

# High-quality organic resources are most efficient in stabilizing Managing soil organic carbon in tropical agroecosystems: Evidence from four long-term experiments in Kenya.

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## Abstract.

In sub-Saharan Africa, maize is one of the most important staple crops, but long-term maize cropping with low external inputs has been associated with the loss of soil fertility. While adding high-quality organic resources combined with mineral fertilizer has been proposed to counteract this fertility loss, the long-term effectiveness and interactions with site properties still require more understanding. This study used repeated measurements over time to assess the effect of different quantities and qualities of organic resource addition combined with mineral N-nitrogen (N) on the change of soil organic carbon ~~concentrations~~ (SOC) contents over time (and SOC stocks in the year 2021) in four ongoing long-term ~~trials-experiments~~ in Kenya. These ~~trials-experiments~~ were established with identical treatments in moist to dry climates, on coarse to clayey soil textures, and have been managed-conducted for at least 16 years. They received organic resources in quantities equivalent to 1.2 and 4 t-ton C ha<sup>-1</sup> per year in the form of *Tithonia diversifolia* (high quality, fast turnover), *Calliandra calothyrsus* (high quality, intermediate turnover), *Zea mays* stover (low quality, fast turnover), sawdust (low quality, slow turnover) and local farmyard manure (variable quality, intermediate turnover). Furthermore, the addition ~~or absence~~ of 240 kg N ha<sup>-1</sup> per year as mineral N fertilizer or no fertilizer was the split-plot treatment. At all four sites, a loss of SOC ~~, rather than gain,~~ was predominantly observed ~~due to a recent conversion from permanent vegetation to agriculture. The average reduction of SOC concentration,~~ likely because the sites had been converted to cropland only a few decades before the start of the experiments. Across sites, the average decline of SOC content over 19 years in the 0 to 15 cm depth-topsoil layer ranged from 42% to 13% of the initial SOC ~~concentration-content~~ for the control and the farmyard manure treatments at 4 t-ton C ha<sup>-1</sup> yr<sup>-1</sup>, respectively. Adding *Calliandra* or *Tithonia* at 4 t-ton C ha<sup>-1</sup> yr<sup>-1</sup> limited the loss of SOC ~~concentrations-contents~~ to about 24% of initial SOC, while the addition of ~~saw-dust~~ sawdust, maize stover (in ~~3-of-4~~ three of the four sites) and sole mineral N addition ~~, showed~~ no significant reduction in-of SOC loss over the control. ~~Site-specific~~ Site-specific analyses, however, did show, that at the site

with the lowest initial SOC ~~concentration~~ content (about 6 g kg<sup>-1</sup>), the addition of 4 ~~t~~ ton C ha<sup>-1</sup> yr<sup>-1</sup> farmyard manure or *Calliandra* ~~plus~~ with mineral N led to a gain in SOC ~~concentrations~~ contents. ~~All contents~~ The other sites lost SOC in all treatments, albeit at ~~site-specific~~ site-specific rates. While subsoil SOC stocks in 2021 were little affected by organic resource additions (no difference in ~~3 of 4~~ three of the four sites), the topsoil SOC stocks corroborated the results ~~for SOC concentrations~~ obtained  
 25 from the SOC content measurements (0-15 cm) over time. The relative annual change of SOC ~~concentrations~~ contents showed a higher site specificity in ~~high-quality organic resource~~ farmyard manure, *Calliandra* and *Tithonia* treatments than in the control treatment, suggesting that the drivers of site specificity in SOC buildup (~~mineralogy~~ soil mineralogy, soil texture, climate) need to be better understood for effective targeting management of organic resources. ~~Even though farmyard~~ Farmyard manure showed the ~~most~~ highest potential for reducing SOC ~~loss~~ losses, ~~our results clearly show that maintaining SOC with external inputs~~ only is not possible at organic resource rates that are realistic for small-scale farmers. ~~Thus~~ losses, but the necessary quantities  
 30 to build SOC are often not realistic for smallholder farmers in Africa. Therefore, additional agronomic interventions such as intercropping, crop rotations or ~~strong-rooting crops may be the~~ cultivation of crops with extended root systems are necessary to maintain or increase SOC.

## 1 Introduction

35 Maize cropping in Sub-Saharan Africa (SSA) is generally characterized by yields ~~<less than 2~~ t ~~tons (t)~~ ha<sup>-1</sup>, far below the world average of ~~more than about~~ 5.5 t ha<sup>-1</sup> (FAO, 2021). This has to a large extent been attributed to a lack of nutrient inputs ~~into these cropping systems~~ into these cropping systems, leading to nutrient mining and a decrease in soil fertility, including soil organic matter loss (Vanlauwe and Giller, 2006). The longer ~~these~~ these low-input cropping systems are maintained, the stronger the ~~soil fertility decline~~ decline of soil fertility that occurs and the lower the ability of soils ~~becomes~~ becomes to provide nutrients to crops through ~~the~~ the mineralization  
 40 of soil organic matter (Vanlauwe et al., 2015). To counteract this soil fertility decline, sustainable intensification practices ~~are needed~~ are needed, which allow for increased crop yields while simultaneously maintaining or ~~preferably~~ preferably increasing soil fertility (Pretty and Bharucha, 2014), ~~are needed~~ are needed. In the context of smallholder farming systems in SSA, integrated soil fertility management (ISFM) is expected to deliver on both aspects (Gentile et al., 2009; Gram et al., 2020). ISFM implies the use of improved germplasm and mineral fertilizer, which mainly ~~target~~ targets short-term crop productivity, combined with the application of  
 45 organic resources ~~to the soil~~ to the soil (Vanlauwe et al., 2010), which ~~target~~ targets long-term system sustainability by providing the needed ~~C and N~~ carbon (C) and nitrogen (N) inputs (Kong et al., 2005) to replenish soil organic carbon (SOC).

Results from several experiments (Adams et al., 2020; Laub et al., 2022) and a recent ~~meta-analysis~~ meta-analysis (Fujisaki et al., 2018) suggest that, given the right quantity and quality of inputs ~~, management and surrounding soil conditions and depending on soil conditions and management~~ , management and surrounding soil conditions and depending on soil conditions and management, it is possible to increase the SOC contents in tropical soils. However, ~~in some~~ in some  
 50 other experiments in tropical SSA ~~revealed that~~ revealed that it was not ~~even~~ even possible to maintain ~~the SOC concentrations~~ SOC in the long term, even ~~for high-input treatments with high levels of organic inputs~~ for high-input treatments with high levels of organic inputs (Kihara et al., 2020). This has been ~~hypothesized to be a result of~~ hypothesized to be a result of attributed to the initially high SOC levels at the sites, ~~the~~ the favorable conditions for SOC decomposition, and ~~a reduced~~ a reduced the low SOC stabilization capability of the 1:1 kaolinite clay minerals in tropical soils because of their low surface

reactivity (Six et al., 2002; Sommer et al., 2018). Vanlauwe et al. (2015) suggested that differences in local soil conditions  
55 ~~may be responsible for~~ are responsible for the observed differences in the success of SOC building practices, and hence local  
adaptation to different soil conditions is needed. ~~Land cover history also explains site specific SOC dynamics and can help to~~  
~~define the local potential for SOC sequestration. Indeed soils cultivated as pasture or forest have initially high SOC stocks that~~  
~~are difficult to maintain under cultivation (Lal, 2004).~~

Besides the effect of soil geochemistry (Doetterl et al., 2015), the quality of organic resources can play an important role in  
60 SOC formation (Puttaso et al., 2013), but their relative contribution to SOC storage is still insufficiently understood, especially  
in heavily weathered tropical soils. It has been established that the quality of organic resources, through the effect of litter stoi-  
chiometry (Sinsabaugh et al., 2013), ~~initially determines the maximum~~ determines the potential to built new stable ~~microbially~~  
~~derived~~ microbial-derived SOC (Kallenbach et al., 2016). However, it is less certain if the efficiency of microbial processing of  
low-quality organic resources (~~high C/N~~ with high C:N ratio) can somehow be enhanced, for example by increasing the avail-  
65 ability of nutrients from mineral sources. The latter has been suggested by comparing microbial efficiencies at low and high  
~~atmospheric~~ N addition rates in forests (Li et al., 2021a), but has to date not been studied in ~~agroecosystems~~ agro-ecosystems.  
A recent study suggested that the combination of low and high-quality organic resources can enhance the overall microbial  
carbon use efficiency (Pingthaisong and Vityakon, 2021), yet we are not aware of any study that ~~tested~~ investigated whether the  
same ~~was possible~~ occurs when adding mineral ~~mineral~~ N to low-quality organic resources. Also, the interactions of organic  
70 resource quality with geochemistry have been ~~understudied~~ under-investigated. While a recent ~~lab~~ laboratory study indicated  
that geochemistry may be as important as organic resource quality in the formation of new, stable SOC (Bucka et al., 2021),  
both factors and their interactions need to be studied under field conditions. ~~Thus~~ In fact, we are only beginning to understand  
the fundamental principles behind their interactions and a better understanding of ~~all these~~ factors that regulate the microbial  
processing, stabilization and decomposition of SOC in soils is needed as old paradigms get cast into doubt (Cotrufo et al.,  
75 2021). For example, the ~~idea~~ theory that low-quality organic resources, rich in recalcitrant lignin and polyphenol, lead to more  
soil organic matter formation than high-quality resources Palm et al. (2001a, b) has been replaced by concepts that consider  
SOC of microbial origin, which favorably forms from high-quality resources, to be the most stable (Cotrufo et al., 2013; Denef  
et al., 2009). Also, the concept that soil texture plays the dominant role in determining how much SOC can be stabilized in  
a soil (Hassink, 1997) did not hold for ~~soils at a local scale~~ adjacent soils differing in texture but with the same mineralogy  
80 (Schweizer et al., 2021); the latter suggests that the actual driver behind correlations of soil texture with SOC storage across  
large scales may be the different soil mineralogy and not the ~~grain~~ particle size.

As the ~~main long-term~~ goal of ISFM is to increase ~~short-and-short-~~ and long-term soil fertility, in particular through  
increasing SOC, there is a need to better understand the ~~effect of~~ extent to which the rate and quality of organic resource  
additions ~~on SOC dynamics influence the rate of SOC change~~ under different pedo-climatic conditions. This can help to an-  
85 swer the question whether organic resource quality, quantity or site properties are most important in building SOC. The answer  
to this question is crucial for site-specific recommendations. Yet, few studies reported on SOC dynamics following organic  
resource additions over time spans of decades or longer. Besides, it is not clear in how far the combined application of organic  
and mineral N fertilizer affects SOC dynamics. ~~Therefore~~ To shed light on these questions, we analyzed data from four long-

term experiments conducted at ~~four different~~ sites in central and western Kenya (~~established in 2002 and 2005, respectively~~).

90 ~~All four experiments had the same treatments with organic resource additions of the same quality, with exactly the same organic and mineral resource additions~~ at each site. The ~~aims were to study objectives of the study were~~ (i) ~~to quantify~~ the efficiency of SOC build-up ~~under with~~ the addition of organic resources ~~with of~~ different qualities combined with or without mineral N fertilizer ~~across sites as well as, and~~ (ii) ~~to investigate~~ how the efficiency of SOC formation differs between sites. To guide our research, the following hypotheses were formulated:

- 95 1. Addition of high-quality organic resources (low C/N and lignin/N ratios) ~~, which leads to an increase in SOC content, because they~~ are most efficiently processed by soil microbes (Cotrufo et al., 2013), ~~leads to an increase in SOC concentration.~~  
In contrast, low-quality resources ~~do not maintain SOC concentrations maintain less SOC~~ in the long term.
2. Due to a C/N ratio that is too high compared to typical microbial C/N ~~ratios: N ratios of about 8 to 14~~, nitrogen availability is a major limitation for SOC build-up from low-quality organic resources. Therefore, low-quality resources  
100 have a higher SOC build-up if mineral N fertilizer is added compared to when no mineral N is applied.
3. The efficiency with which new SOC is formed is influenced by both the ~~site pedo-climatic~~ conditions and the organic resource quality, ~~but the resource quality plays the strongest role. Hence, we expect a significant interaction of resource quality with site in the efficiency to store new SOC.~~

## 2 Material and methods

### 105 2.1 Site characteristics

This study uses the combined data from four equally designed long-term experiments, located in central and western Kenya (see Table 1). The two experiments in central Kenya (i.e., in Embu and Machanga) were initiated in 2002, whilst the experiments in western Kenya (i.e., in Sidada and Aludeka) were started in 2005. All sites were under continuous maize cropping with two growing seasons per year. The long rainy season lasted usually from March until August/September and the short rainy season  
110 from September/October until January/February (Table 1). The sites were specifically selected to represent different altitudes, levels of precipitation, temperatures and soil conditions. ~~With the The~~ sites in Sidada (1730 mm; 22.6°C) and Aludeka (1660 mm; 24.4°C) ~~having have~~ the longest rainy seasons ~~, the highest amounts of rainfall, and and the highest rainfall amounts of of the four sites, with~~ intermediate mean annual temperatures; ~~thus,~~ representing the most favourable climate for maize production. The Embu site (1175 mm; 20.1°C) is slightly less favorable, while Machanga (795 mm; 23.7°C) represents a dryer  
115 climate, where maize is at considerable risk of crop failure. The soils in Machanga and Aludeka are coarse-textured and have less than 15% of clay, while those in Sidada and Embu both contain more than 55% of clay. The soils at all four sites are heavily weathered, which was also reflected in the low pH values of between 5.3 and 5.5 at the start of the experiments. ~~The soil in Embu is~~ Based on reference soil profiles that were dug before establishing the experiment at each site, the soil in Embu was classified as a Humic Nitisol, which has more weatherable minerals than the Humic Ferralsol in Sidada, a soil that is

**Table 1.** ~~Locations~~[Location](#), soil properties and climatic conditions of the study sites. Soil properties are given for the 0 - 15 cm depth layer, and are based on ~~a measurement~~[measurements](#) before ~~experiment~~[the start of the experiment](#)(1 reference [soil](#) profile per site). Coordinates are given in the WGS 84 reference system. The site names ~~in the table~~ contain a hyperlink to display the ~~location~~[locations](#) on ~~google maps~~[Google Maps](#).

| Soil characteristics                              | Embu          | Machanga      | Sidada          | Aludeka        |
|---------------------------------------------------|---------------|---------------|-----------------|----------------|
| Latitude                                          | -0.517        | -0.793        | 0.143           | 0.574          |
| Longitude                                         | 37.459        | 37.664        | 34.422          | 34.191         |
| Initial soil C (g kg <sup>-1</sup> ) <sup>+</sup> | 29            | 3             | 15              | 8              |
| Initial N (g kg <sup>-1</sup> ) <sup>+</sup>      | 3.0           | 0.2           | 1.2             | 0.8            |
| Initial bulk density (g cm <sup>-3</sup> )        | 1.26          | 1.51          | 1.3             | 1.45           |
| pH (H <sub>2</sub> O)                             | 5.43          | 5.27          | 5.4             | 5.49           |
| Clay (g kg <sup>-1</sup> )                        | 598           | 132           | 557             | 134            |
| Soil type (IUSS Working Group, 2014)              | Humic Nitisol | Ferric Alisol | Humic Ferralsol | Haplic Acrisol |
| Altitude (m)                                      | 1380          | 1022          | 1420            | 1180           |
| Mean annual rainfall (mm) <sup>*</sup>            | 1175          | 795           | 1730            | 1660           |
| Mean annual temperature (°C) <sup>*</sup>         | 20.1          | 23.7          | 22.6            | 24.4           |
| Months of long rainy season                       | 3 - 8/9       | 3 - 8         | 3 - 8           | 3 - 8          |
| Months of short rainy season                      | 10 - 1/2      | 10 - 1/2      | 9 - 1           | 9 - 1          |

<sup>+</sup>By dry combustion (CHN628, LECO Corporation, Michigan, USA) <sup>\*</sup>Means calculated based on measured data from 2005 to 2020

120 dominated by low activity clays as well as iron and aluminium oxides. The Haplic Acrisol in Aludeka and the Ferric Alisol in Machanga are both characterized by illuvial clay accumulation in the subsoil and low base saturation. The clay minerals in the Acrisol [in Aludeka](#) are characterized by lower activity than those in the the Alisol (~~FAO, 1998; IUSS Working Group, 2014~~) ~~in Machanga~~(FAO, 1998; IUSS Working Group, 2014). [All sites have almost flat land surfaces with gentle slopes in all sites except Embu \(Machanga, 2.5%; Sidada, 2%; Aludeka, 1%; Embu 5%\). However, the Embu site has been terraced to reach a flat surface of the plots as at the other sites.](#)

125 The land-use history prior to the establishment of the experiments differed between sites. The sites ~~in at~~ Sidada and Aludeka were savanna-type ecosystems, with ~~shrubs, tree trees, shrubs~~ and witchgrass *Elymus repens*, having a tree cover of about 50%, and had been converted to low-intensity shifting cultivation approximately 25 years prior to the start of the experiments. The Embu site was initially a tropical evergreen forest, that was lost about 50 to 100 years ago, when low-intensity shifting cultivation had started. The site at Machanga was a steppe grassland, with a tree cover of

130 around 20 to 30 %, that was only converted upon the start of the experiment. However, the area around Machanga has been subject to intensive grazing by cattle, so the site was far from undisturbed at the start of the experiment.

### 2.2 Experiment description

All four experiments were conducted by using a split plot design with three replicates ~~(Fig. A1)~~. The main treatments consisted of the addition of five types of organic resources, applied in quantities of 1200 and 4000 kg C ha<sup>-1</sup> yr<sup>-1</sup> on plots of 12x5 m

**Table 2.** Dry matter based ~~mean-measured~~ chemical ~~characteristics-properties~~ (means and 95% confidence intervals in brackets) of the organic resources applied at all sites. Measurements were ~~available from~~ done at Embu and Machanga from 2002 to 2004 and in 2018, all-sites-and at Sidada and Aludeka from 2005 to 2007 and in 2018. Significant differences in ~~residue~~-properties were found between the different organic resources, but not between sites and years. Same letters within the same row indicate the absence of significant differences for that property ( $p < 0.05$ ). Abbreviations: n.c. = not classified.

| Measured property                                           | <i>Tithonia</i>               | <i>Calliandra</i>               | Maize stover                  | Sawdust                          | Farmyard manure               |
|-------------------------------------------------------------|-------------------------------|---------------------------------|-------------------------------|----------------------------------|-------------------------------|
| Abbreviation <sup>x</sup>                                   | TD1.2 (TD4)±N                 | CC1.2 (CC4)±N                   | MS1.2 (MS4)±N                 | SD1.2 (SD4)±N                    | FYM1.2 (FYM4)±N               |
| C (g kg <sup>-1</sup> ) <sup>+</sup>                        | 345 <sup>b</sup> (333-357)    | 396 <sup>c</sup> (383-409)      | 397 <sup>c</sup> (386-408)    | 433 <sup>d</sup> (416-449)       | 234 <sup>a</sup> (213-255)    |
| N (g kg <sup>-1</sup> ) <sup>+</sup>                        | 33.2 <sup>d</sup> (28.9-38.2) | 32.5 <sup>d</sup> (28.3-37.3)   | 7.2 <sup>b</sup> (6.5-8)      | 2.5 <sup>a</sup> (2.1-2.8)       | 18.1 <sup>c</sup> (15-21.8)   |
| C:N ratio                                                   | 12.4 <sup>a</sup> (10.8-14.1) | 13.6 <sup>a</sup> (11.9-15.5)   | 58.7 <sup>b</sup> (52.8-65.2) | 199.1 <sup>c</sup> (174.1-227.7) | 12.3 <sup>a</sup> (9.9-15.4)  |
| P (g kg <sup>-1</sup> ) <sup>#</sup>                        | 2.3 <sup>d</sup> (1.8-2.9)    | 1.1 <sup>c</sup> (0.8-1.5)      | 0.4 <sup>b</sup> (0.3-0.6)    | 0.1 <sup>a</sup> (0-0.2)         | 3.1 <sup>d</sup> (2.3-3.9)    |
| K (g kg <sup>-1</sup> ) <sup>#</sup>                        | 37.2 <sup>c</sup> (21.2-65.2) | 8.7 <sup>b</sup> (5-15.3)       | 9 <sup>b</sup> (6-13.5)       | 2.8 <sup>a</sup> (1.6-4.9)       | 19.4 <sup>bc</sup> (7.8-48.6) |
| Lignin (g kg <sup>-1</sup> ) <sup>#</sup>                   | 90 <sup>ab</sup> (62-117)     | 105 <sup>b</sup> (77-133)       | 48 <sup>a</sup> (37-60)       | 172 <sup>c</sup> (144-199)       | 198 <sup>c</sup> (154-242)    |
| Polyphenols (g kg <sup>-1</sup> ) <sup>#</sup>              | 19 <sup>c</sup> (14.9-24.3)   | 108.7 <sup>d</sup> (85.3-138.6) | 11.3 <sup>b</sup> (9.5-13.6)  | 4.9 <sup>a</sup> (3.8-6.2)       | 7.8 <sup>ab</sup> (5.2-11.5)  |
| Ligin/N ratio                                               | 2.6 <sup>a</sup> (1.8-3.7)    | 3.1 <sup>ab</sup> (2.2-4.3)     | 6.2 <sup>c</sup> (4.8-8)      | 58.3 <sup>d</sup> (41.1-82.8)    | 6.9 <sup>bc</sup> (3.9-12.3)  |
| Quality / turnover speed*                                   | High / fast                   | High / intermediate             | Low / fast                    | Low / slow                       | n.c.                          |
| Class*                                                      | 1                             | 2                               | 3                             | 4                                | n.c.                          |
| kg N in 4.0 t C ha <sup>-1</sup> yr <sup>-1</sup> , -N [+N] | 323 [563]                     | 295 [535]                       | 68 [308]                      | 20 [260]                         | 324 [564]                     |
| kg N in 1.2 t C ha <sup>-1</sup> yr <sup>-1</sup> , -N [+N] | 97 [337]                      | 88 [328]                        | 20 [260]                      | 6 [246]                          | 97 [337]                      |

<sup>x</sup>For 1.2 (or 4.0) t C ha<sup>-1</sup> yr<sup>-1</sup> treatments; <sup>+</sup>By dry combustion (CHN628, LECO Corporation, Michigan, USA); <sup>#</sup>Total digestion (P, K), acid detergent fibre (lignin) and Folin-Denis (polyphenols) according to Anderson and Ingram (1993); \* according to Palm et al. (2001a); +N and -N indicate 120 or 0 kg mineral N fertilizer application per growing season.

135 (Embu) or 12x6 m (other sites). The subplot treatment consisted of the application of 120 kg mineral N ha<sup>-1</sup> season<sup>-1</sup> (+N treatment) compared to a no N input (-N) treatment. Mineral N (CaNH<sub>4</sub>NO<sub>3</sub>) was applied twice during each growing season: the first 40 kg N ha<sup>-1</sup> at planting and the remaining 80 kg N ha<sup>-1</sup> about six weeks later, as top dressing. In each growing season, all plots received a blanket application of 60 kg P ha<sup>-1</sup> as triplesuperphosphate and 60 kg K ha<sup>-1</sup> as muriate of potash at planting. The organic resources were applied once a year just before planting at the start of the long rainy season. The

140 incorporation was done using a hand hoe to a soil depth of about 15 cm. The applied organic resources represented all four quality classes that were defined by Palm et al. (2001a), differing in N, lignin and polyphenol contents (Table 2): pruned leaves including stems of <2cm thickness from *Tithonia diversifolia* (TD; high quality and fast turnover; class 1), pruned leaves including small stems from *Calliandra calothyrsus* (CC; high quality and intermediate turnover; class 2), stover of *Zea mays* (MS; low quality and fast turnover; class 3), sawdust from *Grevillea robusta* trees (SD; low quality and slow turnover; class 4) and locally available farmyard manure (FYM; no defined class, but considered of intermediate to high quality with intermediate turnover; Sileshi et al., 2019; Silva et al., 2014). A treatment without any organic resource addition served as the control (CT). All maize residues were removed from the plots at harvests, so the only C inputs from the maize crop were the

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roots (and root exudates). In addition, a randomly allocated quarter of each split plot was kept as bare fallow throughout the entire duration of the ~~experiment, experiments, receiving the exact same inputs but~~ with no maize planted and with all emerging weeds removed by regular weeding. This was done to study the SOC dynamics without any additional inputs from roots or other plant debris. Further details on ~~agricultural the crop~~ management can be found in Chivenge et al. (2009), Gentile et al. (2011) and ~~Laub et al., (in review)~~ Laub et al. (2023).

### 2.2.1 Soil sampling

We used ~~pooled~~ data from all soil sampling campaigns that were conducted throughout the experiments, the latest sampling being in ~~2021, 2021 (Overview in Tab. A1)~~. In Embu and Machanga, regular soil sampling campaigns were done every two to three years since the first experimental year (2002), while in Sidada and Aludeka, regular soil samplings were only initiated in 2018, due to budget constraints. With the exception of the 2021 sampling, only topsoil (0-15 cm) samples from the cropped plots were taken, by combining a composite sample of six transect insertions along the two diagonals. During the 2021 sampling campaign, soil samples were taken down to 50 cm depth (the depth intervals were 0-15-30-50 cm) and the bare plots were also sampled. The initial aim was to sample even deeper, but due the soil being too hard and crumbly (Embu) or too shallow (Aludeka), it turned out unfeasible to sample from deeper layers. To minimize plot disturbance, especially below ploughing depth, the 2021 samples were collected by only one soil core from the center of each plot, using a gauge auger of 60 mm diameter and 500 mm length ~~in at~~ Embu (Eikelkamp Soil & Water, Giesbeek, Netherlands) and a soil corer of 55 mm diameter and 1000 mm length (Giddings Machine Company, Windsor, USA) ~~in all at the~~ other sites. ~~This~~ The reason we had to use a different and partially open soil auger in Embu was that the other auger type repeatedly broke due to the very hard subsoil. Sampling full cores allowed for bulk density (BD) ~~estimations by weighing fresh samples, determining the soil moisture content based on a sub-sample and subsequently computing the dry soil weight per known core volume. All soil measurements. On the same day of sampling, all soil~~ samples were sieved through an 8 mm sieve ~~immediately after sampling~~ and then air dried for storage until further analysis in the laboratory. ~~Samples were then~~ For further analysis, a sub-sample was broken and crushed by pestle and mortar and sieved through a 2 mm sieve. ~~Prior to~~ Stone contents of both sieving steps were recorded. The soil moisture content of air-dried samples was based on drying a sub-sample at 105°C for 24h and subsequently calculating the dry soil weight per known core volume. Bulk density was determined based on the calculated absolute dry weight of stone-free samples and and the stone-corrected volume of soil cores, assuming a density of stones of 2.65 g cm<sup>-3</sup>. At all sites but Aludeka (with 1, 5 and 12% of stones in 0-15, 15-30 and 30-50 cm depth, respectively), stone content was very low (below 1% on average). Prior to C and N analysis, samples were finely ground with a ball mill, then soil C and N ~~concentrations-contents~~ were measured by dry combustion using an elemental analyzer (CHN628, LECO Corporation, Michigan, USA). In addition, soil pH (H<sub>2</sub>O) was determined on ~~the unmilled~~ 0-15 cm topsoil samples (2-mm sieved) taken in 2018. Because soil pH values lower than 6.5 were observed at all sites, no correction for carbonates was necessary in the calculation of SOC ~~concentrations-contents~~. From additional deeper soil sampling down to 60 cm depth carried out in 2018 (data not shown here), it is known that the soil pH was lower than 6.5 in all depths considered.

## 2.3 Calculation of SOC stocks based on equivalent soil mass

As the measurements conducted in 2021 consisted of both SOC and BD, the equivalent soil mass approach was used to estimate SOC stocks across normalized soil masses (Lee et al., 2009), in addition to SOC ~~concentrations~~ contents. For this, the approach of Wendt and Hauser (2013) was followed, by fitting a cubic spline to measured data in order to re-scale to equivalent soil masses. First, the soil masses and SOC stocks were computed for each soil depth layer that was sampled. Then, cumulative SOC stocks and soil masses were calculated for combined depth layers to increasing depth (0-15, 0-30 and 0-50 cm) by summing the SOC stocks and soil masses of the depth layer with the values from all above layers. Next, a cubic spline with no intercept was fitted to each individual sampled soil core with cumulative SOC stocks as dependent and cumulative soil masses and the squared value thereof as independent variables. From visual inspection of this fit and  $R^2$  values of 0.99, it was concluded that the fitted cubic splines could be used to compute SOC stocks for normalized cumulative soil masses for each sampled soil core. We chose normalized cumulative soil masses of 0-2500 t soil ha<sup>-1</sup> and 2500-7500 t soil ha<sup>-1</sup>, which across the sites roughly corresponded to the mean soil masses in the depths of 0-15 cm and 15-50 cm, respectively. Changes in SOC stocks based on equivalent soil masses are the most reliable way to assess carbon gains or losses, but were only available for 2021. Therefore, we tested by correlation analysis whether the 2021 SOC stocks for the top 2500 t ha<sup>-1</sup> equivalent soil mass were in alignment with the observed temporal trends of the SOC content in the 0 - 15 cm soil layer, i.e., whether soils with the highest losses in SOC content had the lowest SOC stocks in 2021.

## 2.4 Statistical analysis

### 2.4.1 Creation of statistical models

Statistical analyses of SOC and soil total N ~~concentrations~~ contents in 0 to 15 cm depth and their temporal trends were performed using mixed linear models. Random slopes and intercepts of the temporal effect were initially included in a nested structure down to the split plot level. Because of an almost perfect correlation between changes in SOC and soil total N ~~concentrations~~ contents (Fig. ?? contents ( $r > 0.95$ ;  $p < 0.001$ )), the focus of this paper was on SOC ~~concentrations~~ contents. Two different statistical models were developed. One model which focused on site-specific effects (fixed-site model), and another model that focused on the general trend of SOC ~~concentrations~~ contents across sites, in which site was a random effect (random-site model). For the ~~latter random-site~~ model, the SOC data was normalized to the percentage of initial SOC, i.e., data points from each site were divided by the mean SOC ~~concentration~~ content of each experimental replicate (block) at the start of the experiment (as initial measurements were not available for all plots). The initial fixed effects in the ~~model were interactions~~ random-site model were 1) time since the experiment started, 2) the interaction of time since the experiment started with the organic resource treatment, ~~the~~ 3) the interaction of time since the experiment started with the mineral N treatment and ~~the~~ interaction of organic resource treatment with mineral N treatment 4) the three-way interaction of time since the experiment started with organic resource and mineral N treatments. In the ~~site-specific model, all these fixed-site model, the~~ fixed effects were ~~further allowed to be site specific, by adding an interaction with site for each of them. In this model, site was the only fixed effect that was also allowed to have an intercept of its own and not only an interaction with time.~~ 1) the interaction

of site with time since the experiment started, 2) the three-way interaction of site with time since the experiment started and with the organic resource treatment, 3) the three-way interaction of site with time since the experiment started and with the mineral N treatment and 4) the four-way interaction of site with time since the experiment started, organic resource and mineral N treatments. Moreover, the fixed-site model, had a fixed site effect without interactions. This assured that different treatments at the same site would start at the same SOC ~~concentration~~content at the start of the experiment in the ~~site-specific model, and that fixed-site model~~. Because the random-site model only had one common intercept, all treatments and sites in that model would start at initially 100% of SOC~~in the model that treated site as a random effect~~. In both statistical models, random effects were systematically eliminated one by one until the best random effects structure with the lowest Akaike Information Criterion (AIC) was found. This resulted in the removal of the random slope in the models but a random intercept was kept. The nested random intercepts were dataset/blockInSite/Plot and dataset/Site/blockInSite/Plot, for the models without and with site as random effect, respectively. The random effect of "dataset" accounted for potential systematic effects of the soil sampling in different years. Additionally, visual inspection of model residuals revealed variance heterogeneity between sites. Consequently, a site dependent residual variance was allowed in the models. After selecting an appropriate random effects structure and visually checking for normality and homoscedasticity~~(a site-specific variance had to be included)~~, interactions of fixed effects were removed one by one until only significant fixed effects remained, starting at the highest order of interaction. Selection of random effects was done using restricted maximum likelihood, that of fixed effects with maximum likelihood and the final model was then fit again with restricted maximum likelihood (Zuur et al., 2009). Additionally, an alternative version of the site-specific model, using relative SOC data (percent of initial SOC) as dependent variable, was created to compare SOC changes within the same organic resource treatments across sites.

All statistical analyses of the SOC stock data from 2021, based on equivalent soil mass, and pH data from 2018, were done in a similar way as the analyses of SOC ~~concentration~~content. Fixed effects were the organic resource and mineral N treatments, the site and the cropping status (cropped vs bare; in the case of SOC stocks data only) and interactions of all were allowed. Models contained a random nested blockInSite/Plot effect and allowed for variance heterogeneity between sites, while no random effect for "dataset" and no interaction with time was needed, given the single time point.

#### 2.4.2 Estimation of carbon storage efficiency

From the temporal trends of SOC ~~concentration~~content, we further ~~estimated the change of SOC stocks in 0-15 cm depth, with the goal to derive the~~ derived an estimate of the apparent carbon storage efficiency ( $CSE_a$ ) of the different organic resources in the 0-15 cm soil layer, i.e., a measure of efficiency to retain C. The  $CSE_a$  has been defined as the fraction of C inputs contributing to C storage in the soil (Manzoni et al., 2018), e.g., in our case how much the annually added C through organic resources ~~is found in the soil~~ changed the trend of SOC stocks compared to the control treatment. To do so, ~~we multiplied first~~ the least square means of the change in annual SOC ~~concentration~~content obtained by site and treatment from the ~~mixed model, with the mean fixed-site model had to be transformed to mean annual change in SOC stocks. The mean~~ BD of each site estimated from topsoil measurements ~~to obtain the mean annual change in SOC stocks was used for this~~ (treatment-specific differences in BD were absent). These BD estimations were also derived using a mixed linear model, from the available BD

measurements which had been conducted in the experimental year 1, 2 and in the calendar year 2021 at each of the sites. ~~Then, a site- and organic resource-specific linear regression between the estimated~~ This model did not contain any temporal trend; different methods of BD measurements in initial years and in 2021 were used (core method vs direct measurement from the soil augers), so any trend would have likely been an artefact. While assuming this constant BD for SOC stock calculations is only a rough approximation and not fully consistent with the equivalent soil mass approach, we nonetheless considered that it was a valuable approach to quantify CSE<sub>a</sub>. Another potential limitation of these calculations considering only the 0-15 cm soil layer (soil layer in which organic resources were incorporated), is that CSE<sub>a</sub> may be underestimated if significant portions of carbon inputs are stabilized in deeper soil layers from e.g. leached dissolved organic carbon.

In the final step of CSE<sub>a</sub> estimations, a linear regression was fit, with the calculated mean annual change in SOC stocks for 0-15 cm as the response variable and the amount of annual ~~organic resource C applied was fit, using a site-specific intercept.~~ C input as the independent variable (i.e., 0, 1.2 and 4 t C ha<sup>-1</sup> yr<sup>-1</sup>). These regressions were site- and organic resource-specific, so that estimates of CSE<sub>a</sub> per site and organic resources could be compared:

$$dSOC = Site + C_{in} : OR + C_{in} : OR : Site \quad (1)$$

Here, *dSOC* is the mean annual change in SOC stocks in 0-15 cm (t C ha<sup>-1</sup> yr<sup>-1</sup>), *Site* is the site specific intercept, *C<sub>in</sub>* the amount of annual C input (t C ha<sup>-1</sup> yr<sup>-1</sup>) and *OR* the type of organic resources. Note that ":" represents interactions and that there was no *OR*-specific intercept. The intercept was set to site specific, i.e., not allowed to vary between different organic resources at the same site (i.e., the SOC change at 0 t C ha<sup>-1</sup> yr<sup>-1</sup> did not vary between treatments). The slope of this regression was taken on the numerical variable *C<sub>in</sub>* represented the yearly change in SOC stocks (in 0-15 cm) per t C ha<sup>-1</sup> yr<sup>-1</sup> of organic resources added. It was thus interpreted as an estimate for CSE<sub>a</sub> of the different organic resources at the different sites (Manzoni et al., 2018) and we tested whether significant differences existed ~~in the CSE<sub>a</sub> between the~~ between slopes for different organic resources ~~at the and at~~ different sites (i.e., testing for a significant effect of organic resource treatment, site and their interaction). Estimated least-square means of the slope were converted into percent from t C t C<sup>-1</sup> by multiplying them by 100.

### 2.4.3 Statistical software and definitions

The R software version 4.0.4 (R Core Team, 2021) with the 'nlme' package (Pinheiro et al., 2021) was used for all statistical analyses. The post-hoc pairwise comparison of different treatments at different times was done with the 'cld' function (Piepho, 2004) of package 'emmeans' (Lenth, 2021) using the 'containment' method to estimate degrees of freedom. Estimated least square means were computed for SOC at different time points, for the temporal trends of SOC ~~concentrations~~ contents and for SOC stocks from 2021 and pH data at the time of measurement. Note that in the ~~flow following~~ text, the term significant refers to the p < 0.05 threshold, if not specified otherwise.

## 2.5 First-order decay model for comparison of SOC loss with other experiments

Finally, we compared the losses of SOC in the control treatments to losses reported in other experiments. A simple modelling approach was used assuming a yearly 1<sup>st</sup> order kinetic SOC loss:

$$SOC_{loss} = SOC_{initial} * (1 - k * t)^{yr} - SOC_{initial} \quad (2)$$

Here,  $SOC_{loss}$  and  $SOC_{initial}$  correspond to the initial SOC contents and the SOC loss at the end of the experimental period ( $g\ kg^{-1}$ ),  $k$ , is the annual loss of SOC under a base temperature of  $10^{\circ}C$  ( $g\ g^{-1}\ SOC$ ), and  $t$ , a site-specific rate modifier, based on site mean annual temperature (MAT) and a  $Q_{10}$  of 2:

$$t = 2^{(\frac{MAT-10}{10})} \quad (3)$$

The annual turnover of SOC ( $k$ ), was manually calibrated, and the Nash Sutcliffe modelling efficiency was calculated for assessing the goodness of model fit, as follows:

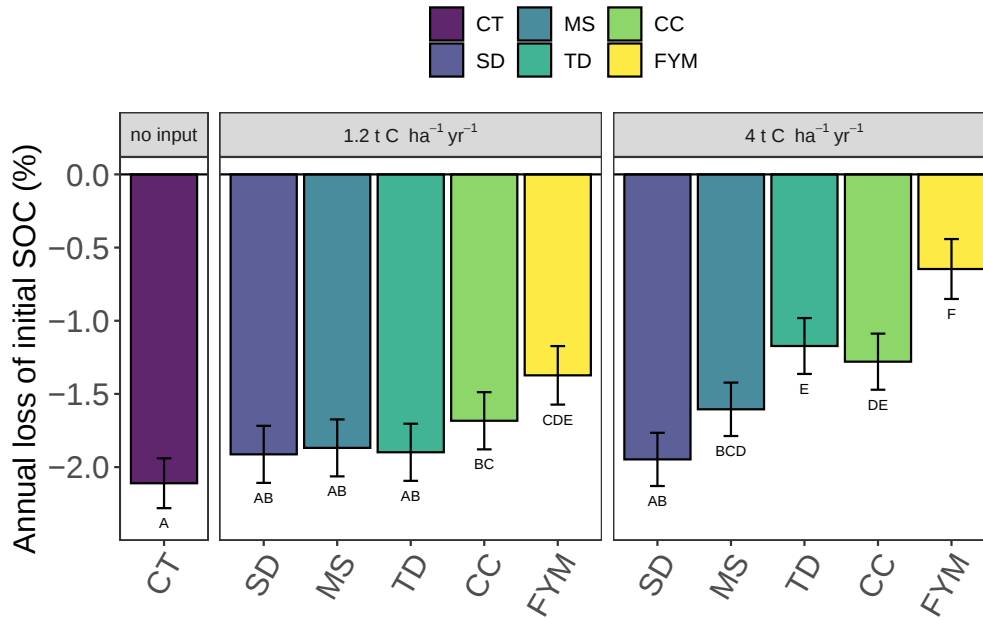
$$EF = \frac{\sum_{z=1}^n (O_z - \bar{O})^2 - \sum_{z=1}^n (O_z - P_z)^2}{\sum_{z=1}^n (O_z - \bar{O})^2} \quad (4)$$

Here,  $EF$  is Nash-Sutcliffe modelling efficiency,  $O_z$  is the measured SOC loss of the  $z$ -th site at experiment end,  $\bar{O}$ , the mean loss of SOC in all experiments and  $P_z$  the simulated value corresponding to  $O_z$ .

## 3 Results

### 3.1 General trends of SOC concentrations contents across sites

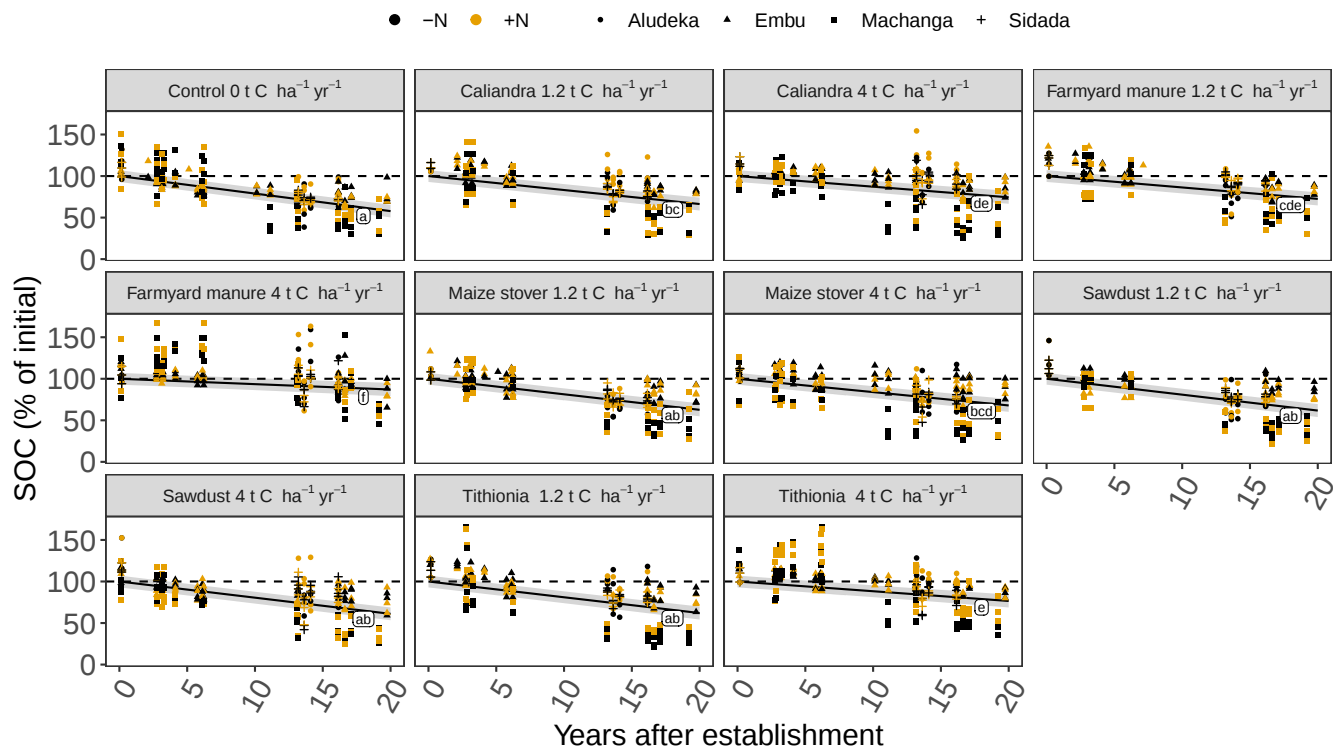
The analysis of relative SOC change across all sites showed that even at high amounts of added organic resources, a decrease of topsoil SOC concentration content occurred over time with continuous maize cropping (Fig. 1 and 2). Significant differences in the magnitude of annual SOC decrease existed between the different organic resource classes applied at the high rate of 4 t C ha<sup>-1</sup> yr<sup>-1</sup> and to a lesser extent at the lower rate of 1.2 t C ha<sup>-1</sup> yr<sup>-1</sup>. In contrast, the application of mineral N did not have a significant effect on the annual decrease of SOC concentration content (Fig. 2), so +N and -N were evaluated together. Across the four sites, SOC concentration content decreased strongest under the CT treatment. On average by 2.1% of initial SOC per year, corresponding to 40% loss over 19 years. Average SOC decreases in all the 4 t C ha<sup>-1</sup> yr<sup>-1</sup> treatments, except SD, were significantly lower than in the control but did not completely halt SOC losses. The FYM and TD treatments resulted in the lowest decrease of SOC concentration content, i.e., 0.6 and 1.2% of initial SOC per year, respectively (12 and 23% over 19 years). Notably, the FYM treatment at 4 t C ha<sup>-1</sup> yr<sup>-1</sup> resulted in a significantly lower SOC decrease than all other treatments, followed by TD at 4 t C ha<sup>-1</sup> yr<sup>-1</sup>, which was significantly lower than all but the CC treatment at 4 t C ha<sup>-1</sup> yr<sup>-1</sup>. The application rates of 1.2 t C ha<sup>-1</sup> yr<sup>-1</sup> were much less effective in reducing SOC decrease; only FYM and CC were significantly different from the control, with average decreases of 1.4 and 1.7% of initial SOC per year, respectively.



**Figure 1.** Annual changes of SOC ~~concentrations~~ contents in the top 0-15 cm soil layer in different organic resource treatments across sites. ~~Annual~~ The annual change in SOC ~~concentrations~~ contents represent the slope of the regression against experimental time from the random-site model, using all available data (i.e., 5 (Sidada, Aludeka) or 14 (Embu, Machanga) repeated measurements from 3 replicates at 4 sites, comprising both mineral N treatments due to absence of mineral N effect). They are displayed as percentage of initial SOC (intercept of the model set to 100%). Treatments ~~that share with the same~~ capital ~~letters~~ letter do not differ significantly from each other in the annual change of SOC (~~all~~ p < 0.05). The error bars indicate the 95% confidence ~~interval~~ intervals for the annual change of SOC. ~~Abbreviations: CT, control; SD, saw dust; MS, maize stover; TD, Tithonia; CC, Calliandra; FYM, farmyard manure.~~ Abbreviations: CT, control; SD, sawdust; MS, maize stover; TD, Tithonia; CC, Calliandra; FYM, farmyard manure.

### 305 3.2 Site specific differences ~~in of the influence~~ effects of organic resource quality on SOC ~~development~~ formation

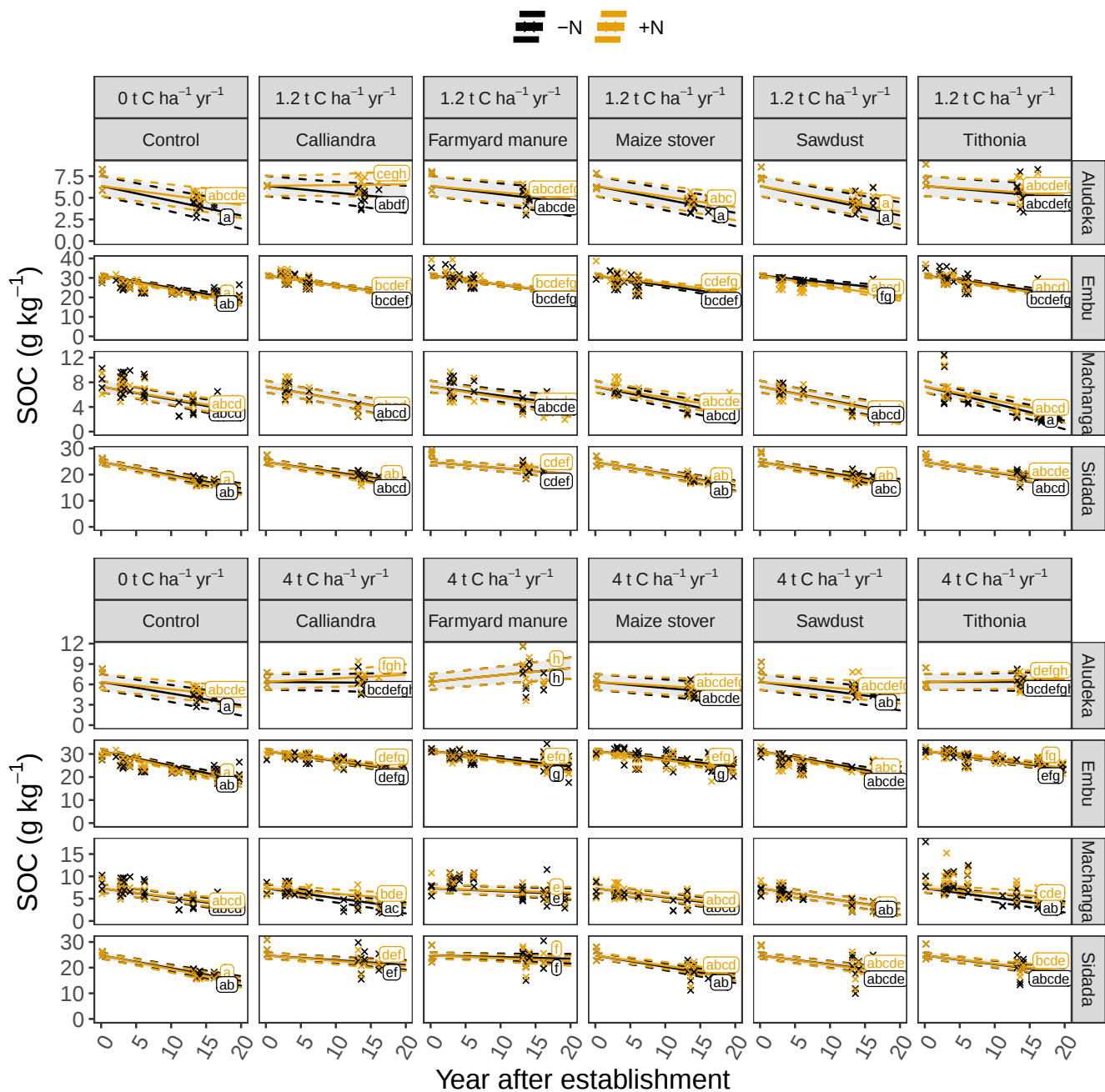
The analysis of SOC ~~concentration~~ content at the site level revealed strong site specificity of temporal changes in relation to the different organic resource treatments. For example ~~in, at~~ in, at Aludeka, the FYM treatment at 4 t C ha<sup>-1</sup> yr<sup>-1</sup> showed a significant gain in SOC of 0.1 g C kg<sup>-1</sup> yr<sup>-1</sup>, while ~~in at~~ in Embu, all treatments lost at least 0.4 g C kg<sup>-1</sup> yr<sup>-1</sup> (Fig. 4). The order of treatments, however, was similar at all sites. The control treatment resulted in the largest decrease in SOC ~~concentration~~ content, while the application of FYM at the rate of 4 t C ha<sup>-1</sup> yr<sup>-1</sup> led to the smallest decrease or even increase ~~in SOC content~~ in SOC content. The SD treatment generally showed similar decreases in SOC ~~concentration~~ content as the control treatment, and CC and TD led to SOC change rates in between the control and FYM treatments. The application of organic resources at the rate of 1.2 t C ha<sup>-1</sup> yr<sup>-1</sup> did, with a few exceptions (e.g., FYM ~~in at~~ in Embu and Sidada), not significantly reduce the decrease in SOC ~~concentration~~ content compared to the control. Notably, the absolute annual SOC ~~concentration~~ content decrease in the control treatment was largest



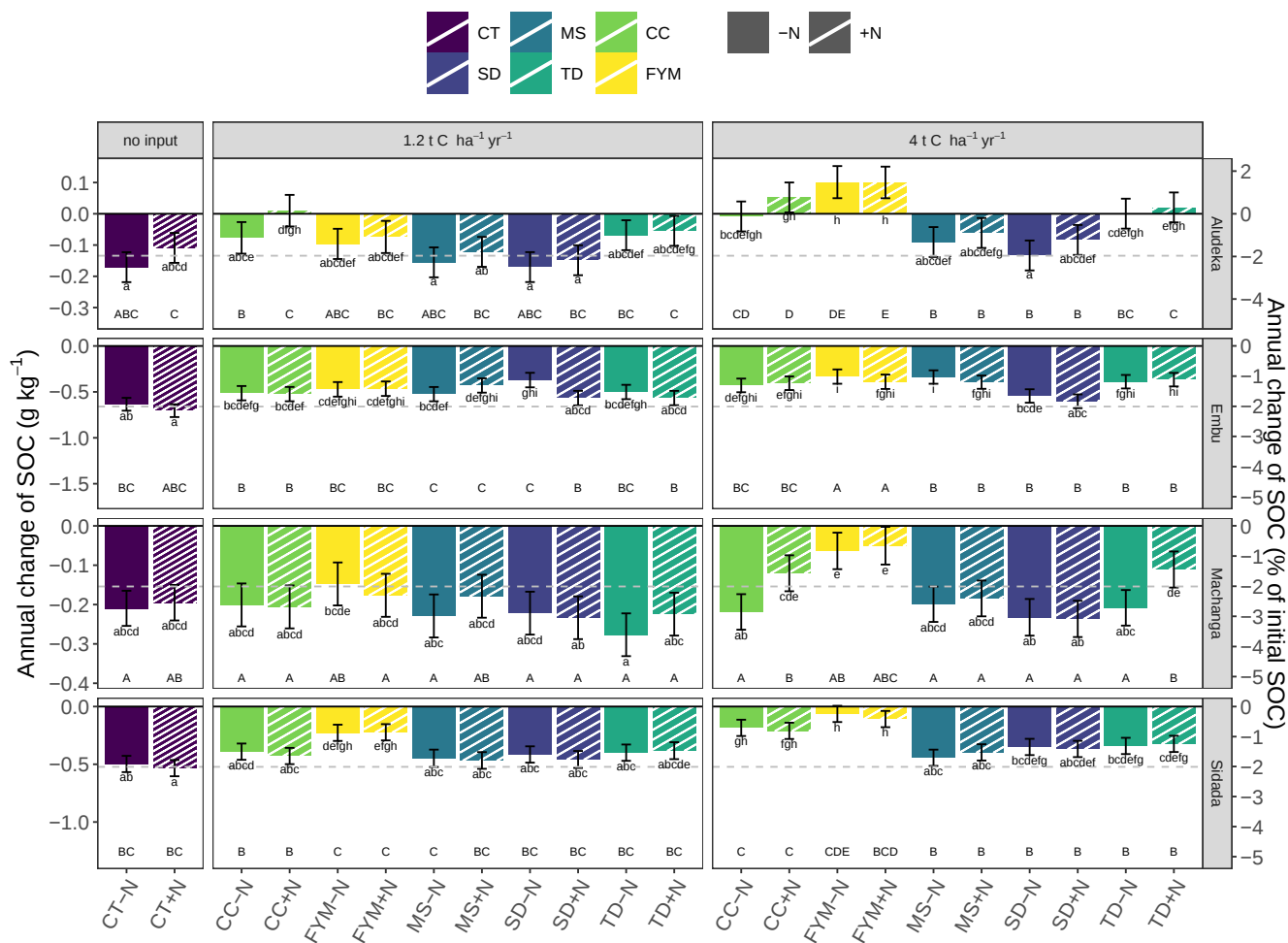
**Figure 2.** General trend in-the-of SOC concentrations-contents in the top 15 cm soil layer under different organic resource treatments across sites. The change in SOC concentrations-contents is shown as a percentage of initial SOC concentrationscontents (mean by site). Same lowercase letters indicate the absence of a significant difference in-the-temporal-development between the different organic resource treatments, while the mineral N additions did not have a significant effect ( $\alpha$ -p < 0.05). Large initial fluctuations in-and values above 100% at the Machanga site are a combined result of measurement variability and low SOC contents. The grey shaded areas constrained-by-the dashed-lines indicate the 95% confidence intervals for true mean of SOC at different times.

315 in-at Embu (about 0.6-0.7 g C kg<sup>-1</sup> yr<sup>-1</sup>), followed by Sidada (about 0.5 g C kg<sup>-1</sup> yr<sup>-1</sup>), and was about 0.2 g C kg<sup>-1</sup> yr<sup>-1</sup> in-at Machanga and Aludeka. The picture was different in In relative terms, with site-specific annual decreases of annual decreases were about 2% to 3% of initial SOC in the control (Fig. 4). The highest relative SOC decrease in-the-control-treatments-was observed-in-was observed at Machanga in the CT-N treatment, which lost almost 3% of initial SOC per year. Within the control treatments across sites, significantly lower decreases than CT-N in-at Machanga were found in CT+N in-at Aludeka, CT-N in 320 at Embu and in both control treatments in-at Sidada, all losing about 2% per year.

In contrast to the control, the FYM treatment at 4 t C ha<sup>-1</sup> yr<sup>-1</sup> lost only about 0.4 g C kg<sup>-1</sup> yr<sup>-1</sup> (corresponding to 1% of initial SOC) in-at Embu, and about 0.1 g C kg<sup>-1</sup> yr<sup>-1</sup> in-at both Machanga (1% of initial SOC) and Sidada (0.3% of initial SOC), while in-at Aludeka it gained about 0.1 g C kg<sup>-1</sup> yr<sup>-1</sup> (1.5% of initial SOC). In terms of relative SOC changes, differences between sites within the same organic resource treatment were observed more frequently than in the control. For example, the SOC



**Figure 3.** Temporal trends of SOC ~~concentrations~~ ~~contents~~ in the top 15 cm ~~of the soil~~ ~~layer~~ by site, organic resource and N treatment, displayed in combination with measured raw data, shown by the crosses. Same lowercase letters indicate the absence of significantly different temporal trends between treatments ( $\alpha p < 0.05$ ). The grey shaded areas ~~constrained by the dashed lines~~ indicate the 95% confidence intervals for the true mean of SOC at different times.



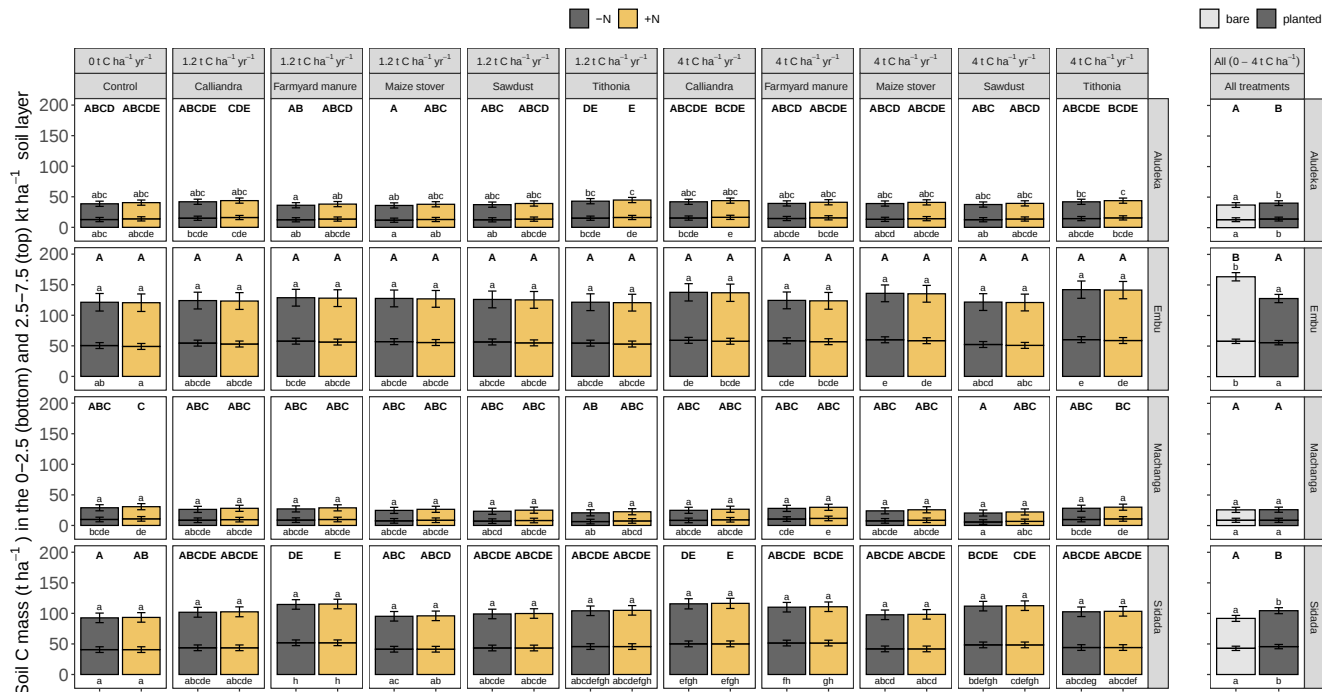
**Figure 4.** Temporal trend of SOC concentrations-contents in the top 15 cm of the soil layer by site, organic resource and N treatment in both absolute and relative values. Bars in lighter colors with grey outlines Filled bars represent the -N treatments and dashed bars in darker colors with black outlines represent the +N treatment. Statistics across sites were done based on the relative SOC changes (all  $p < 0.05$ ). Same lowercase letters indicate the absence of a significant difference in the temporal trend between treatments at the same site. Same uppercase letters indicate the absence of a significant difference within the same organic resource treatment across sites (resource type and amount, including the comparison between +N and -N of the same treatment). As a reference to the general trends, the grey dashed line indicates the mean annual decrease of 2.1% per year, observed in the control treatment across sites. Error bars indicate the 95% confidence intervals. Abbreviations: CT, control; SD, saw dust; MS, maize stover; TD, Tithonia; CC, Calliandra; FYM, farmyard manure. Abbreviations: CT, control; SD, sawdust; MS, maize stover; TD, Tithonia; CC, Calliandra; FYM, farmyard manure.

gains in the FYM treatments at 4 t C ha<sup>-1</sup> yr<sup>-1</sup> ~~in-at~~ Aludeka differed significantly from the losses observed within the same treatments ~~in-at~~ Embu and Machanga. Also, the other 4 t C ha<sup>-1</sup> yr<sup>-1</sup> treatments showed ~~in-most-cases-generally~~ significantly lower relative SOC decreases ~~in-Aludeka-than-in-at Aludeka than at~~ Machanga. Additionally, the +N treatments of CC and TD showed significantly lower relative SOC decreases ~~in-Aludeka-than-in-at Aludeka than at~~ Embu and Sidada (Fig. 4).

In the ~~site-specific-site-specific~~ analysis, the addition of mineral N showed a significant interaction with the organic resource treatment, but, with a few exceptions, significant differences between +N and -N treatments were absent, ~~nonetheless~~. Specifically, the CC treatment at 1.2 t C ha<sup>-1</sup> yr<sup>-1</sup> ~~in-at~~ Aludeka showed no significant SOC decrease in the +N treatment while the corresponding -N treatment lost about 0.1 g C kg<sup>-1</sup> yr<sup>-1</sup>. Similarly, the +N ~~subplots-subject-to-treatments with~~ 4 t C ha<sup>-1</sup> yr<sup>-1</sup> addition of either CC or TD ~~in-Machanga-, at Machanga~~ showed a significantly lower decrease in SOC ~~concentration-content~~ than their -N counterparts. Finally, the SD treatment at 1.2 t C ha<sup>-1</sup> yr<sup>-1</sup> ~~in-Embu-, at Embu~~ had a significantly lower decrease in SOC ~~concentration-content~~ in the -N treatment compared to the +N treatment, but for all other organic resource treatments at all sites, the +N and -N subplots were not significantly different from each other.

### 3.3 SOC stocks at different equivalent soil masses and the ~~correspondence-to-correlation with~~ SOC ~~concentrationscontents~~

~~Changes in SOC stocks based on equivalent soil masses are the most reliable way to assess carbon sequestration or losses, but~~  
~~in-contrast-to-the SOC concentrations of our study, were only available for 2021. Hence, apart from the one time point SOC~~  
~~stock assessment (Fig. 5), we tested whether the~~ A highly significant correlation emerged between losses in SOC content (0-15  
~~cm) and the observed SOC stocks in~~ 2021 SOC stocks for the top 2500 t ha<sup>-1</sup> equivalent soil mass were in alignment with the  
~~observed temporal trends of the SOC concentration in the 0–15 cm soil layer, i.e., whether soils with the highest losses in SOC~~  
~~concentration had the lowest SOC stocks in 2021. A highly significant correlation emerged between these two types of SOC~~  
~~assessment in the planted plots, explaining 81% of total variation for at~~ the clayey sites, Embu and Sidada, and about 56% ~~for~~  
~~the two coarse textured at the two coarse-textured~~ sites, Machanga and Aludeka (Fig. B1). Due to ~~comprising of a one-time~~  
~~output the one sampling point in time~~, less significant differences in SOC stocks were detected between treatments ~~compared~~  
~~to SOC concentration. The 2021 SOC stocks also assessed bare plots, but there was no significant interaction between the plot~~  
~~status (being bare or planted) and the experimental treatments. Yet, the plot status had a site-specific effect on SOC stocks.~~  
~~In Aludeka and Sidada, significantly higher SOC stocks were observed in the planted compared to the bare plots in both the~~  
~~top and subsoil equivalent soil mass layers, while no effect was found in Machanga. Surprisingly, in Embu, the bare plots~~  
~~showed significantly higher SOC stocks than the planted plots in both soil layers (Fig. 5). Yet, subsoil~~ than in trends of SOC  
~~contents. Yet, SOC stocks in the 2500-7500 t-ton ha<sup>-1</sup> soil layer showed no significant differences between treatments in-at  
~~any of the sites, except in-at~~ Aludeka, where the TD treatments at both rates of C input had higher SOC stocks than the FYM  
~~treatment at 1.2 t-ton C ha<sup>-1</sup> yr<sup>-1</sup>. Besides, in contrast to the topsoil SOC stocks, the subsoil SOC stocks were much less related~~  
~~to topsoil trends of SOC concentration, suggesting relatively small interactions between top and subsoil SOC dynamics. In~~  
~~fact, a content. A significant association between the temporal trends of topsoil SOC~~ ~~concentrationscontents~~ and subsoil SOC  
~~stock-stocks~~ was only found ~~for-at~~ two sites, i.e., ~~in-at~~ Machanga (R<sup>2</sup> of 0.24) and Sidada (R<sup>2</sup> of 0.45), ~~yet-but~~ much weaker~~



**Figure 5.** Estimated least square means for the SOC stocks across sites in the cumulative equivalent soil masses of 0 to 2500 and 2500 to 7500 t soil ha<sup>-1</sup> (bottom and top bar, respectively). As there was no significant interaction between the cropping status (being bare or planted) and the organic resource and N treatments, the results are displayed as the estimated least square means for different organic resource and N treatments for planted plots (left plot) and as mean SOC stocks from all organic resource and N treatments in the bare compared to planted plots (right plot). Error bars display the 95% confidence intervals. Same lowercase letters indicate the absence of a significant difference in SOC stocks from-between treatments at the same site and soil mass layer which-share-no-lowercase-letter-are-significantly-different-from-each-other(above bars, 2500 to 7500 t ha<sup>-1</sup>; below bars, 0 to 2500 t ha<sup>-1</sup>; all  $p < 0.05$ ). Same capital-uppercase letters indicate the absence of a significant difference between treatments for the whole 0 to 7500 t ha<sup>-1</sup> equivalent soil mass layer at the same site ( $p < 0.05$ ).

increases of SOC stocks were found for-the-subsoil-compared-to-in the subsoil than in the topsoil (Fig. B1). SOC stocks were also assessed in the bare plots (without maize planted), but there was no significant interaction between the plot status (being bare or planted) and the experimental treatments. Yet, the plot status had a site-specific effect on SOC stocks. At Aludeka and Sidada, significantly higher SOC stocks were observed in the planted compared to the bare plots in both the top- and subsoil equivalent soil mass layers, while no effect was found at Machanga. Surprisingly, at Embu, the bare plots showed significantly higher SOC stocks than the planted plots in both soil layers (Fig. 5).

### 3.4 Apparent carbon storage efficiency and losses as affected by site and organic resource type

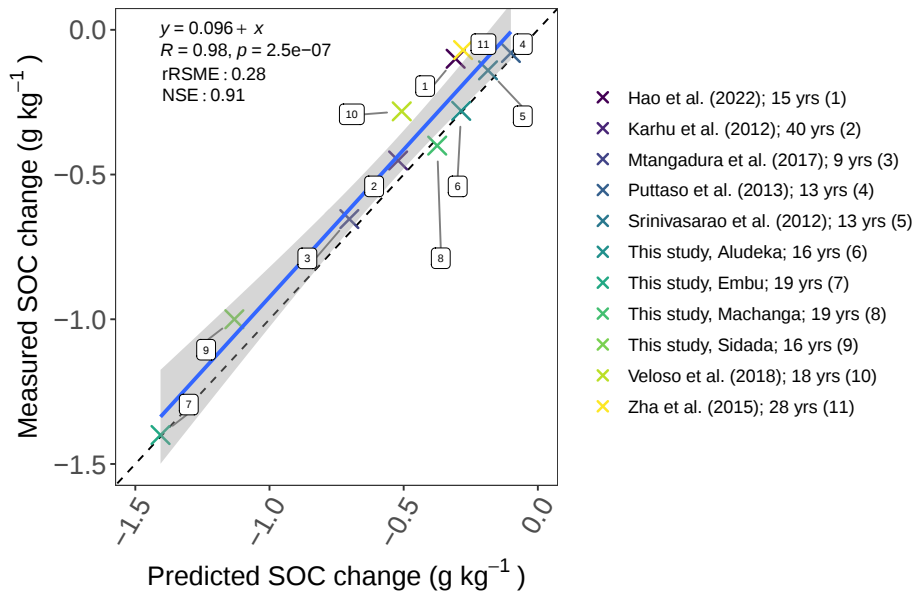
The efficiency with which organic resources were converted into SOC varied by site and treatment (Table 3). The treatments with respectively-the highest and lowest decrease in SOC concentration-content (FYM and SD) -corresponded to the treatments

**Table 3.** Estimated apparent carbon storage efficiency (CSE<sub>a</sub> of SOC formation) by treatment and site. Same lowercase letters at the same site indicate the absence of a significant difference between treatments at that site. Same uppercase letters of the same organic resource indicate the absence of a significant difference between sites within the same organic resource (all  $p < 0.05$ ).

| Residue treatment | Site     | <u>apparent-CSE</u> <u>CSE<sub>a</sub></u> (%) | 95% CI   |
|-------------------|----------|------------------------------------------------|----------|
| CC <sup>B</sup>   | Aludeka  | 10 <sup>c</sup>                                | 6 to 13  |
| FYM <sup>A</sup>  | Aludeka  | 13 <sup>c</sup>                                | 10 to 17 |
| MS <sup>A</sup>   | Aludeka  | 3 <sup>ab</sup>                                | 0 to 7   |
| SD <sup>AB</sup>  | Aludeka  | 1 <sup>a</sup>                                 | -2 to 5  |
| TD <sup>B</sup>   | Aludeka  | 9 <sup>bc</sup>                                | 5 to 12  |
| CC <sup>B</sup>   | Embu     | 10 <sup>ab</sup>                               | 6 to 13  |
| FYM <sup>A</sup>  | Embu     | 13 <sup>b</sup>                                | 9 to 16  |
| MS <sup>B</sup>   | Embu     | 13 <sup>b</sup>                                | 9 to 16  |
| SD <sup>AB</sup>  | Embu     | 4 <sup>a</sup>                                 | 1 to 8   |
| TD <sup>B</sup>   | Embu     | 11 <sup>b</sup>                                | 8 to 15  |
| CC <sup>A</sup>   | Machanga | 2 <sup>a</sup>                                 | -1 to 6  |
| FYM <sup>A</sup>  | Machanga | 9 <sup>b</sup>                                 | 6 to 13  |
| MS <sup>A</sup>   | Machanga | 1 <sup>a</sup>                                 | -2 to 5  |
| SD <sup>A</sup>   | Machanga | -2 <sup>a</sup>                                | -5 to 2  |
| TD <sup>A</sup>   | Machanga | 2 <sup>a</sup>                                 | -1 to 6  |
| CC <sup>B</sup>   | Sidada   | 13 <sup>b</sup>                                | 10 to 16 |
| FYM <sup>B</sup>  | Sidada   | 20 <sup>c</sup>                                | 16 to 23 |
| MS <sup>A</sup>   | Sidada   | 3 <sup>a</sup>                                 | 0 to 7   |
| SD <sup>B</sup>   | Sidada   | 6 <sup>a</sup>                                 | 3 to 9   |
| TD <sup>AB</sup>  | Sidada   | 8 <sup>ab</sup>                                | 4 to 11  |

with the ~~highest and lowest~~ CSE<sub>a</sub> lowest and highest CSE<sub>a</sub>, respectively. The highest CSE<sub>a</sub> for FYM was found ~~in~~ at Sidada (20%), while it was about 13% ~~in~~ at Aludeka and Embu, and 9% ~~in~~ at Machanga. On the other hand, the lowest CSE<sub>a</sub> for SD (-2%) was observed ~~in Machanga, whilst~~ at Machanga, while it was between 1 and 6% ~~in~~ at the other sites. Besides, the differentiation of FYM in terms of CSE<sub>a</sub> ~~compared to~~ from other treatments varied among sites. ~~In~~ At Sidada, the CSE<sub>a</sub> for FYM was significantly higher than ~~in~~ for the other treatments~~except CC, except for CC, at Embu, in Embu~~ it was only significantly higher than ~~that of~~ for SD, and ~~in~~ at Machanga and Aludeka, FYM had a significantly higher CSE<sub>a</sub> than MS and SD. On the other hand, the SD treatment was outperformed in terms of CSE<sub>a</sub> by the CC treatment ~~in~~ at Aludeka and Sidada, and by the TD treatment ~~in~~ at Aludeka and Embu. The MS treatment was in the group of lowest CSE<sub>a</sub> ~~in~~ at all sites (1-3%)~~except in, except at~~ Embu, where, surprisingly, it had a CSE<sub>a</sub> of 13%, which was not significantly different from that for FYM. The CSE<sub>a</sub> within the same organic resource types further varied between sites, as indicated by a significant interaction of organic

Estimated least-square means for the topsoil pH value by site. Error bars display the upper half of the 95% confidence intervals. Organic resource treatments from the same site which share no lower case letter are significantly different from each other ( $p < 0.05$ ). The asterisk (\*), indicates that a similar significant difference existed between the +N and -N treatment for all organic resource treatments and at all sites (due to the lack of a significant interaction of  $\pm N$  with both organic resource treatments and site).



**Figure 6.** Comparison of predicted vs measured SOC change at the end of different experiments without input of external organic matter and mineral fertilizers assuming a 1.5% per year first order decomposition at 10°C mean annual temperature. The decomposition rate was scaled with an exponential temperature function with a  $Q_{10}$  of 2 using the mean annual temperature of each site. The legend shows the different experiments (Srinivasarao et al., 2012; Karhu et al., 2012; Puttaso et al., 2013; Zha et al., 2015; Mtangadura et al., 2017; Veloso et al., 2018; Hao et al., 2022), and their duration in years (yrs). Abbreviations: *rRSME*, relative root mean square error; *NSE*, Nash-Sutcliffe model efficiency.

resource treatment with site. For example, FYM had a significantly higher  $CSE_a$  in Sidada than in at Sidada than at all other sites, while the  $CSE_a$  of for CC and TD was significantly lower in Machanga than in at Machanga than at the other three sites.

### 380 3.5 The effect of organic resources and mineral N on soil pH

In contrast, when assessing SOC losses of the CT treatments compared to control treatments of other long-term experiments using the simple first-order decay modelling approach, we found that the decrease in SOC content in the control was rather uniform across sites. That is, when adjusting the turnover rate by mean annual temperature, a fairly consistent trend of SOC loss across studies was found (Fig. 6), corresponding to a yearly SOC loss of about 1.5% at 10°C mean annual temperature.

385 Mineral N application had a significant negative effect on soil pH, which was consistent across sites, i.e., the +N treatments had a pH of 0.11 units lower than the -N treatments. In addition, there was a highly significant interaction of organic resource

treatment with site. In all sites, soil pH values were highest in the FYM treatment at  $4 \text{ t C ha}^{-1} \text{ yr}^{-1}$ , whereas the lowest pH values were found in the control or CC treatments. Yet, the strength of the differentiation of FYM at  $4 \text{ t C ha}^{-1} \text{ yr}^{-1}$  compared to other treatments differed among sites. For example, the pH values in the FYM treatment at  $4 \text{ t C ha}^{-1} \text{ yr}^{-1}$  in Embu (5.95 in -N) and Machanga (6.96 in -N) were significantly higher than in all other treatments, in Sidada (5.85 in -N) it was higher than all, except in the FYM treatment at  $1.2 \text{ t C ha}^{-1} \text{ yr}^{-1}$ , while in Aludeka (6.15 in -N), it was only significantly higher than that in the control and CC treatments. While the pH values in SD and CC at both C input rates were not significantly different from that in the control in any site, TD at  $4 \text{ t C ha}^{-1} \text{ yr}^{-1}$  had a significantly higher pH value than the control in Machanga, and a significantly higher pH value than those in CC at  $4 \text{ t C ha}^{-1} \text{ yr}^{-1}$  in Aludeka and Sidada, as well as those in CC at  $1.2 \text{ t C ha}^{-1} \text{ yr}^{-1}$  in Machanga and Sidada.

## 4 Discussion

### 4.1 In most sites, even high-quality High-quality organic resource addition ~~could do not completely consistently~~ halt SOC loss

The results of our study ~~showed clearly~~ clearly showed that even at high rates of organic resource addition ( $4 \text{ t C ha}^{-1} \text{ yr}^{-1}$ ), SOC generally decreased (i.e., of the four sites in this study, only Aludeka showed increased SOC; Fig. 4). This was indicated by the changes in SOC ~~concentrations~~ contents in the 0-15 cm topsoil layer and corroborated by their strong correlation with the 2021 topsoil equivalent soil ~~mass-based~~ mass-based SOC stocks. Hence, very high amounts of high-quality organic resource inputs farmyard manure are needed to prevent a loss of SOC ~~concentrations~~ contents, with even  $4 \text{ t C ha}^{-1} \text{ yr}^{-1}$  not being sufficient at Machanga and Embu. The high losses ~~in Machanga, the site with the highest at Machanga, i.e. a~~ relative loss of almost 3% of initial SOC ~~concentrations~~ contents per year, are likely not only ~~from~~ caused by SOC mineralization but also ~~from erosion. It is by erosion. Despite the gentle slope of the experimental site,~~ Machanga showed ~~extremely~~ strong signs of topsoil erosion, ~~which was, however, but this was unfortunately~~ not quantified. ~~For~~ At Embu, the high initial SOC ~~concentrations~~ contents may be responsible for the fact that even the high loads of  $4 \text{ t C ha}^{-1} \text{ yr}^{-1}$  of ~~the farmyard manure treatment~~ farmyard manure could not form enough new SOC to counterbalance the losses. Yet, even ~~in Sidada, a very favorable site at Sidada,~~ considered as having favorable soil conditions for SOC formation, SOC was lost in almost all treatments and none ~~had showed~~ a significant gain. In conclusion, our first hypothesis, that addition of high-quality organic resources ~~increase SOC, increases~~ SOC is rejected for all sites except ~~Aludeka~~ for Aludeka where farmyard manure (but not high-quality Tithonia and Calliandra) could increase SOC.

The generally observed SOC losses in our study corroborate the results of ~~Sommer et al. (2018), who reported similar SOC losses at two sites in western Kenya, both close to Sidada. Another recently published study from a long-term trial of compost application of about  $3 \text{ t C ha}^{-1}$  a year in Ivory coast, could also not maintain initial SOC levels (Cardinael et al., 2022). recent studies under similar conditions (Sommer et al., 2018; Cardinael et al., 2022).~~ It seems thus, that maintaining SOC in arable cropping ~~in tropical systems is very difficult~~ systems in the tropics is challenging, even with high C inputs that are aimed at replenishing SOC. Potential explanations for the high SOC losses in tropical soils are manifold. They have been attributed to

420 ~~the~~ humid climate (Ryan and Law, 2005; Todd-Brown et al., 2013), ~~the~~ high weathering status of the soils and thus limited protection of SOC (Six et al., 2002; Doetterl et al., 2015), and ~~the~~ high temperatures (Davidson and Janssens, 2006; Conant et al., 2011; Wei et al., 2014). ~~If inconsistent but strong rains occur, as was the case in Machanga, erosion can also~~ Besides, if heavy rainfall events occur, erosion can be considerable and wash away the ~~SOC-rich topsoil~~ SOC-rich topsoil, as was the case in Machanga. Furthermore, ~~a~~ loss of SOC usually occurs when natural vegetation is converted to arable land (Sanderman et al., 425 2017) and about 50% of initial ~~carbon~~ C is usually lost (Guo and Gifford, 2002; Lal, 2018), suggesting a better stabilization of SOC under natural vegetation. In the tropics, ~~the SOC loss due to land use change is usually more severe than that SOC loss upon clearing of natural vegetation is usually larger~~, and can be as high as 70-85%, ~~and usually~~ strongest in the initial few years following the land clearing (Solomon et al., 2007). Three sites of this study had been under shifting cultivation with considerable fallow time prior to the ~~experiment establishment~~ establishment of the experiment, while Machanga was 430 even converted from natural vegetation. This suggests that soils were still in the initial rapid ~~loss phase~~ phase of SOC loss, as observed most strongly in the absolute decreases of SOC ~~concentrations in contents at~~ Embu and Sidada, the sites with the highest initial ~~concentrations~~. ~~Hence, if starting at degraded lands with initially low SOC, SOC contents. On the other hand,~~ it may be possible to increase SOC ~~stocks with input~~ with inputs of external organic resources (~~Sommer et al., 2018~~). ~~The example of Machanga on degraded lands with initially low SOC (Sommer et al., 2018). Our results at Machanga indicate,~~ 435 ~~however, indicates, however~~ that low initial SOC is not the only determinant for successful SOC increase and that suitable ~~climate~~ pedo-climatic conditions, as in Aludeka, ~~is also needed~~ are also required.

Depending on the initial levels, it is expected that a certain amount of SOC will initially be lost from soils that were only recently converted from native vegetation. The evidence of this study and others (Sommer et al., 2018; Cardinael et al., 2022) confirm this and indicate that tropical soils under maize monocropping will not be an absolute carbon sink in the sense that 440 they still add CO<sub>2</sub> to the atmosphere even though they might be reducing emissions compared to the business-as-usual cropping systems (i.e. the control treatment). Since it is unrealistic that with a growing population and living standard there is much land in SSA that will be spared from crop production, aiming to halt SOC loss at medium SOC levels may be the only possible scenario to maintain soil fertility. For example, in our experiments, several treatments that experienced a reduction in SOC concentration still experienced a gain in yield with time (Laub et al., ; in review). The results of our study demonstrate that the 445 mitigation potential of improved management can still be substantial, i.e., between one and two thirds of the SOC losses in the control could be avoided by farmyard manure additions of 1.2 and 4 t C ha<sup>-1</sup> yr<sup>-1</sup>, respectively (Fig. 1). The farmyard manure treatments also had the highest yields (Laub et al., ; in review), suggesting an even better performance if emissions are yield sealed as they should be (Clark and Tilman, 2017). If however, the declared goal is an actual absolute removal of CO<sub>2</sub> from the atmosphere, one may need to target soils like in Aludeka, or include other agronomic measures, such as intercropping or 450 agroforestry, that could add more C to the soil (Muchane et al., 2020).

## 4.2 Organic resource quality has a strong effect on SOC buildup and loss

Like in several other studies (e.g. Galicia and García-Oliva, 2011; Laub et al., 2022; Li et al., 2022), our results, despite SOC losses in three of four sites, corroborate the emerging paradigm that organic resources of high quality (i.e., with a low C:N ratio)

are most effective in forming new SOC have the higher potential to form new SOC compared to low-quality organic resources.

455 This paradigm assumes that SOC is mostly of microbial origin (Denef et al., 2009; Cotrufo et al., 2013; Kallenbach et al., 2016) and that high-quality resources are processed with a higher carbon use efficiency (Manzoni et al., 2012; Sinsabaugh et al., 2013). The results of our ~~than~~ low-quality resources, such as ~~saw dust~~ sawdust and maize stover, ~~which that~~ only marginally improved the SOC ~~concentration~~ content over the control, ~~are also in alignment with this paradigm on our study~~ (Manzoni et al., 2012; Sinsabaugh et al., 2013). Yet, farmyard manure, which due to high lignin contents is not necessarily considered high quality, was the most effective

460 treatment. Similar results were reported in another long-term experiment in Thailand, where Tamarind leaves of intermediate C:N and moderate lignin contents performed better than low C:N, low-lignin, groundnut stover (Puttaso et al., 2013). The results of these studies ~~combined~~ suggest that abiotic condensation of humic substances from lignin-rich materials (Frimmel and Christman, 1988) does not play a major role in SOC formation, ~~as contrary to what~~ was postulated in the 1980s (Woomer and Swift, 1994). This finding is highly relevant for ISFM, ~~as~~; earlier hypothesized trade-offs between building SOC and

465 providing plant nutrition by organic resources (Palm et al., 2001b) are in fact ~~falsified~~ not supported by the results from long-term experiments. In contrast to ~~these early hypotheses~~ this earlier hypothesis, organic resources that have a good synchrony of nutrient release with plant demand, such as farmyard manure and *Calliandra*, are also the most effective in SOC formation ~~/maintenance. Together, these and maintenance. Thus, our~~ results show that the concept of ISFM remains highly relevant for crop productivity and soil fertility improvement.

470 Despite the trend of a general loss of SOC ~~concentration~~ content in the topsoil layer (0-15 cm) across sites, the different rates of loss for different organic resources show the importance of their quality in SOC ~~maintenance. This was seen best at formation~~ (Fig. 1). ~~This was observed best with~~ additions of 4 t C ha<sup>-1</sup> yr<sup>-1</sup>, where farmyard manure was most efficient in building SOC at all sites. In contrast, the low-quality ~~saw dust~~ sawdust could not reduce SOC losses compared to the control without organic resource addition at any site. Our findings corroborated the results of several other studies that demonstrated that application

475 of farmyard manure is the best option to sustain SOC (Sileshi et al., 2019; Rusinamhodzi et al., 2013; Mtangadura et al., 2017) ~~and pH~~ (Muecheru-Muna et al., 2014). One important consideration here is that manure quality depends on the animal species and that higher quality feed generally results in higher quality manures (Sileshi et al., 2017). Our study also confirmed that cut-and-carry green manures such as *Calliandra* and *Tithonia* ~~are~~ can be effective in forming new SOC ~~compared to no input~~ (Kunlanit et al., 2014), yet with lower efficiency than farmyard manure. On the other hand, green manures were shown

480 to be more effective in SOC maintenance compared to low-quality maize residues and sawdust, ~~yet not as consistent although~~ not consistently across sites (e.g., not in at Embu). ~~The postulated advantage of class 2 organic resources, such as Calliandra, relative to class 1 organic resources, such as Tithonia~~ (Kunlanit et al., 2014), was, however, not supported by our results. Kunlanit et al. (2014) hypothesized that a higher SOC formation of class 2 organic resources was related to the polyphenol and lignin content, increasing synchronisation between microbial demand and availability by preventing leaching of nutrients and

485 dissolved organic carbon. A potential reason for similar performance of *Calliandra* and *Tithonia* could thus be, that they only ~~differed in terms of polyphenol contents but not in lignin.~~

The question is, why farmyard manure, despite being similar to *Calliandra* in terms of C:N ratio and concentrations of aromatic compounds (Table 2), was better in avoiding SOC losses. It could be that farmyard manure, which constitutes an already

decomposed form of ~~biomass (e.g., about 30% of the original biomass intake by animals; Diekhoefer et al., 2021; Hossain, 2021)~~  
 490 ~~;~~ organic matter contains more stabilized forms of C that can ~~;~~ for example, directly attach to soil minerals (Angst et al., 2021).  
 Alternatively, it is possible that the the high amount of lignin in combination with high nutrient ~~concentration contents~~ (i.e., the  
 regulatory effects of N and aromatic components combined; Kunlanit et al., 2014) or the ~~higher P concentration in farmyard~~  
~~manure high P content~~ (the only ~~treatment organic resource~~ with a C:P ratio < 100) made it most effective in terms of SOC  
 formation. ~~In the first case, lignin but not polyphenols would be responsible for the regulatory effect, as lignin and polyphenols~~  
 495 ~~combined were similar in farmyard manure and Calliandra.~~ Xiao et al. (2021) found that ~~an the~~ increased microbial carbon  
 use efficiency of farmyard manure was not only due to a favorable carbon-to-nutrient ratio, but also because its application in-  
 creased soil pH. ~~Mtangadura et al. (2017) in another long-term ISFM trial, also~~ Similarly, Mtangadura et al. (2017) found that  
 farmyard manure application significantly increased soil pH and SOC. Such a positive effect of farmyard manure on soil pH  
~~compared to the initial measurements~~ was also found ~~in all four of our experiments at all four sites of our study~~ (Fig. C1), and  
 500 it is possible that the pH increase achieved by farmyard manure addition ~~further~~ alleviated constraints on carbon use efficiency  
~~introduced by due to~~ low pH (Malik et al., 2018).

#### 4.3 ~~Effect of~~ No coherent SOC response to mineral N fertilizer

~~Contrasting Contradicting~~ our second hypothesis, low-quality ~~saw dust sawdust~~ or maize stover did not show lower SOC losses  
 in the +N treatment than in the -N treatment (Fig. 4). The ~~impossibility to enhance a poor organic resource stoichiometry failure~~  
 505 ~~to enhance the performance of organic resources with low C:N ratios in building SOC~~ by amending mineral nutrients from  
 external sources ~~either indicates indicates either~~ that organic resource quality is determined by more than just ratios of nutri-  
 ents, or that it is difficult for microbes to counterbalance the poor quality of organic amendments by taking up nutrients in ~~the a~~  
 mineral form. It is furthermore surprising that no differences in SOC loss between the +N and -N treatments of the control were  
 found at any site. ~~For example, Ladha et al. (2011) showed~~ In contrast with our findings, Ladha et al. (2011) found through a  
 510 global analysis that SOC does on average increase when mineral fertilizer is added, ~~yet the effect from organic inputs was~~  
~~considerably stronger. A higher although to a lesser extent than when organic resources are added. Higher~~ maize productivity  
 for +N compared to -N treatments was found in about half of the treatments of ~~this experiment Laub et al., (in review), so~~  
~~the absence of ±N differences in SOC indicates that our experiments Laub et al. (2023), which may suggest that increased~~  
 aboveground biomass productivity ~~cannot automatically be assumed to translate into belowground does not automatically~~  
 515 ~~translate into increased belowground C~~ inputs. Plants may invest less into roots if they are supplied ~~too well well with~~  
nutrients from mineral fertilizer (Prescott et al., 2021). Additionally, ~~Silva-Sánchez et al. (2019), studying forest soils, found a~~  
N fertilizer may affect the microbial carbon use efficiency. For example, Silva-Sánchez et al. (2019) found reduced microbial  
 carbon ~~efficiency in treatments of mineral N addition. This reduced efficiency may be related to root exudate related priming~~  
~~(Kuznyakov et al., 2000), which could have been higher in the +N treatments. Also, while the treatments in our study received~~  
 520 ~~basal P and K, it cannot be ruled out that this unresponsiveness to mineral N addition in the control was due to a lack of other~~  
~~essential nutrients (see e.g. Mtangadura et al., 2017). In this case, higher N availability might lead to higher priming as plants~~  
~~of higher biomass try to obtain other missing nutrients.~~ use efficiency in forest soils that received mineral N fertilizer.

#### 4.4 Organic resource additions reduce SOC loss, but further agronomic measures are needed to increase SOC

While ~~in-at~~ Sidada and Aludeka, SOC losses could be avoided with high inputs of 4 t C ha<sup>-1</sup> yr<sup>-1</sup> of farmyard manure, such  
525 amounts are usually not ~~achievable in smallholder systems available on smallholder farms~~ in SSA (Rufino et al., 2006; Wawire  
et al., 2021). In fact, the limited availability of manure is one of the main factors creating the gradients of soil fertility and SOC  
on smallholder farms (Tittonell et al., 2013). The ~~net primary above-ground~~ biomass production in the study regions is around  
6 t C ha<sup>-1</sup> yr<sup>-1</sup> (Running et al., 2004), ~~so suggesting that~~ the high rates of 4 t C ha<sup>-1</sup> yr<sup>-1</sup> ~~as of~~ manure are exceeding by far what  
is available at the landscape scale (Vanlauwe and Giller, 2006). ~~For example, typical~~ Typical conversion rates of biomass C to  
530 manure C are around 30% (de Azevedo et al., 2021; Dickhoefer et al., 2021), ~~so even implying that e.g.,~~ a ha of grassland can  
maximally produce manure equivalent to 2 t C per year. Increased organic resource inputs at one location will therefore happen  
at the cost of losses at another location, effectively only redistributing SOC in the landscape (Wiesmeier et al., 2020) ~~and the~~.  
~~The~~ typical gradients of lower soil fertility with increasing distance ~~to homesteads (Vanlauwe et al., 2015; Kayani et al., 2021)~~  
~~already from the homesteads (Vanlauwe et al., 2015; Kayani et al., 2021)~~ indicate that this redistribution ~~is current reality has~~  
535 ~~been and continues to happen.~~

This effectively means that to increase SOC at landscape scale, further agronomic practices that increase primary pro-  
duction and biomass availability, such as intercropping, rotations with grasses or agroforestry practices (Corbeels et al.,  
2019; Tessema et al., 2020), are needed. ~~Additionally, the focus should be on direct plant inputs from the roots, which is~~  
~~considered the most efficient pathway of SOC buildup (Prescott et al., 2021; Sokol et al., 2019). Both roots and root exudates~~  
540 ~~are known to~~ Intercropping can produce more biomass than sole crops on the same surface due to complementary use of  
resources (i.e., light, nutrients, water; Malézieux et al., 2009; Bedoussac et al., 2015). Cereal-legume intercropping was shown  
to increase SOC in the long term compared to sole crops (Li et al., 2021b). Recent research (Prescott et al., 2021; Sokol et al., 2019)  
confirms plant C inputs through roots as most effective contributors to SOC increase (Denef and Six, 2006), because they form  
new SOC with higher efficiency than external above-ground organic resource inputs (Rasse et al., 2005; Jackson et al., 2017;  
545 Sokol and Bradford, 2019), ~~partly due to the fact that microbes can easily assimilate root exudates, which contributes to the~~  
~~formation and stabilization of microbial necromass (e.g. Wang et al., 2022), partly due to the proximity of inputs and microbes~~  
~~. The latter. The proximity of microbes to C inputs~~ has been highlighted as the most important factor (Lavalée et al., 2018),  
so focusing only on the quantity of C inputs and ignoring quality may be misleading, as demonstrated by the poor performance  
of adding 4 t C ha<sup>-1</sup> yr<sup>-1</sup> saw dust and maize straw in this study (Fig. 1).

550 ~~In the light of the results of our study, suitable measures should thus increase the input quantity and quality at the same~~  
~~time. For example, intercropping can produce more biomass than sole crops on the same surface due to complementary use of~~  
~~resources (e.g., light, nutrients, water, etc.; Malézieux et al., 2009; Bedoussac et al., 2015). Especially the high-quality inputs~~  
~~from cereal-legume intercropping were shown enhance SOC build up in the long term compared to sole crops (Li et al., 2021b)~~  
~~. If intercropping is not suitable, crop rotations with legumes (Cong et al., 2015) and for SOC formation (Lavalée et al., 2018)~~  
555 ~~. Therefore, the use of crop~~ genotypes with strong root systems ~~(e.g. Van de Broek et al., 2020), may be alternatives. Our data~~  
~~partly shows the importance of roots by the~~ is seen as a good option to build SOC (e.g. Van de Broek et al., 2020) The higher

SOC stocks in planted compared to bare plots at ~~both equivalent soil mass layers in~~ Aludeka and Sidada (Fig. 5) ~~–both of these suggest the importance of roots for building SOC; both sites had the highest maize biomass productivity of all four sites (data not shown, here; Laub et al., in review; Laub et al. (2023)).~~ However, the ~~contrasting results from Embu, with higher SOC stocks in bare compared to planted plots do not fully align with this. We cannot exclude that this was an artifact of the different and partially open soil auger we had to use in Embu for the 2021 sample – the other auger type repeatedly broke due to the very hard subsoil. In any case at Embu do not corroborate these findings.~~ Besides, the strong losses of SOC in the control treatments with and without mineral N fertilizer at all sites indicate that ~~despite the presence of root inputs, maize monocropping root inputs from maize monocropping systems cannot counterbalance SOC loss. This is in alignment with estimates of losses. It is estimated that root C inputs represent less than 1 t C ha<sup>-1</sup> and year of root inputs and rhizodeposits combined for a similar experiment yr<sup>-1</sup> in this type of cropping systems~~ (Cardinael et al., 2022).

#### 4.5 Site specificity in the formation of new SOC

~~Comparison of predicted vs measured SOC change at the end of different experiments without input of external organic matter and fertilizers assuming a 1.5% per year first order decomposition at 10°C mean annual temperature. The decomposition rate was scaled with an exponential temperature function with a Q<sub>10</sub> of 2 using the mean annual temperature of each site. The legend shows the different experiments (Srinivasarao et al., 2012; Karhu et al., 2012; Puttaso et al., 2013; Zha et al., 2015; Mtangadura et al., 2017) and their duration in years (yrs). Abbreviations: rRSM, relative root mean square error; NSE, Nash-Sutcliffe model efficiency.~~

~~An interesting pattern of site specificity emerged from our data.~~ The decrease in SOC concentration content in the control treatment without addition of organic resources was rather uniform among the sites, i.e., e.g., about 2% of initial SOC per year, with Machanga being ~~one exception as a result an exception, probably because~~ of erosion losses (-N treatment; 3%). ~~This seems not to be limited to our sites, because comparing our data with published data of SOC loss from no-input experiments around the globe; Fig. 4). Comparing our data with data from other experiments worldwide showed a fairly consistent trend rate of SOC loss across studies if temperature effects were accounted for (Fig. 6). In contrast to this rather uniform loss, the response of SOC concentrations the rather uniform rates of SOC loss, SOC responses to high-quality organic resource and farmyard manure additions were more site specific. This In our study this was indicated by large differences between sites in SOC formation in predominantly in the farmyard manure treatment, but also in the Tithonia and Calliandra treatments at 4 t C ha<sup>-1</sup> yr<sup>-1</sup> between sites (Fig. 4). Also, the CSE<sub>a</sub> of values were site specific for all organic resources was site-specific (i.e., significantly lower in at Machanga compared to the other sites, and in the case of farmyard manure, higher in at Sidada compared to the other sites, (Table 3). This cannot be due to texture alone These site differences can however not solely be attributed to soil texture differences: Aludeka and Machanga have a similar soil texture but a significantly higher CSE<sub>a</sub> was found for Calliandra and Tithonia in Aludeka compared to at Aludeka than at Machanga. Also, Sidada and Embu are similar in texture, but have similar soil textures, but the CSE<sub>a</sub> of farmyard manure was significantly higher in Sidada than in at Sidada than at Embu. Thus, we reject confirm our third hypothesis that organic resource quality is of higher soil properties are of similar importance for SOC formation than soil properties, as the difference between sites as organic resource quality; the differences in CSE<sub>a</sub> was between sites were as large as those between organic resources.~~

Possibly, there are differences in Possibly, differences in soil mineralogy between sites, which, under similar inputs, are the main explanatory factor for differences in SOC stocks (Zech et al., 1997; Reichenbach et al., 2021; Keller et al., 2022), next to texture (Mainka et al., 2022). For example, mafic parent material has shown a higher potential to store SOC in aggregates than felsic material (Reichenbach et al., 2021). As a consequence, it is unlikely that rainfall and temperature combined with texture  
595 are sufficient to explain differences in  $CSE_a$ , as put forward by some authors (Schimel et al., 1994; Smith and Waring, 2019). Even a temperature-dependent  $CSE_a$  (Frey et al., 2013) would not suffice to explain the higher  $CSE_a$  observed in Aludeka compared to Machanga (*Calliandra*, *Tithonia*) or in Sidada compared to Embu (farmyard manure): Sidada has higher temperatures than Embu and in Aludeka and Machanga temperatures are similar. Clearly, a better understanding of the interactions of play a  
600 be considered as effectively C saturated under the current maize monocropping (Castellano et al., 2015). Overall, there is a lack  
of understanding on how  $CSE_a$  is influenced by the interactions between organic resource quality, soil carbon saturation, climate and soil mineralogy are needed to effectively target soils that have the potential to sequester SOC. This calls for standardized  
manipulation experiments with a range of different quality inputs across a range of mineralogies and climates. Standardized  
605 dissimilar. Only an improved in tropical soils, and this calls for more research efforts. Only a better understanding of all factors influencing  $CSE_a$  can help to satisfy the local adaptation criterion of ISFM (Vanlauwe et al., 2015) so that smallholder  
farmers can inform local adaptation of ISFM practices (Vanlauwe et al., 2015) in order for smallholder farmers to apply organic  
resources and mineral fertilizer fertilizers where they are most effective.

## 5 Conclusions

610 This study showed that continuous maize cropping in sub-Saharan Africa without organic resource inputs are subject to a  
significant loss is subject to significant SOC losses of on average 2% per year of their initial SOC concentrations initial SOC  
contents (ca. 40% over 19 years). Site specificity existed: While the addition of 4 t C ha<sup>-1</sup> yr<sup>-1</sup> of high-quality organic resources  
farmyard manure, and to a certain degree Tithonia and Calliandra, could counteract the losses in all four study sites, a complete  
halt of SOC loss or even achieving achieving SOC gains, was only possible at two sites. Farmyard manure application was the  
615 most effective treatment, but. On the other hand, the addition of 4 t C ha<sup>-1</sup> yr<sup>-1</sup> of green manures from *Calliandra* and *Tithonia*  
still had a significantly lower SOC concentration losses than the control. With the exception of maize stover at one site reduced  
significantly SOC losses compared to the control treatment. In contrast, the application of low-quality organic resources such  
as saw dust sawdust and maize stover did not help to reduce SOC losses, even at rates of 4 t C ha<sup>-1</sup> yr<sup>-1</sup>. Mineral Similarly,  
mineral N application did also not help much to reduce not lower SOC losses, and it did not improve carbon nor improve SOC  
620 stabilization from low-quality organic resources. Differences between sites in the efficiency of high-quality organic resources  
to form new SOC were found to be stronger than differences in loss of SOC in the control, which showed While the extent  
of SOC losses in the control treatments was similar across sites, formation efficiency of new SOC from organic resources was  
found to differ between sites, highlighting the importance of soil properties in the effectiveness of ISFM practices. Despite

625 ~~site-specificity in the efficiency to from new SOC~~Overall, our results ~~showed clearly~~, clearly show that farmyard manure application was the most effective ~~treatment option~~ for SOC formation at all sites. ~~The application of farmyard manure, which is realistically only possible at rates around 1.2 t C ha<sup>-1</sup> yr<sup>-1</sup>, should thus be a priority~~ Farmyard manure is a key resource to maintain SOC stocks and soil fertility in tropical soils. In addition crops that increase carbon inputs through roots may be needed if a complete halt of SOC loss is the aim on smallholder farms in sub-Saharan Africa. However, the required quantities to maintain or build SOC are often not realistic for smallholder farmers. Additional agronomic interventions that enhance in-situ biomass production, such as intercropping, crop rotations or the cultivation of crops with extended root systems are therefore necessary to maintain or increase SOC in the tropical soils of smallholder farms.

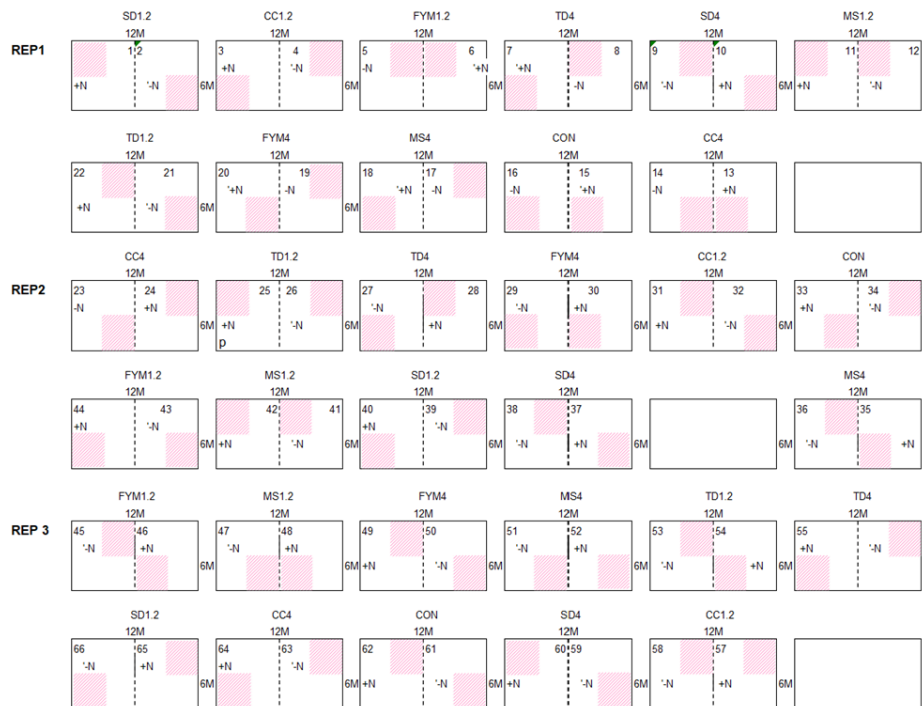
*Data availability.* The dataset used for this study, including SOC and N is made available under the IITA data repository <https://doi.org/10.25502/wdh5-6c13/d>

635 *Author contributions.* BV and JS designed the research. MWMM, DM, SMN and WW managed and maintained the long-term trials. ML, AC, SMN, MN, WW, MvdB and JS were involved in the various sampling campaigns. MC, MN, BV and JS acquired funding for the research. ML summarized the data, did the statistical analysis and prepared the original draft. All coauthors contributed in writing and editing of the final submitted article.

*Competing interests.* Johan Six is an executive editor of SOIL. All the other authors declare that they have no conflict of interest.

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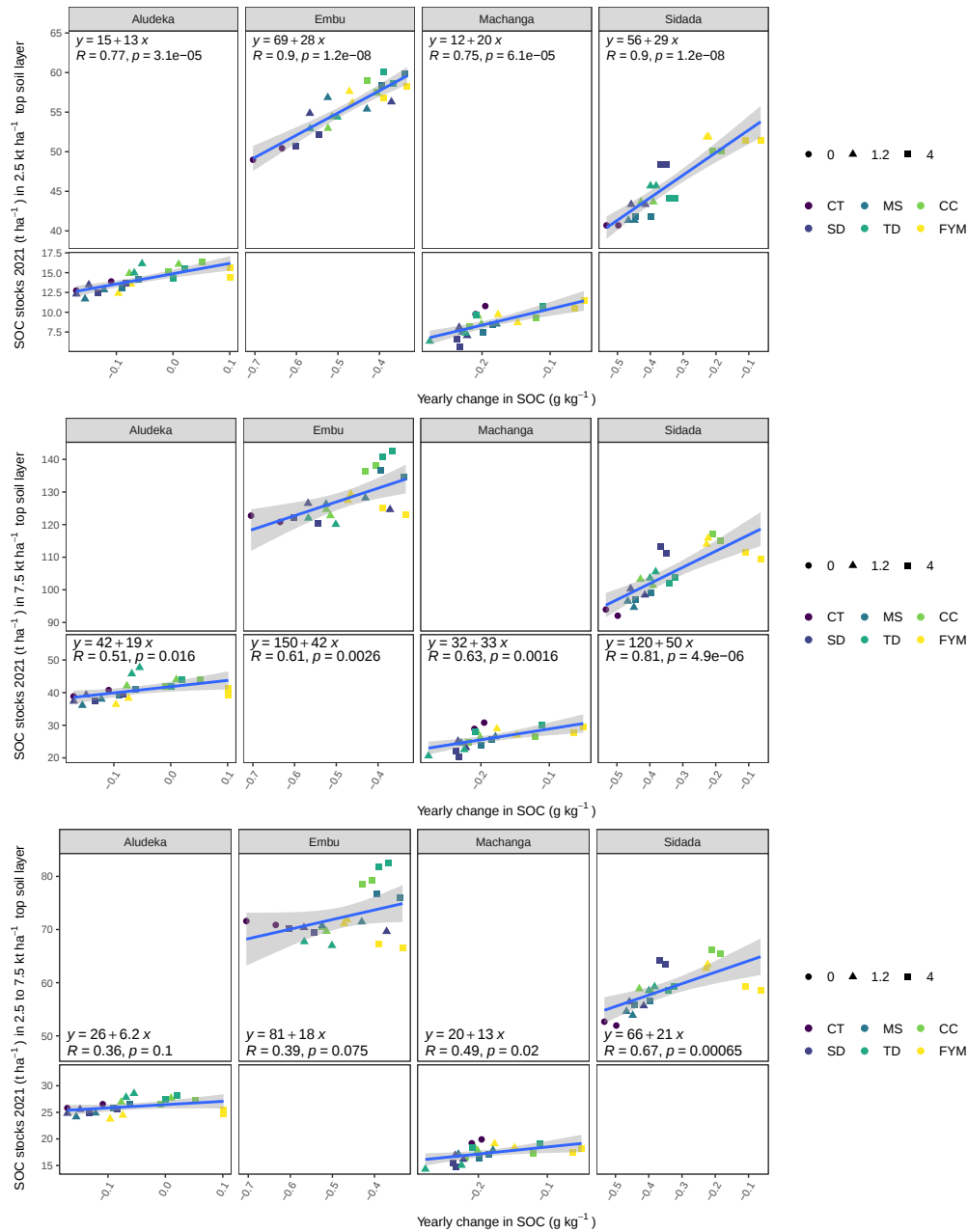
650 throughout many years.



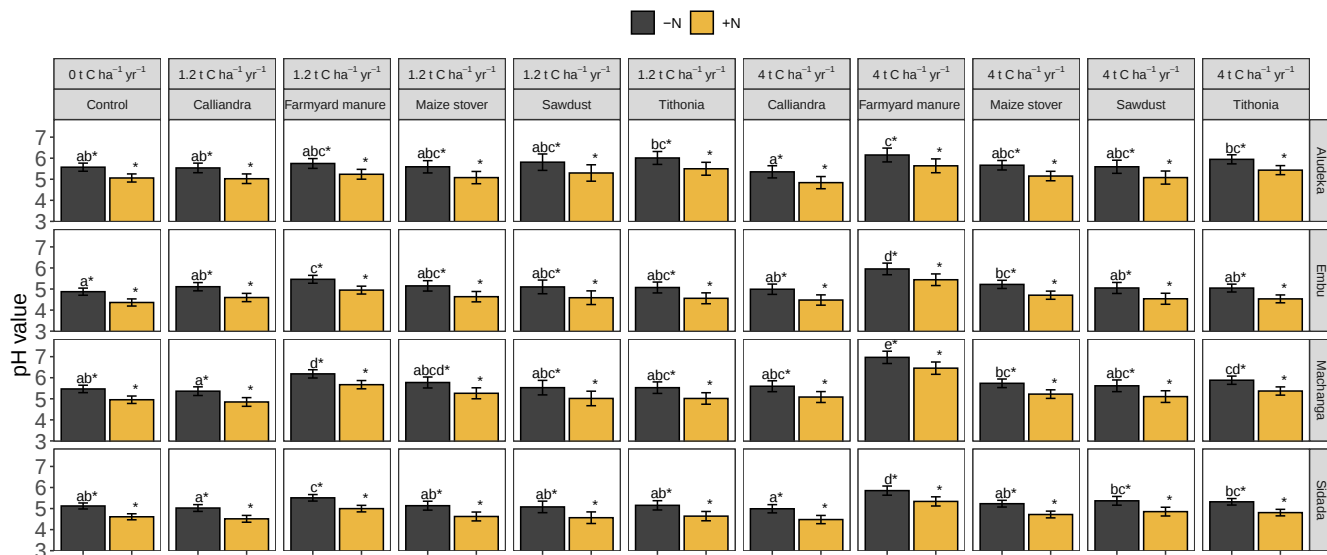
**Figure A1.** Correlation-Example of temporal trends the split plot design of SOC and TN the long-term trials: Aludeka. Displayed are Red areas indicate the site-specific least square means for both bare-fallow plot.

**Table A1.** Overview of the soil data that were available for this study.

| Sampling dates | Sites sampled                   | Properties samples | Depth         |
|----------------|---------------------------------|--------------------|---------------|
| 2002           | Embu, Machanga                  | SOC, BD            | 0-15 cm       |
| 2004           | Embu, Machanga                  | SOC, BD            | 0-15 cm       |
| 2005           | Embu, Machanga                  | SOC, BD            | 0-15 cm       |
| 2005           | Aludeka, Sidada                 | SOC                | 0-15 cm       |
| 2006           | Embu, Machanga                  | SOC                | 0-15 cm       |
| 2008           | Embu, Machanga                  | SOC                | 0-15 cm       |
| 2012           | Embu                            | SOC                | 0-15 cm       |
| 2013           | Embu, Machanga                  | SOC                | 0-15 cm       |
| 2015           | Embu, Machanga                  | SOC                | 0-15 cm       |
| 2018           | Embu, Machanga, Aludeka, Sidada | SOC                | 0-15 cm       |
| 2019           | Embu, Machanga, Aludeka, Sidada | SOC                | 0-15 cm       |
| 2021           | Embu, Machanga, Aludeka, Sidada | SOC, BD            | 0-15-30-50 cm |



**Figure B1.** Estimated least square means of the SOC stocks in the cumulative equivalent soil masses 0 to 2500, 0 to 7500 and 2500 to 7500 t ha<sup>-1</sup> soil mass (top, middle and bottom graph, respectively) plotted against the least square means for the temporal trend of SOC concentrations in the 0-15cm topsoil horizon



**Figure C1.** Estimated least square means for the topsoil pH value by site. Error bars display the 95% confidence intervals. Organic resource treatments from the same site which share no lowercase letter are significantly different from each other ( $p < 0.05$ ). The asterisk (\*), indicates that a similar significant difference existed between the +N and -N treatment for all organic resource treatments and at all sites (due to the lack of a significant interaction of  $\pm N$  with both organic resource treatments and site).

## B1 The effect of organic resources and mineral N on soil pH

Mineral N application had a significant negative effect on soil pH, which was consistent across sites, i.e., the +N treatments had a pH of 0.11 units lower than the -N treatments. In addition, there was a highly significant interaction of organic resource treatment with site. In all sites, soil pH values were highest in the FYM treatment at  $4 \text{ t C ha}^{-1} \text{ yr}^{-1}$ , whereas the lowest pH values were found in the control or CC treatments. Yet, the strength of the differentiation of FYM at  $4 \text{ t C ha}^{-1} \text{ yr}^{-1}$  compared to other treatments differed among sites. For example, the pH values in the FYM treatment at  $4 \text{ t C ha}^{-1} \text{ yr}^{-1}$  in Embu (5.95 in -N) and Machanga (6.96 in -N) were significantly higher than in all other treatments, in Sidada (5.85 in -N) it was higher than all, except in the FYM treatment at  $1.2 \text{ t C ha}^{-1} \text{ yr}^{-1}$ , while in Aludeka (6.15 in -N), it was only significantly higher than that in the control and CC treatments. While the pH values in SD and CC at both C input rates were not significantly different from that in the control in any site, TD at  $4 \text{ t C ha}^{-1} \text{ yr}^{-1}$  had a significantly higher pH value than the control in Machanga, and a significantly higher pH value than those in CC at  $4 \text{ t C ha}^{-1} \text{ yr}^{-1}$  in Aludeka and Sidada, as well as those in CC at  $1.2 \text{ t C ha}^{-1} \text{ yr}^{-1}$  in Machanga and Sidada.

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