Diversifying with grain legumes amplifies carbon in management-sensitive soil organic carbon pools on smallholder farms

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ABSTRACT

Crop diversification with grain legumes has been advocated as a means to increase agroecological resilience, diversify livelihoods, boost household nutrition, and enhance soil health and fertility in cereal-based cropping systems in sub-Saharan Africa and around the world. Soil organic carbon (SOC) is a primary indicator of soil health and there is limited data regarding SOC pools and grain legume diversification on smallholder farms where soils are often marginal. In Malawi, a range of legume diversification options are under investigation, including rotations and a doubled-up legume rotation (DLR) system in which two compatible legumes are intercropped and then rotated with a cereal. The impact of the DLR system on SOC has not yet been determined, and there is a lack of evidence regarding SOC status over a gradient of simple to complex grain legume diversified systems. We address this knowledge gap by evaluating these systems in comparison to continuous sole maize (Zea mays L.) at three on-farm trial sites in central Malawi. After six years of trial establishment, we measured SOC in bulk soils and aggregate fractions and in faster cycling SOC pools that respond more rapidly to management practices, including water extractable organic carbon (WEOC), particulate organic matter carbon (POM-C), potentially mineralizable carbon (C), and macroaggregate C. Cropping treatment differences were not seen in bulk SOC or total N, but they were apparent in SOC pools with a shorter turnover time. The DLR system of intercropped pigeonpea (Cajanus cajan (L.) Millsp.) and groundnut (Arachis hypogaea L.) rotated with maize had higher WEOC, POM-C, potentially mineralizable C, macroaggregate and microaggregate C values than continuous maize. Of the single legume rotations, the pigeonpea-maize rotation had more mineralizable C and microaggregate C compared to continuous maize, while the groundnut-maize rotation had similar C values to the maize system. Overall, this study shows the potential for crop rotations diversified with grain legumes to enhance C in management sensitive SOC pools, and it is one of the first reports to show this effect on smallholder farm sites.

1. Introduction

An indicator of soil health, soil organic carbon (SOC) is integral to the soil biological, chemical, and physical properties and processes that sustain productive agricultural soils (Lal, 2011). Soil organic carbon is the primary constituent and a relatively easily measured component of soil organic matter (SOM), which is critical for soil nutrient and water holding capacity, soil structure, and microbial diversity, abundance, and species composition (Powlson et al., 2011). Increasing agroecosystem plant diversity can increase both aboveground and belowground net primary productivity and plant carbon (C) inputs, potentially creating a feedback loop of increased plant biomass and organic inputs into the soil and more efficient microbial nutrient cycling (Lange et al., 2015). Crop rotations can boost belowground C inputs and microbial contributions to soil C stocks through biotic and physical changes stimulated by the addition of diverse plant residues, root morphologies, root biomass, rhizodeposition, and root exudates (Kong and Six, 2010; McDaniel et al., 2014; Tiemann et al., 2015). A meta-analysis by McDaniel et al. (2014) demonstrates the positive impacts of increasing crop diversity on soil C stocks across a wide range of systems, but also highlights the much greater impacts of increased crop diversity when a legume is included in the system.

Integration of grain legumes into maize-based cropping systems has been advocated, particularly in regions like sub-Saharan Africa (SSA), as
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a means to enhance nutrition and farmer livelihoods through production of nutritious grains and fodder, as well as diversify systems with the goal of improving soil fertility and agroecological resilience through increased C and nitrogen (N) inputs (Kuyah et al., 2021; Smith et al., 2016; Snapp et al., 1998). Agroforestry systems with legume-maize intercrops have been shown to enhance SOC status in SSA (Beedy et al., 2010), yet the effects of grain legume diversification and continuous monocropping of maize (Zea mays L.) are less known. Grain legumes in rotation with cereals in general have positive effects on yields, attributed to the extra N availability generated through legumes’ capacity for biological nitrogen fixation (BNF) and of the resulting N-rich, low C:N residues, as well as other rotation effects, such as the breakup of pest and disease cycles (Franke et al., 2018). Grain legumes’ impacts on SOC are less well-established compared to impacts on N supply as detectable changes in SOC accrual require longer-term experiments (Franke et al., 2018).

Grain legume and cereal characteristics (e.g., harvest index, maturation time, seasonal leaf fall, and N-partitioning), in combination with management practices (e.g., timing of planting and harvesting, harvesting methods, and residue retention), and climatic conditions, (e.g., mean annual precipitation and mean annual temperature), can strongly influence C and N inputs to soil and subsequent changes in SOC and N (Giller, 2001; Kumar and Goh, 2000). Multi-year studies in SSA that quantified SOC and N in grain legume-cereal crop rotations compared to monocropping of maize (Zea mays L.), are generally as associated with C that is more recent, readily decomposable, and susceptible to microbial activity, as well as other rotation effects, such as the breakup of pest and disease cycles (Franke et al., 2018). Grain legumes’ impacts on SOC are less well-established compared to impacts on N supply as detectable changes in SOC accrual require longer-term experiments (Franke et al., 2018).

Because changes in total SOC stocks may take decades to detect, and with a dearth of longer-term studies of SOC under different management practices in SSA (Franke et al., 2018), we can turn to assessing more management-sensitive, dynamic and quickly changing SOC pools as potential indicators of change. We measured biologically active and rapid-cycling SOC pools, including water extractable organic carbon (WEOC), particulate organic matter carbon (POM-C), and short-term soil respiration C (CO₂-C), as well as more protected and longer-lived SOC pools within different aggregate size fractions. Water extractable organic carbon (WEOC) is an indicator for dissolved organic carbon, which while considered the most labile, mobile, bioavailable C pool, often regarded as the primary C source for decomposers, is also a contributor to slow-cycling mineral-associated organic matter (Haney et al., 2012; von Lützow et al., 2007). Particulate organic matter carbon (POM-C) consists largely of plant-derived material that can be biochemically accessible but physically protected in aggregates, and therefore persists in soils, although it is vulnerable to disturbance (Cotrufo et al., 2019). Total C respired after dry soils are re-wet and from short-term lab incubations are functionally relevant SOC pools, highly sensitive to management and indicative of nutrient dynamics and the potential for SOC accrual (Calman et al., 2013; Franzluebbers et al., 2000). Finally, soil biological and physical aggregate fractionation separates SOC into pools associated with distinct aggregate sizes and biological and physical protection mechanisms (Six et al., 2000; von Lützow et al., 2007). Macroaggregates (>250 µm), are generally associated with C that is more recent, readily decomposable, and susceptible to microbial activity, as well as other rotation effects, such as the breakup of pest and disease cycles (Franke et al., 2018). Grain legumes’ impacts on SOC are less well-established compared to impacts on N supply as detectable changes in SOC accrual require longer-term experiments (Franke et al., 2018). Grain legumes’ impacts on SOC are less well-established compared to impacts on N supply as detectable changes in SOC accrual require longer-term experiments (Franke et al., 2018).

2. Methods

2.1. Study sites

In November of 2012, the Africa RISING (Research in Sustainable Intensification for the Next Generation) project established multi-location, on-farm experiments that broadly integrated maize with grain legumes across three sites (Linthipe, Kandeu and Nsipe; Fig. 1). The sites encompass a range of agricultural production potential as follows: Linthipe is a high elevation site with generally well-distributed rainfall and high agricultural potential, while Kandeu and Nsipe are mid-elevation with intermediate rainfall distribution and medium potential (Table 1; Mungai et al., 2016; Smith et al., 2016; Snapp et al., 2018). All three sites are sub-humid tropical. Malawi has a unimodal rainfall regime with a rainy season extending from November to April and a dry season from May to October (Table 1; Jury and Mwafuulira, 2002). In all the three sites, annual precipitation exhibits strong inter-annual variability in both distribution and quantity (Mungai et al., 2016; Snapp et al., 2018).
each site, experiments with 10 treatments replicated three times were established in a nonrandomized block design. Plots were 5 × 5 m at the site in Linthipe, 6 × 5 m in Kandeu, and 8 × 5 m in Nsipe. The detailed description of these participatory action research experiments is described in Mungai et al. (2016) and Snapp et al. (2018). In this study, we focus on soils sampled from a subset of four treatments: (1) groundnut-maize rotation (Gnut), (2) “doubled-up legume” rotation (DLR) consisting of a pigeonpea-groundnut intercrop rotated with maize, (3) pigeonpea-maize rotation (PP) and (4) a continuous, sole maize (Maize) (Table 2). In brief, the continuous fully fertilized maize treatment received the recommended rate of full 69 kg N ha⁻¹ and 9.2 kg phosphorus (P) ha⁻¹ annually, while a half-rate was applied to maize grown in rotation or as an intercrop with a legume (Ministry of Agriculture and Food Security, 2012; Table 2). All the P was applied through a NP compound fertilizer (23:21) at 100 or 50 kg ha⁻¹ at planting, with the respective outstanding N applied as urea at six weeks after crop emergence. Each crop was grown according to its respective recommended planting density, in-row spacing, and planting arrangement with 0.75 m between planting ridges and all ridging done by hand-hoe (Snapp et al., 2018). All crops were planted on the same day at each site; however, planting dates differed among sites as effective rainfall was received on different dates. During the six cropping seasons, maize and groundnut were harvested between April 15 and May 10. Crop residues were left in the field after harvest, and, during the dry season, were partly consumed by free-ranging livestock (goats and cattle).

### 2.3. Soil sampling, handling, and aggregate fractionation

We sampled soils in June 2018 at the conclusion of the sixth growing season after all rotations had been planted to maize; each rotation treatment had completed three full rotations (i.e., three maize harvests and three legume harvests). Using a 6.35 cm diameter PVC corer, we collected three soil cores at random to 10 cm depth from ridges within each of the three replicate plots for the four different cropping treatments. Therefore, we took 108 samples. Soil cores were sealed in plastic bags with a cushion of air to minimize compression and disruption of aggregates. Field moist samples were transported to a soils lab at University of Malawi’s Chancellor College in Zomba, where each core was weighed, analyzed for gravimetric soil moisture, and separated into aggregate size fractions. Bulk density was measured using the core

### Table 1

| Characteristics of the three Africa RISING trial sites in Central Malawi. |
|-----------------|----------------|----------------|
| Site            | Linthipe       | Kandeu         | Nsipe          |
| Latitude/Longitude | -14.20 S/ 34.11 E | -14.63 S/ 34.60 E | -14.93 S/ 34.75 E |
| Elevation (m)   | 1221           | 921            | 864            |
| Mean annual rainfall (2012-2018 mm) | 979 | 969 | 992 |
| Total rainfall 1/1/2018-June 2018 sampling (mm) | 656.8 | 552.6 | 562.1 |

*Climate Hazards InfraRed Precipitation with Station (CHIRPS) (Funk et al., 2015) data attained via Google Earth Engine (Gorelick et al., 2017)*

2016; Snapp et al., 2018). Soils vary by study site. Linthipe is largely dominated by ferric luvisols, and Kandeu and Nsipe have a mix of chromic luvisols and orthic ferralsols (Lowole, 1983).

### 2.2. Trial establishment and management

At each of the three sites, a uniform field was identified to host the trials. In order to be an acceptable experiment site, the entire area to be covered by the trial was supposed to have been cropped with maize, at least for the previous cropping season. The selected fields were prepared for planting by manually making raised ridges with a hand hoe, 0.75 m apart, in line with the primary land preparation practice in Malawi. At

### Table 2

| Cropping systems examined in this study with abbreviations and description of management practices. |
|-----------------|----------------|----------------|
| Cropping system/treatment | Fertilizer (kg ha⁻¹ y⁻¹) | Planting density (plants ha⁻¹) | Plant spacing |
| Maize | Sole maize, fully fertilized | (69 N, 9.2 P) | 53,000 | 25 cm within row |
| Gnut | Groundnut-maize rotation | Gnut= 17.3 N, 2.3 P; maize= 34.5 N, 4.6 P | Gnut= 88,000, maize= 53,000 | Gnut = 10–15 cm within row & 2 rows per ridge |
| PP | Pigeonpea-maize rotation | PP= 17.3 N, 2.3 P; maize= 34.5 N, 4.6 P | PP= 44,000, maize= 53,000 | PP = 90 cm within row & 3 plants per station |
| DLR | Doubled-up legume rotation (Pigeonpea/groundnut intercrop rotated with maize) | PP= 44,000, Gnut= 79,000, maize= 53,000 | PP = 90 cm within row & 3 plants per station Gnut = 9 cm within row; 2 rows per ridge |
method (Grossman and Reinsch, 2002). The three replicate soil cores from each plot were analyzed individually and not combined for analyses. Field moist cores were gently broken by hand to obtain aggregates smaller than 8 mm diameter. Gravimetric soil moisture was determined by weighing 5 g subsamples into tins, drying the samples for 24 h at 105 °C in a drying oven, then reweighing the dried samples. Upon determining that soils were at maximum friability for dry sieving (Kristiansen et al., 2006), a 200 g subsample was passed through a series of three sieves using a portable sieve shaker (Gilson Wet/Dry Sieve Vibrator SS-23, Lewis Center, Ohio) and separated into four fractions: > 2000 μm (large macroaggregates), 2000–250 μm (small macroaggregates), 250–53 μm (microaggregates), and < 53 μm (silt and clay). We ran the sieve shaker for 2 min with the full stack of sieves then removed the 2 mm sieve, ran it for 1.5 min and then removed the 250 μm sieve, and finally ran it for 3 min with the remaining 53 μm sieve (Bach and Hofmoeckel, 2014). Aggregates and remaining whole, i.e., bulk, soils were subsequently air-dried, packed into coolers and shipped to Michigan State University in East Lansing, MI, for further analysis. Prior to analysis, whole soils were passed through a 2000 μm sieve.

We determined the sand content of each aggregate fraction by dispersing 4 g subsamples in 0.5% sodium hexametaphosphate solution, shaking on a reciprocal shaker for 18 h, and washing samples through a 53 μm sieve with deionized water (Elliott et al., 1991; Grandy and Robertson, 2007). The particles remaining on the 53 μm sieve were washed into pre-weighed tins and dried at 60 °C for 48 h. We used the following equations to sand-correct the aggregate distribution:

\[
\text{Sand-corrected aggregation} = \frac{\text{Weight}_{\text{aggregate size fraction}} - \text{Weight}_{\text{aggregate - sand}}}{\sum_{\text{All fractions}} \text{Weight}_{\text{sand-corrected aggregate size}}} \times 100
\]  

(1)

and to calculate the sand-free aggregate-associated C and N (Denef and Six, 2005):

\[
\text{Sand-free (C or N) fraction} = \frac{\text{C or N}_{\text{fraction}}}{1 - (\text{sand proportion})_{\text{fraction}}}
\]  

(2)

2.4. In-situ soil respiration and water infiltration

We measured in-situ soil respiration and water infiltration on the day that we collected soil samples at each respective site. We measured in-situ soil respiration immediately before and approximately two hours after adding 2 L water to a 23.7 cm diameter ring set at least 3 cm into planting ridge soils. We took three respiration measurements per replicate using a portable CO₂ gas analyzer (PP Systems EGMM-5, Amesbury, MA). Concurrently, we measured water infiltration as the time taken for the water added to the ring to percolate into the soil with no surficial water remaining (Franzluebbers, 2002).

2.5. Water-extractable organic carbon

To measure water extractable organic carbon (WEOC), we weighed 4 g of air-dried bulk soils into 50 ml centrifuge tubes and added 40 ml of deionized water. Tubes were capped and shaken at 30 rpm for 10 min on a reciprocal shaker, after which they were centrifuged for 5 min at 3500 rpm, and the resulting supernatant was filtered through Whatman 2 V filter paper (Haney et al., 2012). Triplicate 10 ml samples were analyzed with a TOC analyzer (vario TOC cube, Elementar, Ronkonkoma, NY).

2.6. Particulate organic matter carbon

Particulate organic matter carbon (POM-C) was determined by dispersing 10 g of air-dried bulk soil with 30 ml of 5% sodium hexametaphosphate and shaking for 18 h on a reciprocal shaker at 180 rpm (Robertson et al., 1999). The material remaining on the 53 μm sieve was classified as particulate organic matter and sand and was dried at 105 °C, ground to a fine powder with a ball mill, and analyzed for organic C and total N concentration by dry combustion in an elemental analyzer (Costech ECS 4010, Costech Analytical Technologies, Valencia, CA).

2.7. Laboratory incubation

In preparation for the incubation, water holding capacity (WHC) was determined on a subset of four bulk soils per site (Robertson et al., 1999), with 5 g of soil placed into a funnel lined with Whatman #1 filter paper. The weight of the funnel and its contents was recorded, and soils were subsequently fully soaked with water. The funnels were wrapped with plastic wrap and left to drain for 24 h, after which the weight was again recorded. To obtain the WHC, the initial dry weight was subtracted from the final wet weight of the funnel and soil. An average WHC was calculated for each site and from this the amount of water needed to bring soils to 65% WHC. For the incubation experiment, 5 g of soil was added to 250 ml jars, soils were brought to 65% WHC, and jars were capped with rubber stoppers. To measure CO₂ respiration, jars were uncapped, flushed with lab air, recapped and a 5 ml gas sample was removed from the headspace using a syringe and injected into an infrared gas analyzer (Li-Cor Inc., Lincoln, NE). After allowing the capped soils to sit and accumulate CO₂ in the headspace, a second sample was collected and analyzed. Soils were sampled on days 1, 2, 3, 6, and 12 with a corresponding increase in time between the first and second gas samples, respectively, 3, 5, 8, 24, and 48 h. The difference between the two sampling points was calculated as respiration potential over time (Robertson et al., 1999). We determined cumulative CO₂-C by integrating the respiration rates for the total incubation time period.

2.8. Soil organic carbon (SOC) and total N

Soil organic carbon and total N were determined for bulk soil samples, macroaggregates (2000–250 μm), and microaggregates (250–53 μm). Approximately 5 g subsamples were weighing into scintillation vials, oven dried at 105 °C, and ground to a fine powder on a roller mill. Samples weighing 15–20 mg were packed into tins and analyzed via elemental analyzer (Costech ECS 4010, Costech Analytical Technologies, Valencia, CA). Using bulk density measurements, mean SOC and N stocks were calculated to 10 cm depth as: SOC or N stock (Mg ha⁻¹) = SOC or N (%) x bulk density x 10.

2.9. Statistical Analysis

To compare treatment differences across all three sites and account for the nonrandomized design, we first transformed all variables using a normal quantile transformation, also known as a normal scores transformation (SAS PROC RANK with Blom option for the normal scores, SAS Institute, Cary, NC) (Conover, 2012; Conover and Iman, 1981; Montgomery, 2005). Transformed variables were analyzed by additive
two-way ANOVA with treatment and site as the main effects (SAS PROC MIXED); interaction effects were not significant. Post-hoc testing of differences between means used the pdiff option of the LSMEANS statement in PROC MIXED, and we used the PDMIX800 macro (Saxton, 1998) to assign letters for mean separation. Preplanned contrasts, i.e., treatment comparisons specified in the initial study design and prior to data analysis, were used to compare (1) continuous maize vs legume-maize rotations, (2) DLR vs single legume rotations (Gnut and PP), and (3) treatments with pigeonpea vs treatments without pigeonpea.

3. Results

3.1. Aggregation and soil physical properties

At all sites, ~40% of soil aggregates were in the 2000–250 µm size fraction (Fig. 2). The next highest distribution (~30%) was the 250–53 µm aggregate fraction, and the > 2000 and < 53 size classes had low aggregate mass. Only the < 53 µm fraction exhibited a response to cropping treatment, where PP was significantly greater (P < 0.05) than the Maize and Gnut treatments (Fig. 2). Linthipe and Nsipe soils had a greater proportion of > 2000 µm aggregates than Kandeu, though Kandeu had more 250–53 µm and < 53 µm aggregates. The proportion of small macroaggregates (2000–250 µm) was similar across sites.

We saw no influence of cropping treatment on the rate of water infiltration, bulk density or aggregate mean weight diameter (MWD). Kandeu had higher infiltration rates than Linthipe, which in turn were greater than Nsipe, whereas both bulk density and aggregate MWD were greater in Nsipe and Linthipe than in Kandeu (Table 3).

3.2. In-situ respiration and infiltration

In-situ respiration rates were not significantly different among treatments (Supplementary Fig. S1A). The percent change from respiration rate prior to water addition to post water addition was also not significantly different by treatment (Supplementary Fig. S1B). However, rates were significantly different among trial sites. Nsipe had the highest in-situ respiration rates and the greatest changes in respiration rate after water addition, Linthipe followed, and lastly Kandeu exhibited the lowest respiration rates and differences in the rate of CO₂ respired pre- and post-water addition.

3.3. Water extractable organic carbon (WEOC)

Water extractable organic carbon was significantly higher in DLR compared to all other treatments (P < 0.01), and significantly greater at Linthipe than Kandeu and Nsipe (Fig. 3A). Relative to bulk SOC, we saw no treatment differences in WEOC, and Nsipe had significantly more WEOC than Linthipe, which had more than Kandeu (Fig. 3B). Water extractable organic carbon was approximately 0.83–0.97% of bulk SOC.

3.4. Particulate organic matter carbon (POM-C)

POM-C was higher in the DLR and the single-legume rotations compared to maize (P < 0.05, Table 4), but differences were not apparent for POM-C relative to total SOC (Fig. 4B). POM-C comprised approximately 25–29% of total SOC. Linthipe and Kandeu had significantly more POM-C relative to bulk soil than Nsipe (Fig. 4A), but relative to bulk SOC, Kandeu soils had significantly more than Linthipe and Nsipe (Fig. 4B).

3.5. Potentially mineralizable C – laboratory incubation

We observed significant effects of treatment on total C respired during a 12-day incubation with highest total C respired in DLR soils,
Kandeu soils respired greater total C per bulk SOC than Linthipe soils. SOC exhibited no significant treatment differences, and Nsipe and compared to Nsipe (Fig. 5A). In contrast, total C respired relative to bulk Table 4. Kandeu and Linthipe had significantly greater total C respired—followed by PP, Gnut, and lowest in Maize soils (P < 0.05, Table 4). Planned contrasts to differentiate effects of maize vs legume rotations, DLR vs single legume rotations, and treatments containing pigeonpea (PP, DLR) vs treatments without (Gnut, Maize), on SOC pools, or soil C:N ratios (Table 3). Bulk SOC was highest in Linthipe, followed by Kandeu, and lowest in Nsipe. Kandeu and Linthipe were higher in total N and SOC and total N stocks than Nsipe. Linthipe had the widest C:N ratio, while Kandeu and Nsipe had narrower C:N values. In total C stocks, or soil C:N ratios (Table 3). Bulk SOC was highest in Linthipe, followed by Kandeu, and lowest in Nsipe. Kandeu and Linthipe were higher in total N and SOC and total N stocks than Nsipe. Linthipe had the widest C:N ratio, while Kandeu and Nsipe had narrower C:N values (Table 3).

3.6. Total soil organic carbon (SOC) and nitrogen (N)

We did not observe a treatment response on bulk SOC, total N, SOC stocks, or soil C:N ratios (Table 3). Bulk SOC was highest in Linthipe, followed by Kandeu, and lowest in Nsipe. Kandeu and Linthipe were higher in total N and SOC and total N stocks than Nsipe. Linthipe had the widest C:N ratio, while Kandeu and Nsipe had narrower C:N values (Table 3).

### 3.7. Aggregate C and N

Carbon within small macroaggregates (2000–250 μm) and microaggregates (250–53 μm) was impacted by treatment (P < 0.05, Figs. 6A and 6B).
and different among sites. For both size classes, planned contrasts indicated the maize-legume rotations were different in C than the maize ($P < 0.01$ and $P < 0.05$, respectively, Table 4). Within the small macroaggregates, we found treatment effects on total N, which was significantly lower in the continuous maize treatment compared to the rotations containing legumes ($P < 0.01$, Fig. 6B); however, we did not find treatment effects on total N within the microaggregates (Fig. 6E). Small macroaggregate and microaggregate C and total N were all highest in Kandeu, next highest in Linthipe, and lowest in Nsipe. C:N values within small macroaggregates and microaggregates showed no treatment impact, and sites followed different patterns than the SOC and total N; Linthipe soils had the widest C:N ratios in both fractions (Figs. 6C and 6F).

4. Discussion

Grain legume diversification had positive impacts on management-sensitive SOC pools after six years of cropping trials on smallholder farms in three differing sites in central Malawi. Compared to continuous maize, the DLR had greater C within labile C pools – POM-C, WEOC, mineralizable C, and macroaggregates, as well as in the more stable microaggregates. Rotational diversity did not impact aggregate distribution, but it did affect aggregate associated C and N. For the single legume rotations, PP and Gnut had fairly similar C values within the different pools, though PP had higher mineralizable and microaggregate C compared to Maize. Planned contrasts differentiated legume-maize rotations from Maize in all pools except WEOC, and further differentiated DLR from the single legume rotations in WEOC, mineralizable C, and macroaggregate C. These results are consistent with the idea that diversifying crop rotations with grain legumes will have the potential to enhance C in management sensitive SOC pools, and this is one of the first reports to show this effect on smallholder farm sites. As expected, there were differences by site for almost all variables measured.

4.1. Bulk soil SOC and related soil physical characteristics not different by treatment

Across all sites, treatment differences were not apparent in bulk SOC and total N concentrations or stocks or C:N ratios suggesting that changes seen in the other C pools are too small or incremental to be captured at the larger, bulk soil scale (Table 3). These results are consistent with other studies in SSA that did not detect changes when comparing bulk SOC and total N in legume-maize rotations to continuous sole maize (Anyanzwa et al., 2010; Batino and Naare, 2000; Franke et al., 2008; Yusuf et al., 2009). Substantial changes in SOC stocks are required to impact soil physical properties like bulk density, MWD, and infiltration, and with little change in total SOC, we also did not see impacts of legumes on these parameters (Table 3). Although it has been theorized that decomposition of pigeonpea’s large coarse roots could create deep channels that enhance rainfall infiltration (Chikowo...
et al., 2020), we did not see evidence of increased water infiltration rates in PP or DLR after three rotation cycles. Overall, we did not find differences in bulk or total pools of C and N with legumes after three rotation cycles, but the treatment differences seen in more dynamic C pools suggest that over time differences among treatments could be apparent in bulk SOC.

4.2. Aggregates in legume treatments higher in SOC

Aggregation did not vary by treatment for the >53 µm size classes (Fig. 2), likely because all treatments and sites were intensively tilled with hand hoes and ridged on an annual basis. Tillage reduces the number and stability of soil aggregates and through aeration promotes rapid mineralization of SOC (Six et al., 2000). Aggregate disturbance is often greater in coarse-textured, sandy soils (Feller and Beare, 1997). Sandier soils at Kandeu may have contributed to lower numbers of large macroaggregates (>2000) compared to Linthipe and Nsipe. However, Kandeu had a larger proportion of microaggregates and <53 size fraction, which are size classes that are less susceptible to tillage disturbance.

DLR had the highest C in both small macroaggregates and microaggregates, PP and Gnut followed, and Maize had the lowest C. This is
consistent with legume diversified systems being associated with more stabilized SOC and accrual in aggregates, relative to sole maize (Figs. 6A and 6D). In a container experiment using soil from the Linthipe trial site, pigeonpea was shown to be associated with SOC accrual in small macroaggregates relative to sole maize (Garland et al., 2018). While all treatments received proportional amounts of N-fertilizer (Table 2), the greatest inputs were to the Maize treatment, which also had the lowest SOC. In trials located in similar pedo-climatic conditions in Kenya, N-fertilizer addition was shown to increase C mineralization and macroaggregate turnover, resulting in lower SOC (Chivenge et al., 2011). A meta-analysis of N addition in China found that N addition in croplands did not significantly affect macroaggregate, microaggregate, or silt-clay associated C (Lu et al., 2021). The sole maize treatment received more N-fertilizer, but the rotations with legumes had significantly more N in macroaggregates, perhaps due to N-rich litter and root inputs from legumes contributing to macroaggregate formation. Like the SOC, the N in macroaggregates is more easily mineralized, and therefore it is not surprising that the total N within microaggregates was not higher for legume treatments as the N was mineralized before reaching that stage of stabilization.

4.3. Active C pools higher in legume rotations

The POM-C fraction exhibited the same pattern of treatment differences as the macroaggregates, which follows as POM can act as a “seed” in macroaggregate formation, and can be indicative of macroaggregate generation and C concentration (Six et al., 2000). POM-C is understood to be largely plant-derived, thus larger amounts of POM-C are linked to changes in the quantity or quality of plant matter inputs. N-rich, low C:N legume residues are expected to decompose faster than high C:N maize residues (Cotrufo et al., 2019). It is possible that the higher POM-C content in the DLR and single legume rotations is actually associated with maize residue inputs, but with maize being only present in one phase of the two-year rotation, it is likely that POM-C is associated with legume residues. This is consistent with isotopic studies showing soil C accrual being specifically associated with legume root residues (Puget and Drinkwater, 2001).

Partially in-line with our hypotheses, we found higher WEOC to be associated with DLR but not single legume rotations relative to continuous maize. In contrast, another study in SSA did find water soluble carbon, i.e., WEOC, to be higher in single legume-maize rotations compared to continuous maize (Yusuf et al., 2009). The range of WEOC values (Fig. 3) are lower than those obtained by Yusuf et al. (2009) but comparable to other values reported in the literature (Schiedung et al., 2017). The concentration of WEOC can vary based on the season, duration of extraction, soil-to-water ratio, and air-drying of soils (Chantigny, 2003; Kaiser et al., 2015; Schiedung et al., 2017). We sampled at one time point during the dry season, after maize harvest, and values may be affected by seasonal fluctuations, soil handling, and measurement methods.

We explored whether measuring respiration before and after wetting soils in situ could effectively capture a CO₂ burst, but no treatment differences were seen (Supplementary Fig. S1). At all sites, the soils were extremely dry and there had been no rainfall for over one month prior to adding the water for the burst tests at Linthipe and Nsipe, and only one 5 mm rainfall event recorded at Kandeu more than two weeks prior to sampling (Climate Hazards InfraRed Precipitation with Station (CHIRPS), Funk et al., 2015). Due to logistical time and equipment constraints, we were limited to measuring respiration two hours after water addition. There were dramatic fluxes of CO₂ after soil rewetting (Supplementary Fig. S1), but due to the short time frame, it is unlikely that we captured a flush indicative of longer-term C mineralization (Canarini et al., 2017; Franzluebbers et al., 2009). Multiple measurements over a longer time period post-rewetting could be more effective. Nsipe, which had the lowest total SOC and lowest SOC in all other measurements relative to the other sites, had the highest in-situ soil respiration rates and change in soil respiration post-water addition, suggesting that at Nsipe SOC exists in fragile, rapidly mineralized pools.

Although treatment differences were not discernible in the field-based respiration test, in our 12-day laboratory incubation, cumulative respiration trended highest for DLR followed by PP, Gnut, and maize on a bulk soil basis. These data support other analyses of bioaccessible soil C accrual with legumes and highlight that at least some of the SOC in the DLR and the single legume treatments was not stabilized and was easily mineralized. However, when quantifying cumulative respiration relative to bulk SOC as opposed to bulk soil, there were no treatment differences, which is consistent with there being more SOC, and potentially more stable SOC, in the DLR than in the continuous maize.

4.4. Variable SOC and N across sites

As expected, the impact of cropping system treatment on SOC and N pools varied by site. The Agricultural Production Systems Simulator (APSIM) modeling study that examined SOC and N changes at Africa RISING trial sites including Linthipe and Kandeu, predicted slightly negative bulk SOC and N trends in DLR and a strongly negative trend in Gnut and Maize at Linthipe, while at Kandeu it predicted a positive trend for SOC and N in the DLR and fairly constant values in Gnut and Maize (Smith et al., 2016). We did not see treatment differences in bulk SOC and total N concentrations or stocks across trial sites in Kandeu, Linthipe, and Nsipe, but we observed that Kandeu had higher SOC and N in the more rapid-cycling macroaggregate, microaggregate, and POM-C pools (Figs. 6 and 4), which appears to support the APSIM predictions. In these same pools DLR had the highest values followed closely by either PP or Gnut, which lends some support to Smith et al.’s (2016) observation that the addition of pigeonpea to the cropping system model caused SOC and N to increase at Kandeu and remain constant at Linthipe. Nsipe, which was not included in the APSIM study, had the lowest bulk SOC and N values (Table 3), but the highest in situ respiration rates (Supplementary Fig. S1), lab incubation cumulative respiration relative to bulk SOC (Fig. 5), and WEOC relative to bulk SOC, which suggests that the low concentration of SOC at Nsipe was also easily mineralized and unstable. Based on soil and environmental indicators, Mungai et al. (2016) classified the agricultural land potential at Linthipe as highly suitable, while Kandeu and Nsipe were marginally suitable, but our findings and those of Smith et al. (2016) highlight the potential for Kandeu, a more marginal site, to achieve higher SOC and N gains, relative to Linthipe and Nsipe.

4.5. Doubled-up legume rotations elevated in SOC pools

Higher POM-C, mineralizable C, small macroaggregate C, and microaggregate C in the maize-legume rotations compared to continuous maize suggest that diversifying with legumes can lead to greater SOC accumulation and stabilization over time (Table 4). The DLR had higher WEOC, mineralizable C, and macroaggregate C than the single legume rotations (Table 4), exhibiting a trend of slightly elevated values. Our results are consistent with those of a global meta-analysis that examined the effects of legume incorporation into cropping systems on SOC fractions and found significant increases in SOC with grain legumes in all fractions considered (Li et al., 2023). In contrast, a literature study that looked at bulk SOC concentrations found that including a grain legume in rotation decreased SOC by 5.3% and that increasing the species diversity of the rotations only affected SOC if a perennial or cover crop were added (King and Blesh, 2018); however, this study was heavily dominated by experiments located in the United States (with no studies from Africa) and focused on maize, soybean, and wheat. Legume crop species is important as compared to soybean, pigeonpea produces much more biomass and while it was grown as an annual legume in our trials, it is a woody perennial. Because of pigeonpea’s longer time in the field, greater biomass, and more extensive roots, we hypothesized that PP would accrue more SOC than Gnut, yet PP and Gnut were not
significantly different in any of the SOC pools. In comparing rotations containing pigeonpea (PP, DLR) to rotations that did not (Gnut), only WEOC exhibited an effect due to pigeonpea inclusion (Table 4). These results suggest that the DLR’s advantage lies in the combined effect of intercropped pigeonpea and groundnut, which together increase ground cover, biological nitrogen fixation inputs, and add both higher quality and higher quantities of C to soil (Chikowo et al., 2020; Snapp et al., 2002). Doubled-up legume technology has the potential to build soil health and benefit smallholder farmers through increased yields, improved nutrition, and diversity of marketable grains. Further research is needed to assess these benefits and to test the impacts of DLR on SOC and soil health in the long term.

5. Conclusion

Integration of grain legume rotations into continuous maize has the potential to enhance SOC pools compared to sole maize. This study is the first evidence for soil health services being associated with the doubled-up grain legume diversified maize system under smallholder farm conditions. Intercropped pigeonpea and ‘groundnut in the DLR system had more C in WEOC, POM-C, potentially mineralizable C, macroaggregate and microaggregate C pools than sole maize, and greater WEOC than the single legume rotations. Of the single legume-maize rotations, PP had more mineralizable and microaggregate C compared to continuous maize, while Gnut had similar C values to the maize system. Cropping treatment differences were not seen in bulk SOC or total N after six years of trial establishment, but they were apparent in SOC pools with a shorter turnover time. Readily decomposable and biologically active C pools like aggregate-associated SOC, POM-C, WEOC, and soil respiration indicated positive effects of crop rotation and diversification on SOC dynamics. We recommend that in addition to measuring bulk SOC and total N, future studies examining the impacts of management practices on SOC or soil health, measure these or other rapid-cycling, active SOC pools.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2023.108611.

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