl functional
43 groups increased, and the proportion of carbohydrate groups remained relatively constant. In
44 summary, our results indicate that initial associations between minerals and root-derived organic
45 matter are significant and form rapidly; by 4 weeks, a substantial amount (17%) of the total
46 plant-derived ^{13}C had become associated with the heavy fraction (HF) of soil. While the majority

47 of annual C input cycles rapidly (<2-year timescale), a sizeable proportion (~12% of the original
48 inputs) persisted for 2 years.

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51 **1. Introduction**

52 California annual grasslands occupy over 10 million ha (Heady et al., 1992), and have
53 been predicted to be a more resilient and therefore effective carbon sink than California's fire-
54 prone forests (Dass et al., 2018). According to a recent metaanalysis, grassland soils also have an
55 increased capacity to store soil carbon in the face of increasing atmospheric CO₂ concentrations
56 (Terrer-Moreno et al., 2020). As such, these ecosystems have become an attractive target for
57 California state policy initiatives aimed at mitigating atmospheric CO₂ levels through soil carbon
58 sequestration (Biggs & Huntsinger, 2021; Baker et al., 2020). However, our scientific
59 understanding of soil carbon cycling, the fate of plant residues belowground, and how annual
60 grasslands can broadly act as a viable carbon sink remains incomplete (Bradford et al., 2019). To
61 effectively manage carbon stocks, further research is required to better understand the ecological
62 parameters that regulate soil carbon cycling in these globally significant ecosystems.

63 Belowground carbon dynamics in California annual grasslands reflect the annual plant
64 life cycle (Eviner & Firestone, 2007). Plant communities are dominated by annual grasses and
65 forbs, species whose reproductive strategies are well-adapted to a Mediterranean climate where
66 seasonal moisture limitations largely regulate nutrient cycling. This climate is characterized by
67 cool wet winters and a warm summer period with no rainfall, a defining feature of which is the
68 misalignment of two critical conditions for plant growth: rainfall and solar energy. Moisture

69 limits plant growth in the summers, and sunlight and temperature limit plant growth in the
70 winters (Bartolome et al., 2007; Eviner and Firestone, 2007). The plant life cycle begins with the
71 first germinating rainfall in the autumn, proceeds slowly through the winter due to sunlight and
72 temperature limitations, peaks in the spring when both soil moisture and sunlight are usually
73 optimized, and ends with plant senescence once soil moisture has declined with the onset of the
74 summer dry period (Eviner 2016; Eviner and Firestone, 2007). Throughout the winter/spring
75 growing season, carbon enters the soil primarily via a constant “drip” of plant root exudation
76 (Sokol & Bradford, 2019; Pett-Ridge et al., 2021); over the summer dry period, plant senescence
77 and subsequent litter input, coupled with low-moisture conditions drives the transient
78 accumulation of litter; in the autumn at the onset of the rainy season, a large portion of
79 accumulated carbon is rapidly released as CO₂ in a decomposition “pulse” (Blankinship &
80 Schimel, 2018).

81 The characteristic seasonal soil moisture variability in Mediterranean climates can impact
82 the persistence of soil organic matter (SOM) via multiple mechanisms. Following 4-6 months of
83 no precipitation, autumn’s first major rainfall event strongly impacts the soil biota, physically
84 stresses soil aggregates, chemically alters soil minerals, and destabilizes SOM, driving a pulse of
85 respiration known as the Birch Effect (Barnard et al., 2020; Blazewicz et al., 2020; Birch, 1958).
86 This release of CO₂ is generally understood to result from the stimulation of microbial
87 respiration and high availability of substrate (Schimel, 2018; Barnard et al., 2020). Plant
88 germination usually occurs in the Autumn, temperature limits growth in the winter, and the peak
89 period of plant growth occurs in the spring when both temperature and moisture are optimized.
90 The majority of rhizosphere-derived SOC generation occurs during this spring growth period.
91 Then, in late spring when soil moisture conditions no longer support growth, new root C inputs

92 decline as plant senescence occurs and annual species set seed. During this dry-down period,
93 decreasing soil moisture reduces hydrological connectivity between soil pores, isolating
94 microbes from carbon substrates and resulting in a decline in ecosystem activity (Manzoni and
95 Katul, 2014; Barnard et al., 2013; Blankinship and Schimel, 2018). Decomposition of plant
96 biomass and transfer of carbon belowground continue to be limited throughout the summer dry
97 period (Chou et al., 2008; Eviner and Firestone, 2007). While not fully understood, these wet-dry
98 cycles characteristic of California annual grasslands may be important drivers of SOM
99 persistence, and may be susceptible to future climate conditions that either amplify or minimize
100 their stabilizing or destabilizing effects (Bailey et al., 2019).

101 Soil carbon is often considered in terms of distinct soil organic matter “pools”: either
102 associated with minerals, occluded within microaggregates, or “free” (not subject to either
103 chemical or physical protection) (Poeplau et al., 2018). Recent studies have shown that labile
104 carbon substrates can play an important role in SOM formation and stabilization in both
105 physically (occluded) and chemically (mineral-associated) pools (Cotrufo et al., 2015; Totsche et
106 al., 2017; Villarino et al., 2021), while plant litter is generally understood to largely comprise the
107 free-light fraction material. The mineral-associated or “heavy fraction” is of particular interest
108 for soil organic carbon persistence; it is typically the oldest distinct pool (Torn et al., 1997) and
109 represents carbon stabilized via mineral sorption and co-precipitation mechanisms (Kogel-
110 Knabner et al., 2008). This fraction of SOM, commonly termed MAOM (mineral-associated
111 organic matter) (Cotrufo et al., 2019), is largely of microbial origin and thought to be derived
112 from relatively labile C substrates (Clemente et al., 2011; Cotrufo et al., 2013; Villarino et al.,
113 2021). Recent work has suggested that microbial necromass may be a primary precursor to stable
114 organic matter in grasslands (Angst et al., 2021).

115 In this study, we followed plant-fixed C entering the soil and moving into various soil
116 organic matter pools, and also tracked its form and transformations over the course of multiple
117 growing seasons. Initially, we exposed soil to the stable isotope ^{13}C , via a 5-day $^{13}\text{CO}_2$ field
118 labeling of a California annual grassland plant community. To quantify the distribution and two-
119 year dynamics of added ^{13}C tracer in aboveground plant biomass, plant roots, soil, and various
120 soil organic matter pools, we sampled biomass and soil at multiple timepoints following the field
121 pulse labeling (immediately, 3 days, 4 weeks, 6 months, 1 year, and 2 years), and physically
122 fractionated soil samples with a sodium polytungstate density gradient procedure. We isolated 3
123 soil density fractions: (HF) the heavy fraction (mineral-associated soil organic matter, MAOM),
124 (OLF) the occluded-light fraction (micro-aggregate occluded soil organic matter), and (FLF) the
125 free-light fraction (accessible soil organic matter debris) (Golchin et al., 1994; Sollins et al.,
126 2009). We further characterized the chemistry of the heavy fraction ^{13}C using solid state CPMAS
127 ^{13}C NMR spectroscopy, in order to better understand both the composition of carbon newly
128 incorporated into this fraction, as well as the influence of new inputs on the total mineral-
129 associated carbon pool. By combining ^{13}C labeling, soil density fractionation, and solid-state ^{13}C
130 NMR, we sought to temporally characterize the flow and fate of newly fixed C entering this
131 annual grassland soil and understand the dynamics of plant-derived soil organic carbon
132 formation and persistence.

133 **2. Methods**

134 **2.1 Field site description**

135 Field work was conducted at the University of California Hopland Research and
136 Extension Center (HREC), in southwestern Mendocino County, CA (39.004056, -123.085872).

137 Climatic conditions at the field site are similar to conditions across Mediterranean California,
138 with cool and wet winters, and hot, dry summers. Germination of the annual plant community
139 occurs in the fall, following the first significant rainfall of the winter rainy season. Growth is
140 limited throughout much of the winter by sunlight and temperature, and then peaks in the spring,
141 followed by seed production and senescence by early summer (Becchetti et al., 2016). The
142 vegetation community is dominated by naturalized annual grass and forb species including
143 *Avena spp.*, *Festuca spp.*, *Erodium spp.*, and *Bromus spp.* (Bartolome et al., 2007). Today,
144 HREC is operated by the UC system as a working sheep ranch. Our field plots have been fenced
145 off from grazing for >20 years. The soil at our field site belongs to the Squawrock-Witherell
146 complex, a loamy-skeletal, mixed, superactive, thermic Typic Haploxeralf. Underlying parent
147 material is colluvium derived from sandstone (Soil Survey Staff, 2020). Our measurements
148 suggest the dominant clays are muscovite, chlorite, and kaolinite; dominant non-clay minerals
149 are quartz and plagioclase (Table 1).

150 **2.2 Experimental design**

151 Samples collected for this study are a subset from a larger $^{13}\text{CO}_2$ labeling and
152 precipitation manipulation field experiment. In the spring of 2017, sixteen 3.24 m² plots were
153 established, each delineated by a 1-m deep plastic liner to limit soil water equilibration with
154 surrounding soils, and each containing six 40-cm diameter circular subplots. Circular subplots
155 were surrounded by a 15cm deep PVC “collar” designed to be fitted with an above-ground
156 cylindrical chamber for labelling plants with either ^{12}C or ^{13}C -CO₂ (Supplemental Figure 1A).
157 Each circular subplot was subdivided into four 15-cm deep sections via plexiglass dividers; this
158 “wedge” design allowed us to destructively harvest $^{13}\text{CO}_2$ -labeled soil and biomass from a single
159 circular subplot at multiple timepoints following a labeling event (Supplemental Figure 1B).

160 Removable rain-exclusion shelters were installed above all plots and precipitation manipulation
161 began in the fall of 2017 and continued through the 2019-2020 growing season. Of the 16 plots,
162 eight received 50% of average annual precipitation and eight received 100%, where average
163 annual rainfall was calculated from precipitation data collected at HREC dating back to 1951
164 (Supplemental Figure 2). Depending on the timing and quantity of natural rainfall events, field
165 plot precipitation was augmented via manual watering with natural spring water and limited
166 through application of the rain-out shelters.

167 Experimental plots were labeled with ^{12}C or ^{13}C -CO₂ (99 atm %, Cambridge Isotopes) for
168 5 days from February 11-15, 2018, timed to correspond with the expected maximum root
169 development phase of *Avena spp.* plant growth (the plots were seeded the prior year to encourage
170 a *Avena spp.* dominated community). CO₂ levels during the pulse labeling event were monitored
171 with a Picaroon G2200-I analyzer (for ^{13}C) and infrared gas analyzer (IRGA) (for ^{12}C). For the
172 labeling, well-sealed cylindrical chambers made of PAR transmissive PVC film were fitted over
173 two circular subplots per plot (Supplemental Figure 1A). Plants within these chambers were
174 exposed to either $^{12}\text{CO}_2$ or an isotopically labeled analog, $^{13}\text{CO}_2$, resulting in 16 $^{12}\text{CO}_2$ -labeled
175 ‘control’ subplots and 16 $^{13}\text{CO}_2$ -labeled subplots. The headspace CO₂ concentrations within the
176 chambers were maintained between 400-1500ppm during the daytime, and chambers were
177 removed from plots at night. ^{13}C enrichment of $^{13}\text{CO}_2$ -labeled subplots was maintained between
178 30-75 atom%. Within each CO₂ group, 8 of the 16 subplots were under the 50% precipitation
179 regimen, and 8 under the 100% regimen.

180 **2.3 Field harvests of ^{13}C labeled biomass and soil**

181 Soil was harvested at six times following the $^{13}\text{CO}_2$ labeling event: (1) immediately after
182 the 5-day labeling period, (2) three days later, (3) four weeks after the first harvest, (4) six
183 months after the first harvest, (5) one year after the first harvest, and (6) two years after the first
184 harvest. We subsequently refer to these harvest sampling times both by the time elapsed since the
185 ^{13}C labeling event (0 days, 3 days, 4 weeks, 6 months, 1 year, and 2 years), as well as by the
186 season in which the harvest occurred: Spring '18 (4 weeks), Fall '18, Spring '19, and Spring '20.
187 At each sampling time, we harvested 4 replicate samples from each experimental treatment: ^{12}C -
188 labeled, ^{13}C -labeled, 50% precipitation, 100% precipitation. We did not detect a statistically
189 significant ($p < 0.05$) precipitation treatment effect on any soil or plant characteristics measured
190 in this study; thus, replicates from these treatments were pooled for final analyses such that $n=8$
191 for most of the analyses presented.

192 Root and shoot biomass samples were collected at three times following the $^{13}\text{CO}_2$ pulse
193 labeling: (1) at zero days, when we expected the maximum ^{13}C -labeled shoot biomass, (2) at
194 three days, and (3) at four weeks, by which point we expected a measurable fraction of ^{13}C label
195 to have been translocated to root biomass and the surrounding soil. Shoot biomass was collected
196 by clipping all live aboveground plant tissue from a single subplot “wedge”, and then scaling up
197 to g / m^2 . Root biomass was collected from three 2.45-cm cores that were installed in each wedge
198 at the time of harvest and scaling up to the full wedge volume (1/4 X 40cm subplot X 15cm
199 depth), and then g / m^2 . Prior to the 6-month sampling time (Fall18), aboveground litter
200 remaining from the 2017-2018 growing season was removed from the plots.

201 At each of the first four sampling times, we harvested 16 “wedges” of soil. Once soil
202 wedges were harvested, visible roots were removed and soil was homogenized, 500g sub-
203 samples were collected, air-dried, and stored at ambient temperature for further analysis. At each

204 of the final two sampling times (Spring19 and Spring20), we harvested two cores per wedge
205 (2.54 cm diameter X 15cm deep) rather than the whole wedge to preserve the remaining soil for
206 future experiments, however, we harvested the same number of samples as in the previous two
207 harvests. In these two cores we removed roots and homogenized the soil (~300g) as above, then
208 air dried and stored the soil for further analyses. Additional subsamples at each of the four
209 harvests were immediately aliquoted from homogenized soil for analyses requiring fresh soil.

210 Altogether, eight ^{13}C -labeled and eight ^{12}C -labeled shoot, root, and soil samples were
211 harvested at 0 days, 3 days, and 4 weeks after labeling. Senescence of ^{13}C -labeled plants
212 occurred over the summer of 2018, between the 4-week and 6-month sampling times. Therefore,
213 for the remaining sampling times (6-month, 1-year, and 2-year), only soil samples were collected
214 (eight ^{13}C -labeled and eight ^{12}C -labeled).

215 **2.4 Processing and diagnostic analyses of soil, root, and shoot biomass**

216 Aboveground biomass dry weight was determined by drying harvested biomass at 65 °C
217 until a stable dried weight was achieved. Root biomass dry weight was determined by hand
218 picking live roots from collected root biomass cores, washing, and drying at 65 °C until dried
219 weights reached a stable plateau. Dry root biomass per wedge was then calculated by scaling the
220 dried root weights to the volume of the wedge.

221 Soil pH was determined in a 1:1 ratio of fresh homogenized soil to 0.01M CaCl_2 . Soil
222 gravimetric water content was assessed by drying a 10g subsample of fresh homogenized soil at
223 105 °C until dried weights reached a stable plateau when weighed, then calculating percent
224 water. DOC was extracted from 5g fresh soil with 20ml 0.5M K_2SO_4 ; DOC and ^{13}C -DOC was
225 assessed by the Yale Analytical and Stable Isotope Center (YASIC) via wet oxidation method

226 (Lang et al., 2012). Remaining analyses were conducted on homogenized air-dry soil. Soil
227 texture analysis was conducted by the UC Davis Analytical Lab via the hydrometer method
228 (Sheldrick et al., 1993). Aggregate stability was conducted using a wet sieving method
229 previously adapted by Sher et al. (2020), using a custom wet-sieve apparatus (Singer et al., 1992;
230 Sher et al., 2020).

231 **2.5 Soil quantitative X-ray diffraction analysis**

232 Soil mineralogy was determined at Lawrence Livermore National Lab (Zhou et al.,
233 2018). Soil samples were dried, crushed and passed through a 500 μm sieve. Then 3 grams of
234 soil was ground with 15 mL of methanol in a McCrone mill with corundum grinding elements
235 for 5 minutes. The sample was transferred into a plastic tray, air dried and homogenized on a
236 vortex mixer with 10 mm plastic beads for 3 minutes (Bakker et al., 2018). The random powders
237 were then side loaded into XRD sample holders and analyzed on a Bruker D8 advance XRD
238 scanning from 3 to 65° 2Θ with a step size of 0.011° at a rate of 5 seconds per step. Quantitative
239 analysis was done using BGMN Rietveld refinement and the Profex interface software (Doebelin
240 et al., 2015). The XRD patterns were refined to fit crystal unit cell parameters, size, site
241 occupancy and preferred orientation.

242 **2.6 Soil density fractionation**

243 Soil density fractionation was performed on samples collected at 4-weeks, 6-months, 1-
244 year, and 2-year sampling times. Air-dried soil was sieved to 2mm before being density
245 fractionated into three discrete pools of soil organic matter using a sodium polytungstate (SPT)
246 density gradient: free-light fraction (FLF, $\rho < 1.75\text{g}\cdot\text{cm}^{-3}$), occluded-light fraction (OLF, $\rho <$
247 $1.75\text{g}\cdot\text{cm}^{-3}$), and mineral-associated or heavy fraction (HF, $\rho > 1.75\text{g}\cdot\text{cm}^{-3}$). The density of

248 1.75g-cm⁻³ was chosen due to the similarities in mineralogy and soil physical characteristics
249 between this sampling site and the site sampled in *Neurath et al., 2021*, which used this same
250 SPT approach.

251 The method for density fractionation used in our study was adapted from Hicks Pries et
252 al. (2017), previously adapted from Strickland & Sollins (1987). For each sample, 50mL of
253 sodium polytungstate (SPT-0, Geoliquids) prepared to a density of 1.75g-cm⁻³ was added to a
254 250mL centrifuge tube containing 20g of air-dried soil. The mixture was inverted by hand to
255 ensure all soil came into contact with the SPT; soil remaining on the lid and sides was rinsed
256 down with an additional 50mL of SPT. The SPT-soil solutions were allowed to settle for 1 hour,
257 then centrifuged for 1 hour at 3,700 RCF in a swinging bucket rotor (Beckman Avant J-20 Floor
258 Centrifuge with JS-5.3 rotor). Following centrifugation, samples were allowed to settle again
259 until no particles remained suspended within the SPT solution. Particles floating on top of the
260 SPT solution were defined as the FLF and were isolated by aspirating onto a 0.7µm glass
261 microfiber filter (Wattman), and rinsed with MilliQ water to remove residual SPT. Then, the
262 FLF filters were transferred into drying tins in a 55 °C oven until standing water had evaporated.

263 To release the OLF from microaggregates, the remainder of the soil-SPT mixture was
264 mixed with a benchtop mixer for 1 minute, followed by sonication for 90 seconds. As above, the
265 soil was then rinsed, allowed to settle, centrifuged for 1 hour, and the floating fraction isolated
266 via aspiration and dried.

267 The remaining sediment ($\rho > 1.75\text{g-cm}^{-3}$) was defined as the HF. This fraction was rinsed
268 with 150mL Milli-Q H₂O, vigorously shaken by hand, centrifuged for 20 minutes, followed by
269 aspiration and disposal of the supernatant. This was repeated 5 times, or until the density of the

270 supernatant $\sim 1\text{g}\cdot\text{cm}^{-3}$. The HF was transferred into drying tins and dried at 55 °C. Once standing
271 water had evaporated, all three fractions were transferred into a 105 °C oven for 48 hours. Oven-
272 dried samples were cooled in a desiccator before being weighed, ground with a mortar and
273 pestle, and stored in glass vials.

274 **2.7 Isotopic and elemental analysis**

275 Prior to elemental analysis, samples were ground to a fine powder and weighed into
276 aluminum tins, with sample weight proportional to expected carbon content (4mf for the FLF,
277 2mg for the OLF, 50mg for the HF and bulk soil, 0.35mg for shoot biomass, and 0.30mg for root
278 biomass samples). Bulk and density fractionated soil were ground using a mortar and pestle,
279 aboveground biomass was ground using a coffee grinder, and root biomass was ground by hand.
280 Total C, N, and ^{13}C enrichment were measured via total combustion using an elemental analyzer
281 coupled with a continuous flow Isotope Ratio Mass Spectrometer (IRMS) at the Stable Isotope
282 Facility at the University of California, Davis (AOAC Official Method 972.43).

283 **2.8 ^{13}C -NMR**

284 Subsamples of heavy fraction (HF) material were analyzed by ^{13}C -NMR to assess broad
285 chemical composition of mineral-associated organic matter. Solid-state ^{13}C cross polarization
286 magic angle spinning (CPMAS) spectroscopy was run on one ^{13}C -labeled HF sub-sample from
287 the 4-week, 1-year, and 2-year sampling times, as well as one ^{12}C -labeled control HF subsample
288 from the 4-week timepoint as a control comparison. These four spectra were acquired using a
289 4mm log-gamma CPMAS probe on a 500 MHz Bruker Avance 1 NMR spectrometer at the UC
290 Davis NMR facility. Samples were spun at 10 kHz with an acquisition time of 41 ms. Scan
291 number ranged from 75,000 – 102,400. Glycine (176ppm) was used as the external reference.

292 Using Topspin software, data were zero filled to 8k; an exponential function with 500 Hz of line
293 broadening was used for signal processing, with zero order phase correction, followed by manual
294 baseline correction.

295 Broad C functional groups were defined based on the following chemical shift regions:
296 aliphatic/alkyl C (0-45ppm), O-alkyl C (45-110ppm), aromatic and aryl C (110-162ppm), and
297 carbonyl C (162-190ppm) (Helfrich, 2006). Integration of chemical shift regions was conducted
298 using Topspin 3 software to calculate relative contribution of different functional group regions
299 to total peak area (0-190ppm).

300 **2.9 Statistical analyses**

301 Effect of sampling time on C, N, and ^{13}C content was determined using ANOVA.
302 Statistical significance was determined using Tukey's HSD post-hoc test with the R package
303 'agricolae' (Mendiburu, 2015). Data was visualized using the R package 'ggplot2' (Wickham,
304 2016).

305 **2.10 Calculation of ecosystem ^{13}C assimilation**

306 We define ecosystem assimilated ^{13}C as that quantifiable in (1) aboveground (plant
307 biomass) and (2) belowground (soil + root biomass) pools, in other words net ^{13}C gain rather
308 than gross ecosystem exchange. Ecosystem ^{13}C assimilation was calculated by converting the ^{13}C
309 concentration of each pool (aboveground biomass, root biomass, soil in mg / g) to quantity (g ^{13}C
310 / m² over a 15cm sampling depth) and then summing. For simplicity, the sum of ^{13}C recovered in
311 aboveground biomass plus soil and root biomass immediately after the pulse labeling period (0-
312 days) was interpreted to equal 100% of assimilated ^{13}C . The percentage of assimilated ^{13}C
313 remaining at each subsequent sampling timepoint was then calculated relative to this original

314 amount (Table 2). We note that, at the 6-month, 1-year and 2-year sampling times, ¹³C-labeled
315 aboveground biomass and roots had senesced and the aboveground pool was not measured, and
316 roots were not physically separated from the soil as was done for the 0-days and 4-weeks
317 timepoints. Average ¹³C recovery based on summing the FLF + OLF + HF was approximately
318 72% that of the bulk soil, which is within the range for density fractionated soil C recovery
319 values cited in the literature (Crow et al., 2007; Cusack et al., 2018).

320 **3. Results**

321 **3.1 Ecosystem ¹³C Incorporation**

322 Immediately following the ¹³CO₂ labeling period, all four ecosystem pools that we
323 analyzed (soil, shoot and root biomass, DOC) were enriched in ¹³C (Figures 1, 2). We defined
324 the total amount of ¹³C present at the 0-day to be 100% and calculated pools thereafter relative to
325 this starting point. During the 5-day labeling period, over 12g ¹³C / m² derived from plant
326 photosynthate had accumulated in these ecosystem pools, accounting for roughly 0.2% of total
327 ecosystem C content (aboveground biomass, root biomass, and soil in the 0-15cm depth
328 horizon). By 4 weeks, shoot biomass ¹³C content had declined to 61.7% of the initial amount (p
329 = 0.015) (Figure 1, Table 2). Transfer of plant-fixed ¹³C to belowground pools was immediate,
330 accounting for 40% of total ecosystem ¹³C immediately after the labeling. Belowground ¹³C
331 content reached a peak 6 months post-labeling. Between the 6-month, 1-year, and 2-year
332 sampling times, assimilated ¹³C in the ecosystem decreased stepwise: 64% of the original ¹³C
333 remained at 6 months, 37% at 1 year, and 23% at 2 years (Figure 1, Table 2).

334 Samples collected at 0 days, 3 days, and 4 weeks following ¹³CO₂ labeling were used to
335 assess the initial dynamics of ¹³C in shoots, roots and DOC (Figure 2). Shoot ¹³C enrichment (g

336 $^{13}\text{C} / \text{m}^2$) declined significantly over the 4 weeks post labeling. While not statistically significant
337 at $p < 0.05$, mean shoot biomass ^{13}C appeared to decline slightly by 3 days post-labeling, despite
338 no detectable change in shoot biomass--possibly due to loss of recently fixed ^{13}C via plant
339 respiration. Allocation of ^{13}C -labeled photosynthate to the roots occurred immediately, and did
340 not significantly change over the 4 weeks, despite expected dilution of the atom-% ^{13}C by
341 continued root growth. ^{13}C enrichment of the DOC pool did not significantly change between 0
342 days, 3 days, and 4 weeks following the $^{13}\text{CO}_2$ labeling event.

343 **3.2 Soil Density Fractions**

344 We used density fractionation to assess changes in the quantity of newly-fixed plant-
345 derived C in soil samples collected in March of 2018 (Spring18 = 4-week sampling time), the
346 Fall of 2018 (Fall18 = 6-month), April of 2019 (Spring19 ~ 1-year), and March of 2020
347 (Spring20 ~ 2-year). Of the three fractions assessed, the HF accounted for roughly 99% of the
348 total recovered soil mass (Table 3) and contained the highest amount of C, accounting for 66%
349 of the total on average, while the OLF was 20%, and FLF 14% (Figure 3). The distribution of
350 total N followed a similar pattern to total C, but with an even greater proportion of N
351 accumulating in the HF (Table 3): 83% in the HF, 10% in the OLF, and 7% in the FLF. The C:N
352 ratio varied significantly by fraction type: highest in FLF (~20:1), intermediate in OLF (~18:1),
353 and lowest in HF (~8:1), and these differences were statistically significant ($p < 0.05$). Total C,
354 N, and C:N (Table 3, Figure 3) did not exhibit detectable seasonal fluctuations between the
355 Spring and Fall sampling times. For all density fractions, total C was generally lower at the
356 Spring20 sampling than at other sampling times (Figure 3).

357 The ^{13}C labeling event occurred during the growing season in February 2018, and
358 samplings occurred in Spring 2018, Fall 2018, Spring 2019, and Spring 2020. Four weeks after
359 the ^{13}C labeling, 24% of the initial ecosystem ^{13}C was recovered in soil density fractions (Table
360 2). Of that, significantly more was recovered in the HF than in the OLF or FLF. While
361 accumulation of total C was higher in the OLF than FLF, ^{13}C was in the FLF at the 4-week
362 sample time. ^{13}C enrichment (atom-% ^{13}C) was generally highest in the FLF and lowest in the
363 OLF. Between the 4-week and 6-month sampling times, soil organic ^{13}C -labeled carbon (SO^{13}C)
364 recovered in the density fractions roughly doubled, with the majority of additional SO^{13}C
365 accumulating in the FLF (Figure 3, Table 2). At 6 months, the amount of ^{13}C recovered in the
366 FLF was similar to that in the HF. Between 6 months and 1 year after labeling, SO^{13}C content of
367 the density fractions declined by 40%, with a particularly large decrease observed in FLF
368 material. The 2-year ^{13}C recovery in the soil density fractions was 55% of that recovered in the
369 1-year samples. By Spring20, over two years after the original ^{13}C addition, isotopically labeled
370 carbon persisted in all three fractions, with over 50% of the remaining ecosystem ^{13}C
371 (representing 13.5% of the initial ecosystem ^{13}C content) found in protected forms, either
372 occluded within soil microaggregates (OLF) or associated with soil minerals (HF) (Table 2).

373 **3.3 ^{13}C NMR analysis of mineral-associated carbon**

374 Heavy fraction (HF) material from 4-week, 1-year, and 2-year sampling times was
375 analyzed by ^{13}C NMR to assess the molecular forms taken by the newly fixed ^{13}C and present in
376 the HF. Likely due to the presence of paramagnetic minerals in these soils, the peaks in the ^{13}C
377 NMR spectra were broad and hence we were not able to identify specific compounds. Instead,
378 we assessed relative proportions of broad chemical classes based on chemical shift regions in the

379 NMR spectra: alkyl C 0-45ppm, O-alkyl C 45-110ppm, aromatic C 110-162ppm, and carbonyl C
380 162-190ppm (Figure 4, Supplemental Figure 5).

381 Labeling plants with $^{13}\text{CO}_2$ allowed us to follow the functional group characteristics of
382 newly fixed C incorporated into the mineral-associated pool (Figure 4). The SO^{13}C -HF appeared
383 to be relatively enriched in alkyl C with lesser amounts of aromatic and carboxyl C. In the ^{13}C
384 NMR spectra, the relative proportion of alkyl C declined over time from 4 weeks to 1 year to 2
385 years after the ^{13}C labeling period. Over this same period, the relative proportion of carboxyl C
386 increased, while the relative proportion of aromatic C and O-alkyl C remained constant. While
387 the proportion of C functional groups in the ^{13}C -labeled HF material was distinct from the ^{12}C -
388 labeled control HF material at all sampling times, the relative proportions of C functional groups
389 in the ^{13}C -labeled material appeared to become more similar to those observed in the control
390 material over time.

391 **4. Discussion**

392 **4.1 Ecosystem ^{13}C Incorporation**

393 In this California annual grassland soil, growing plants quickly allocated a substantial
394 proportion of photosynthate belowground. We traced the translocation of plant photosynthate to
395 belowground carbon pools during a 5-day $^{13}\text{CO}_2$ field labeling (Figure 1). Our labeling period
396 occurred in late winter (February 11-15, 2018), which was intended to correspond to the plant
397 growth stage of maximum allocation of aboveground photosynthate C to root growth (Jackson et
398 al., 1989). By the end of this 5-day period, 40% of assimilated ^{13}C had been allocated
399 belowground (Figure 1).

400 ^{13}C recovery in plant roots is a clear indication of the incorporation of fresh plant-derived
401 carbon inputs into the soil, particularly as these ^{13}C -labeled roots decompose over time. We
402 observed evidence of this phenomenon at the 6-month sampling time, when ^{13}C recovery in FLF
403 material reached its peak. We presume this was primarily due to incorporation of ^{13}C labeled root
404 litter from the previous growing season. However, at the 4-week sampling time, we already
405 observed high ^{13}C recovery (nearly 38%) in all our soil density fractions, from which we had
406 removed live roots. This suggests that substantial ^{13}C was exuded into the soil by the roots
407 during this period of plant growth, or that other types of rhizodeposits (sloughed root tip cells,
408 cell hairs) had become part of the soil's organic matter pools (Table 2).

409 In our study, the ^{13}C enrichment of root tissue was similar to the enrichment of the DOC
410 pool when measured either immediately or 3 days after the end of the labeling period (Figure 2).
411 This suggests that root exudates were in equilibrium with root biomass enrichment during this
412 plant growth stage. By the 4-week sampling time, ^{13}C enrichment appears to slightly increase in
413 plant roots and decline in soil DOC (although neither increase nor decrease was statistically
414 significant), which could imply a shift of plant C allocation from labile root exudates, to root
415 structural compounds. Furthermore, we observed a slight decline in belowground ^{13}C content
416 between 0 days and 4 weeks following the ^{13}C labeling (Figure 1), which is likely from root and
417 microbial respiration. These findings indicate that as plants continue to grow, recently-fixed
418 photosynthate was rapidly translocated within the plant and released into the soil as labile C
419 compounds exuded by roots (and perhaps also by associated arbuscular mycorrhizal fungi
420 (AMF) (Kakouridis et al., 2021)). Almost just as rapidly, these labile exudates are metabolized
421 by the active rhizosphere microbial communities (Waldrop & Firestone, 2006).

422 We found that total ecosystem ^{13}C assimilated (above + belowground ^{13}C content) at the
423 4-week sampling time was statistically indistinguishable from the belowground assimilated ^{13}C
424 at the 6-month sampling time. Root growth of annual species characteristic of these systems has
425 been shown to decline by March (Jackson et al., 1989), suggesting that the ^{13}C recovered in the
426 belowground pool at the 4-week sampling time may have been primarily composed of structural
427 plant root compounds. Additionally, under typical rainfall conditions, soil respiration in
428 California annual grasslands has been shown to greatly decline by early April (Eviner, 2001), as
429 sources of labile C substrates dwindle, and soil moisture begins to decline. This could explain
430 why we saw little evidence of late growing-season decomposition or loss of ^{13}C . ^{13}C recovery in
431 the belowground pool at our 6-month sampling time was 64% of the total ^{13}C present
432 immediately following the labeling period. Other studies in California annual grassland
433 ecosystems have described similarly high accumulations of C (Schaeffer et al., 2017), and added
434 ^{13}C (Castanha et al., 2018) over the summer dry period. This appears to occur because of the
435 large C input as dead root litter (following annual plant senescence and death in June-July each
436 year), and the reduced ability of microbes to access and decompose this C due to the very low
437 soil moisture characteristic of the Mediterranean-type summer; desiccation results in very low
438 activity of decomposers as well as physical isolation from C substrates (Blankinship & Schimel,
439 2018).

440 **4.2 Soil density fractions**

441 In our study, the vast majority of soil organic carbon was recovered in the HF pool. We
442 assume the heavy fraction is primarily composed of mineral-associated organic matter (MAOM),
443 i.e. organic matter stabilized via mineral sorption and co-precipitation mechanisms (Kogel-
444 Knabner et al., 2008). Mineral-associated SOM generally has a C:N ratio that aligns with the

445 C:N ratio of microbes (~10), as opposed to living plant material (>20), (Clemente et al., 2011).
446 The C:N ratio of the HF in our study was consistently near 9:1, suggesting this HF carbon is
447 largely of microbial origin. It has been shown that microbial products are more efficiently and
448 effectively adsorbed to and thus stabilized by soil minerals than are plant-derived compounds
449 (Lavalley et al., 2019). Furthermore, microbial transformation of detrital carbon inputs has been
450 shown to be a critical precursor to long-term carbon stabilization (Cotrufo et al., 2013).

451 The C:N ratios of the FLF and OLF were approximately 20:1 and 17.5:1 respectively,
452 reflecting a more plant-like signature than the HF material, and the C:N ratio of the OLF was
453 consistently slightly lower than that of the FLF. As well, we found slightly higher total carbon in
454 the OLF than in the FLF. California annual grasslands are typically dominated by annual
455 herbaceous plant communities whose litter decomposes completely within three years (Eviner &
456 Firestone, 2007), resulting in minimal FLF accumulation compared to other ecosystems (Crow et
457 al., 2007). C-rich fungal hyphae, bacterial EPS, and plant mucilage promote soil aggregation
458 both as physical structuring agents as well as major contributors to aggregate-associated soil
459 carbon (Six et al. 2004). OLF material has been found to be largely dominated by fine root
460 fragments and fungal hyphae (Kakouridis et al., 2021). The intermediate OLF C:N ratio we
461 measured (between that of FLF and HF) suggests that microbial constituents and aggregation
462 mechanisms such as fungal hyphae and bacterial EPS may have contributed to OLF formation in
463 our samples, although extensive microbial processing of OLF material is likely limited due to
464 physical protection mechanisms. If we interpret C:N ratios as an indicator of decomposition, then
465 our data suggests increasing decomposition progressing from FLF to OLF to HF (Hyvonen et al.,
466 1996).

467 The source compounds of ^{13}C recovered in the density fractions likely differs by season.
468 ^{13}C recovered from Spring18-harvested soil likely represent mostly rhizodeposits and labile
469 carbon substrates exuded by roots and possibly consumed by root-associated bacteria and fungi
470 as well as AMF-mediated carbon flow from $^{13}\text{CO}_2$ -labeled plants (Kakouridis et al., 2021). By
471 our Fall18 sampling, which occurred at the end of the summer dry period, much of the ^{13}C in
472 FLF would have been composed of senesced/dead $^{13}\text{CO}_2$ - labeled root detritus; we assume that
473 most of the ^{13}C in the HF and OLF was root-derived (Jackson et al., 2017). The onset of the
474 2018-2019 growing season (due to fall and winter rainfall), likely triggered decomposition of this
475 senesced ^{13}C - labeled shoot material derived from the previous growing season. Some of this
476 decomposing ^{13}C -labeled material could have been incorporated into the soil, representing an
477 additional potential ^{13}C source by the Spring19 sampling time. By Spring20 we assume that ^{13}C
478 recovered in the soil density fractions largely represents soil organic carbon that persisted from
479 Spring19.

480 ^{13}C -SOC was recovered in all three density fractions at 4 weeks, 6 months, 1 year, and 2
481 years after ^{13}C labeling (Figure 3). We observed a conspicuous increase in FLF ^{13}C content
482 between 4-weeks and 6-months sampling times due to rapid incorporation of ^{13}C -labeled root
483 detritus into this fraction following plant senescence and death, as well as likely incorporation of
484 some aboveground litter; however, an equally conspicuous decrease in ^{13}C within this fraction
485 was observed between 6 months and 1 year after ^{13}C labeling. Between the Fall of 2018 and
486 Spring of 2019, about 38% of FLF material was either converted into more protected forms or
487 lost as CO_2 . Such seasonal transience was not as apparent in the OLF or HF.

488 Additionally, of the three fractions, ^{13}C enrichment was generally lowest in the OLF,
489 indicating that this fraction is not as dynamic as the FLF, or perhaps even as the HF. This could

490 suggest that the incorporation of “new” carbon into soil aggregates occurs less rapidly than does
491 association of this carbon with minerals, or that stable aggregate formation requires repeated
492 iterations of certain environmental conditions, such as annual plant growth periods or seasonal
493 wet-dry cycles, the extent of which were not captured in the timespan of this study (Totsche et
494 al., 2018). However, over this 2-year study, we see the distribution of ^{13}C among the density
495 fractions approaching that of total C; it is likely that with additional time, the quantity of ^{13}C
496 remaining in the OLF will exceed that remaining in the FLF (Figure 3).

497 The rapid association of rhizosphere-derived carbon with mineral surfaces was also
498 observed by *Neurath et al. 2021*, in similar soils, who characterized the short-term dynamics (3
499 months) of root-input carbon. In that study, over the course of a 2-month incubation, the flux of
500 new carbon onto and off of the mineral surfaces was substantial (accounting for over 6% of total
501 C) while total mineral-associated C remained constant. For our study, this finding implies that
502 some of the HF association with ^{13}C OM could in fact be more dynamic than what we observed.
503 By our two-year sampling, 77% of initial ^{13}C stock had been lost from the system. However, of
504 the carbon remaining, over 50% was in a protected form (occluded within soil microaggregates
505 or associated with soil minerals) (Table 2). Furthermore, by four weeks after ^{13}C labeling, we
506 recovered 61% of soil ^{13}C in the HF (Table 2), and quantity of ^{13}C recovered in HF material did
507 not detectably decline over the course of the study. This observation supports the importance of
508 root and rhizosphere-derived carbon inputs in SOM formation (Sokol & Bradford, 2019; Pett-
509 Ridge & Firestone, 2017), and is consistent with rapid microbially-mediated stabilization of
510 carbon onto mineral surfaces (Kallenbach et al., 2016).

511 Our work indicates that initial associations between minerals and root-derived organic
512 matter are significant and form rapidly. While the majority of annual C inputs to soil cycle

513 rapidly (<2-year timescale), a sizeable proportion (11.5% original ^{13}C inputs persist in HF by
514 year 2) can potentially persist longer. In a study modeling soil carbon turnover in a California
515 grassland site with similar physical characteristics to ours, Torn et al. (2013) calculated that 7%
516 of HF carbon sampled from the 1-15cm depth was “fast” cycling (<2 year turnover) and 93%
517 cycled on a centennial timescale. Our observations provide support for the existence of this small
518 yet rapidly cycling HF carbon pool, but also suggest that the HF does not represent a single C
519 pool operating on a single timescale, but rather multiple pools operating on multiple timescales
520 (Lehmann & Kleber, 2015).

521 **4.3 ^{13}C NMR on heavy fraction**

522 The combination of techniques used in this study provides a window into the dynamics
523 and chemical characteristics of MAOM formation. The labeling of field plots with $^{13}\text{CO}_2$ with
524 subsequent sampling and soil density fractionation allowed us to trace the flow and fate of plant-
525 derived inputs into organo-mineral associations. By applying ^{13}C CPMAS NMR spectroscopy to
526 our ^{13}C labeled heavy fraction samples, we sought to further resolve the chemistry of “new”
527 plant-derived carbon in the heavy fraction.

528 It is generally accepted that microbial products are a dominant source of mineral-
529 stabilized organic matter that builds up in heavy fraction material over time (Preston et al., 2009;
530 Creamer et al., 2019). The alkyl C functional group may represent a variety of microbial and
531 plant-derived aliphatic compounds such as lipids, proteins, and waxes (Kögel-Knabner, 1997). In
532 fact, in HF material isolated from a similar site at HREC, Neurath et al. found mineral-associated
533 lipids were largely microbially derived (Neurath et al., 2021). Peaks in the O-Alkyl C region may
534 represent various carbohydrates, proteins, and amino acids (Mathers et al., 2007), including

535 plant-derived carbohydrates such as cellulose and hemicellulose (Kögel-Knabner, 1997), as well
536 as N-rich proteins and root-exudate derived sugars (Angst et al., 2018). However, O-Alkyl C
537 proportions in HF samples have also been attributed to carbohydrates of microbial origin
538 (Schöning et al., 2006). The aromatic C functional group can contain plant-derived phenolic
539 compounds such as lignin and tannins, aromatic portions of proteins and amino acids, as well as
540 condensed, chemically resistant “black carbon” derived from historically frequent wildfires that
541 occurred in California annual grasslands (Sanderman et al., 2008; Czimczik & Masiello, 2007).
542 The carboxyl C functional group has been described to encompass highly oxidized C forms such
543 as organic acids, ketones, and aldehydes (Mathers et al., 2007), and can be used as an indicator
544 of microbial processing (Ng et al., 2014).

545 ^{13}C NMR spectra are only sensitive to molecules containing ^{13}C . In Spring, 2018, we
546 introduced ^{13}C into the soil. The NMR spectra of the mineral-associated samples collected over
547 the following two years show a decline in the proportion of ^{13}C in the alkyl C functional group,
548 and an increase in the proportion of ^{13}C in the carboxyl C, aromatic C, and carboxyl C functional
549 groups. The relatively constant quantity of ^{13}C present in the HF over the course of the study
550 suggests that alkyl ^{13}C is being converted to carboxyl ^{13}C , or that perhaps, that a portion of HF
551 ^{13}C is transient as suggested by Neurath et al., (2021), and that over the course of our study,
552 some alkyl ^{13}C is lost and some carboxyl ^{13}C is accumulated. Carboxyl C content can be
553 indicative of highly oxidized organic matter (Kogel-Knabner et al., 2008). As such, the increase
554 in the relative proportion of carboxyl C we see over the 2-year study period could have resulted
555 from the oxidation of organic matter during the process of decomposition (Baldock et al., 1992).
556 The fact that we see a higher proportion of alkyl C shortly after the $^{13}\text{CO}_2$ labeling period than
557 we do two years later suggests that rhizodeposit C is a substantial source of rapidly forming

558 mineral-alkyl C associations, whether as plant waxes such as cutin, or microbial lipids as
559 suggested by Neurath et al., (2021).

560 Within a two-year period, we see an evolution of the effect of newly introduced ^{13}C on
561 the ^{13}C NMR spectra of the heavy fraction (Figure 4). We expect that some of the added ^{13}C in
562 this fraction underwent chemical transformations during this period and that over time, only the
563 most persistent ^{13}C -HF associations will be retained as less persistent associations disappear.
564 This could leave a legacy of the added ^{13}C label that persists for decades or even centuries
565 (Baisden et al., 2002), while the overall effect of the added ^{13}C label should decline over time.

566 **5. Conclusion and future directions**

567 Understanding the patterns and control of soil organic carbon cycling in California annual
568 grasslands is a critical precursor to any soil carbon management efforts within these ecosystems.
569 Our study traced soil organic carbon formation from plant photosynthesis through its movement
570 into and between soil fractions—and chemical forms in the heavy fraction—for two years. By
571 applying a combination of analytical and spectroscopic techniques, we followed plant carbon
572 from living roots to root detritus to occluded carbon and mineral-associated pools. About a
573 quarter of the C fixed during the 5-day labeling was still present two years after the labeling;
574 most of that “2-year old” carbon was found in the mineral associated heavy fraction. Solid-state
575 ^{13}C NMR spectroscopy was sufficiently sensitive to the ^{13}C introduced that we were able to
576 detect the photosynthetically derived carbon movement into and through the components of the
577 mineral-associated carbon pool. This “new” carbon appears to have a distinct chemical
578 fingerprint from the total background C; that spectroscopic profile declines over the 2 years in
579 the field.

580 ^{13}C analysis and ^{13}C NMR both revealed that the movement of carbon into the heavy
581 fraction occurred rapidly in our system, within four weeks of plant photosynthesis, and that the
582 influence of this “new” carbon on the total background C persisted over the course of our study.
583 Future research that distinguishes between the labile vs. litter inputs on stabilized soil organic
584 carbon formation, and that tracks those dynamics over decadal timescales, would further resolve
585 how plant community characteristics (plant growth stage, lifecycle, seasonal climate parameters)
586 influence the accrual and persistence of soil organic carbon and help us to better predict the
587 responsiveness of annual grassland ecosystems to soil carbon management.

588

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599 Angst, G., Messinger, J., Greiner, M., Häusler, W., Hertel, D., Kirfel, K., ... Mueller, C. W.
600 (2018). Soil organic carbon stocks in topsoil and subsoil controlled by parent material,

601 carbon input in the rhizosphere, and microbial-derived compounds. *Soil Biology and*
602 *Biochemistry*, 122, 19–30. <https://doi.org/10.1016/j.soilbio.2018.03.026>

603 Angst, G., Mueller, K. E., Klass, G. J. N., Simpson, M. J. (2021). Plant- or microbial-derived? A
604 review on the molecular composition of stabilized soil organic matter. *Soil Biology and*
605 *Biochemistry*, 156. <https://doi.org/10.1016/j.soilbio.2021.108189>

606 AOAC Official Method 972.43, Microchemical Determination of Carbon, Hydrogen, and
607 Nitrogen, Automated Method, in *Official Methods of Analysis of AOAC International*,
608 16th Edition (1997), Chapter 12, pp. 5-6, AOAC International, Arlington, VA.

609 Bailey, V. L., Pries, C. H., & Lajtha, K. (2019). What do we know about soil carbon
610 destabilization? *Environmental Research Letters*, 14(8), 083004.
611 <https://doi.org/10.1088/1748-9326/ab2c11>

612 Baker, S. E., Peridas, G., Stolaroff, J. K., Goldstein, H. M., Pang, S. H., Lucci, F. R., Li, W.,
613 Slessarev, E. W., Pett-Ridge, J., Ryerson, F. R., and Aines, R. D., (2019). Getting to
614 Neutral: Options for Negative Carbon Emissions in California (No. LLNL-TR-796100).
615 Lawrence Livermore National Laboratory (LLNL), Livermore, CA (United States).

616 Baisden, W. T., Amundson, R., Cook, A. C., & Brenner, D. L. (2002). Turnover and storage of C
617 and N in five density fractions from California annual grassland surface soils. *Global*
618 *Biogeochemical Cycles*, 16(4), 64–1. <https://doi.org/10.1029/2001gb001822>

619 Bakker, E.; Hubert, F.; Wander, M.M.; Lanson, B. Soil Development under Continuous
620 Agriculture at the Morrow Plots Experimental Fields from X-ray Diffraction Profile
621 Modelling. *Soil Syst.* 2018, 2, 46. <https://doi.org/10.3390/soilsystems2030046>

- 622 Baldock, J. A., Oades, J. M., Waters, A. G., Peng, X., Vassallo, A. M., Wilson, M. A. (1992).
623 Aspects of the chemical structure of soil organic materials as revealed by solid-state ¹³C
624 NMR spectroscopy. *Biogeochemistry*, 16(1), 1-42.
- 625 Barnard, R., Blazewicz, S., and Firestone, M. (2020). Rewetting of soil: revisiting the origin of
626 soil CO₂ emissions. *Soil Biol Biochem.* <https://doi.org/10.1016/j.soilbio.2020.107819>
627
628
- 629 Barnard, R. L., Osborne, C. A., & Firestone, M. K. (2013). Responses of soil bacterial and fungal
630 communities to extreme desiccation and rewetting. *The ISME Journal*, 7(11), 2229-2241.
631 <https://doi.org/10.1038/ismej.2013.104>
- 632 Bartolome, J. W., J. Barry, T. Griggs, and P. Hopkinson. (2007). Valley Grasslands. M. G.
633 Barbour, T. Keeler-Wolf, and A. A. Schoenherr (Ed.), *Terrestrial Vegetation of*
634 *California* (pp. 367-393). University of California Press, Berkeley, California.
- 635 Becchetti, T., George, M., et al. (2016). Rangeland Management Series: Annual Range Forage
636 Production. University of California ANR Publication 8018, 1-12.
- 637 Biggs, N. B., Huntsinger, L. (2021). Managed grazing on California annual rangelands in the
638 context of state climate policy. *Rangeland Ecology and Management*, 76(1), 56-68.
639 <https://doi.org/10.1016/j.rama.2021.01.007>
- 640 Birch, H. F. (1958). The Effect of soil drying on humus decomposition and nitrogen availability.
641 *Plant and Soil*, 10(1), 9-31. <https://doi.org/10.1007/bf01343734>
- 642 Blankinship, J., & Schimel, J. (2018). Biotic versus Abiotic Controls on Bioavailable Soil
643 Organic Carbon. *Soil Systems*, 2(1), 10. <https://doi.org/10.3390/soilsystems2010010>
- 644 Blazewicz, S. J., Hungate, B. A., Koch, B. J., Nuccio, E. E., Morrissey, E., Brodie, E. L., ...
645 Firestone, M. K. (2020). Taxon-specific microbial growth and mortality patterns reveal

646 distinct temporal population responses to rewetting in a California grassland soil. The
647 ISME Journal. <https://doi.org/10.1038/s41396-020-0617-3>

648 Bradford, M. A., Carey, C. J., Atwood, L., Bossio, D., Fenichel, E. P., Gennet, S., ... Wood, S. A.
649 (2019). Soil carbon science for policy and practice. *Nature Sustainability*, 2(12), 1070–
650 1072. <https://doi.org/10.1038/s41893-019-0431-y>

651 Castanha, C., Zhu, B., Hicks Pries, C. E., Georgiou, K., Torn, M. S. (2018). The effects of
652 heating, rhizosphere, and depth on root litter decomposition are mediated by soil
653 moisture. *Biogeochemistry*, 137(1), 267-279. <https://doi.org/10.1007/s10533-017-0418-6>

654 Chou, W. W., Silver, W. L., Jackson, R. D., Thompson, A. W., & Allen-Diaz, B. (2008). The
655 sensitivity of annual grassland carbon cycling to the quantity and timing of rainfall.
656 *Global Change Biology*, 14(6), 1382-1394. [https://doi.org/10.1111/j.1365-
657 2486.2008.01572.x](https://doi.org/10.1111/j.1365-2486.2008.01572.x)

658 Clemente, J. S., Simpson, A. J., & Simpson, M. J. (2011). Association of specific organic matter
659 compounds in size fractions of soils under different environmental controls. *Organic
660 Geochemistry*, 42(10), 1169–1180. <https://doi.org/10.1016/j.orggeochem.2011.08.010>

661 Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial
662 Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition
663 with soil organic matter stabilization: do labile plant inputs form stable soil organic
664 matter? *Global Change Biology*, 19(4), 988–995. <https://doi.org/10.1111/gcb.12113>

665 Cotrufo, M. F., Soong, J. L., Horton, A. J. Campbell, E. E., Haddix, M. L., Wall, D. H., &
666 Parton, W. J. (2015) Formation of soil organic matter via biochemical and physical
667 pathways of litter mass lost. *Nature Geoscience*, 8(10), 776-779.
668 <https://doi.org/10.1038/ngeo2520>

- 669 Cotrufo, M. F., Ranalli, M. G., Haddix, M. L., Six, J., & Lugato, E. (2019). Soil carbon storage
670 informed by particulate and mineral-associated organic matter. *Nature Geoscience*,
671 12(12), 989–994. <https://doi.org/10.1038/s41561-019-0484-6>
- 672 Creamer, C. A., Foster, A. L., Lawrence, C., McFarland, J., Schulz, M., Waldrop, M. P. (2019).
673 Mineralogy dictates the initial mechanism of microbial necromass association. *Geochim
674 Cosmochim Acta*, 260, 161-176. <https://doi.org/10.1016/j.gca.2019.06.028>
- 675 Crow, S. E., Swanston, C. W., Lajtha, K., Brooks, J. R., Keirstead, H. (2007). Density
676 fractionation of forest soils: methodological questions and interpretation of incubation
677 results and turnover time in an ecosystem context. *Biogeochemistry*, 85, 69-90.
678 <https://doi.org/10.1007/s10533-007-9100-8>
- 679 Czimczik, C. I. & Maseillo, C. A. (2007). *Global Biogeochemical Cycles*, 21(1), GB3005.
680 <https://doi.org/10.1029/2006GB002798>
- 681 Dass, P., Houlton, B. Z., Wang, Y., Warlind, D. (2018). Grasslands may be more resilient carbon
682 sinks than forests in California. *Environ. Res. Lett.* 13(7). [https://doi.org/10.1088/1748-
9326/aacb39](https://doi.org/10.1088/1748-
683 9326/aacb39)
- 684 Doebelin, N.; Kleeberg, R. (2015). Profex: A graphical user interface for the Rietveld refinement
685 program BGMN. *J. Appl. Cryst.* 48, 1573–1580.
- 686 Eviner, V. (2001). Linking plant community composition and ecosystem dynamics: interactions
687 of plant traits determine the ecosystem effects of plant species and plant species mixtures.
688 PhD Dissertation. Integrative Biology. The University of California, Berkeley. 404 pp.
- 689 Eviner, V. T., & Firestone, M. K. (2007). Mechanisms Determining Patterns of Nutrient
690 Dynamics. M. Stromberg, J. Corbin, and C. D'Antonio (Ed.), *California Grasslands:*

- 691 *Ecology and Management*, (pp. 94-106). University of California Press, Berkeley,
692 California
- 693 Golchin, A., Oades, J., Skjemstad, J., Clarke, P. (1994). Study of free and occluded particulate
694 organic matter in soils by solid state ^{13}C CP/MAS NMR spectroscopy and scanning
695 electron microscopy. *Soil Res.* 32, 285-309. <https://doi.org/10.1071/SR9940285>
- 696 Heady, H. F., Bartolome, J. W., Pitt, M. D., Savelle, G. D., Stroud, M. C. (1992). California
697 Prairie. Pages 313-335 in R. T. Coupland, editor. *Natural Grasslands. Ecosystems of the*
698 World 8A. Elsevier, New York, New York.
- 699 Helfrich, M., Ludwig, B., Buurman, P., & Flessa, H. (2006). Effect of land use on the
700 composition of soil organic matter in density and aggregate fractions as revealed by
701 solid-state ^{13}C NMR spectroscopy. *Geoderma*, 136(1-2), 331-341.
702 <https://doi.org/10.1016/j.geoderma.2006.03.048>
- 703 Hicks Pries, C. E., Bird, J. A., Castanha, C., Hatton, P.-J., & Torn, M. S. (2017). Long term
704 decomposition: the influence of litter type and soil horizon on retention of plant carbon
705 and nitrogen in soils. *Biogeochemistry*, 134(1-2), 5-16. [https://doi.org/10.1007/s10533-](https://doi.org/10.1007/s10533-017-0345-6)
706 017-0345-6
- 707 Hyvonen, R., Agren, G. I., & Andren, O. (1996). Modelling Long-Term Carbon and Nitrogen
708 Dynamics in an Arable Soil Receiving Organic Matter. *Ecological Applications*, 6(4),
709 1345-1354. <https://doi.org/10.2307/2269612>

- 710 Jackson, L. E., Schimel, J. P., & Firestone, M. K. (1989). Short-term partitioning of ammonium
711 and nitrate between plants and microbes in an annual grassland. *Soil Biology and*
712 *Biochemistry*, 21(3), 409-415. [https://doi.org/10.1016/0038-0717\(89\)90152-1](https://doi.org/10.1016/0038-0717(89)90152-1)
- 713 Jackson, R. B., Lajtha, K., Crow, S. E., Hugelius, G., Kramer, M. G., & Piñeiro, G. (2017). The
714 Ecology of Soil Carbon: Pools, Vulnerabilities, and Biotic and Abiotic Controls. *Annual*
715 *Review of Ecology, Evolution, and Systematics*, 48(1), 419–445.
716 <https://doi.org/10.1146/annurev-ecolsys-112414-054234>
- 717 Kakouridis, A., Yuan, M., Hagen, J., Fossum, C., Moore, M., Herman, D., Nico, P., Weber, P.,
718 Pett-Ridge, J., Firestone, M. (2021). AMF transport of plant carbon into soil alters the
719 characteristics of soil organic carbon as well as the soil microbial community. *bioRxiv*
720 <https://doi.org/10.1101/2020.09.21.305409>
- 721 Kallenbach, C. M., Frey, S. D., & Grandy, A. S. (2016). Direct evidence for microbial-derived
722 soil organic matter formation and its ecophysiological controls. *Nature Communications*,
723 7(1). <https://doi.org/10.1038/ncomms13630>
- 724 Kögel-Knabner, I. (1997). ¹³C and ¹⁵N NMR spectroscopy as a tool in soil organic matter
725 studies. *Geoderma*, 80(1), 243-260.
- 726 Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S.,
727 Eusterhues, K., Leinweber, P. (2008). Organo-mineral associations in temperate soils:
728 Integrating biology, mineralogy, and organic matter chemistry. *Journal of Plant Nutrition*
729 *and Soil Science*, 171(1), 61–82. <https://doi.org/10.1002/jpln.200700048>

- 730 Lang, S. Q., Bernasconi, S. M., & Fröh-Green, G. L. (2012). Stable isotope analysis of organic
731 carbon in small ($\mu\text{g C}$) samples and dissolved organic matter using a GasBench
732 preparation device. *Rapid Commun. Mass Spectrom*, 26(1), 9-16.
733 <https://doi.org/10.1002/rcm.5287>
- 734 Lavallee, J. M., Soong, J. L., & Cotrufo, M. F. (2019). Conceptualizing soil organic matter into
735 particulate and mineral-associated forms to address global change in the 21st century.
736 *Global Change Biology*, 26(1), 261–273. <https://doi.org/10.1111/gcb.14859>
- 737 Lehmann, J., & Kleber, M. (2015). The contentious nature of soil organic matter. *Nature*,
738 528(7580), 60–68. <https://doi.org/10.1038/nature16069>
- 739 Mathers, N. J., Jalota, R. K., Dalal, R. C., Boyd, S. E. (2007). ^{13}C -NMR analysis of decomposing
740 litter and fine roots in the semi-arid Mulga Lands of southern Queensland. *Soil Biology*
741 *and Biochemistry*, 39(1), 993-1006. <https://doi.org/10.1016/j.soilbio.2006.11.009>
- 742 Manzoni, S., & Katul, G. (2014). Invariant soil water potential at zero microbial respiration
743 explained by hydrological discontinuity in dry soils. *Geophysical Research Letters*,
744 41(20), 7151-7158. <https://doi.org/10.1002/2014g1061467>
- 745 Mendiburu, F. D. (2015). *Agricolae: statistical procedures for agricultural research*. Version 1.2-
746 3. <http://CRAN.R-project.org/package=agricolae>
- 747 Neurath, R., Pett-Ridge, J., Chu-Jacoby, I., Herman, D., Whitman, T., Nico, P., Lipton, A. S.,
748 Kyle, J., Tfaily, M. M., Thompson, A., Firestone, M. K. (2021). Root carbon interaction
749 with soil minerals is dynamic, leaving a legacy of microbially-derived residues. *bioRxiv*.
750 <https://doi.org/10.1101/2021.03.23.436628>
- 751 Ng, E.-L., Patti, A. F., Rose, M. T., Schefe, C. R., Wilkinson, K., Smernik, R. J., Cavagnaro, T.
752 R. (2014). Does the chemical nature of soil organic carbon drive the structure and

- 753 functioning of soil microbial communities? *Soil Biology & Biochemistry*, 70(1). 54-61.
754 <http://dx.doi.org/10.1016/j.soilbio.2013.12.004>
- 755 Pett-Ridge, J., & Firestone, M. K. (2017). Using stable isotopes to explore root-microbe-mineral
756 interactions in soil. *Rhizosphere*, 3, 244–253. <https://doi.org/10.1016/j.rhisph.2017.04.016>
- 757 Pett-Ridge, J., Shi, J., Estera-Molina, K., Nuccio, E. E., Yuan, M., Rijkers, R., Swenson, T.,
758 Zhalnina, K., Northern, T., Zhou, J., Firestone, M. K. (2021). Rhizosphere Carbon
759 Turnover from Cradle to Grave: The Role of Microbe-Plant Interactions. In: Gupta V.,
760 Sharma A. (eds) *Rhizosphere Biology: Interactions Between Microbes and Plants*.
761 *Rhizosphere Biology*. Springer, Singapore. https://doi.org/10.1007/978-981-15-6125-2_2.
- 762 Poeplau, C., Don, A., Six, J., Kaiser, M., Benbi, D., Chenu, C., ... Nieder, R. (2018). Isolating
763 organic carbon fractions with varying turnover rates in temperate agricultural soils – A
764 comprehensive method comparison. *Soil Biology and Biochemistry*, 125, 10–26.
765 <https://doi.org/10.1016/j.soilbio.2018.06.025>
- 766 Preston, C. M., Nault, J. R., & Trofymow, J. A. (2009). Chemical changes during 6 years of
767 decomposition of 11 litter in some Canadian forest sites. Part 2. ¹³C abundance, solid-
768 state ¹³C NMR spectroscopy and the meaning of “lignin”. *Ecosystems*, 12(1), 1078-1102.
769 <https://doi.org/10.1007/s10021-009-9267-z>
- 770 Sanderman, J., Baldock, J. A., & Amundson, R. (2008). Dissolved organic carbon chemistry and
771 dynamics in contrasting forest and grassland soils. *Biogeochemistry*, 89(2), 181-198.
- 772 Schaeffer, S. M., Homyak, P. M., Boot, C. M., Roux-Michollet, D., & Schimel, J. P. (2017). Soil
773 carbon and nitrogen dynamics throughout the summer drought in a California annual
774 grassland. *Soil Biology and Biochemistry*, 115, 54–62.
775 <https://doi.org/10.1016/j.soilbio.2017.08.009>

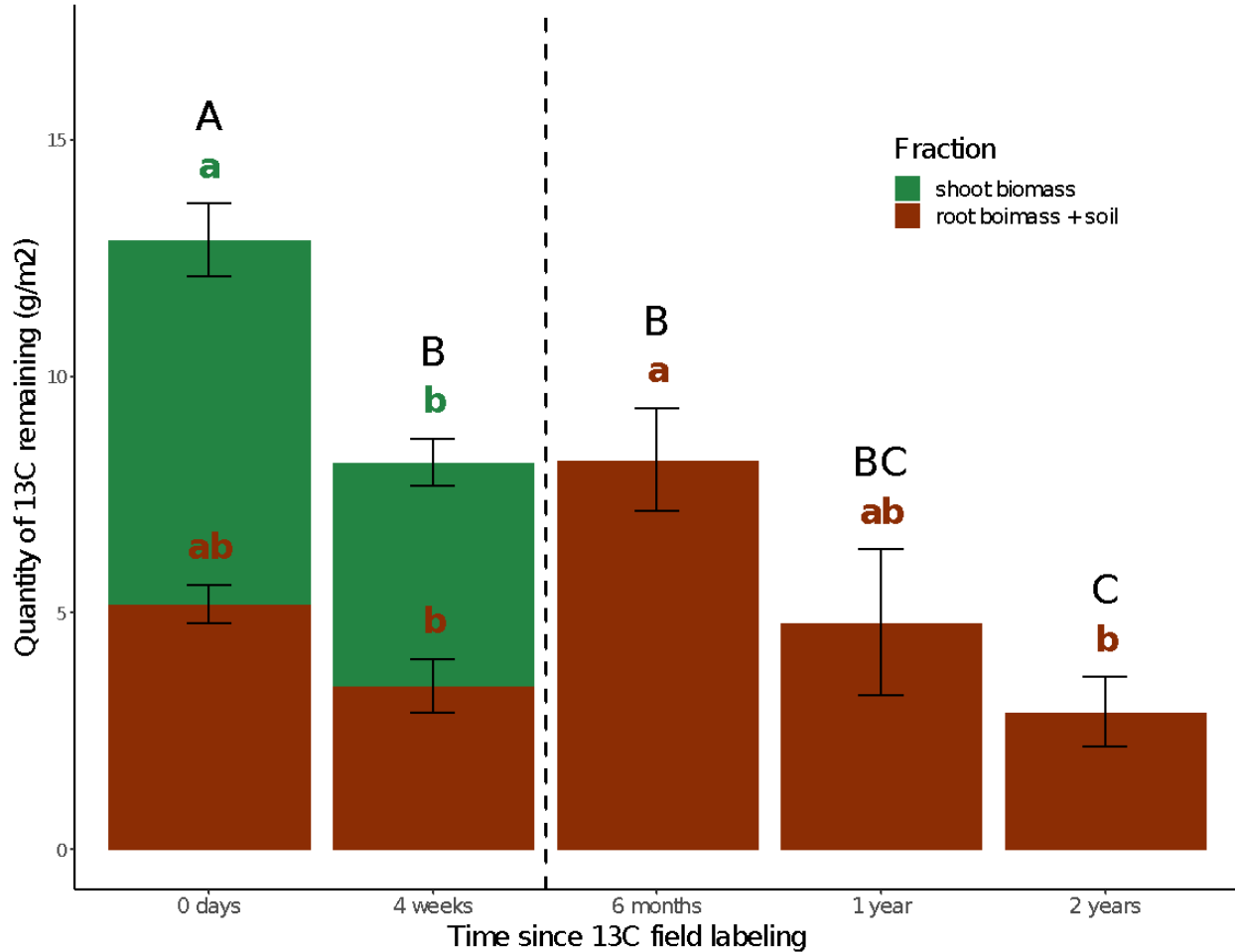
- 776 Schimel, J. P. (2018). Life in Dry Soils: Effects of Drought on Soil Microbial Communities and
777 Processes. *Annual Review of Ecology, Evolution, and Systematics*, 49(1), 409-432.
778 <https://doi.org/10.1146/annurev-ecolsys-110617-062614>
- 779 Schöning, I. & Kögel-Knabner, I. (2006). Chemical composition of young and old carbon pools
780 throughout Cambisol and Luvisol profiles under forests. *Soil Biology and Biochemistry*,
781 38(1), 2411-2424. <https://doi.org/10.1016/j.soilbio.2006.03.005>
- 782 Sheldrick, B. H. and Wang, C. 1993. Particle-size Distribution. pp. 499-511. In: Carter, M. R.
783 (ed), *Soil Sampling and Methods of Analysis*, Canadian Society of Soil Science, Lewis
784 Publishers, Ann Arbor, MI.
- 785 Sher, Y., Baker, N. R., Herman, D., Fossum, C., Hale, L., Zhang, X., ... Firestone, M. (2020).
786 Microbial extracellular polysaccharide production and aggregate stability controlled by
787 switchgrass (*Panicum virgatum*) root biomass and soil water potential. *Soil Biology and*
788 *Biochemistry*, 143, 107742. <https://doi.org/10.1016/j.soilbio.2020.107742>
- 789 Singer, M. J., Southard, R. J., Warrington, D. N., & Janitzky, P. (1992). Stability of Synthetic
790 Sand-Clay Aggregates after Wetting and Drying Cycles. *Soil Science Society of America*
791 *Journal*, 56(6), 1843–1848. <https://doi.org/10.2136/sssaj1992.03615995005600060032x>
- 792 Six, J., Bossuyt, H., Degryze, S., & Denef, K. (2004). A history of research on the link between
793 (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil and Tillage*
794 *Research*, 79(1), 7–31. <https://doi.org/10.1016/j.still.2004.03.008>
- 795 Soil Survey Staff. *Web Soil Survey*. Natural Resources Conservation Service, United States
796 Department of Agriculture. Available online June 1, 2020.

- 797 Sokol, N. W., Sanderman, J., & Bradford, M. A. (2018). Pathways of mineral-associated soil
798 organic matter formation: Integrating the role of plant carbon source, chemistry, and
799 point of entry. *Global Change Biology*, 25(1), 12–24. <https://doi.org/10.1111/gcb.14482>
- 800 Sokol, N. W., & Bradford, M. A. (2019). Microbial formation of stable soil carbon is more
801 efficient from belowground than aboveground input. *Nature Geoscience*, 12(1), 46–53.
802 <https://doi.org/10.1038/s41561-018-0258-6>
- 803 Sollins, P., Kramer, M. G., Swanston, C., Lajtha, K., Filley, T., Aufdenkampe, A. K., ... Bowden,
804 R. D. (2009). Sequential density fractionation across soils of contrasting mineralogy:
805 evidence for both microbial- and mineral-controlled soil organic matter stabilization.
806 *Biogeochemistry*, 96(1–3), 209–231. <https://doi.org/10.1007/s10533-009-9359-z>
- 807 Strickland, T. C., & Sollins, P. (1987). Improved Method for Separating Light- and Heavy-
808 Fraction Organic Material from Soil. *Soil Science Society of America Journal*, 51(5),
809 1390–1393. <https://doi.org/10.2136/sssaj1987.03615995005100050056x>
- 810 Terrer, C., R. P., Phillips, B. A., Hungate, J. Rosendale, J. Pett-Ridge, M. Craig, K. J. van
811 Groenigen, T. F. Keenan, B. N. Sulman, B. D. Stocker, P. B. Reich, A. F. A. Pellegrini,
812 E. Pendall, H. Zhang, R. D. Evans, Y. Carrillo, J. B. Fisher, R. B. Jackson. (2020). A
813 trade-off between plant and soil carbon under elevated CO₂. *Nature* 591: 599-603.
814 doi.org/10.1038/s41586-021-03306-8
- 815 Torn, M. S., Trumbore, S. E., Chadwick, O. A., Vitousek, P. M., and Hendricks, D. M. (1997).
816 Mineral control of soil organic carbon storage and turnover. *Nature*, 389(6647), 170-173.
817 <https://doi.org/10.1038/38260>

- 818 Torn, M. S., Kleber, M., Zavaleta, E. S., Zhu, B., Field, C. B., Trumbore, S. E. (2013). A dual
819 isotope approach to isolate soil carbon pools of different turnover times. *Biogeosciences*,
820 10(1), 8067-8081. <https://doi.org/10.5194/bg-10-8067-2013>
- 821 Totsche, K. U., Amelung, W., Gerzabek, M. H., Guggenberger, G., Klumpp, E., Knief, C., ...
822 Kögel-Knabner, I. (2017). Microaggregates in soils. *Journal of Plant Nutrition and Soil*
823 *Science*, 181(1), 104–136. <https://doi.org/10.1002/jpln.201600451>
- 824 Villarino, S. H., Pinto, P., Jackson, R. B., Piñeiro, G. (2021). Plant rhizodeposition: A key factor
825 for soil organic matter formation in stable fractions. *Science Advances*, 7(16), 1-13.
826 [10.1126/sciadv.abd3176](https://doi.org/10.1126/sciadv.abd3176)
- 827 Waldrop, M. P., & Firestone, M. K. (2006). Response of Microbial Community Composition and
828 Function to Soil Climate Change. *Microbial Ecology*, 52(4), 716–724.
829 <https://doi.org/10.1007/s00248-006-9103-3>
- 830 Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.
831 ISBN 978-3-319-24277-4, <https://ggplot2.tidyverse.org>
- 832 Zhou, X., Liu, D., Bu, H., Deng, L., Liu, H., Yuan, P., Du, P., & Song, H. (2018). XRD-based
833 quantitative analysis of clay minerals using reference intensity ratios, mineral intensity
834 factors, Rietveld, and full pattern summation methods: A critical review. *Solid Earth*
835 *Sciences*, 3, 16-29. <https://doi.org/10.1016/j.sesci.2017.12.002>

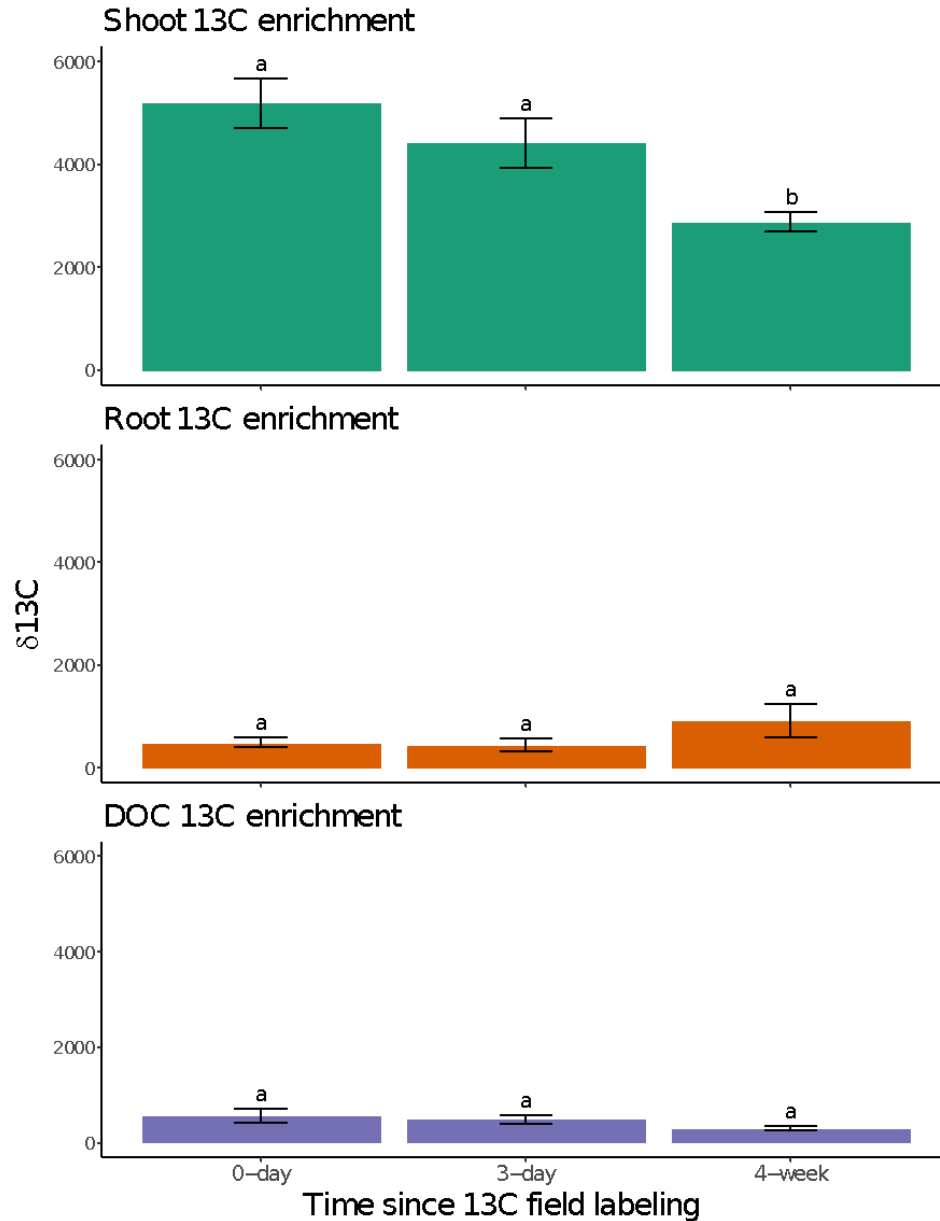
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837 **6. Figures & Tables**



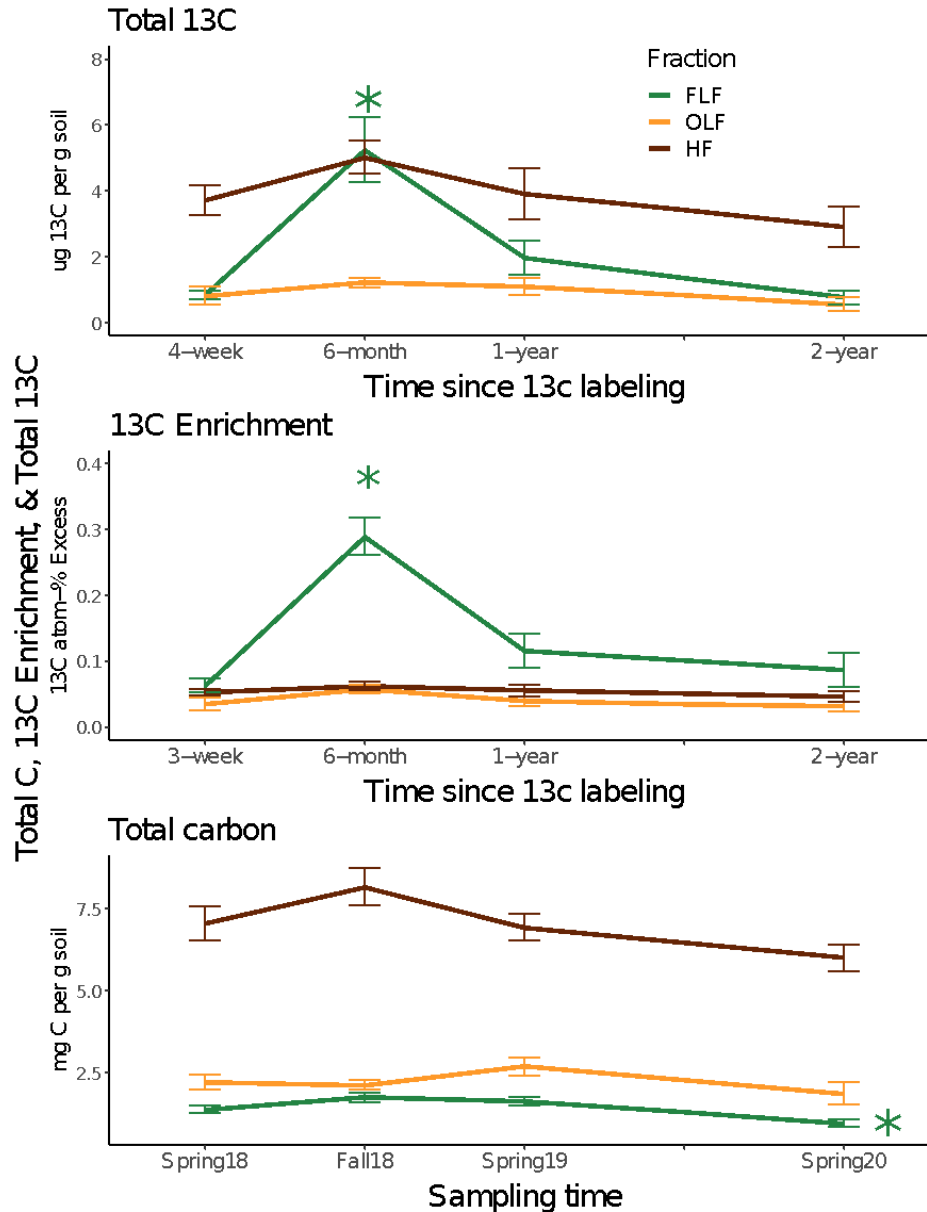
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Figure 1. Ecosystem ^{13}C assimilation. Total excess ^{13}C measured in shoot biomass (green) and root biomass + soil (brown). The 0-day timepoint occurred immediately following completion of the 5-day $^{13}\text{CO}_2$ labeling. Vertical black dashed line between the 4-weeks and 6-months timepoint marks plant senescence at the end of the spring growing season—hence, no shoot biomass ^{13}C measurements occurred beyond that point and previously living root ^{13}C biomass has become part of the soil ^{13}C . Letters indicate significant differences within a given ecosystem fraction (shoots in green vs. root biomass + soil in brown text), and overall (in black text) between timepoints by Tukey’s HSD (p-value = 0.05). Error bars represent 1 SE.



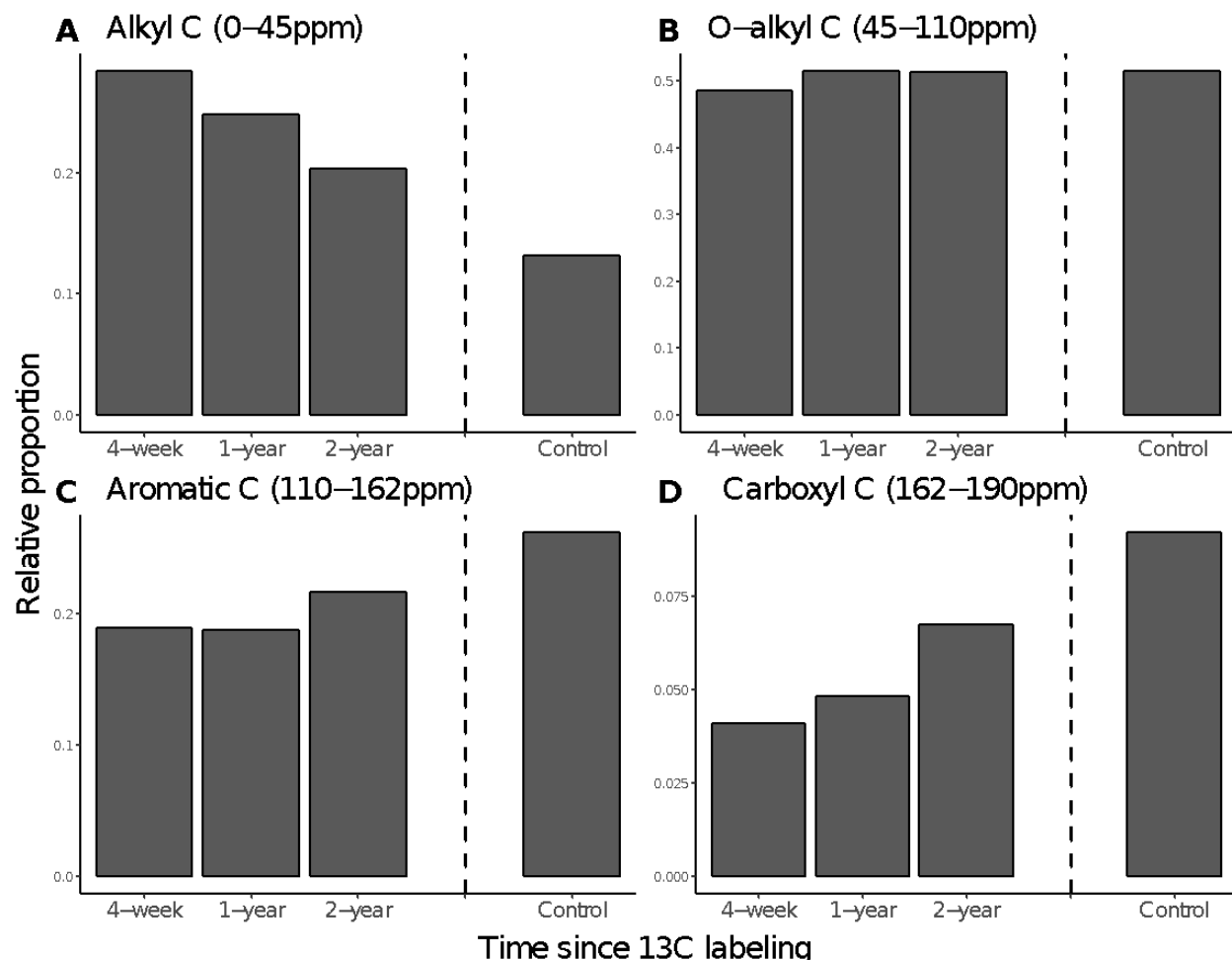
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849 **Figure 2. Short-term ecosystem ^{13}C dynamics.** Delta ^{13}C values measured in shoot biomass
850 (top), root biomass (middle), and soil DOC (bottom). Timepoints correspond to 0 days, 3 days,
851 and 4 weeks following the completion of the 5-day $^{13}\text{CO}_2$ field labeling. Letters indicate
852 significant differences within a given ecosystem fraction (aboveground biomass, roots, DOC)
853 between timepoints by Tukey's HSD (p -value = 0.05). Error bars represent 1 SE.

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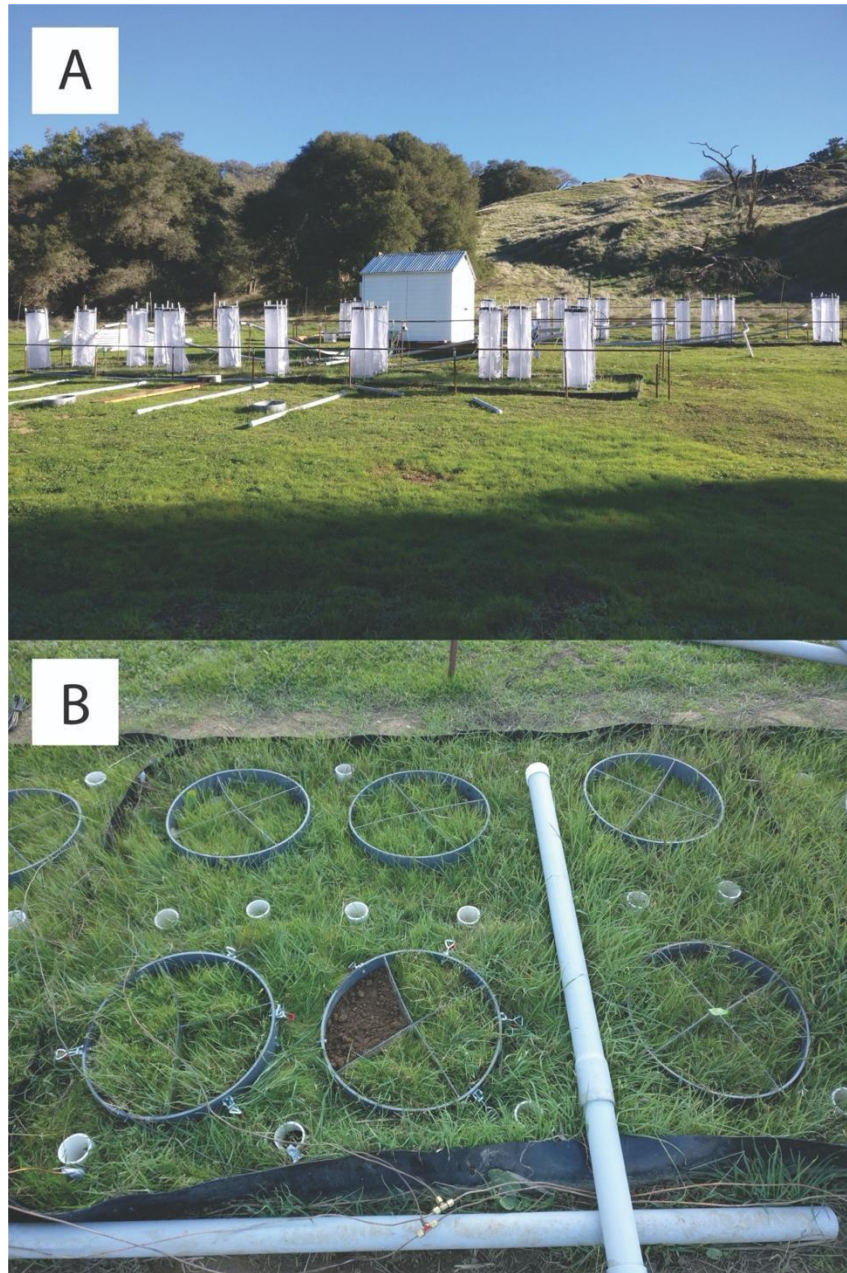
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 861 **Figure 3. Total ¹³C, ¹³C enrichment, and total C distribution among soil density fractions.**
 862 Total ¹³C (ug / g soil), ¹³C enrichment (atom-% excess), and total carbon (mg / g soil) measured
 863 for soil density fractions: free-light fraction (FLF), occluded-light fraction (OLF), and heavy
 864 fraction (HF). Soil density fractions were determined in Spring18, Fall18, Spring19, and
 865 Spring20. These sampling times correspond to 4-weeks, 6-months, 1-year, and 2-years following
 866 the ¹³CO₂ field labeling. Error bars represent 1 SE. Significant differences by Tukey's HSD (p-
 867 value = 0.05) between times are indicated by stars with color coordinating to respective soil
 868 density fraction.

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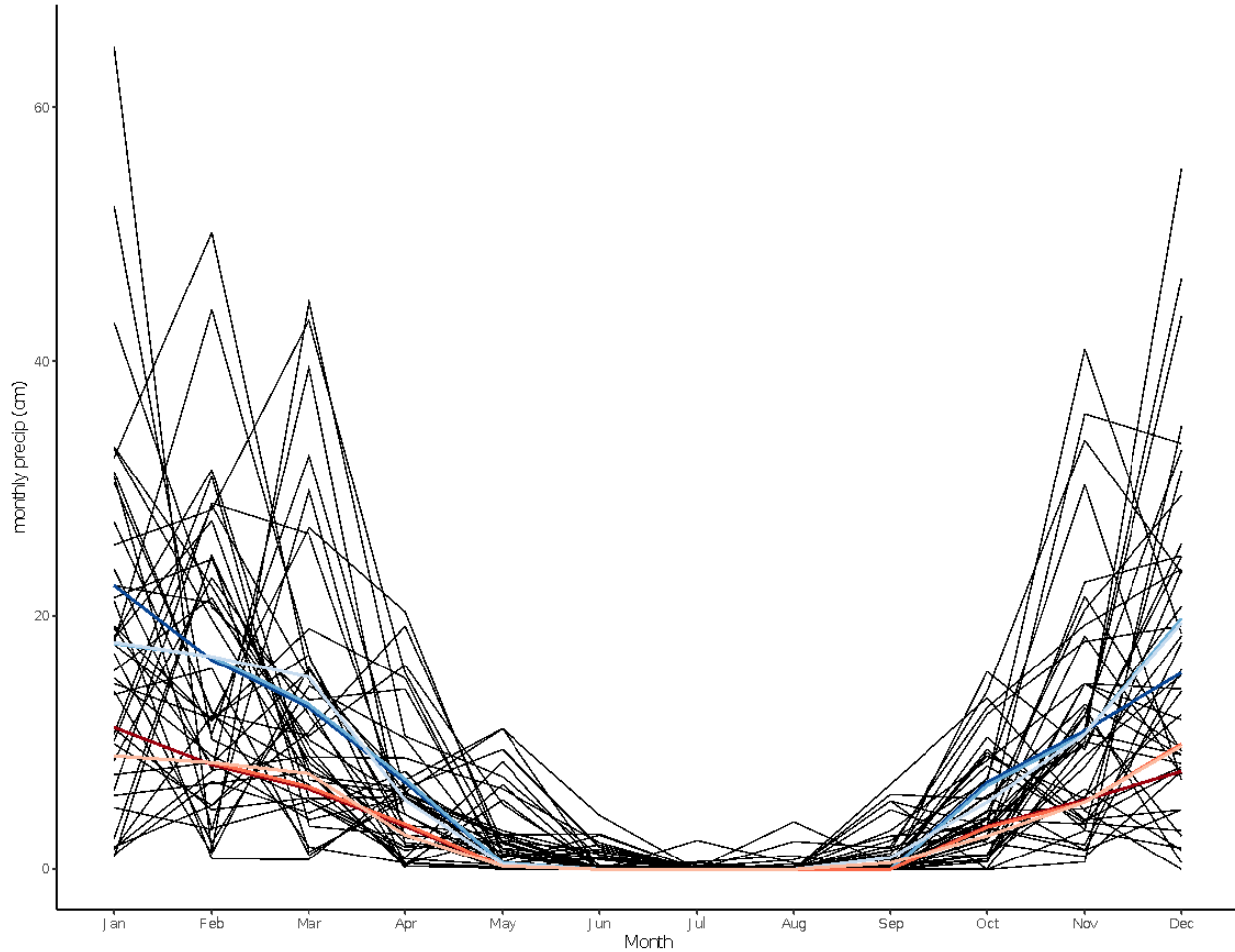
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872 **Figure 4: Heavy fraction ^{13}C functional groups.** Relative proportion of four major carbon
873 functional groups in heavy fraction separated from soil calculated from ^{13}C NMR spectra.
874 Proportions were calculated by integrating functional group regions A. alkyl C, B. O-alkyl C, C.
875 aromatic C, and D. carboxyl C using Topspin software, and analyzed separately for each
876 spectrum. X axis refers to spectra obtained for ^{13}C -labeled Heavy Fraction soil 4 weeks, 1 year,
877 and 2 years following the ^{13}C -labeling, as well as a ^{12}C -labeled control sample collected in the
878 Spring of 2018. Atom percent ^{13}C for the 4 samples (4-wk, 1 year, 2 years, and control) was
879 1.145, 1.139, 1.133, and 1.076 respectively.

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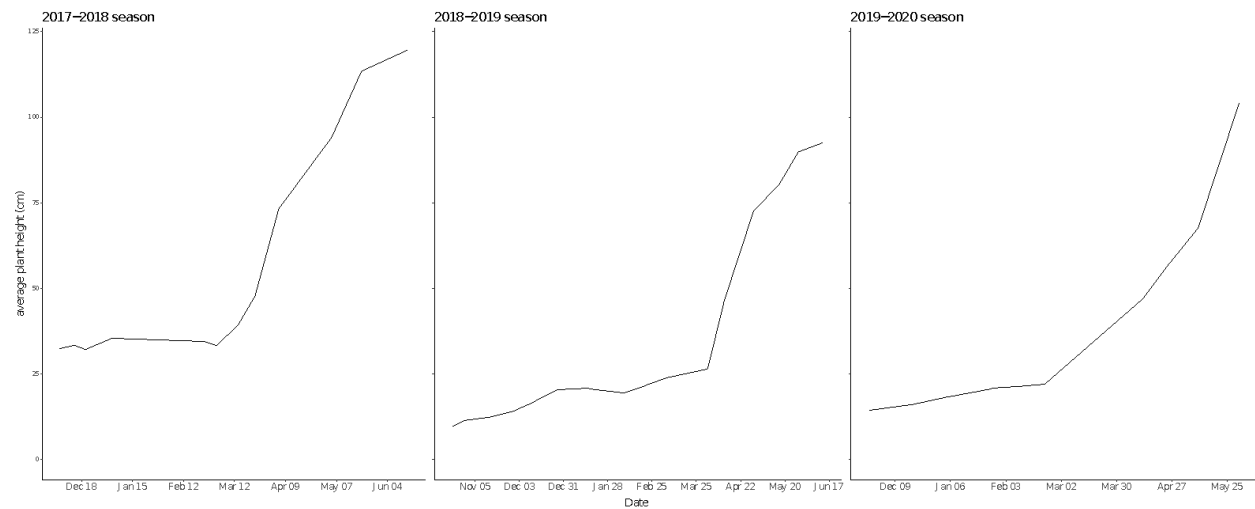
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Supplemental Figure 1. Field site $^{13}\text{CO}_2$ labeling and sampling design. (A) $^{13}\text{CO}_2$ labeling chambers, (B) Field plot with 6 circular subplots; subplots divided into 4 “wedges” for destructive sampling—ex. Bottom row center.



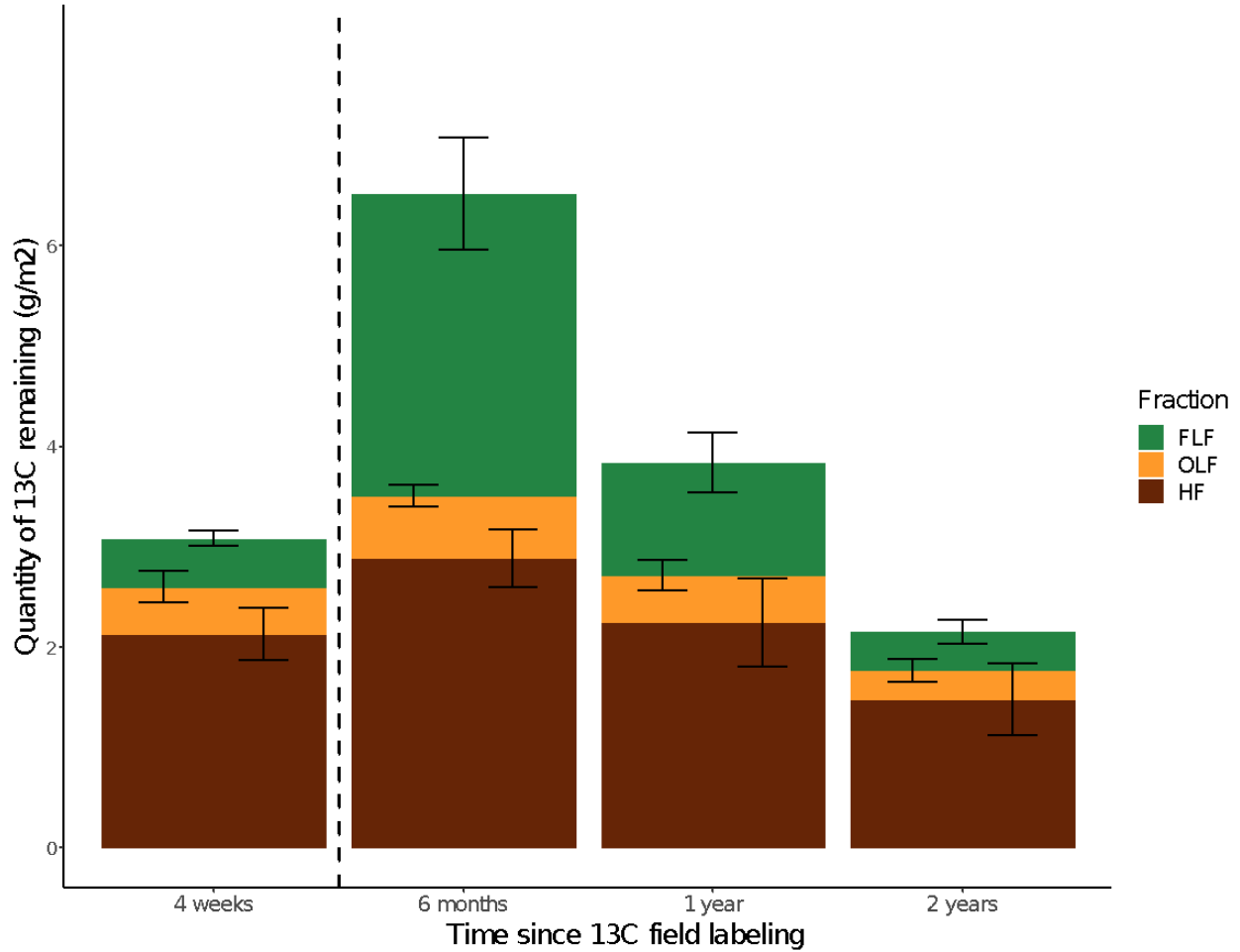
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889 **Supplemental Figure 2. 50-year annual precipitation + Annual rainfall for 50% and 100%**
890 **precipitation treatment plots for 2017-2018, 2018-2019, and 2019-2020 growing seasons.**
891 Black lines indicate monthly precipitation at Hopland Research and Extension Center (HREC),
892 our study site, dating back to 1970. Blue lines indicate manipulated rainfall received by our
893 100% of average annual precipitation treatment plots and red lines indicate manipulated rainfall
894 received by our 50% of average annual precipitation treatment plots. Color shade scales from
895 dark light with darkest blues and reds corresponding to the 2017-2018 growing season, and
896 lightest shades corresponding to the 2019-2020 growing season.

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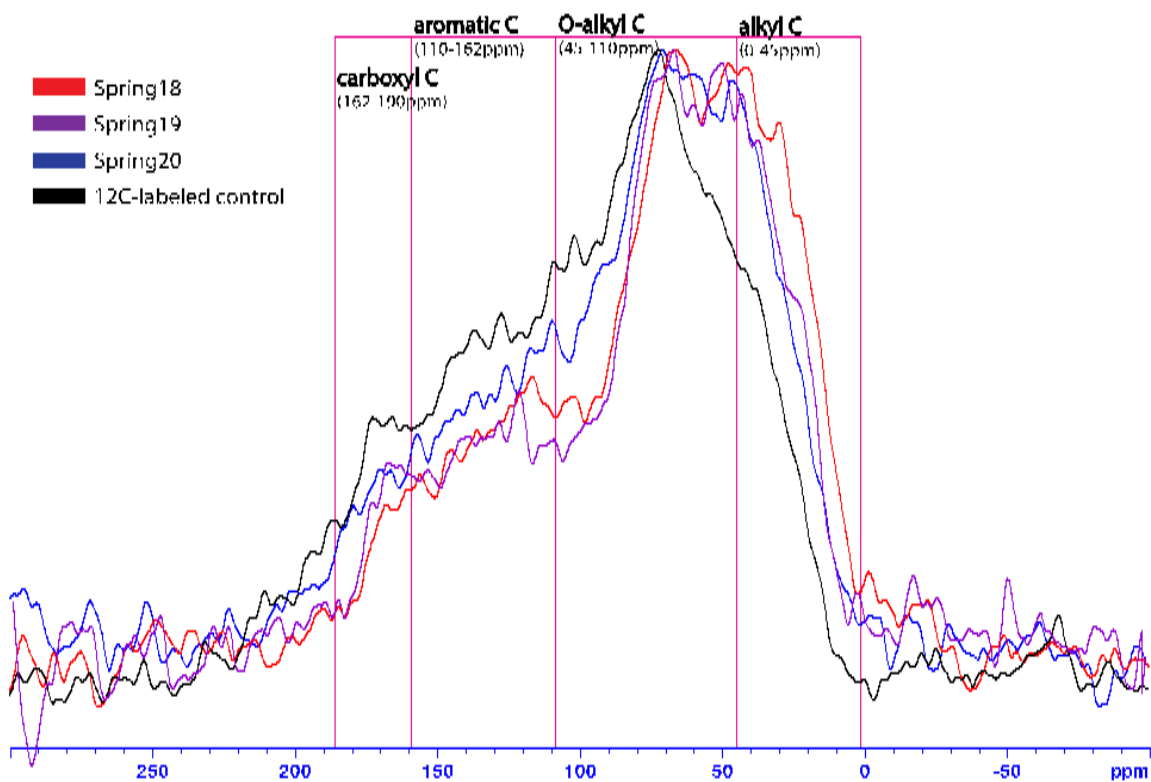
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Supplement Figure 3. Average plant heights per season. Lines represent average maximum plant height throughout the three growing seasons. Maximum plant height was measured for each plot (n=16) and was measured in cm.

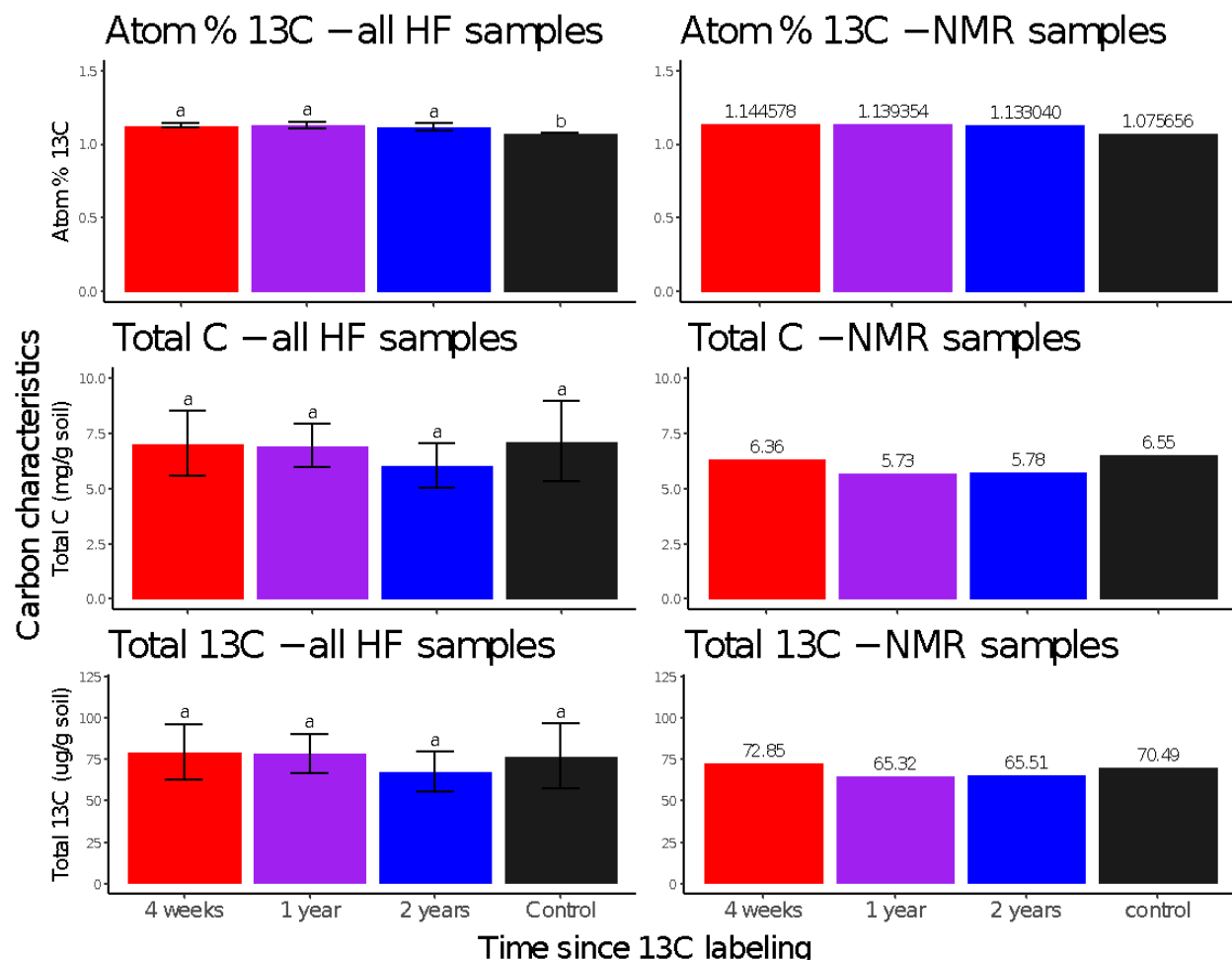


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Supplemental Figure 4. ^{13}C assimilation among soil density fractions. Total excess ^{13}C content of soil density fractions: free-light fraction (FLF); occluded-light fraction (OLF) and heavy fraction (HF), scaled to soil volume (15cm sampling depth). Error bars represent 1 SE.



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929 **Supplemental Figure 5. ^{13}C CPMAS NMR Spectra on Heavy Fraction 4 weeks, 1 year, and**
930 **2 years after ^{13}C labeling relative to control sample. ^{13}C NMR spectra of heavy fraction soil**
931 **collected in Spring18 (4 weeks after ^{13}C field labeling), Spring19 (1 year after), Spring20 (2**
932 **years after), and a ^{12}C -labeled control (collected in Spring18). Carbon functional groups**
933 **outlined in pink.**
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 936 **Supplemental Figure 6. Heavy fraction variability and ¹³C content.** ¹³C content (calculated
 937 from total C content X atom-% ¹³C) measured for all 8 HF replicates of individual samples for
 938 which ¹³C NMR spectra were acquired (left column), and for individual samples for which ¹³C
 939 NMR spectra were acquired (right column). Error bars represent 1 SD. Significant differences by
 940 Tukey's HSD (p-value = 0.05) between timepoints are indicated by letters. Atom-% ¹³C, total C,
 941 and total ¹³C values for the HF samples for which ¹³C NMR spectra were acquired (right column)
 942 label each bar. Colors represent timepoints and correspond to spectra represented in
 943 Supplemental Figure 2.

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Characteristic

Field Site: HREC

Location	39°00'14.6"N 123°05'09.1"W
Elevation (m)	244
MAT (°C)	14
MAP (cm)	94
Soil type	Typic Haploxeralf
Series name	Squawrock-Witherell Complex
Bulk density	1.457 g/cm ³
Aggregate stability	39 +/- 7
CEC (meq/100g)	18.75
SOC (mg/g soil)	13.1 +/- 1.7
TN (mg/g soil)	1.3 +/- .1
C/N	9.7 +/- .4
Texture	48, 35, 17
Clays (30%)	Muscovite (17.8%) Chlorite (11.8%) Kaolinite (0.7%)
Non-clays (70%)	Quartz (47.3%) Plagioclase (22.4%)
pH (Spring)	5.9 +/- .1
pH (Fall)	7.3 +/- .1
Soil Moisture % (Spring)	14.3 +/- 2.6
Soil Moisture % (Fall)	1.9 +/- .3

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Table 1. Site characteristics and soil physicochemical properties. Location and soil pedological information determined via NRCS Web Soil Survey. Elevation and climate characteristics described by HREC. Soil texture and CEC determined by UC Davis analytical lab. Mineralogy determined at LLNL. Aggregate stability defined as % water-stable aggregates, was measured on soil collected March 2018. DOC, SOC, TN, C/N was collected in Spring 2018; pH and soil moisture was sampled in Spring 2018 during the rainy season and fall 2018 during the dry season.

Time after 13C labeling	Ecosystem pool	Total C pool (g / m ²)	Total 13C pool (mg 13c / m ²)	% of plant-fixed 13CO ₂ remaining
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	Total	7152 +/- 899	12887 +/- 3321	100%
0 days	Aboveground	146 +/- 39	7699 +/- 3066	60%
	Belowground (roots + soil)	7006 +/- 888	5187 +/- 1160	40%
	Total	7464 +/- 997	8188 +/- 2307	64%
4 weeks	Aboveground	158 +/- 60	4729 +/- 1969	37%
	Belowground (roots + soil)	7307 +/- 1007	3459 +/- 1588	27%
	FLF	795 +/- 186	489 +/- 217	(4%)
	OLF	1274 +/- 356	468 +/- 449	(3.6%)
	HF	4043 +/- 849	2130 +/- 740	(16.5%)
	Total (soil)	9058 +/- 874	8247 +/- 3058	64%
6 months	FLF	1003 +/- 230	3012 +/- 1588	(23.4%)
	OLF	1236 +/- 217	627 +/- 318	(5%)
	HF	4691 +/- 922	2883 +/- 808	(22.4%)
	Total (soil)	8267 +/- 1319	4797 +/- 3778	37%
1 year	FLF	936 +/- 202	1128 +/- 725	(8.8%)
	OLF	1428 +/- 407	467 +/- 429	(3.6%)
	HF	3982 +/- 570	2246 +/- 1080	17.4%)
	Total (soil)	7072 +/- 999	2910 +/- 2072	23%
2 years	FLF	534 +/- 155	390 +/- 338	(3%)
	OLF	1090 +/- 496	285 +/- 322	(2%)
	HF	3468 +/- 583	1480 +/- 1010	(11.5%)

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Table 2: Distribution of ¹³C label within ecosystem pools. We defined 100% remaining plant-fixed ¹³CO₂ as that present in the system (aboveground + belowground pools) immediately after the ¹³CO₂ labeling period (“0 days” timepoint). The aboveground pool consists of aboveground plant biomass, and the belowground pool consists of root biomass + bulk soil. For the “6 months”, “1 year” and “2 year” timepoints, ¹³C-labeled roots had senesced and so were not measured separately from the bulk soil was done for the “0 days” and “4 weeks” timepoints. The

983 bulk soil was further separated into three sub-pools via soil density fractionation, yielding the
 984 free-light fraction (FLF), occluded-light fraction (OLF), and heavy fraction (HF). Average ¹³C
 985 recovery based on FLF + OLF + HF was about 72% that of the bulk soil, which is within the
 986 range for C recovery in density fractionated soil generally cited in the literature (Crow et al.,
 987 2007; Cusack et al., 2018).

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Sampling time	Density Fraction	Total C (mg/g)	Total N (mg/g)	C/N Ratio	δ ¹³ C	Total ¹³ C (ug/g)	Dry mass (g)
Spring 2018	FLF	1.6 +/- .5 b	.08 +/- .03 b	19.9 +/- 3.0 c	30.1 +/- 26.6	0.9 +/- 0.4 b	.1 +/- .03
	OLF	2.1 +/- .6 b	.1 +/- .03 b	17.5 +/- 1.1 b	-0.8 +/- 18.7	0.8 +/- 0.8 b	.1 +/- .03
	HF	7.1 +/- 1.6 a	.9 +/- .1 a	8.2 +/- .6 a	21.9 +/- 14.4	3.7 +/- 1.3 a	19.5 +/- .2
Fall 2018	FLF	1.9 +/- .6 b	.09 +/- .03 b	20.7 +/- 2.8 c	238.0 +/- 74.4	5.3 +/- 2.8 a	.2 +/- .04
	OLF	2.3 +/- .6 b	.1 +/- .04 b	17.5 +/- 1.0 b	23.2 +/- 26.9	1.2 +/- 0.4 b	.1 +/- .03
	HF	7.8 +/- 1.6 a	.9 +/- .1 a	8.5 +/- .5 a	30.7 +/- 17.1	5.0 +/- 1.4 a	19.4 +/- .2
Spring 2019	FLF	1.6 +/- .4 b	.09 +/- .02 b	19.1 +/- 1.8 c	78.3 +/- 57.2	2.0 +/- 1.3 ab	.1 +/- .04
	OLF	2.4 +/- .6 b	.1 +/- .04 b	17.3 +/- .9 b	12.9 +/- 17.9	1.1 +/- 0.6 b	.1 +/- .04
	HF	7.1 +/- 1.6 a	.8 +/- .1 a	8.5 +/- .8 a	24.5 +/- 20.5	3.9 +/- 1.9 a	19.4 +/- .2
Spring 2020	FLF	0.9 +/- .2 c	.05 +/- .01 b	18.7 +/- 2.1 b	51.3 +/- 64.4	0.8 +/- 0.6 b	.1 +/- .03
	OLF	1.8 +/- .7 b	.1 +/- .04 b	17.8 +/- .8 b	0.4 +/- 16.6	0.6 +/- 0.6 b	.1 +/- .04
	HF	6.3 +/- 1.1 a	.8 +/- .1 a	8.0 +/- .6 a	15.8 +/- 19.3	2.9 +/- 1.6 a	19.6 +/- .2

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991 **Table 3: Characteristics of soil density fractions.** Means shown +/- 1SD. Elemental
 992 characteristics are shown in units of quantity of element per gram of soil. δ¹³C values represent
 993 absolute ¹³C enrichment. Total ¹³C values represent total ¹³C added during the ¹³C labeling
 994 period (calculated from atom-% excess ¹³C). Dry mass (g) value represents quantity of starting
 995 soil sample (~20g) recovered in each density fraction.

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