ORIGINAL ARTICLE

Long-term application of low C:N residues enhances maize yield and soil nutrient pools across Kenya

Christine D. Sprunger · Steve W. Culman · Cheryl A. Palm · Moses Thuita · Bernard Vanlauwe

Abstract Declines in soil fertility and limited access to inorganic nitrogen (N) fertilizer constrain crop production in Sub-Saharan Africa. The incorporation of organic residues could increase nutrient mineralization and replenish soil carbon (C), however, the effect that long-term residue management (10+ years) has on maize (*Zea mays* L.) yields and soil nutrient pools (C and N) is largely unknown. In four identical long-term trials in Kenya that differ by soil type and climate, we compared maize yield and soil C (0–0.15 m) across four treatments comparing organic inputs of contrasting C:N ratios: tithonia [*Tithonia diversifolia* (Hemsl.) A. Gray] + N (120 kg N ha^{-1}); tithonia $-$ N (0 kg N ha^{-1}); maize stover + N (120 kg N ha^{-1}); maize stover $-$ N (0 kg N ha^{-1}).

On average, maize yields were 92% greater under tithonia compared to maize stover at the sandy sites and in general followed this trend: tithonia + N > tithonia $-$ N = stover + N > stover $-$ N. The continuous application of tithonia also increased soil C and N pools; for instance, mineralizable C was up to 57% greater than that of maize stover at the sandy sites. Increases in yield and nutrient pools under tithonia were less apparent at the clay sites. When exploring the relationship between soil C and maize yield, we found that both mineralizable and processed pools were related to agronomic performance. Our findings demonstrate that low C:N residues could significantly benefit crop production and enhance soil organic matter at sandy sites across Kenya.

Keywords Maize · Crop production · Soil carbon pools · Nutrient cycling · Agroecosystems

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Introduction

Soil organic matter is important for short-term benefits, such as crop productivity; long-term benefits include soil carbon (C) accumulation and enhanced soil fertility, which have lasting impacts on agroecosystems beyond a given growing season (Lal 2004; Johnston et al. 2009; O'Rourke et al. 2015). The dynamics of soil organic matter are such that inputs are decomposing at different rates, either due to their

chemical recalcitrance (Rasse et al. 2005) or physical protection from decomposers (Lehmann and Kleber 2015). The soil organic matter that is composed of newly deposited material and that is readily accessible to microbial activity is considered a short-term mineralizable pool that supplies nutrients for crop growth (Franzluebbers et al. 2000). Soil organic matter that has longer mean residence times due to physiochemical reactivity is considered a more processed or stable pool of C and is often associated with a soil's physical status, long-term soil fertility, and C stabilization (Wander 2004).

Striking a balance between mineralization and stabilization of soil organic matter is a challenging aspect of increasing soil C in agroecosystems. For example, Integrated Soil Fertility Management (ISFM) is one strategy that has been promoted to optimize mineralization for enhanced crop productivity while also targeting C stabilization for long-term soil fertility by combining inorganic fertilizers with organic residues (Palm et al. 2001; Vanlauwe et al. 2005, 2010). This management strategy is promoted in Sub-Saharan Africa (SSA), where yields have stagnated largely due to a decline in soil fertility and lack of access to nitrogen fertilizers (Sanchez 2002). Thus, by applying both organic and inorganic resources, farmers have a better chance of meeting nutrient demands, while simultaneously building soil organic matter. Furthermore, the combination of the inorganic nitrogen (N) and organic residues facilitates enhanced N synchrony since the combined release of nutrients is slower and availability occurs closer to high crop demand as opposed to when inorganic fertilizer is the sole source of nutrients (Chivenge et al. 2009).

The extent to which organic residues facilitate C and N mineralization and contribute to soil C accumulation is largely dependent on the chemical composition of a given organic residue, which is strongly determined by C:N ratios (Palm et al. 2001). Past research at our long-term trials in Kenya has shown that the lower C:N ratios of tithonia lead to greater N mineralization, while the higher C:N ratios of maize stover lead to immobilization (Vanlauwe et al. 2005). Even when inorganic fertilizer is added, residue type dictates N availability at these sites. For example, Gentile et al. (2011a) found that when the organic residues are applied with inorganic fertilizer, maize stover leads to a reduced available N pool. Residue with varying C:N ratios also have contrasting effects on soil C dynamics. Gentile et al. (2011a) found that residues with greater N concentrations increased soil C accumulation in the short-term by 9% relative to residues consisting of lower N concentrations. However, to date, there is little evidence that residue type and ISFM can effectively influence long-term C stabilization (Gentile et al. 2011b). Rather, it is clear that ISFM leads to tradeoffs associated with short and long-term nutrient dynamics (Gentile et al. 2011a). We have limited understanding of how these associated nutrient trade-offs influence crop yield over time.

In recent years, there has been a concerted effort to determine how different soil C pools are related to crop productivity, which has important implications for how an agroecosystem could be managed for short-term and long-term benefits (Culman et al. 2013; Hurisso et al. 2016; Wood et al. 2016; Cates and Ruark 2017). Recent work has shown that C pools reflecting short-term mineralization are positively correlated with maize yield. For example, Culman et al. (2013) demonstrate that both mineralizable C and permanganate oxidizable C are positively correlated with maize yields. On the other hand, particulate organic matter fractionation correlations with yield have been mixed. Wood et al. (2016) found that a faster cycling fraction of POM was positively correlated with yield, while the mineral associated POM was negatively correlated with yield. Cates and Ruark (2017) also demonstrated that aggregate occluded fractions of POM that are more closely associated with soil C sequestration were typically associated with lower maize grain yields. Such findings are at odds with those that argue that C stabilization is important for crop productivity (Lal 2004).

Consistent and continuous application of a given management practice is often needed to fully understand the mechanisms driving soil C accumulation and turnover in agroecosystems. Moreover, assessing the relationship between soil C and crop productivity over time is necessary to account for varying climatic conditions and changes in nutrient dynamics over time. Thus, the establishment and maintenance of long-term trials are crucial for quantifying soil C dynamics over time. However, trials that have been in existence for more than 5 years are rare, especially in SSA (Sedogo and Konate 2012). Long-term trials are arguably needed the most in nutrient poor regions of the world where soil fertility continues to decline and

where soil rehabilitation can take decades (Sedogo and Konate 2012).

Here we evaluate how maize yield and soil C and N indicators respond to ISFM (combination of inorganic fertilizer and organic residues) across four long-term trials in Kenya. The four sites are identical in ISFM treatments, but differ in soil type and climate. Previous studies have focused on maize yield and nutrient dynamics from these sites when the trials were in their infancy. Findings from year three of these long-term trials showed that residues differing in C:N ratios influenced C and N mineralization but had no influence on soil C stabilization (Gentile et al. 2011b). The lack of residue type effect on C stabilization might have been a function of time since it can take several years for soil C accumulation to occur (Smith 2004). In addition, Gentile et al. (2011b) only used total soil C as an indicator for C stabilization. To date, a suite of new tools have been developed to assess soil C pools that can be used to predict stabilization of C before changes in total soil C are detectable (Leifeld and Kögel-Knabner 2005; Culman et al. 2012). Our paper builds on these past studies to assess how long-term (10+ years) ISFM influences crop productivity, nutrient mineralization, and C stabilization. We report maize yields from eight growing seasons over the course of 4 years and a suite of soil health indicators of C and N dynamics from one sampling time point when the trials were 11+ years old. Our objectives were to (1) determine how long-term ISFM influences maize yield over time across sites varying in soil type and climate, (2) evaluate how these long-term ISFM practices are related to short-term C and N mineralization and more processed C pools, and (3) determine which C and N indicators are best related to agronomic performance in contrasting soils.

Materials and methods

Study site

This study was carried out at four identical long-term field trials across Kenya that ranged in soil fertility and climate (Table 1 and Fig. 1). The first pair of sites is located in central Kenya: Embu (0°30′S, 37°40′E), hereafter referred to as *Clay Central*, and Machanga (0°47′S, 37°40′E), hereafter referred to as *Sandy Central*. The second pair of sites is located in Western

Kenya: Nyabeda (0°8′N, 34°25′E), hereafter referred to as *Clay West*, and Teso (0°34′N, 34°11′E), hereafter referred to as *Sandy West*. The soils at Clay Central are classified as Humic Nitisol, while soils at Sandy Central are classified as a Ferrallic Arenosol. At Clay West, soils are of the Eutriic Ferrasol, while soils at Sandy West are classified as Ferric Acrisols.

Each site has two distinct rainy seasons including a long rainy season from March to mid-August and a short rainy season from September to January (Fig. 1). The twenty-year mean annual rainfall is 1108 mm, 800 mm, 1740 mm, and 1690 mm for Clay Central, Sandy Central, Clay West, and Sandy West, respectively (CHIRPS v 2.0). The mean annual temperature is 20.2 °C for Clay Central, 26 °C for Sandy Central, 23.2 °C or Clay West, and 22.2 °C for Sandy West.

Experimental design and management

These four field trials were established to evaluate repeated application of organic residues differing in C:N ratios in the presence and absence inorganic N fertilizer. The experiments in central Kenya and western Kenya were established in 2002 and 2004, respectively. The maize-based field experiments were designed as a split-plot with three replicate blocks. The main factor in the experiment is organic residue (6 m by 12 m) and the subplot factor is N fertilizer (6 m by 6 m). The two organic residues compared in this study are (1) *Tithonia diversifolia* (low C:N), an annual non-leguminous shrub and (2) *Zea mays* (maize stover; high C:N). Each organic residue was applied with (120 kg N ha^{-1}) or without (0 kg N ha^{-1}) inorganic nitrogen (Tithonia + N, Tithonia − N, Stover + N, Stover − N). Organic residues were analyzed for dry matter, total C and N, as well as % lignin, each year prior to application. Residue type characteristics have remained very consistent over the course of the trials. Tithonia has a mean C:N ratio of 12 and maize stover has a mean C:N ratio of 60; more extensive residue type characteristics are reported in Gentile et al. (2011b). Planting times varied due to differences in climate at each site (Fig. 1).

Prior to planting, field preparation was conducted using hand hoes. At every planting, the organic residues were broadcast at a rate of 4 tons per hectare and incorporated to a depth of 0.15 m using a hand hoe. Inorganic N fertilizer (120 kg N ha^{-1}) was applied every growing season (twice per year) to half

Table 1 Characteristics of soils (0–0.15 m) at each site taken in the summer of 2015 prior to the short rainy season

[a]Phosphorus soil test values quantified with a Mehlich 3 extraction

Soil property	Clay central	Clay west	Sandy central	Sandy west
Organic Matter (g kg^{-1})	38.0	25.0	4.0	4.0
CEC (mmol$_c$ kg^{-1})	141	116	53	53
[a]P (mg kg^{-1})	80	33	193	154
Soil pH	5.0	5.2	5.3	5.5
Clay (g kg^{-1})	524	648	148	120
Silt (g kg^{-1})	204	224	112	164
Sand (g kg^{-1})	272	128	740	716
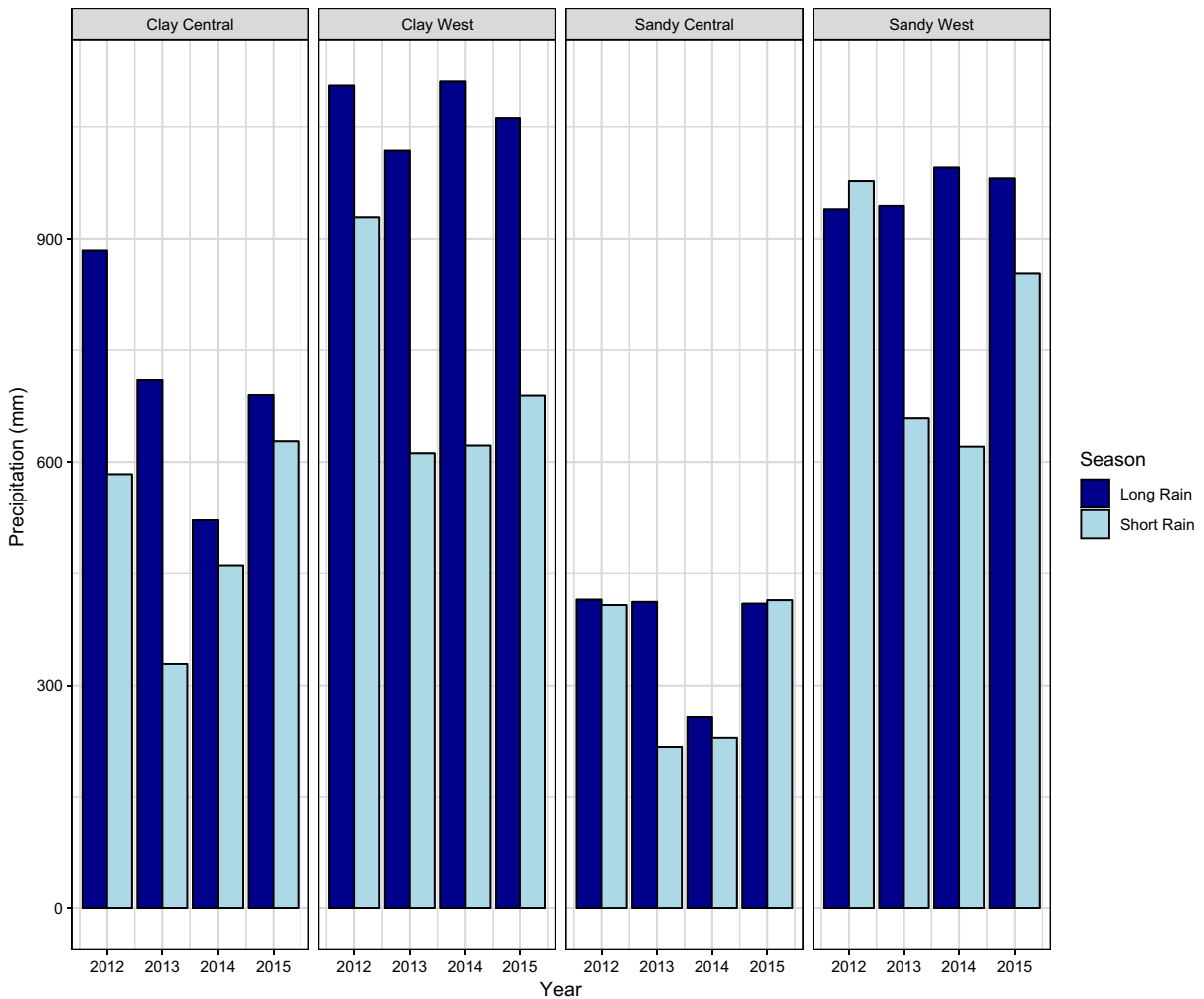
Fig. 1 Total precipitation for the short and long rainy season at four long-term trials in Kenya from 2012 to 2015

of the plots, while the other half received no inorganic fertilizer. The inorganic N fertilizer was applied as a split application, where one third of the fertilizer was broadcast at planting and the remainder was top-dressed approximately 8 weeks after planting. In addition, all plots received a blanket basal application of triple superphosphate at the rate of 60 kg P ha^{-1} and muriate of potash at the rate of 60 kg K ha^{-1} as at planting by broadcasting along with the first N

application. The fertilizers were then incorporated into the soil.

Maize was planted shortly after the onset of either the short or long rainy season each year (Fig. 1). The same 120-day to maturity maize variety was used at each site (DH04). The maize was planted with a between row spacing of 0.75 m and a within row spacing of 0.25 m. The target planting density at each site was 53,333 plants ha^{-1}. Two seeds were planted per hill and thinned down to one after emergence. Weed management consisted of hand weeding plots three times throughout the growing season.

Field sampling

Maize yield was measured at maturity and was taken from the center 13.5 m^2 of each plot. The harvest grain subsamples were dried in the oven at 60 °C until constant weight. Maize grain yield was calculated at 12.5% moisture content.

Prior to applying organic residues and planting, soil samples were collected in August 2015 at each site. A soil core 0.05 m in diameter was used to take samples from 0 to 0.15 m. Five cores were taken per plot following a systematic scheme across both diagonals of each plot. Once collected, the five cores were composited and gently sieved to 2 mm.

Laboratory analysis

Short-term mineralizable carbon

Short-term mineralizable carbon reflects the pool of C that is most accessible to microbial activity and is most sensitive to changes in management. To measure mineralizable C, a 24-h mineralizable C assay was used to determine CO_2 respired from rewetted soils based on Franzluebbers et al. (2000), Haney et al. (2001) and Hurisso et al. (2016). Ten gram of soil were placed in 50 ml polypropylene centrifuge tubes and brought to 50% water holding capacity. Each centrifuge tube was tightly capped with a lid fitted with a rubber septum. A time zero CO_2 reading was taken immediately after capping by injecting 0.5 ml of headspace into a Li-Cor LI-820 infrared gas analyzer. Next, the centrifuge tubes were stored in the dark for 24 h at 25 °C. Following the 24-h incubations, a second CO_2 reading was taken following the same procedure. Short-term mineralizable C was determined as the difference between time zero and 24 h CO_2 concentration.

Permanganate oxidizable carbon

POXC is an indicator of the theoretical 'active C pool'; however, it reflects a more processed pool compared to mineralizable C and can provide an early indication of soil C stabilization (Culman et al. 2012; Hurisso et al. 2016). POXC is based on a chemical oxidation of organic matter by a weak potassium permanganate solution (Weil et al. 2003; Culman et al. 2012). Briefly, 2.5 g of air-dried soil were placed in 50 ml polypropylene centrifuge tubes. Next, each tube received 20 ml of 0.02 mol L^{-1} KMnO$_4$. The tubes were shaken for exactly 2 min at 240 oscillations min^{-1} and then sat undisturbed to ensure settling for exactly 10 min. Following the 10 min, 0.5 ml of the supernatant were transferred into a second 50 ml centrifuge tube containing 49.5 ml of deionized water. From this dilution, 200 µL from each sample was loaded into a 96-well plate. A spectrophotometer was used to read sample absorbance at 550 nm, and POXC (mg kg^{-1} soil) was calculated according to Weil et al. (2003).

Particulate organic matter and total C and N

Physical size fractionation was used to determine particulate organic matter (POM), which has been shown to reflect both short-term mineralization and more processed C pools, depending on particle size (Cambardella and Elliot 1992). Ten gram of air-dried soil were combined with 30 mL of 0.05 sodium hexametaphosphate in 50 mL centrifuge tubes. Tubes were then placed on a shaker for 8 h at 120 oscillations min^{-1}. Next, two POM fractions were isolated, large (2 mm–250 µm) and medium (53–250 µm), to capture both short-term mineralization and more processed soil C pools, respectively. Deionized water was used to pass the mixture of soil and sodium hexametaphosphate through stacked 250 µm and 53 µm sieves. The materials that were retained on each sieve included fine roots and large sand particles. POM fractions were oven dried at 55 °C until constant weight. Dried samples were then ground using a mortar and pestle and analyzed for C and N with a CHNSO Analyzer (Costech ECS 4010, Costech

Analytical Technologies, Valencia, CA). Whole soils were also analyzed for total C and N.

Soil protein

Soil protein was measured to determine the size of the organically bound N pool in soils (Hurisso et al. 2018). This measurement reflects a pool of N that is sensitive to changes in management. First, 3 g of air-dried soil and 24 ml of 20 mM sodium citrate, pH 7.0) were added to 50 ml glass extraction tubes. Samples were shaken at 180 strokes per minute for 5 minutes and then placed in the autoclave for 30 min at 121 °C and 1.02 atm. After cooling, the soil was re-suspended by shaking the tubes for 1 min at 180 strokes per minute. Then, 1.75 ml of the mixture was transferred to a 2 ml microcentrifuge tube and centrifuged at $10,000 \times g$ for 3 min. Ten μl of the clarified extract were transferred from the centrifuge tubes into a 96-well microplate for a standard colorimetric protein quantification assay (Thermo Pierce BCA Protein Assay). Two hundred μl of the working reagent were added to each well of the micro-plate. The plate was then sealed and incubated on a heating plate for 60 min at 60 °C. The plate was read at 562 nm. The extractable protein content of the soil was calculated by multiplying the protein concentration of the extract by the volume of extractant used and dividing that product by the number of grams of soil used.

Soil characteristics

Soil organic matter was determined using loss on ignition where soils were placed in a muffle furnace at 360 °C for 2 h (Combs and Nathan 1998). Soil water pH was determined with a glass electrode in a 1:1 soil/water (w/v) slurry. Buffer pH was determined with a Sikora buffer solution (pH of 7.7) (Sikora 2006). CEC was calculated by summation of cations. Extractable soil P was determined using a Melich-3 extractant (Mehlich 1984). Particle size analysis using a hydrometer was used to quantify the proportions of sand, silt, and clay.

Statistical analyses

All crop and soil response variables were analyzed using Proc Mixed of SAS (version 9.4; Institute, Cary, NC, USA). Site, season, organic residue, fertilizer, and treatment interactions were treated as fixed effects, while block and treatment by block interactions were treated as a random effect. When maize yield was analyzed over multiple growing seasons, year was treated as a repeated measure with a compound symmetry covariance structure (TYPE = csh). Significant differences were determined at α = 0.05. Means were compared with an adjusted Tukey's pairwise means comparison. Our statistical analysis includes yields from plots receiving either organic residue ammendments combined with inorganic fertilizers and organic residue ammendments applied without inorganic fertilizers during both the short and rainy seasons over a 4-year period (2012–2015).

We conducted correlation tests to determine the relationship between individual soil C and N indicators and maize yields using the pairs function in R (R Core Team 2017). The *cor.test()* function was used to determine the significance of the correlations at α = 0.05.

Stepwise multiple linear regression was used to assess which soil C indicators best predicted maize yields. This regression analysis was conducted using the *regsubsets()* function within the leaps package in R. The regsubsets function performs an exhaustive search, considering all variables (x) and then ranks each variable based on their ability to best predict (y). We used adjusted R^2 to determine the best model fit.

We also wanted to explore how the application of organic residues with or without fertilizer related to POXC and mineralizable C as a means of exploring stabilization and mineralization processes. To execute this, we adopted a framework developed by Hurisso et al. (2016) that calculates the average residuals of a linear regression model to determine how different management practices impact POXC and mineralizable C and assesses the relationship between these two indicators. Here, a linear model was fit at each site and across all sites to identify how residue type was related to POXC and mineralizable C. In the model, mineralizable C was designated as the predictor variable, while POXC was designated as the response variable. We calculated residuals of the linear model across all sites and within each site. Next, we examined residuals from the model whereby observations with greater than predicted POXC values had positive residuals, while observations with greater than predicted mineralizable C values had negative residuals.

Results

Maize grain yield

Overall, there was a significant residue effect on maize grain yield at each site, except Clay Central during both seasons and Clay West during the short rainy season ($P < 0.05$, Fig. 2). The addition of fertilizer only impacted yields at the sandy sites (Fig. 2, Table 2). There was also a strong year effect at each site for both the short and rainy seasons (Fig. 2, Table 2). Finally, there were fertilizer by year interactions at both the Sandy Central and Sandy West sites during the long rainy season ($P < 0.05$, Table 2).

Differences between the two residues were most pronounced at the sandy sites, where the addition of tithonia consistently led to greater yields compared to maize stover (Fig. 2). The four-year mean grain yields at the Sandy West site followed the ranking: 4.9 (Tithionia + N) > 4.0 (Tithionia − N) = 3.2 (Stover + N) > 2.0 Mg ha^{-1} (Stover − N) (Table 3). The treatment means were significant from one another, except for the Tithionia − N and the Stover + N treatments. Similar trends were visible at the Sandy Central site where the tithonia treatments typically had

greater yields compared to the stover treatments. Exceptions to this trend were evident during the short rainy season in both 2013 and 2014, and during the long rainy season in 2015, when the site experienced periods of drought (Figs. 1, 2). The lack of differences between the treatments in 2014 is likely the cause of the significant residue by year effect at the Sandy Central site.

Differences were muted between treatments at the clay sites, with the exception of the Clay West site during the long rainy season (Fig. 2), where there was a significant residue effect ($P < 0.01$, Table 3). During the long rainy season at Clay West, both tithonia treatments were consistently greater than both stover treatments in 2012 and 2013. In 2014, tithonia − N was significantly greater than all other treatments (Fig. 2). There were no residue effects at the Clay Central site during either rainy season ($P > 0.05$, Table 2).

Year to year differences in yield were evident at all sites; however, the western sites had more stable yields over time compared to the central sites, reflecting more consistent cumulative and seasonal precipitation trends (Table 2). Maize grain yields were lowest at the
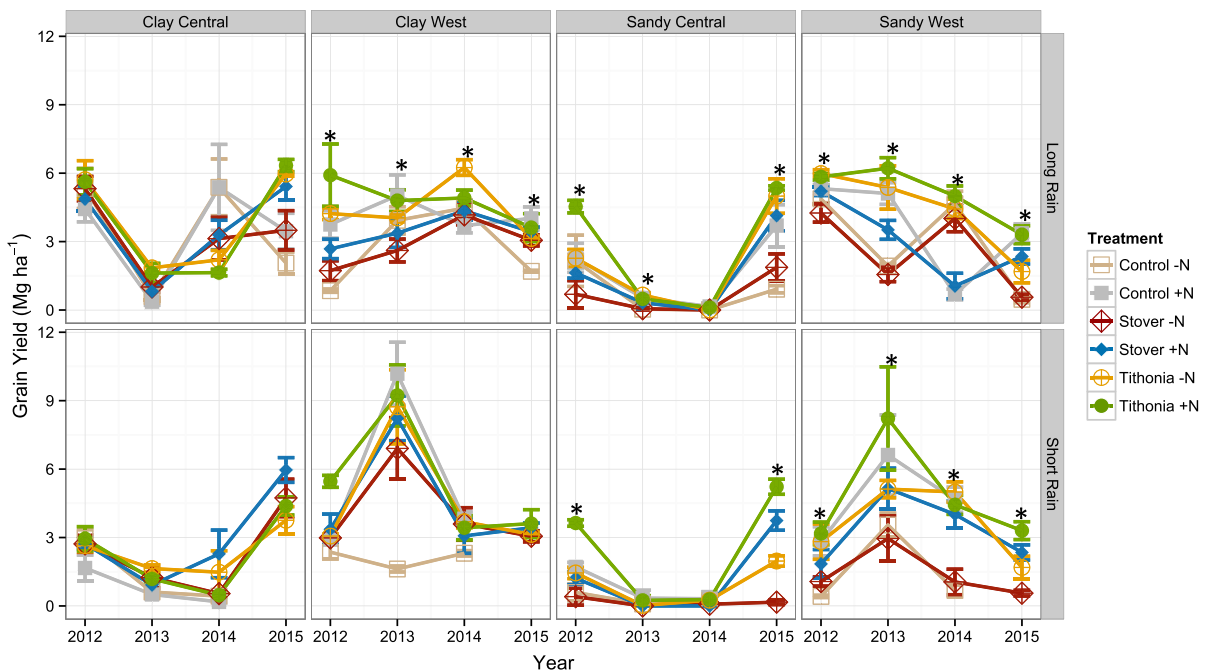
Fig. 2 Mean maize yields from the long rainy season (top panel) and the short rainy seaon (bottom panel) over the course of 4 years (2012–2015) from systems amended with organic residues differing in C:N ratios (maize stover and tithonia) in the presence

Table 2 Maize yield F-statistics and significance from a repeated measures ANOVA across 4 years (eight growing seasons)

Source	Long rainy season				Short rainy season			
	Clay central	Clay west	Sandy central	Sandy west	Clay central	Clay west	Sandy central	Sandy west
Residue (R)	1.0	15.5**	55.4***	69.55***	1.5	2.6	76.7***	14.1**
Fertilizer (F)	0.3	2.3	21.3**	6.31*	1.0	2.7	138.4***	13.8*
R × F	1.3	0.1	0.5	0.39	2.2	0.3	2.6	0.9
Year (Y)	116.1***	17.6**	115.8***	107.15***	64.8***	16.3**	111.1***	14.5**
R × Y	5.1*	5.6*	16.9**	6.0*	2.5	1.7	13.3**	1.3
F × Y	3.0	3.2	6.8*	8.7**	2.2	2.7	38.9***	1.5
R × F × Y	1.0	1.4	2.5	4.9*	1.8	1.8	1.5	2.7

*Significance level: $P < 0.05$

**Significance level: $P < 0.01$

***Significance level: $P < 0.0001$

Table 3 Means (and standard error) of maize grain yield ($n = 8$) and soil C and N indicators ($n = 3$) from maize systems amended with organic residues (maize stover and tithonia) applied at a rate of 4 Mg C ha^{-1} with 120 kg N ha^{-1} (+) of inorganic fertilizers or 0 kg N ha^{-1} (−)

Site	Residue	Yield	Mineralizable C	POXC	Soil Protein	250 μm-2 mm POM	53–250 μm POM	Total C
	(Plus or minus fertilizer)	Mg ha^{-1}	mg C kg^{-1} soil day^{-1}	mg kg^{-1} soil		g C kg^{-1} POM		g C kg^{-1} soil
Clay central	Stover (+ N)	3.3 (0.5)	36.5 (3.4)	388 (14)	8.7 (0.3)	9.5 (7.7)	9.70 (7.9)	17.2 (2.4)
	Stover (− N)	2.8 (0.5)	30.9 (2.6)	377 (40)	7.9 (0.3)	11.6 (7.1)	10.0 (6.7)	15.4 (3.9)
	Tithonia (+ N)	3.0 (0.3)	29.9 (4.7)	339 (32)	7.9 (0.9)	15.7 (5.9)	16.3 (6.7)	18.5 (3.7)
	Tithonia (− N)	3.2 (0.4)	39.6 (3.0)	362 (49)	8.3 (0.7)	16.6 (6.6)	16.5 (6.8)	18.9 (2.5)
Clay west	Stover (+ N)	3.9 (0.5)^{ab}	28.6 (4.9)	430 (42)	7.7 (0.2)^b	35.9 (6.4)	27.8 (1.3)^b	13.6 (1.4)
	Stover (− N)	3.5 (0.5)^b	27.9 (3.6)	464 (55)	8.1 (0.6)^b	42.2 (8.3)	42.2 (5.0)^a	16.2 (0.9)
	Tithonia (+ N)	5.1 (0.7)^a	32.6 (2.1)	435 (113)	8.2 (0.9)^b	34.3 (5.7)	41.3 (6.0)^a	13.7 (1.7)
	Tithonia (− N)	4.5 (0.4)^{ab}	29.3 (3.4)	472 (130)	10.3(1.1)^a	32.2 (3.7)	36.7 (8.8)^{ab}	17.2 (1.4)
Sandy central	Stover (+ N)	1.6 (0.3)^{ab}	12.0 (2.3)^a	86 (19)	2.0 (0.2)^{bc}	11.7 (5.1)	11.0 (4.3)	1.6 (0.2)
	Stover (− N)	0.5 (0.3)^c	9.3 (2.9)^b	84(26)	1.9 (0.4)^c	11.7 (5.4)	11.1 (4.8)	1.6 (0.5)
	Tithonia (+ N)	2.8 (0.2)^a	14.3 (2.7)^a	117 (32)	3.0 (0.2)^a	15.8 (4.9)	17.9 (6.2)	1.4 (0.1)
	Tithonia (− N)	1.7 (0.3)^b	16.9 (5.0)^a	111 (23)	2.5 (0.1)^b	15.3 (4.9)	14.7 (4.8)	2.0 (0.2)
Sandy west	Stover (+ N)	3.2 (0.5)^b	13.7 (1.4)^b	125 (13)^b	2.4 (0.2)^b	1.3 (0.5)	0.8 (0.03)^b	1.3 (0.1)
	Stover (− N)	2.0 (0.4)^c	15.2 (1.1)^b	137 (16)^b	2.6 (0.1)^b	1.0 (0.1)	0.9 (0.10)^{ab}	1.2 (0.2)
	Tithonia (+ N)	4.9 (0.6)^a	22.1 (3.5)^a	208 (29)^a	3.6 (0.2)^a	3.1 (0.9)	1.6 (0.50)^a	1.5 (0.2)
	Tithonia (− N)	4.0 (0.5)^b	22.0 (3.5)^a	238 (51)^a	3.6 (0.1)^a	1.9 (1.2)	1.0 (0.03)^{ab}	1.8 (0.6)

Yield values represent a 4 year average across the short and long rainy season. Different lower case letters denote significant differences across organic residues and fertilizer additions within a given site

Central Sandy site, which was the site most affected by drought in 2013 and 2014.

The site effect was included in the statistical model for the 4-year mean maize grain yield and was statistically significant at $P < 0.05$ (Tables 3, 4). In general, the western sites had greater yields compared to their eastern counterparts (Tables 4). Averaged across treatment, the Clay West site was the most

Table 4 F-statistics of maize yield, soil C and soil N indicators

Source	Yield	Mineralizable C	POXC	Soil protein	250 μm–2 mm POM	53–250 μm POM	Total C
Site (S)	43.1***	36.5***	45.0***	101.2***	9.3**	10.8**	96.0***
Residue (R)	43.5**	12.6**	0.02	8.7*	0.4	3.3[*]	0.9
S × R	5.94*	0.8	3.6[*]	1.8	1.7	0.4	0.4
Fertilizer (F)	54.7***	0.0	1.9	1.0	0.4	0.9	0.6
S × F	5.0*	0.3	0.4	2.7[t]	0.5	2.3	0.8
R × F	1.3	1.2	0.7	1.8	1.8	9.6**	0.4
S × R × F	0.7	1.7	0.5	1.5	0.9	5.7**	0.1

[*]Significance level: $P < 0.1$

*Significance level: $P < 0.05$

**Significance level: $P < 0.01$

***Significance level: $P < 0.0001$

productive site, with an average yield of 4.25 Mg ha^{-1}, followed by the Sandy West site (3.52 Mg ha^{-1}), the Clay Central site (3.10 Mg ha^{-1}), and the Sandy Central site (1.65 Mg ha^{-1}). The significant site by residue and site by fertilizer interactions were likely caused by the strong residue and fertilizer effects at the sandy sites in contrast to no significant residue or fertilizer effects for the 4-year mean maize yield at the clay sites.

Average yields from the control plots that did not receive any type of input were 2.5 Mg ha^{-1} ± 0.5, 2.5 Mg ha ± 0.3, 0.6 Mg ha^{-1} ± 0.2, and 2.1 Mg ha ± 0.4 for Clay Central, Clay West, Sandy Central, and Sandy West, respectively. Yields from the plots that received 120 kg N ha but no organic residues were 2.3 Mg ha^{-1} ± 0.5, 4.8 Mg ha^{-1} ± 0.6, 1.3 Mg ha^{-1} ± 0.3, and 4.1 Mg ha^{-1} ± 0.5 for Clay Central, Clay West, Sandy Central, and Sandy West, respectively. These control plot maize yields (no input, and only inorganic fertilizer) were not included in the statistical model and are reported here for comparison purposes only. For instance, we compared yields from the Tithonia − N plots and the inorganic only plots (control + N) to understand which source of N was more important for yield. We found that in three out of the four sites, the Tithonia − N plots had equal to or greater yields compared to the plots only receiving inorganic N.

Soil carbon and nitrogen pools

Tithonia increased mineralizable C relative to maize stover, but only at the sandy sites (Table 3). For instance, at the Sandy West site, the tithonia treatments had mineralizable C rates that were 53% greater than that of the stover treatments. A similar disparity between the two treatments was evident at the Sandy Central site, where the tithonia treatments had 49% greater mineralizable C compared to maize stover treatments. There were no significant differences between the tithonia and stover treatments at either of the clay sites. The addition of inorganic N fertilizer had no effect on mineralizable C (Table 4; $P > 0.1$). Mineralizable C was also strongly influenced by a site effect (Table 4; $P < 0.0001$). When averaged across treatment, mineralizable C was greatest at the Clay Central site (34.2 mg C kg^{-1} soil day^{-1}), followed by Clay West (29.6 mg C kg^{-1} soil day^{-1}), Sandy West (18.3 mg C kg^{-1} soil day^{-1}), and Sandy Central (13.1 mg C kg^{-1} soil day^{-1}).

Although there was no residue or fertilizer effect on POXC (Table 4; $P > 0.05$), there were noteworthy differences between the two residue treatments at the Sandy West site, which likely caused the marginally significant site by residue interaction (Table 3). POXC was significantly different across sites (Table 4; $P < 0.0001$); for instance, POXC values were ∼ 3 times greater in the clay sites compared to the sandy sites. Fertilizer additions had no effect on POXC (Table 4; $P > 0.05$).

On average, tithonia treatments had 44% more soil protein compared to the maize stover treatments at the sandy site (Table 3). For example, at the Sandy West site, both the Tithonia + N (3.6 mg kg^{-1} soil) and the Tithonia − N (3.6 mg kg^{-1} soil) treatments were significantly greater than the Stover + N (2.4 mg kg^{-1} soil) and Stover − N (2.6 mg kg^{-1} soil) treatments. While there was no overall fertilizer effect (Table 4; $P > 0.05$), there was a marginally significant fertilizer by site interaction (Table 4; $P < 0.1$), which was likely the result of significant differences in soil protein between the Tithonia + N and Tithonia − N at both the Sandy Central and Clay West sites. There was also an overall significant site effect (Table 4; $P < 0.0001$), where soil protein values at the clay sites were three times greater than those at the sandy sites.

The large POM C fraction (250 μm–2 mm) was significantly influenced by site but not by residue or fertilizer (Table 4; $P < 0.001$). Noteworthy differences were visible between soil type and region. In particular, Clay West had 2.7 times more C in the large POM than that of the Clay Central site (Table 3). There was also a noticeable difference in large POM C values between the sandy sites. Sandy West had substantially lower large POM C values than the Sandy Central site (1.8 vs. 13.62 g C kg^{-1} POM).

Medium POM C (53–250 μm) values trended higher in the tithonia treatments relative to maize stover at each site, except Clay West (Table 3). Furthermore, Tithonia + N had significantly greater medium POM C than the Stover − N treatment at both the Sandy West and Clay West sites (Table 3). The Clay West site had substantially greater medium POM C values compared to the other sites (Table 3). The significant site by residue by fertilizer interaction (Table 4; $P < 0.01$) was likely caused by significant differences amongst the treatments at the western sites, while there were no significant differences amongst the treatments at the central sites.

The clay sites had over ten times the amount of total C compared to the sandy sites, causing an overall significant site effect (Tables 3, 4; $P < 0.0001$). The values at the clay sites ranged from 13.7 to 18.9 g C kg^{-1} soil, while total C values at the sandy sites ranged from 1.2 to 1.6 g C kg^{-1} soil. There were no significant differences between fertilizer rates or residue type (Table 4).

Correlations between maize yield and soil C and N pools

We correlated all six soil C indicators with yield across all four sites as well as within each individual site. When combining all sites together, we found positive and significant correlations between all six soil C indicators and maize yield ($P < 0.01$, Table 5), but soil protein, mineralizable C, and POXC were more strongly correlated to yield than total C, medium POM C, and large POM C. Trends were substantially different when correlations were calculated within individual sites and inconsistent among sites. Across the four individual sites, the number of times a soil measurement was significantly related to yield ranked as follows: soil protein (3), POXC (2), mineralizable C (1), large POM C (1), medium POM C (1), Total C (0) (Table 5).

Soil C and N pools as indicators of agronomic performance

Using the subset regression analysis, we ranked the top indicators on their ability to predict maize yields across all sites and within each site (Table 6). When the four sites were combined, the top three ranked indicators of maize yield were soil protein, total C, and mineralizable C. When looking at each site individually, the rankings of the predictor variables varied; however, there were commonalities based on soil type and region. For instance, medium POM C was ranked in the top three in all but the Clay Central site. Mineralizable C was in the top two rankings for both of the clay sites, and both soil protein and medium POM C were in the top two for the sandy sites (Table 6). Total C was ranked in top three for Clay Central and Sandy West; however, the correlation between total C and yield at Clay Central was negative (Table 6).

Organic residue impacts on stabilization and mineralization processes

In Fig. 3, we present average residuals, where positive residuals are an indication that a given treatment is more closely related to POXC relatitve to mineralizable C and thus reflects more processed C (stabilization), whereas negative residuals indicate a greater relationship to mineralizable C relative to POXC and

Table 5 Pearson's correlations and significance between soil nutrient (C and N) indicators and maize yields

Soil C indicators	All sites combined	Clay central	Clay west	Sandy central	Sandy west
Correlation coefficient, r					
Mineralizable C	0.54***	0.63*	0.41	0.40	0.40
POXC	0.52**	0.50[*]	− 0.40	0.49[*]	0.02
Soil Protein	0.56***	0.60*	0.20	0.74**	0.64*
Large POM C	0.34*	0.36	0.07	0.26	0.64*
Medium POM C	0.37**	0.38	0.40	0.41	0.67*
Total C	0.38**	− 0.12	0.15	− 0.26	0.39

[*]Significance level: $P < 0.1$

*Significance level: $P < 0.05$

**Significance level: $P < 0.01$

***Significance level: $P < 0.0001$

Table 6 Soil C and N indicators are ranked based on their capacity to predict maize yields determined by subset regression analysis

Ranked indicators	All sites combined	Clay central	Clay west	Sandy central	Sandy west
First	Soil protein	Mineralizable C	Medium POM C	Soil protein	Medium POM C
Second	Total C	Total C	Mineralizable C	Medium POM C	Soil protein
Third	Mineralizable C	*	Large POM C	POXC	Total C

(kg N ha^{-1}) and absence (0 kg N ha^{-1}) of inorganic N fertilizer. Asterisks reflect significant differences amongst the organic residue treatments as control plots were not included in the statistical model

*Only two indicators were included in the best fit model

thus reflects more mineralization processes (Hurisso et al. 2016). When all sites were combined, Stover + N and Stover − N had positive residuals, while Tithiona + N and Tithonia − N treatments had negative residuals. Aside from Clay West, trends across the individual sites were less clear (Figure S1). However, in general, Stover − N was positive, except at Sandy West, and tithonia + N was negative at every site, except at Sandy Central.

Discussion

Integrated soil fertility management effects on maize yields

In general, tithonia had greater maize yields relative to maize stover at the sandy sites, which is likely the result of greater available N. For instance, prior research from these trials report that maize residues released 142 mg N kg^{-1} less N than tithonia and that the addition of inorganic fertilizer N did not increase the release of N (Gentile et al. 2011a). Furthermore, combining inorganic fertilizer N with maize residues led to a net N immobilization and reduced overall N availability. In this study, we assessed the organically bound pool of N by measuring soil protein and found that at the sandy sites, tithonia consistently had greater values relative to maize stover.

However, we found exceptions to this trend when yields were analyzed by individual growing seasons. For example, at the Sandy Central site, during the short rainy season of 2013 and both rainy seasons in 2014, we found no difference across the four treatments. The lack of difference in maize yields between the two organic residue treatments was likely the consequence of a drought that occurred during 2013 and 2014, where precipitation was 21% and 39% lower than the 30-year annual mean (Fig. 1). A lack of response to N additions during growing seasons with lower rainfall is

Fig. 3 Average residuals from linear regression models used to explore treatment influences on POXC versus Mineralizable C from all sites combined. Positive residuals indicate that organic residues were more related to POXC (stabilization), while negative residuals, indicate that organic residues are more related to Mineralizable C (mineralization processes). Residuals from linear regression models exploring treatment influences on POXC versus mineralizable C from each individual site are included in the supplementary online material
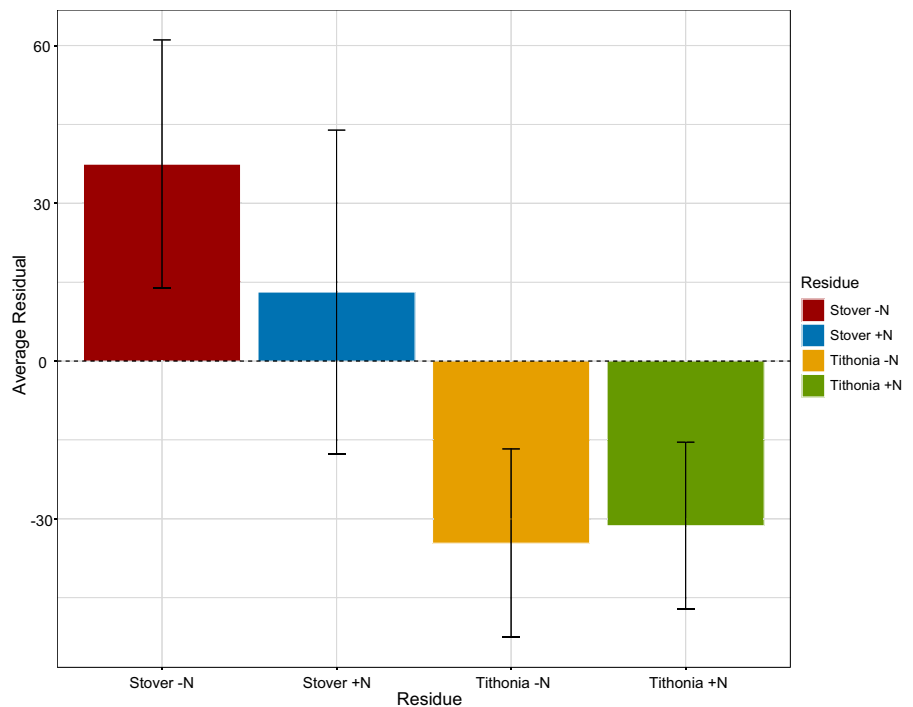
common in this region and is likely an indication that water limitations have a greater impact on maize yields compared to N limitations (Tully et al. 2015).

In contrast to the sandy sites, residue type had little to no effect on maize yields at the clay sites. The only exception to this trend was during the long rainy season at the Clay West site, where tithonia increased maize yield compared to maize stover each year (Fig. 2). Our results slightly differ from Chivenge et al. (2009) who reported maize yields from 2002 to 2006 for the central sites, where tithonia led to greater maize yields at both the Clay Central and Sandy Central. However, Chivenge et al. (2009) also found a lack of inorganic fertilizer effects at the Clay Central site, indicating that organic sources of N had a greater impact on yields compared to inorganic N additions alone at this site.

Larger yield responses to organic residues and inorganic N at the sandy sites compared to the clay sites could be explained by a difference in fertility and nutrient limitations between the two soil types. For example, Clay Central, and Clay West had soil organic matter levels of 3.8% and 2.5%, respectively, while both sandy sites had levels of 0.4%. In addition, the clay sites had two to three times more total soil N compared to the sandy sites (data not shown). Sandy

Central, Sandy West, and Clay West all appear to be N limited, as there is a clear response to N fertilizer additions compared to the no input control. In contrast, yields are greater in the no input plots compared to the inorganic only plots at the Clay Central site, indicating no response to fertilizer. Non-responsiveness to organic and inorganic N has been reported in SSA and was associated with micronutrient deficiencies or lower pH values (Kihara et al. 2016). Thus, the more acidic soil status of Clay Central (5.0), compared to the other sites, could be a key reason for this non-responsiveness to sources of N.

Organic residue effects on soil carbon and nitrogen pools

Plots with the tithonia treatment had greater mineralizable C and soil protein at the sandy sites and thus demonstrates that low C:N residues can enhance nutrient mineralization more so than maize stover. For instance, mineralizable C has been shown to be highly associated with organic matter mineralization and nutrient availability (Hurisso et al. 2016). Similarly, soil protein reflects a readily mineralizable pool of N that supplies plant available N throughout a given growing season (Hurisso et al. 2018). Our results

complement previous research at these trials, which found that tithonia was effective at increasing short-term C mineralization relative to maize stover (Gentile et al. 2011a). In addition, these results follow the same trend as maize yield in that residue type influenced mineralizable C and soil protein at sandy sites, but had little to no effect on these indicators at the clay sites.

There were certain instances where tithonia also increased soil C indicators that reflect more processed pools of C, including POXC and medium POM C. For instance, tithonia increased POXC at the Sandy West site and POXC values trended higher in the tithonia treatments compared to maize stover at the Sandy Central site. In addition, Tithonia + N significantly increased medium POM C (53–250 µm) at the Sandy West site compared to Stover + N. Earlier work from these trials, when plots only had 3 years of treatment applications, found that organic residue type had no influence on even the most sensitive soil aggregates (Gentile et al. 2011a, b). Thus, the accumulation of more processed C under tithonia relative to maize stover is a new finding at these trials and is an indication that the continuous application of tithonia could lead to long-term C stabilization at the sandy sites. POXC is a chemically extracted fraction of soil C that is closely related to smaller and heavier particulate organic C fractions (Culman et al. 2012), thus, is closely associated with more processed or stable pools of C (Culman et al. 2013; Hurisso et al. 2016). In addition, the medium POM C fraction that consisted of smaller sized organic particles tended to have significantly greater C concentrations compared to the large POM C fraction, perhaps indicating a more processed form of C. Total C was not significantly influenced by either residue type or fertilizer.

Despite the lack of differences in total soil C between the two residues, the framework from Hurisso et al. (2016) allowed us to explore how treatments impact POXC relative to mineralizable C, which indicates if a treatment is more stronlgy related to more processed C or mineralization processes. Such analyses can reflect short and long-term trajectories of soil C. When taking into account all of the sites, maize stover residues related more to stabilization processes (POXC), while tithonia related more to mineralization processes (mineralizable C) relative to more processed C (POXC). However, when looking at individual sites, results were less decisive, especially at the sandy sites, where there was no discernable trend. It is important to

recognize that POXC and mineralizable C both represent fractions within the theoretical 'active C' pool and are often similarly related to a given treatment, which is likely the case at the sandy sites.

Perhaps the relationship between C mineralization and POXC indicates that the long-term application of tithonia leads to greater quantities of soil C (pool size) with potentially shorter mean residence times given the larger rates of C mineralization. In contrast, the long-term application of maize stover leads to less short-term mineralization, but consists of C that may have longer mean residence times as it has a stronger influence on stabilization. These functional processes are reasonable given the C:N ratios of both tithonia (12) and maize stover (60). However, we also know that C decomposition is driven by a filtering effect where particulate C is futher reduced, leading to more stable C pools that consist of microbial biomass and byproducts (Grandy and Neff 2008). Simply stated, the chemical composition of organic residues is not the sole determinant of C stabilization; rather, recent work argues that mineral associations are more important for C stabilization because aggregation increases physical protection and limits accessibility to microbes (Grandy and Neff 2008). Continuous monitoring of soil C dynamics and quantifying accumulation and turnover rates in these systems are needed to further understand residue effects on stabilization processes and soil C build-up.

Soil carbon and nitrogen pools as indicators of yield

Our findings suggest that greater C and N mineralization are likely the mechanisms that led to greater maize yields within the tithonia treatments at the sandy sites. Thus, we wanted to further explore the relationship between different soil C pools and maize yields across different soil types. We found that the relationship between maize yield and soil C pools was heavily dependent on soil type. For example, we examined correlations with all sites combined and found positive and significant correlations for each indicator; however, when correlations were examined for individual sites, important distinctions across the different soil types were visible. At the Clay Central site, mineralizable C had the strongest correlation with maize yield, which is in line with findings from long-term trials in Michigan that also indicate that

mineralizable C is more closely related to agronomic performance compared to other soil C indicators (Culman et al. 2013). In contrast, medium POM C had the strongest correlation with yield at the Sandy West Site. To provide more clarity on how different indicators were influencing yield we used subset regression to rank the different indicators and their ability to predict maize yield at each site. An important and noteworthy trend emerged from the subset regression analysis, as we found that indicators reflecting both mineralization and stabilization were ranked in the top two at each site. Our findings demonstrate that at the Clay West, Sandy Central, and Sandy West sites, indicators that reflected both mineralization and stabilization were important predictors of maize yield. These findings are a departure from other studies that report negative associations between yield and stabilization (Wood et al. 2016; Cates and Ruark 2017).

Our findings are novel in that we found positive associations for indicators that reflect both mineralization and stabilization at each site, except Clay Central, perhaps suggesting that some C stabilization will enhance agronomic performance. A difference in methodology could partially explain our contrasting results from the aforementioned studies. While we measured medium POM C (53–250 μm), which contains smaller organic particles and likely more processed C, we did not measure < 53 μm POM C that is even more processed. Thus, medium POM C is not as far along on the C stabilization trajectory as mineral-associated organic matter or microaggregate C that were used by Wood et al. (2016) and Cates and Ruark (2017). Another reason for contrasting results is a difference in experimental design and temporal scale; while our work reflects the long-term and continuous application of residues in replicated trials, Wood et al. (2016) report data 1–2 years post residue application. Thus, it could be that the long-term application of residues could alter C accumulation and turnover of different soil C pools and create different feedbacks between soil C and maize yields (O'Rourke et al. 2015).

Our work highlights the importance of examining the relationship between maize yield and soil C and N indicators by soil type, as varying mechanisms control soil nutrient pools and their impacts on maize yield. In general, we did not find trade-offs between mineralization and C stabilization across the different sites.

That said, it appears as if short-term C and N mineralization is most important for crop productivity at sites that have higher percentages of clay, while yields at sandy sites benefit from both nutrient mineralization and C stabilization processes. This study also has practical implications for smallholder farmers in SSA, who are managing for increased yields in the short-term, while simultaneously working to improve soil fertility over time. Our work demonstrates that the continued application of tithonia increases yields and soil nutrient pools relative to maize stover but only at the sandy sites. Applying low rates of tithonia at sites with several nutrient deficiencies has led to profitability on farms in western Kenya (Jama et al. 1999). Thus, we can recommend that farmers apply tithonia on farms with low soil fertility.

Conclusions

Soil nutrient depletion and a lack of access to inorganic fertilizers has led to chronically low soil fertility and reduced yields across much of SSA. Applying organic residues in combination with inorganic fertilizers is a strategy that can be used to increase yields and improve soil fertility. We found that the long-term application of tithonia leads to substantial increases in yield and soil nutrient pools relative to maize stover at sandy sites, indicating that low C:N residues could significantly benefit crop production and enhance soil organic matter at low fertility soils. This means that small-holder farmers with poor fertility soils need to consider applying residues with low C:N ratios, such as tithonia, rather than maize stover to meet crop nutrient needs. We also found that in general, tithonia is more effective at building C pools that reflect short-term mineralization, a mechanism that is important for enhancing maize yields. Maize stover, on the other hand, has less short-term mineralization, but may have slower turnover times, which could have important implications for C stabilization and soil fertility over time. Future work at these long-term trials should address rates of soil C accumulation and turnover to determine which residues are most effective for long-term C stabilization.

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