

1 **Examining the contributions of maize shoots, roots, and manure to stable soil organic**
2 **carbon pools in tropical smallholder farming soils**

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11 **Abstract**

12 Continuous inputs of organic matter are vital for sustaining soil organic carbon (SOC) and productivity of
13 soils in smallholder crop-livestock systems. However, the dynamics of the different inputs i.e. maize shoots,
14 roots and manure used are poorly understood. Along with organic inputs, use of mineral fertilizers can alter
15 the nutrient stoichiometry of organic matter inputs and have implications for SOC turnover. This study
16 sought to understand how maize-based inputs and alterations to nutrient stoichiometry contribute to stable
17 SOC pools. We hypothesized that higher quality litter (i.e., manure) contributes more than maize residues
18 to stable SOC pools and that N, P and S additions, designed to balance the stoichiometry of inputs to reflect
19 the stable fine fraction of soil organic matter (C:N:P:S-10,000:833:200:143) results in greater SOC
20 stabilization. We used a ¹³C natural abundance approach, where the C₄ maize residues were incubated for
21 11 months to trace C stabilization into different SOC pools within a C₃ soil. Contrary to our expectations,
22 we observed greater recovery and stabilization of shoot-derived C (2 X more than manure and 1.63 X more
23 than roots) in the mineral-associated organic matter (MAOM) fraction. Mineral N, P and S additions
24 reduced new C recovery in MAOM by 40% compared to no mineral nutrient's additions. Our study
25 highlights the importance of residue retention as a strategy to maintain SOC and soil health in smallholder
26 systems, and our results challenge the idea that nutrient additions increase C stabilization of added residues.

27 **Key words:** mineral-associated organic matter, C stabilization, root derived C, ¹³C natural abundance

28 **1 Introduction**

29 Smallholder farming systems often face challenges of low availability of organic matter
30 and nutrient inputs, with long-term implications for soil health and soil organic carbon (SOC)
31 stocks (Tittonell et al. 2005; Rusinamhodzi et al. 2015). As a key indicator of soil fertility, SOC is
32 crucial for the maintenance of soil health and for supporting crop yields in smallholder farming
33 communities (Batiano et al. 2007). Concerns over current soil health trends have led to the
34 promotion of agricultural practices such as reduced tillage, organic matter additions, residue
35 retention, legume incorporation and agroforestry to help reverse soil degradation (Ojiem et al.
36 2006; Mungai et al. 2016). By enhancing organic matter inputs and/or slowing the loss of SOC via
37 decomposition, these practices have the potential to significantly improve long-term SOC
38 dynamics in smallholder soils (Chenu et al. 2019).

39 Smallholder farmers often manage an array of organic inputs in their fields, but maintaining
40 SOC stocks, even under conservation agriculture and other SOC supporting practices mentioned
41 above, can still present a significant challenge (Sommer et al. 2018). In many smallholder systems
42 of sub-Saharan Africa, crop residues are often retained in-field or transferred to other plots to
43 support soil fertility and SOC (Rusinamhodzi et al. 2015; Berazneva, et al. 2018). In mixed crop-
44 livestock systems, however, there is competition for residues that often favors feeding livestock
45 and then applying the manure to the field (Castellanos-Navarrete et al. 2015). The fate of crop
46 residues likely has important implications for SOC dynamics; however, it remains unclear whether
47 applying crop residues directly to fields is more effective at building SOC than feeding this
48 material to animals and then returning what C remains to the field as manure (Rufino et al. 2011;
49 Rodriguez et al. 2017). Moreover, there continues to be considerable uncertainty surrounding the
50 role of belowground inputs (i.e., roots and other rhizodeposits) in maintaining SOC, even though

51 roots may be the most important source of C inputs in many farming systems. Root-derived C is
52 thought to be preferentially stabilized in soils due to the release of labile C exudates and the
53 presence of aliphatic compounds that are more easily assimilated in microbial biomass C (Rasse
54 et al. 2005; Jackson et al. 2017), as well as their close proximity with soil particles which allows
55 them to more likely become associated with mineral surfaces (Schmidt et al. 2011). While our
56 knowledge for SOC dynamics and stabilization from different C sources is improving, tropical
57 soils and smallholder agricultural systems remain understudied and additional research is needed
58 to generate more concrete residue management recommendations for supporting soil health and
59 long-term productivity on smallholder farms.

60 Residue inputs are known to contribute to SOC and overall soil health, however,
61 knowledge gaps remain on the role of residue quality and the influence of mineral fertilizer
62 additions on SOC dynamics. Studies by Chivenge et al. (2011) and Puttaso et al. (2011) noted that
63 the quality of organic inputs influenced stabilization of C in slow and passive SOC pools.
64 Furthermore, Chivenge et al, (2011) suggested that smallholder farmers have more access to low
65 quality organic resources (in terms of C:N ratio) such as maize shoots, but these are often presumed
66 to contribute less to SOC compared to residues with higher N content, such as manure (Kapkiyai
67 et al. 1999). It should also be noted that the choice to apply manure versus shoots for supporting
68 soil health is not just governed by the need to improve soil fertility, but by the need for livestock
69 feed and other competing uses of residue and norms management (Rodriguez et al. 2017;
70 Nyamasoka-Magonziwa et al. 2021). Related to the role of residue quality, additions of mineral
71 fertilizer can influence SOC dynamics. For example, Kirkby et al. (2013; 2014) found that
72 additions of mineral N, P, and S together with organic inputs can increase the amount of new C
73 stabilized in soil. They suggested that strategic application of mineral nutrients to match the

74 C:N:P:S stoichiometry of the stable fine-fraction of soil organic matter (i.e., C:N:P:S-
75 10,000:833:200:143) would result in the greatest degree of SOC stabilization. Generally speaking,
76 residues with nutrient stoichiometries that more closely match that of microbial biomass are
77 thought to be more efficiently assimilated by microbes; in essence, when residues are overly C-
78 rich, microbes are more likely respire off this 'extra' C in order to better match their stoichiometry
79 with that of their substrate. The fine-fraction refers to the SOC pool that has reached near constant
80 ratios of C:N:P:S and is very slow to decompose (Kirby et al. 2013; Cotrufo et al. 2015; Basile-
81 Doelsch et al. 2020). This pool is thought to be largely microbially-derived, therefore Kirby et al.
82 (2013) suggest that by using nutrient additions to better match the stoichiometry of residues with
83 microbial biomass (and the stable carbon fraction) more residue C becomes assimilated within the
84 microbial biomass, and thus eventually becomes part of the stable SOC pool. However, this
85 mechanism of stabilization remains poorly understood and has received little attention in tropical
86 smallholder contexts.

87 Soil organic matter (SOM) is comprised of distinct pools, which can be distinguished via
88 diverse fractionation approaches that can provide insight on the overall dynamics of SOC. For
89 example, density-based fractionations typically involve separation of a light fraction, which is a
90 more active C pool of less-decomposed plant material and can be easily degraded by microbes,
91 versus a heavy fraction, which represents a more passive SOC pool and is thought to be more
92 microbially-derived and associated with mineral surfaces (Cotrufo et al. 2020; Lavelle et al. 2019).
93 While the active SOM pool is important for providing crops with readily available nutrients and
94 perhaps offers a more rapid assessment of soil health changes in the short-term (Barrios et al. 1997;
95 Nyamasoka-Magonziwa et al. 2020), it is the passive pools that reflect more stable C and long-
96 term SOM accrual. Beyond heavy and light fractions, soil aggregation can also play an important

97 role in SOC turnover, and it can be helpful to further separate organic matter that is occluded
98 within aggregates versus that occurring freely in the soil (Six et al. 2002). In this study we focus
99 on three fractions that reflect both density and size separations: 1) a light fraction, comprised of
100 free particulate organic matter (fPOM), 2) a heavy fraction comprised of organic matter occluded
101 within aggregates (i.e. occluded particulate organic matter; oPOM), and 3) mineral-associated
102 organic matter (MAOM) that is smaller in size and not necessarily occluded within aggregates.
103 Free POM is mostly plant-derived and formed from the fragmentation and depolymerization of
104 organic inputs, with a mean residence time of less than a decade (Cotrufo et al. 2015). MAOM on
105 the other hand, is derived more from microbially-processed organic matter and has a mean
106 residence time of decades to centuries. At the same time oPOM, is thought to be less readily
107 accessible to microbes than fPOM and likely represents a pool with intermediate rates of turnover
108 and nutrient release (Wander and Yang, 2000).

109 Despite the documented benefits of crop residue inputs in smallholder farming contexts
110 (Turmel et al. 2014; Rusinamhodzi et al. 2016), numerous questions remain as to the most effective
111 ways to build SOM and efficiently manage tradeoffs in organic resources within many smallholder
112 systems. Improved clarity on the possible mechanisms for increasing SOC stabilization (i.e., via
113 crop residue type and addition or balancing of nutrient stoichiometry) is crucial to building SOC
114 and supporting long-term soil health and productivity in smallholder systems in the tropics.
115 Therefore, the goal of this study was to understand how varying forms of maize-based organic
116 matter inputs contribute to SOC dynamics in smallholder farming contexts. Specifically, we used
117 an incubation experiment and a ^{13}C natural abundance approach to: 1) assess the incorporation
118 of maize shoots versus maize-derived cattle manure into distinct soil SOC pools (mentioned
119 above); 2) compare the potential contribution of maize root-derived C relative to maize shoots to

120 key soil C pools; and 3) determine if balancing the stoichiometry of residue inputs by adding
121 mineral forms of N, P, and S, to match that of the stable fine-fraction, improves the stabilization
122 of SOC in a tropical soil. We hypothesized that manure derived-C is stabilized more readily
123 compared to maize shoots due to the higher quality (lower C:N ratio) of this material. We also
124 hypothesized that that additions of N, P and S enhance C stabilization for all types of inputs, and
125 especially for the MAOM pool that is thought to be more dependent on microbial activity.

126

127 **2 Materials and Methods**

128 **2.1 Study approach**

129 An incubation experiment was carried out at the Kenya Agriculture and Livestock
130 Research Organization (KALRO) center in Kibos, Kenya. The experiment relied on ¹³C natural
131 abundance differences between residues derived from maize (*Zea mays*), a C4 plant, and soil from
132 a nearby forest dominated by C3 vegetation.

133 Soil was collected from the A horizon (5-20 cm depth) at a site in the Nandi Hills (N
134 00°05.034' and E 034°58.580'), which was under relatively undisturbed C3 forest for at least 100
135 years prior to sampling. At roughly 2000 m in elevation, this site has an annual precipitation of
136 1800 mm and temperature range of 18-24 °C. Soils from this region are generally classified as
137 ferralsols or nitisols (FAO 1988). Upon return to the laboratory, soil was passed through a 2 mm
138 sieve, thoroughly mixed, and air-dried prior to the start of the incubation. The collected soil had
139 an SOC content of 4.2 %, $\delta^{13}\text{C}$ signature of -24, and a pH of 4.6 (measured in a 2:1 deionised
140 water:soil suspension).

141 **2.2 Experimental design and establishment**

142 The experiment was comprised of three residue treatments with different types of maize-
143 based residues incorporated into small pots containing the forest soil mentioned above. These
144 treatments comprised additions of: 1) maize shoots, 2) maize roots (collected from a nearby field),
145 3) manure (from cattle fed solely with maize shoots), and 4) a control with no residue additions
146 Additionally, each of the residue treatments were applied with or without mineral fertilizer
147 additions to achieve a C:N:P:S stoichiometry of 10,000:833:200:143 for each residue type (see
148 Table 1 and additional detail below). The fertilized control (i.e., no residue) treatment received N,
149 P, and S additions equivalent to the maize shoot treatment, the latter requiring the greatest addition
150 of mineral N, P, and S additions to achieve the desired stoichiometry. This resulted in full factorial
151 design with eight treatments (four residue treatments x two fertilizer/stoichiometry levels) each
152 with five replicates and arranged in a completely randomized design.

153 Forty plastic 4 L pots (20 cm height x 15.5 cm diameter) were each filled with 3.6 kg of a
154 soil-sand mixture (2:1 soil:sand ratio by volume; the sand was added to help provide drainage) and
155 the mixture packed down gently to achieve a bulk density of approximately 1.2 g cm⁻³. The forty
156 pots were maintained without any residue additions during a 5-week preincubation stage. All pots
157 were maintained at similar moisture and temperature levels by weighing a representative sub-
158 sample of pots on a weekly basis and adding the average amount of water required to achieve 80
159 % of field capacity.

160 For treatments with maize shoots and roots, mature maize plants were collected from a
161 farm in Nandi (close to where forest soil was collected) by excavating and uprooting the whole
162 plant. The roots were cleaned by rinsing with tap water and passing the soil water slurry over a
163 fine mesh and then air-dried. A subsample of the roots and shoots were then oven-dried at 60 °C
164 to determine the air-dried to oven-dry biomass conversion for roots and shoots, separately.

165 Remaining air-dried shoots and roots were chopped to < 8 mm in size. In order to obtain manure
166 with a pure C4 signature, two cows under zero-grazing were fed with pure maize stover for 3 days.
167 The manure produced in the first 2 days was discarded and manure produced on the third day was
168 collected, assuming that most of this would reflect the maize consumed over the previous 2 days.
169 This material was air-dried, broken apart by hand and passed through an 8 mm sieve, while a sub-
170 sample was oven-dried to determine the moisture content.

171 Samples of each residue type were sent to a commercial laboratory in Nairobi, Kenya, for
172 characterization of total N using Kjeldahl acid digestion, total P and total S using microwave
173 digestion with nitric acid and hydrochloric acid and analyzed with Optical Emission spectrometry
174 (ICP-OES), as well as total C according to the Walkley-Black chromic acid wet oxidation method.
175 Results from the laboratory were used to determine rates of N, P and S additions for all residue
176 treatments (Table 1).

177 After 5 weeks for preincubation, organic inputs (shoots, manure, roots) were incorporated
178 at an oven-dry rate equivalent to 12 Mg ha⁻¹ (or 27.7 g air dried biomass pot⁻¹) to pots with the
179 homogenized C3 forest soil. Treatment specific additions of N, P and S (Table 1) were applied to
180 half of the pots at the same time as the residues to achieve uniform stoichiometry across all
181 treatments equivalent to C:N:P:S- 10,000:833:200:143, so as to mirror the stoichiometry of the
182 stable fine fraction of soil organic matter as reported by Kirkby et al. (2013). Mineral N, P, and S
183 were comprised of triple super phosphate (TSP), calcium ammonium nitrate (CAN), and
184 ammonium sulfate fertilizer. Pelletized fertilizer was ground and weighed separately for each
185 treatment prior to application. All pots were mixed with a trowel, including the control treatment,
186 to incorporate residue and mineral nutrient additions and approximate a uniform level of soil
187 disturbance across all treatments.

188 Upon full treatment implementation, the pots were kept in a dark room and maintained at
189 roughly uniform moisture by weighing a sub-sample of pots from each treatment and adding water
190 to bring them up to 60 % field capacity every two weeks for a period of 48 weeks. While the room
191 was not climate controlled, the space was selected to approximate outside temperatures of a typical
192 shaded surface soil in the region.

193

194 **2.3 Sampling and analysis**

195 At harvest, each pot was emptied out and passed through an 8 mm sieve by gently breaking
196 large soil aggregates along natural planes of weakness. The soils were then air-dried, and a
197 representative sub-sample shipped to Colorado State University and quarantined until further
198 processing. The samples were de-quarantined by transferring soils to pre-weighed aluminum pans,
199 weighing and heating for 24 hours at 115°C, then cooled and then sieved to 2 mm. This follows
200 methods outlined by Haddix et al. (2020), who found the heating treatment to not significantly
201 affect C content of the different fractions.

202 **2.4 Fractionation procedure**

203 In order to better understand the decomposition and stabilization dynamics of the added
204 residues we separated the soils into three C density/size fractions (Fig. 1) according to Soong and
205 Cotrufo (2015). In brief, a 10.5 g sub-sample of soil was separated by density fractionation using
206 sodium polytungstate (SPT) at 1.85 g cm⁻³ to isolate the free particulate organic matter (fPOM)
207 that floated to the top after 30 min. in a centrifuge (at 3400 rpm) at 20 °C. The fPOM floating at
208 top was aspirated and collected on a 20 µm nylon filter and allowed to oven dry at 60 °C. The
209 denser material settling at the bottom was then shaken with 0.5 % sodium hexametaphosphate for

210 18 hours to disperse aggregates and then passed through a 53 μm sieve to separated occluded POM
211 (oPOM), which is $>53 \mu\text{m}$, from the mineral-associated organic matter (MAOM), which is <53
212 μm . Occluded POM is thought to represent POM that was trapped in aggregates (along with some
213 sand particles). The fractions were collected in aluminum pans and dried in an oven at 60 °C.

214 **2.5 Isotopic analyses and calculations**

215 Subsamples from all fractions and the bulk soil were ground and sent to the UC Davis
216 Stable Isotope Facility for analysis of ^{13}C , as well as total C and N using a Micro Cube elemental
217 analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-
218 20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Subsamples of the different
219 organic residues and C3 forest soil were also sent to the UC Davis Stable Isotope Facility for
220 isotopic and elemental analysis.

221 A mixing model was used to determine the proportion of maize-derived (C4) C present in
222 the soil fractions and bulk soil (equation 3.1).

$$223 \quad f \text{ value} = (\delta_{\text{soil}} - \delta_{\text{na}}) / (\delta_{\text{input}} - \delta_{\text{na}}) \quad (3.1)$$

224 where f value is the proportion of C from the C4-derived organic residue, δ_{soil} is the ^{13}C signature
225 of the soil fraction or bulk soil after incubation, δ_{na} is the ^{13}C value for the relevant soil fraction
226 or bulk soil in the control treatment (no residues added), δ_{input} is the ^{13}C of the added maize-
227 derived residue.

228 In order to account for the different amounts of C added in each residue treatment (due to
229 varying C concentrations of the three residue types; Table 1) the proportion of maize-derived C
230 that was stabilized for each fraction was corrected to account for the total amount of C added in
231 each treatment and calculated as follows:

232
$$\text{New } C = (C \text{ concentration} * f \text{ value}) / C \text{ input} \quad (3. 2)$$

233 where *New C* is the proportion of added C that was stabilized, *C concentration* is the concentration
234 of C of each fraction (g C kg⁻¹ soil), and *C input* is the amount of C that was added in each
235 treatment, on a g C kg⁻¹ whole soil basis. This new C was calculated both on per soil fraction basis
236 (i.e. fPOM, oPOM, and MAOM), which allowed for a mechanistic understanding of dynamics in
237 individual fractions, as well as on a whole soil basis, to understand how these changes related to
238 overall SOC dynamics.

239 **2.6 Statistical analysis**

240 Two-way ANOVA was used to examine the effect of residue type, nutrient addition, and the
241 nutrient by residue interaction on C content, f values, New C and the C:N ratio of each soil fraction
242 and the bulk soil. Assumptions of ANOVA were examined, and ln or square root transformations
243 applied as needed. All analyses were conducted using JMP Pro 15.0 software (SAS Institute,
244 2019).

245 **3 Results**

246 **3.1 Treatment effects on total C and C:N ratio**

247 Of the three fractions, fPOM was generally the most enriched in total C (range: 204-232 g
248 C kg⁻¹ soil fraction), followed by MAOM (range: 43.5-45.3 g C kg⁻¹ soil fraction) and then oPOM
249 (range: 4.80-5.85 g C kg⁻¹ soil fraction), while bulk soil ranged from 26.7-31.1 g C kg⁻¹. Residue
250 type and nutrient addition did not significantly affect total soil C concentrations for any of the
251 fractions (Table 2). Also, we note that total C within the different fractions on a whole soil basis
252 did not differ significantly among treatments. Overall, the MAOM fraction contained most of the
253 SOC (i.e., over 80 % of total SOC across all treatments).

254 Nutrient additions lowered the C:N ratio of bulk soil ($p = 0.005$; Table 2) with a tendency
255 for manure and root inputs to be reduced more than shoots by nutrient additions (nutrient by residue
256 interaction, $p = 0.062$). At the same time no effects of residue type nor nutrient additions were
257 evident for the C:N ratio of the fPOM, and oPOM fractions. MAOM had the lowest and narrowest
258 range of C:N ratios (12.0-12.3) compared to oPOM (12.6-13.3) and fPOM (19.4-24.1).

259 **3.2 Recovery of maize-derived C in soil fractions**

260 Residue type had significant effects on $\delta^{13}\text{C}$ values in MAOM and bulk soil fractions, such
261 that the manure and root treatments (with and without fertilizer) had lower maize-derived residue
262 (indicated by more negative values) than maize shoots (Table 3). Nutrient additions only had
263 significant effects in the MAOM fraction, such that adding nutrients led to slightly lower maize-
264 derived C (more negative $\delta^{13}\text{C}$ values) than without nutrient additions. There was a significant
265 residue type by nutrient interaction for the oPOM fraction ($p = 0.02$), such that the addition of
266 nutrients resulted in less maize-derived C for the root and manure treatments, but more maize
267 derived C when maize shoots were added.

268 Following from the observed differences in ^{13}C values and different amounts of C input
269 across treatments, the proportion of C from the added inputs (i.e., f value; Table 3) was
270 significantly affected by residue type in MAOM and bulk fraction, where manure treatments had
271 lower f values compared to the other treatments. Contrary to our hypothesis, nutrient additions
272 resulted in significantly lower f values ($p = 0.012$) across treatments for the MAOM fraction. There
273 was also a significant residue by nutrient interaction for oPOM ($p = 0.014$), such that a higher
274 proportion of shoot-derived C was found in this fraction in the presence of nutrient additions, while
275 nutrient additions tended to decrease f values for root- and manure-derived C inputs.

276 When looking at new C derived from residue additions in the different C pools on a per
277 fraction basis (and corrected for differences in total C input), fPOM was highly variable within
278 and across treatments ranging from 7.2 to 19.1 g C kg⁻¹ fraction g⁻¹ C added with no significant
279 treatment effects (Table 3). For the oPOM fraction, there were no simple effects of residue type or
280 nutrient addition, but there was a significant residue by nutrient interaction ($p = 0.044$), where
281 nutrient additions tended to increase the amount new C recovered in maize shoot fraction, but
282 decreased new C for manure and roots. New C in the MAOM fraction was significantly influenced
283 by both residue type ($p = 0.008$) and nutrient additions ($p = 0.009$; Fig. 2). Averaged across nutrient
284 additions, shoot-derived C was stabilized into MAOM at twice the rate of manure-derived C and
285 1.6 times more than root C (Fig. 2a), while nutrient additions (averaged across residue type)
286 decreased C stabilization in MAOM by roughly 40% relative to that observed in the absence of
287 nutrients (Fig. 2b).

288 New C on a whole soil basis (i.e., taking into account the relative contribution of each
289 fraction to the whole soil mass) did not differ significantly for fPOM and oPOM, but residue type
290 influenced new C present in the MAOM fraction ($p = 0.011$) with shoots having the highest amount
291 of new C stabilized (2.2 and 1.7 times more new C than for manure and ex-situ roots; respectively),
292 while addition of nutrients showed a marginally significant ($p = 0.068$) effect on C stabilization in
293 MAOM, indicated a 30% reduction on a whole soil basis, relative to soils with no fertilizer addition
294 (Table 3). We note that most new C in manure and root treatments was found in fPOM, while most
295 new C from shoots was found in the MAOM fraction.

296

297 **4 Discussion**

298 Our study sought to understand how different types of maize-based organic matter inputs
299 (shoots, roots, and manure) contribute to distinct soil fractions and overall SOC stabilization within
300 smallholder farming contexts and whether nutrient additions to balance the C:N:P:S stoichiometry
301 of residues and optimize their incorporation into microbial biomass would enhance C stabilization
302 of distinct organic inputs.

303 **4.1 Residue quality as a driver of SOC dynamics**

304 Our findings indicate that while different organic residue types resulted in minimal impacts
305 on fPOM and oPOM fractions, maize shoot-derived C appears to be preferentially retained in the
306 MAOM fraction relative to C derived from maize roots or manure. This is relevant, since MAOM
307 has the slowest turnover of SOC for the soil fractions we evaluated, and C recovered in this fraction
308 can be thought of as stabilized SOC. It is also worth noting that we found the MAOM fraction to
309 comprise ~80% of the total bulk SOC. Because MAOM is the largest SOC pool, it has been noted
310 to be highly and positively correlated to total SOC (Cotrufo et al. 2019), such that behavior of C
311 in the MAOM fraction likely reflects the behavior of SOC in the whole soil.

312 The higher stabilization of shoot-derived C in MAOM may be attributed to several
313 mechanisms related to the biochemical composition and overall quality of residues. For example,
314 some authors have suggested that materials with lower C:N ratios, such as manure in this study,
315 may be more readily stabilized compared to lower quality residues (e.g., coarse roots and shoots)
316 since they tend to result in a high microbial C use efficiency (CUE), i.e., greater assimilation of C
317 within microbes and decreased losses via respiration (e.g., Cotrufo et al. 2013; Dannehl et al.
318 2017). At the same time, Kallenbach et al. (2019) noted that in some cases, lower quality substrates
319 can result in higher CUE at the community level by selecting for microbial taxa (e.g., fungi) that
320 are more efficient at assimilating C. Other studies have noted that residue quality to have no effects

321 on the stabilization of C in different fractions (Gentile et al. 2011). These apparently conflicting
322 findings suggest that diverse mechanisms are at play and that organic matter turnover in soils is
323 likely controlled by a variety of factors.

324 Additionally, residue quality involves more than just simple stoichiometric measures such as
325 the C:N ratio, and refers also to the structural composition of organic matter (e.g., lignin content).
326 Our study supports this idea since we balanced the stoichiometry to have equivalent C:N:P:S ratios
327 across residue types and still found differences in C stabilization between manure and roots versus
328 shoots. Therefore, there are likely other characteristics that influence SOC turnover and
329 stabilization by soil microbes. Similar to our findings, Fulton-Smith and Cotrufo (2019) conducted
330 an incubation with sorghum shoot versus ex-situ root residues and found that shoots resulted in
331 greater new C in MAOM than roots. They attributed this to litter chemistry, in that shoots had a
332 lower lignocellulose index (i.e., ratio of acid non-hydrolysable to non-hydrolysable + hydrolysable
333 products) than roots and may thus affect microbial decay of residues and CUE. Both the
334 lignocellulose index and lignin content have been shown to be the good predictors of organic
335 matter decomposition processes (Palm et al. 2001; Moorhead et al. 2014). This emphasizes the
336 importance of considering lignin content, or the lignin:N ratio, and not just the C:N ratio in models
337 predicting C stabilization. While not measured in this study, manure generally has higher lignin
338 content (18.2 %-50 %) than maize shoots and maize roots (12 % to 18 %; Abiven et al. 2011;
339 Beyaert and Voroney 2011, Yan et al. 2018; Zhu et al. 2020) and this generally aligns well with
340 the patterns we observed for SOC dynamics in our study.

341 The finding of relatively lower stabilization of root-derived C compared to shoot-derived
342 C contrasts with other studies (e.g., Rasse et al. 2005; Kong and Six 2010; Sokol et al. 2018; Hui
343 Xu et al.2019; Sokol and Bradford 2019), which highlight the importance of root-derived C, and

344 suggest a 2 to 13-fold greater stabilization for root than for shoot-derived C. The lower stabilization
345 of root-derived C in our study is likely related to the fact that we used ex-situ roots. Important
346 mechanisms of SOC stabilization that are unique to roots growing in place, such as being in close
347 proximity to soil particles, effects on aggregation and release of high-quality root exudates (and
348 other rhizodeposits), were not tested here and therefore limit our conclusions regarding the
349 contributions of roots to stable SOC.

350 **4.2 Balancing C:N:P:S stichometry**

351 In contrast to our hypothesis, we found that adding mineral forms of N, P, and S to balance
352 the C:N:P:S stoichiometry of the added residues led to a general decrease in C stabilization in the
353 MAOM fraction. Therefore, our results appear to contradict those of Kirkby et al. (2013), who
354 found that balancing the stoichiometry resulted in more C stabilization of wheat straw in the fine
355 stable fraction (comparable to MAOM in this study) during an 84-day incubation in soils from
356 Australia. While few studies have examined the co-application of N, P and S on SOC dynamics,
357 our results are broadly in line with the findings of Chivenge et al. (2011), who found that adding
358 N fertilizer with residues resulted in lower SOC stabilization in stable aggregate fractions.
359 Similarly, Fonte et al. (2009) reported a reduction in aggregate-associated C with fertilizer
360 additions to soil in Ghana. Along these same lines, Chen et al. (2020) reported that addition of
361 mineral N led to a decrease in C in the mineral-associated fraction due to the inhibition of microbial
362 activity and hence a reduction in microbial biomass C. While these studies did not seek to perfectly
363 balance nutrient stoichiometry as was done in this experiment, others have found that doing so can
364 lead to a decreased C in soils in the long term. For example, Fang et al. (2019) applied N, P and S
365 during a 245-day incubation and suggested that while balancing stoichiometry may result in better
366 nutrient use efficiency and increased microbial biomass in the short term; there might be increased

367 decomposition of the C in the long-term due to more active microbial populations, leading to an
368 overall decrease in SOC. Our study was 335 days, had we measured the effect of nutrient additions
369 earlier in the experiment, we might have seen greater stabilization with N, P, and S additions, as
370 reported by Kirby et al. (2013).

371

372 **4.3 Implications for crop-livestock systems in smallholder farming systems**

373 Farmers often feed crop residues to their animals and then apply manure to their cropping
374 fields rather than retaining the residues in the field (Castellanos-Navarrete et al. 2015; Nyamasoka-
375 Magonziwa et al. 2021). This choice has potentially important implications for maintaining SOM
376 and the long-term productivity of soils. However, surprisingly little is known about the fate of
377 residue versus manure C additions to soil fractions, and which type of input best supports SOC in
378 the long-term. We must also consider that feeding crop residues to animals decreases the amount
379 of C being returned to soils, since livestock assimilate and respire much of what they eat, and only
380 about 45% of the C fed to animals can be returned to the soil as manure (Nennich et al. 2006).
381 Nevertheless, even beyond this initial C loss due to livestock respiration and assimilation, our
382 findings suggest that C added to the soil as manure may actually result in lower stabilization of
383 SOC than C added in stover. If we further consider the benefits of maintaining crop residues on
384 the soil surface, especially as mulch (e.g., erosion control, reduced evaporative losses), then this
385 implies clear tradeoffs for soil health. Considering, however, that livestock are important (for
386 household nutrition, food security, investments, etc.) and crop residues may provide critical feed
387 during times of forage scarcity, the decision to retain residues or feed to livestock is not
388 straightforward. While our findings are not conclusive, they raise important concerns about what
389 residue management strategies should be promoted by extension and development organizations

390 to support soil health (Rodriguez et al. 2017). Despite our findings indicating lower stabilization
391 of manure derived-C, manure can still play important role in managing soil health, as manure is
392 likely to support overall crop nutrition and key soil properties (e.g., soil aggregation) better than
393 crop residues (Dunjana et al. 2012; Miller et al. 2012; Yagüe et al. 2016). Manures, at least those
394 of high quality, also tend to release nutrients faster than crop residues (Reddy et al. 2008) and thus
395 can provide better synchrony between nutrient release and crop nutrient demands, especially in the
396 absence of fertilizer inputs.

397 It should also be noted that in smallholder systems not all shoots produced on farm are
398 cycled within the farm and that manure is often available only in small quantities. Therefore, root
399 derived-C is the most reliable C source in these systems, as it is left in field after the shoots are
400 harvested or added as exudates during the growing season. Considering the noted potential of root-
401 derived C to contribute to SOC stabilization, efforts should be made to better understand this key
402 organic matter input and explore options to increase root biomass in smallholder farming systems.
403 This can be done by adopting systems that allow for active roots to be maintained during a greater
404 portion of the year (e.g., crop rotations with perennial forages) or crop varieties with more vigorous
405 roots systems, but without compromising crop yields. Finally, while mineral fertilizer additions
406 may play a positive role in building SOC by supporting crop growth and increased overall C inputs
407 (Zhang et al. 2015), the effects of nutrient additions on MAOM observed here (and in other studies
408 noted above) may counteract potential increases to productivity and C inputs.

409 **5 Conclusion**

410 Our findings indicate that strategies for SOC stabilization in smallholder systems are not
411 always straightforward and are often complicated by tradeoffs in resource allocation. Contrary to
412 expectations, our findings indicate that maize shoots may contribute more to stable SOC pools

413 (i.e., MAOM) than ex-situ roots or manure. This highlights the potential importance of residue
414 retention as a strategy to increase SOC content and long-term soil health. We note that this finding
415 is still not conclusive, and we emphasize the need for additional research using ¹³C enrichment
416 approaches to provide more definitive information about the long-term fate of different residue
417 types in soils, especially root-derived C and inputs from actively growing roots. Additionally, there
418 is still need to further examine the effects of different residue types and nutrient additions on other
419 soil health attributes and overall productivity, so as to have a more systems-oriented understanding
420 of likely impacts on smallholder soils.

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427 **Data Availability**

428 The datasets generated during and/or analysed during the current study are available from the
429 corresponding author on reasonable request.

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593 **Table 1:** Nutrient content and ^{13}C isotopic signature of maize-based organic inputs and
 594 associated nutrient additions used in a mesocosm incubation experiment in western Kenya.

| Organic input | Organic inputs added | | | | $\delta^{13}\text{C}$ | Nutrient additions | | |
|----------------------|-----------------------------|----------|----------|----------|-----------------------|---------------------------------|----------|----------|
| | C | N | P | S | | N | P | S |
| | ----- % ----- | | | | | ----- g pot ⁻¹ ----- | | |
| Shoots | 52.7 | 0.79 | 0.07 | 0.09 | -13.33 | 0.98 | 0.27 | 0.18 |
| Manure | 43.3 | 1.83 | 0.66 | 0.17 | -13.21 | 0.48 | 0.06 | 0.12 |
| Ex-situ Roots | 53.3 | 0.62 | 0.10 | 0.12 | -12.44 | 0.88 | 0.24 | 0.16 |
| Control | N/A | N/A | N/A | N/A | N/A | 0.98 | 0.27 | 0.18 |

Table 2: Total carbon per soil fraction and whole soil basis, and the carbon to nitrogen ratio of soil organic matter fractions and bulk soil following an 11- month long incubation with maize shoots, roots, and maize-derived cattle manure as well as nutrient additions in a tropical soil in western Kenya. Values presented represent the treatment mean with standard errors presented below each mean in parentheses.

| Residue Type | Nutrient Additions | Total C per soil fraction | | | | Total C per whole soil | | | Carbon to Nitrogen ratio | | | |
|-------------------------------------|--------------------|--|-----------------|----------------|----------------|---|----------------|----------------|--------------------------|----------------|----------------|----------------|
| | | fPOM* | oPOM | MAOM | Bulk Soil | fPOM | oPOM | MAOM | fPOM | oPOM | MAOM | Bulk Soil |
| | | ----- g C kg ⁻¹ soil fraction ----- | | | | ----- g C kg ⁻¹ whole soil ----- | | | | | | |
| Shoots | No | 211 (15.4) | 5.41 (0.255) | 43.5 (1.68) | 29.6 (1.64) | 1.12 (0.16) | 2.62 (0.18) | 21.9 (1.12) | 19.4 (1.24) | 13.3 (0.47) | 12.0 (0.04) | 11.6 (0.23) |
| Shoots | Yes | 204 (17.6) | 5.32 (0.402) | 45.3 (0.27) | 31.1 (1.85) | 1.46 (0.33) | 2.54 (0.18) | 23.1 (1.76) | 23.1 (1.52) | 13.2 (0.11) | 12.1 (0.06) | 11.5 (0.17) |
| Manure | No | 206 (18.4) | 4.80 (0.292) | 44.9 (0.31) | 26.7 (1.12) | 1.95 (0.21) | 2.69 (0.17) | 19.4 (0.44) | 19.6 (1.18) | 13.0 (0.18) | 12.1 (0.08) | 11.4 (0.16) |
| Manure | Yes | 232 (20.9) | 5.85 (0.607) | 44.7 (0.20) | 27.7 (1.71) | 1.94 (0.49) | 2.97 (0.41) | 21.6 (1.71) | 20.3 (1.86) | 13.0 (0.10) | 12.3 (0.03) | 11.0 (0.25) |
| Roots | No | 227 (3.8) | 5.54 (0.311) | 44.5 (0.35) | 28.8 (1.26) | 1.70 (0.20) | 2.75 (0.31) | 22.3 (1.63) | 24.1 (1.55) | 12.6 (0.81) | 12.1 (0.04) | 11.8 (0.09) |
| Roots | Yes | 216 (31.4) | 5.09 (0.115) | 45.1 (0.37) | 30.7 (0.79) | 1.86 (0.27) | 2.52 (0.16) | 22.3 (1.39) | 21.1 (1.57) | 13.2 (0.33) | 12.1 (0.13) | 10.9 (0.22) |
| Treatment Effects (p-values) | | | | | | | | | | | | |
| <i>Residue</i> | | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>0.092</i> | <i>0.064</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> |
| <i>Fertilizer</i> | | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>0.005</i> |
| <i>Residue x Fertilizer</i> | | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>0.098</i> | <i>ns</i> | <i>ns</i> | <i>0.062</i> |

*fPOM is free particulate organic matter; oPOM is occluded particulate organic matter, MAOM is mineral-associated organic matter. Numbers show treatments means (n=5) followed by standard error of means in parenthesis.

Table 3: Isotopic values, f values and New Carbon derived from residue additions on a per soil fractions and whole soil basis following an 11-month incubation experiment using ^{13}C natural abundance approach, with a C4 maize-based residues (shoots, manure, roots) and nutrient additions incorporated into a C3, forest-derived, soil in western Kenya. Values presented represent the treatment mean with standard errors presented below each mean in parentheses.

| Residue Type | Nutrient Additions | ^{13}C | | | | f value | | | | New Carbon on a per Soil Fraction Basis | | | New Carbon on a Whole Soil Basis (Proportion of Added C Recovered in Each Fraction) | | |
|-------------------------------------|--------------------|-----------------|-----------------|-----------------|-----------------|-----------------|------------------|------------------|------------------|---|------------------|------------------|--|------------------|------------------|
| | | fPOM* | oPOM | MAOM | Bulk Soil | fPOM | oPOM | MAOM | Bulk Soil | fPOM | oPOM | MAOM | fPOM | oPOM | MAOM |
| | | -----‰----- | | | | | | | | g new C kg ⁻¹ soil fraction g ⁻¹ C added kg ⁻¹ whole soil | | | g new C kg ⁻¹ whole soil g ⁻¹ C added kg ⁻¹ whole soil | | |
| Shoots | No | -23.4 (0.50) | -24.1 (0.07) | -23.7 (0.02) | -23.4 (0.06) | 0.169 (0.04) | 0.056 (0.006) | 0.018 (0.002) | 0.030 (0.006) | 9.03 (2.04) | 0.076 (0.007) | 0.198 (0.027) | 0.040 (0.014) | 0.037 (0.004) | 0.099 (0.011) |
| Shoots | Yes | -20.9 (1.30) | -23.8 (0.06) | -23.7 (0.03) | -23.3 (0.13) | 0.364 (0.11) | 0.098 (0.005) | 0.014 (0.003) | 0.045 (0.012) | 17.35 (4.60) | 0.131 (0.013) | 0.157 (0.036) | 0.108 (0.025) | 0.063 (0.008) | 0.084 (0.024) |
| Manure | No | -22.1 (0.83) | -24.0 (0.08) | -23.7 (0.01) | -23.7 (0.05) | 0.267 (0.07) | 0.082 (0.007) | 0.010 (0.001) | 0.006 (0.004) | 16.57 (4.77) | 0.120 (0.013) | 0.135 (0.014) | 0.163 (0.054) | 0.067 (0.008) | 0.058 (0.006) |
| Manure | Yes | -22.9 (0.47) | -24.0 (0.06) | -23.8 (0.03) | -23.3 (0.17) | 0.198 (0.04) | 0.070 (0.005) | 0.003 (0.001) | 0.045 (0.016) | 13.33 (1.84) | 0.126 (0.016) | 0.042 (0.020) | 0.111 (0.026) | 0.063 (0.008) | 0.023 (0.013) |
| Roots | No | -20.9 (0.53) | -23.8 (0.16) | -23.7 (0.05) | -22.9 (0.19) | 0.339 (0.04) | 0.091 (0.013) | 0.011 (0.005) | 0.073 (0.017) | 19.07 (2.22) | 0.127 (0.022) | 0.135 (0.036) | 0.146 (0.031) | 0.063 (0.014) | 0.065 (0.018) |
| Roots | Yes | -21.1 (1.00) | -24.0 (0.21) | -23.8 (0.03) | -23.2 (0.19) | 0.320 (0.08) | 0.075 (0.017) | 0.007 (0.003) | 0.050 (0.017) | 18.06 (5.31) | 0.094 (0.021) | 0.082 (0.022) | 0.158 (0.052) | 0.048 (0.013) | 0.042 (0.012) |
| Treatment Effects (p-values) | | | | | | | | | | | | | | | |
| Residue | | <i>ns</i> | <i>ns</i> | 0.013 | 0.019 | <i>ns</i> | <i>ns</i> | 0.002 | 0.033 | <i>ns</i> | <i>ns</i> | 0.008 | <i>ns</i> | <i>ns</i> | 0.011 |
| Fertilizer | | <i>ns</i> | <i>ns</i> | 0.046 | <i>ns</i> | <i>ns</i> | <i>ns</i> | 0.012 | <i>ns</i> | <i>ns</i> | <i>ns</i> | 0.009 | <i>ns</i> | <i>ns</i> | 0.068 |
| Residue x Fertilizer | | <i>ns</i> | 0.019 | <i>ns</i> | 0.078 | <i>ns</i> | 0.014 | <i>ns</i> | 0.025 | <i>ns</i> | 0.044 | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> |

*fPOM is free organic matter; oPOM is occluded organic matter, MAOM is mineral associated organic matter. Numbers show treatments means (n=5) and numbers in parenthesis are standard error of treatment means. NS is treatment differences that are not significantly different at p<0.05. f value is the proportion of C from the C4-derived organic residue.

Figure Captions

Figure 1: Fractionation of soil by density and size following an 11-month long incubation of different types of organic input in western Kenya. fPOM is free particulate organic matter, oPOM is occluded particulate organic matter, and MAOM is mineral associated organic matter and SPT is sodium polytungstate. Adapted from Fang et al. (2019).

Figure 2: Impacts of a) residue type and b) nutrient additions on new C stabilized in the mineral-associated organic matter (MAOM) soil fraction following an 11-month incubation experiment, using a ^{13}C natural abundance approach. Maize-based C4 residues (shoots, roots, manure) were incorporated into a C3 forest soil in western Kenya with or without the addition of mineral N, P and S to balance the stoichiometry of the residues. Values represent the mean and error bars reflect the standard error.

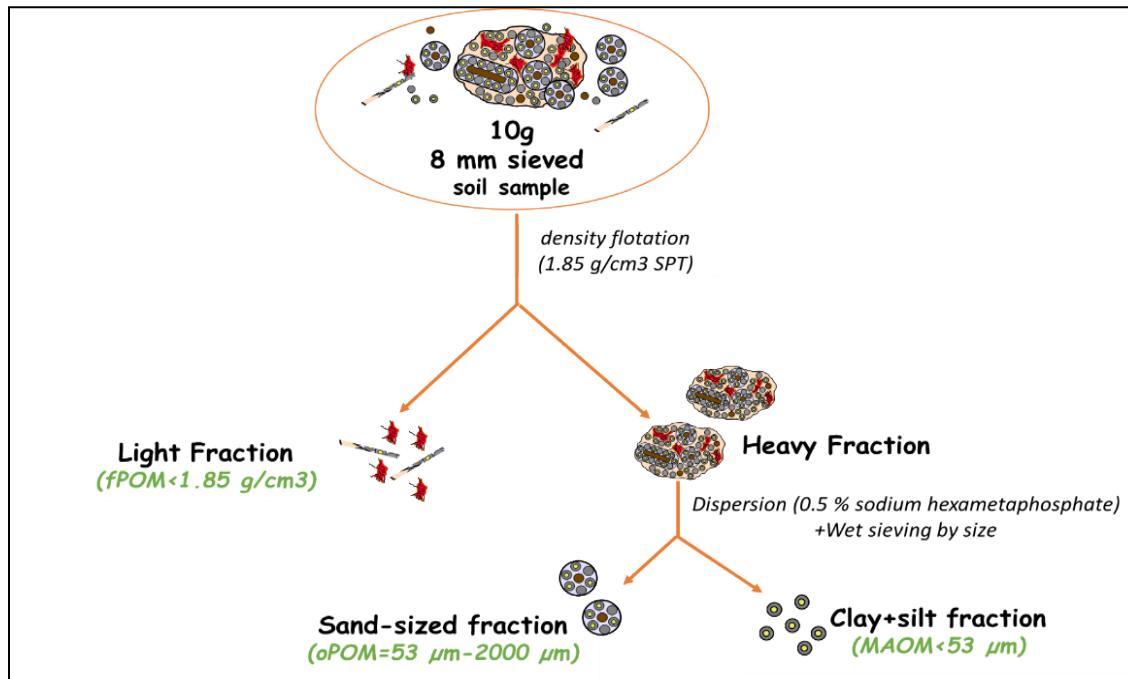


Figure 1: Fractionation of soil by density and size following an 11-month long incubation of different types of organic input in western Kenya. fPOM is free particulate organic matter, oPOM is occluded particulate organic matter, and MAOM is mineral associated organic matter and SPT is sodium polytungstate. Adapted from Fang et al. (2019).

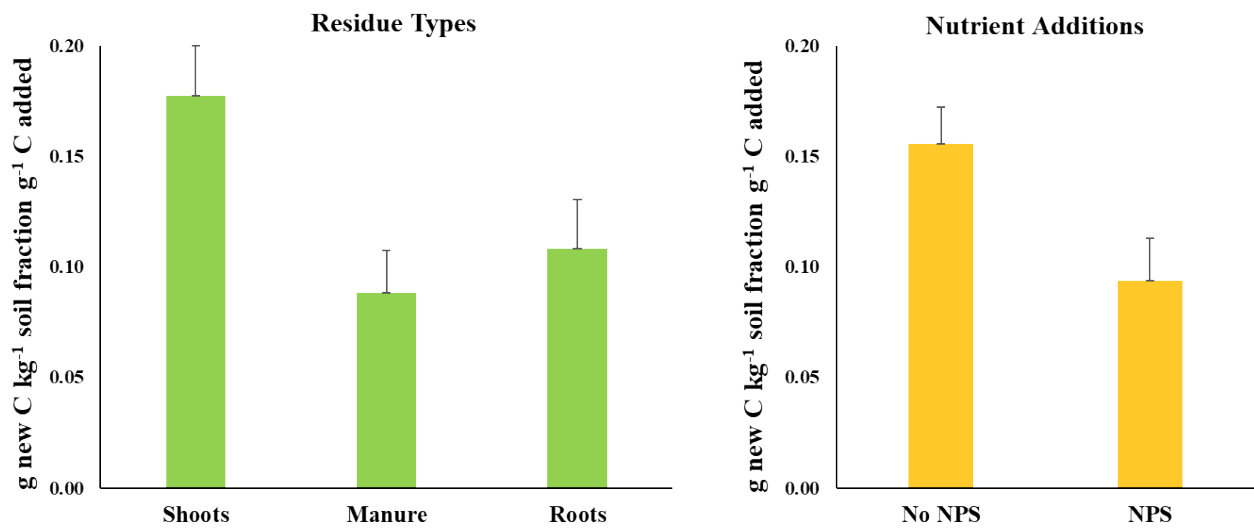


Figure 2: Impacts of a) residue type and b) nutrient additions on new C stabilized in the mineral-associated organic matter (MAOM) soil fraction following an 11-month incubation experiment, using a ¹³C natural abundance approach. Maize-based C₄ residues (shoots, roots, manure) were incorporated into a C₃ forest soil in western Kenya with or without the addition of mineral N, P and S to balance the stoichiometry of the residues. Values represent the mean and error bars reflect the standard error.

