

## Role of fertilization regime on soil carbon sequestration and crop yield in a maize-cowpea intercropping system on low fertility soils

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### ABSTRACT

Achieving food security through intensive agricultural practices on low fertility soils is challenging as crop productivity is increasingly curtailed by the loss of soil structural stability and rapid depletion of soil organic carbon (SOC). As such, the conversion from traditional mono-cropping to legume-cereal intercropping, especially with integrated fertilization, may increase crop yields with the least ecological footprint. We set up a 2-year field experiment in a split-plot design with cowpea-maize monoculture and intercropping under different organic-inorganic fertilization regimes, including no fertilization (control), organic input only (compost), chemical input only (NPK), and multi-nutrient enriched compost (NPKEC). We observed that intercropped maize had a significantly higher biomass yield compared to the corresponding monoculture when fertilized with NPKEC fertilizer. However, cowpea biomass yield differences between monoculture and intercropped plots were comparable under all fertilization regimes. In contrast, the grain yield advantage of both maize and cowpea was significantly enhanced under the intercropping system compared to monoculture, with NPKEC showing the most significant effect among all fertilization regimes. When comparing the relative contribution of the fertilization regime to SOC, the NPKEC fertilizer provided the highest SOC-sequestration (0.30 Mg C/ha yr<sup>-1</sup>). At the same time, the effect of the cropping system on C-sequestration showed that intercropping provided the highest C-sequestration (0.17 Mg C/ha yr<sup>-1</sup>) compared to monocultures of both crops. Although compost application significantly increased mineral associated (MAOC) and particulate associated organic carbon (PAOC) concentrations compared to unfertilized control plots, NPKEC fertilization with intercropping system was the most effective combination causing the greatest increase of both soil C pools over time. Based on redundancy analysis (RDA), the positive association of MAOC and PAOC with C-sequestration suggests the importance of both organic fractions as primary C reservoirs conducting SOC storage. Importantly, although compost alone in association with intercropping had a lower C-sequestration, it was associated to a better soil structure as confirmed by its positive relationship with macro-and micro-aggregation, water stable aggregates (WSA), and mean weight diameter (MDA). Overall, our results indicate the importance of restoring soil structure in degraded soils through appropriate land management solutions, such as stoichiometrically balanced fertilization practices (NPKEC) and crop diversification (intercropping), in order to achieve significant gains in SOC storage and, ultimately, improve crop productivity.

**Abbreviations:** SOC, Soil organic carbon; NPK, Chemical input; NPKEC, Multi nutrient enriched compost; MAOC, Mineral associated organic carbon; PAOC, Particulate associated organic carbon; RDA, Redundancy analysis; WSA, Water stable aggregates; MM, Maize monoculture; CC, Cowpea monoculture; MC, Maize-cowpea intercropping; SSP, Single super phosphate; BD, Bulk density; MWD, Mean weight diameter; Mic\_agg\_F, Micro aggregate fraction; Mac\_agg\_F, Macro aggregate fraction; SOC\_S, Soil organic carbon stocks; C-seq, Carbon sequestration.

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## 1. Introduction

Globally, the agricultural system is facing a sustainability crisis to meet the rising food demand of 9 billion people by 2050 (van Dijk et al., 2021). From now on, crop yields will need to increase by more than 70 % to address this looming food security challenge (Hunter et al., 2017; Putelat et al., 2021). Indeed, agricultural intensification (i.e., mono-cropping and excessive chemical inputs) can lead to land degradation, a decline in freshwater resources, and frequent disease outbreaks, resulting in unstable crop yields (Jang et al., 2021). For this reason, the application of sustainable agricultural practices can serve as an effective tool for sustainable land management while ensuring higher crop productivity on regular basis.

Soil organic carbon (SOC) is of paramount importance for achieving and maintaining high crop productivity (Bünemann et al., 2018), as SOC is central to the maintenance of several physical, chemical, and biological functions of soils (Page et al., 2020). Indeed, rapid depletion of SOC could result in poor crop productivity due to its close association with soil fertility (Yadav et al., 2018; Sharma et al., 2021). As such, increasing and/or maintaining SOC content in agricultural soils should be a top priority, as this can greatly contribute to achieving several important sustainable development goals, including resilience to climate change and food security (Rumpel et al., 2020). Soil can be an important sink of atmospheric CO<sub>2</sub>; however, the potential of agricultural soils to store more C, better known as C sequestration, depends primarily on farming practices, nutrient inputs, and climatic conditions (Waqas et al., 2020; Gross and Glaser, 2021). So far, the use of unsustainable agricultural practices has led to significant depletion of SOC (25–75 %) worldwide (Lal, 2013), which would otherwise have contributed to soil fertility and productivity (Liang et al., 2021) and positively impacted various ecosystem services (Bossio et al., 2020). Globally, arable soils can accumulate 0.25–1.0 Mg C/ha yr<sup>-1</sup> (Lal, 2018), if appropriate land use management practices are adopted (Alam et al., 2019).

Soil structural stability is a crucial indicator of soil quality due to its significant role in water and nutrient retention as well as in C storage (Chen et al., 2021). In this context, soil aggregation is one of the major components of soil structure as it provides SOC physical protection from decomposition (Ozlu and Arriaga, 2021). According to the traditional model of aggregate formation, the provision of C substrate ensures adhesive strength for silt and clay particles that form micro-aggregates (Zhou et al., 2016), whereas the formation of macroaggregates from microaggregates is primarily facilitated by various metabolites of plant and microbial origin (Baumert et al., 2018). In recent years, intensive mono-cropping coupled with mechanical interventions has led to the deterioration of soil structure, which could reduce the stability of aggregates, resulting in poor soil fertility and restricting the normal growth of crops (Zhang et al., 2020).

In semi-arid regions, frequent soil structural disturbances and rapid SOC depletion combined with poor fertility have led to widespread degradation of agricultural land (Práválie et al., 2021). In most cases, the use of mineral fertilizers is the best strategy to improve nutrient availability in these low fertility soils, although this is often at the expense of low nutrient use efficiency by crops and soil structure degradation, further leading to poor soil quality (Buvaneshwari et al., 2020; Xu et al., 2020). In addition, the irregular distribution of rainfall and frequent droughts pose another major challenge by exacerbating the impact of declining SOC content on crop productivity. Indeed, it is important to note that the type of fertilizer and cropping systems have been shown to influence both the degree of soil aggregation and the fate of C stability in agroecosystems (Wan et al., 2020; Jin et al., 2021). Hence, it is imperative to find a suitable crop rotation as well as an effective fertilization regime that favor higher aggregate stability, SOC accrual, and better nutrient supply, which ultimately has a positive impact on crop yields.

The quest for higher crop yields and better soil quality brings

intercropping, i.e., the simultaneous cultivation of two or more crops in a mixed sequence, back into contention in order to improve food supplies, human health, and environmental quality (Tilman, 2020). On average, intercropping can produce about 15–25 % more food on a given surface of cropland area compared to mono-cropping (Vandermeer, 2011). Among the major cereals, maize (*Zea mays* L.) is the most commonly intercropped staple food crop in the world, especially in cropping systems with limited external inputs and extensive land fragmentation (Lopez-Ridaura et al., 2021). Integrating legumes with cereals as intercrops can be an important component of ecological intensification to promote sustainable agricultural development, as their interspecific facilitation leads to better resource utilization and higher yield stability than monocropping (Xu et al., 2022; Zhang et al., 2022). Grain legumes are the most popular intercrops in the Indus-Ganges region, mainly to ensure food security and provide a better source of income for smallholder farmers. Cowpea (*Vigna unguiculata* L.), also known as an orphan legume, is one of the most neglected minor crops in global cropping systems due to its limited use and supply constraints (Cullis and Kunert, 2017). Despite its enormous potential as a multi-purpose crop to meet dietary protein and livestock feed needs, it has so far received little attention from researchers and industry. Above all, cowpea is better suited to nutrient-poor soils and thrives well under harsh climatic conditions than most legume crops (Horn and Shimelis, 2020; Kumar and Bhalothia, 2020). Indeed, this supports the fact that cowpea can be a vital component of legume-cereal intercropping in semi-arid regions, where achieving higher crop productivity with soil conservation measures is key to future food security scenarios.

Nevertheless, such production gains in an intercropping system may be limited if intensive fertilization and agronomic practices continue (Brooker et al., 2015). Therefore, the choice of fertilization must also be carefully considered, especially in semi-arid regions, where low organic matter together with low fertility is one of the main obstacles to optimal crop production. In this context, the integration of organic–inorganic material, such as compost with mineral fertilizers, can be an effective means not only for improving the availability of yield-limiting nutrients, but also for using a value-added amendment to improve soil structure and C storage. Previous studies have found a positive effect of organic fertilization on soil structure stability and SOC content, suggesting its importance in improving SOC stocks and stability while neutralizing both the land degradation and CO<sub>2</sub> emissions challenges (Zhang et al., 2020; Liu et al., 2021). However, the impact of different organic–inorganic fertilization practices on soil C pools, aggregate stability, SOC sequestration, and crop yield in intercropping systems is yet to be fully clarified.

To this goal, we conducted a short-term (2-year) intercropping trial of a cowpea-maize combination under different compost-based, organic–inorganic fertilization practices in order to replace historical monocultures in a degraded semi-arid agroecosystem. Our main objectives were:

- 1) To assess the impact of different fertilization regimes in combination with cropping system on crop yield, SOC-sequestration and SOC pool distribution (i.e., PAOC and MAOC) on a degraded semi-arid soil
- 2) To disentangle the relevance of improved soil structural stability and SOC sequestration for higher crop productivity under different fertilization regimes and cropping systems

## 2. Materials and methods

### 2.1. Study site

A two-year field experiment with farmers' participation was conducted in the Faisalabad district (31°33' N, 73° 07' E) in the Punjab province of Pakistan during the 2015–16 and 2016–17 cropping seasons. The study site, a sandy clay loam soil (Haplic Cambisol), has been traditionally used for intensive mono-cropping (i.e., wheat-maize) over

the last two decades (IUSS, 2014). Soil texture and particle size distribution were determined using the Bouyoucos hydrometer method (Bouyoucos,1962). Soil pH and EC were analyzed using a soil-to-water ratio of 1:5 (w/v) with a portable combination meter (HI9813-5, Hanna, Germany). Total soil N content was determined by the Kjeldahl digestion and distillation method (Bremner and Tabatabai,1972). Extractable NO<sub>3</sub>-N and NH<sub>4</sub><sup>+</sup>-N were extracted with 2 M KCl and analyzed colorimetrically on microplates using the vanadium chloride method (NO<sub>3</sub>) and the salicylate nitroprusside method (NH<sub>4</sub><sup>+</sup>) as described by Mulvaney et al. (1996). Total phosphorus (P) in soil was determined by the molybdenum-antimony blue method, whereas available P concentration (i.e., NaHCO<sub>3</sub>-P and H<sub>2</sub>O-P) was determined sequentially by the modified method of Sui et al.1999. Soil total and available potassium (K) (i.e., ammonium acetate extracted-K and water extractable-K) concentrations were determined following the method adopted by He et al. 2016. Overall, soil of the study site contains an inherently low organic matter content and is characterized by poor fertility (Supplementary Table 1).

The mean annual temperature varies between the highest 50 °C in summer (August) and the lowest 5 °C in winter (January). Summers are scorching hot and humid, determined mainly by the prevailing monsoon, while winters are predominantly cold and dry. An irregular annual precipitation pattern characterizes the experimental site, ranging from the highest (175 mm) in summer to the lowest (93 mm) in winter, with the monsoon seasons (July-September) making the most significant contribution to the total annual rainfall (average: 138 mm).

## 2.2. Compost, fertilization and experimental design

A composite nutrient (i.e., NPK) enriched compost was prepared from collected mixed food wastes as described by Roohi et al. (2020). Briefly, compostable feedstock was ground to obtain a uniform particle size (2 mm) after initial air and oven drying. Sawdust (1:10) was added as a bulking agent for optimum moisture conditions and microbial proliferation. Composting was performed over a period of 9 weeks in an on-site fabricated reactor system at a flow rate of 30 L min<sup>-1</sup> with repeated agitation at 15 min h<sup>-1</sup>. An automated temperature sensor was used to record the average temperature twice a day during the mesophilic and thermophilic phases of composting. For fertilizer enrichment in the maize plots, NPK was added at the rate of 350–200–200 g kg<sup>-1</sup> compost, whereas the cowpea compost was supplemented with NPK at the rate of 5–12–12 g kg<sup>-1</sup> compost. In the present study, compost was applied at a rate of 500 kg ha<sup>-1</sup> to compensate for the experimental soil's low organic matter content and poor fertility status. Both the physical and chemical properties of compost products were assayed as described previously by Gómez-Muñoz et al. 2017, whereas lignocellulosic composition of compost was determined according to methods adopted by Sluiter et al. 2010. Different physico-chemical characteristics of raw and NPK enriched compost are presented in Supplementary Table 2.

The experimental design consisted of three cropping systems, i.e., maize monoculture (MM), cowpea monoculture (CC), and maize-cowpea intercropping (MC), and four fertilization regimes, i.e., no fertilizer (control), synthetic mineral fertilizer (NPK), organic fertilizer

**Table 1**

Multiple analysis of variance (MANOVA) for crop yield, soil physical stability, C-sequestration and associated pools affected by cropping system (MM: maize monoculture, CC: cowpea monoculture, MC: maize intercropped with cowpea) and fertilization regimes (Control: no fertilization, Compost: organic fertilization, NPK: mineral nitrogen, phosphorus, potassium fertilization, NPKEC: multi-nutrient enriched compost fertilization).

Nutrient System	Compost		Control		NPK		NPKEC					
	MC	CC	MM	MC	CC	MM	MC	MM				
PAOC	3.36 ± 0.05	3.08 ± 0.05	3.17 ± 0.1	2.17 ± 0.07	1.69 ± 0.05	1.87 ± 0.05	2.23 ± 0.08	1.73 ± 0.03	1.9 ± 0.05	4.89 ± 0.14	4.09 ± 0.07	4.1 ± 0.06
MAOC	4.78 ± 0.32	4.0 ± 0.16	4.16 ± 0.13	2.5 ± 0.12	2.19 ± 0.07	2.23 ± 0.09	2.55 ± 0.13	2.12 ± 0.08	2.27 ± 0.11	6.01 ± 0.05	5.06 ± 0.22	5.22 ± 0.32
SOC	9.9 ± 0.30	8.8 ± 0.2	9.1 ± 0.1	6.4 ± 0.2	5.6 ± 0.20	5.8 ± 0.1	6.5 ± 0.2	5.5 ± 0.10	5.9 ± 0.1	12.5 ± 0.1	10.7 ± 0.3	10.8 ± 0.4
SOC_S	2.2 ± 0.08	1.98 ± 0.05	2.03 ± 0.04	1.45 ± 0.03	1.28 ± 0.03	1.32 ± 0.01	1.47 ± 0.03	1.27 ± 0.02	1.33 ± 0.03	2.72 ± 0.04	2.36 ± 0.07	2.39 ± 0.07
C.Seq	0.46 ± 0.04	0.35 ± 0.02	0.37 ± 0.02	0.08 ± 0.01	0.01 ± 0.0	0.02 ± 0.01	0.09 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.72 ± 0.02	0.54 ± 0.04	0.55 ± 0.03
CN	18.1 ± 0.3	17.6 ± 0.4	17.6 ± 0.3	16.1 ± 0.7	15 ± 0.50	15 ± 1.4	16.4 ± 0.3	15.1 ± 0.5	15.2 ± 0.7	19.3 ± 0.6	17.7 ± 0.4	17.9 ± 0.6
BD	1.48 ± 0.02	1.5 ± 0.02	1.5 ± 0.01	1.51 ± 0.02	1.53 ± 0.02	1.52 ± 0.02	1.51 ± 0.02	1.53 ± 0.02	1.52 ± 0.01	1.45 ± 0.01	1.48 ± 0.01	1.47 ± 0.01
Mac_agg_F	46.7 ± 1.4	38 ± 1.1	41.2 ± 1.2	37.1 ± 1.0	33 ± 0.9	34.9 ± 1.3	37.2 ± 1.1	34.3 ± 1.0	36.5 ± 1.1	49.5 ± 1.5	39.3 ± 1.2	42.5 ± 1.2
Mic_agg_F	30.3 ± 1.5	24.8 ± 1.2	26.6 ± 1.3	23.4 ± 1.2	21.1 ± 1	22.7 ± 1.1	23.6 ± 1.2	21.6 ± 1.1	23.3 ± 1.1	29.6 ± 1.4	26.3 ± 1.3	26.9 ± 1.3
WSA	39.6 ± 1.5	33.8 ± 1.1	35.1 ± 1.4	25.5 ± 1.0	23.2 ± 1.1	24.1 ± 1.1	26 ± 1.0	23.5 ± 1.0	25 ± 0.9	43.2 ± 1.3	36.8 ± 1.5	38.1 ± 1.2
MWD	0.73 ± 0.02	0.68 ± 0.02	0.71 ± 0.02	0.49 ± 0.02	0.41 ± 0.01	0.44 ± 0.01	0.5 ± 0.02	0.41 ± 0.01	0.46 ± 0.01	0.77 ± 0.02	0.7 ± 0.02	0.72 ± 0.02
	Data transf.	Test	Nutrient	System	Interaction	MC	CC	MM	compost	control	NPK	NPKEC
PAOC		ANOVA	***	***	**	b	a	a	b	a	a	c
MAOC	log	ANOVA	***	***	ns	b	a	a	b	a	a	c
SOC	log	ANOVA	***	***	ns	b	a	a	b	a	a	c
SOC_S	log	ANOVA	***	***	ns	b	a	a	b	a	a	c
C.Seq		Perm Test	***	ns	ns				b	a	a	c
CN		KW	***	ns	ns				b	a	a	b
BD		ANOVA	***	ns	ns				ab	b	b	a
Mac_agg_F		ANOVA	***	***	ns	c	a	b	b	a	a	b
Mic_agg_F		ANOVA	***	**	ns	b	a	ab	b	a	a	b
WSA		ANOVA	***	***	ns	b	a	a	b	a	a	c
MWD	log	ANOVA	***	***	ns	c	a	b	b	a	a	b

Particulate-associated organic C (PAOC); Mineral-associated organic C (MAOC); Soil organic carbon (SOC); Soil organic carbon stocks (SOC\_S); C storage rate (C.Seq); C:N ratio (CN); Bulk density (BD); micro- (Mic\_agg\_F) and macro- (Mac\_agg\_F) aggregate fraction; Water stable aggregates (WSA); Mean weight diameter (MWD).

(compost), and NPK enriched compost (NPKEC), that were laid out in a split plot design and replicated four times. Cropping systems were established on main plots (22 × 6 m), while subplots (6 × 5 m) received selected organic–inorganic fertilization regimes. Maize (*Zea mays* L. cv. Pioneer P1543) and cowpea (*Vigna unguiculata* L. cv. White Star) were sown on the elevation ridges after soil preparation with a disk plow. In the intercropping system, two rows of maize followed by two rows of cowpea were established for a total of 8 intercropped rows. An inter-plant distance of 20 cm with a row spacing of 70 cm was uniformly maintained in both crop systems. All experimental plots were laid out in an East-to-West orientation to ensure uniform sunlight distribution for each cropping system (Fig. 1).

All NPK fertilization was done with urea (N), single superphosphate (SSP), and potassium sulfate (K). Due to the lower fertilizer requirement of legumes, cowpea received 25–60–60 kg ha<sup>-1</sup> of NPK fertilizer, while maize was fertilized with 175–100–100 kg ha<sup>-1</sup> of NPK. In the NPK treatment, all P and K fertilizers and half of the recommended N fertilizer were uniformly applied and plowed into the soil before sowing the maize. Moreover, the other half of the N fertilizer was used in two parts at stem elongation and then at flowering stage. On the other hand, cowpea received all the designated NPK as basal fertilizer before sowing. In the case of composite NPKEC, all the recommended fertilization was applied at the time of seeding because the nutrients enriched with compost are generally released slowly into the soil. The total amounts of nutrients applied in each treatment are shown in Supplementary Table 3. During the growth period, weed emergence was routinely monitored and mechanically controlled by hand sorting before scheduled irrigation. Every week, canal water was supplied as the primary source of irrigation for both mono and intercrop experimental plots. A

flood irrigation method was employed across all experimental plots. At the head of the ridge, a cut-throat flume (90 cm × 20 cm) was installed to apply a measured amount of irrigation water to each cropping system, as described by Skogerboe et al. (1993). To avoid water loss, the first two rows, either in monoculture or in the intercropping system, were irrigated at once, while the water outlets of the other rows remained closed (Fig. 2). Water requirement for the selected cropping systems was calculated based on soil moisture depletion in the root zone one day before the scheduled irrigation as described by Abyaneh et al (2017). The flow rate of irrigation water from the installed cut-throat flume (diameter = 10 in.) into the experimental plot was calculated to be 0.062 ± 0.004 m<sup>3</sup>/sec. The average irrigation application efficiency was determined to be 63 ± 3.91 % and 65 ± 3.65 % for 2016 and 2017, respectively. For the maize crop, the total delta of water was 7403.47 m<sup>3</sup> ha<sup>-3</sup> in 2016 and 7982.34 m<sup>3</sup> ha<sup>-3</sup> in 2017, including pre-soaking irrigation. On the other hand, the entire delta of water for cowpea was 6428.52 and 6641.80 m<sup>3</sup> ha<sup>-3</sup>, including pre-soaking irrigation during 2016 and 2017, respectively.

### 2.3. Crop productivity data collection

At the time of physiological maturity, i.e., during the first week of August each year, three adjacent rows of maize and cowpea were selected for monocrop and intercrop harvesting. In both systems, the first guard row on each side of the plot was excluded for data collection to avoid the edge effects of the treatment plot. All harvested samples were air-dried for two weeks and manually separated by threshing to calculate crop yield. The productivity of each cropping system was determined by summing plant biomass and grain yield during a cropping

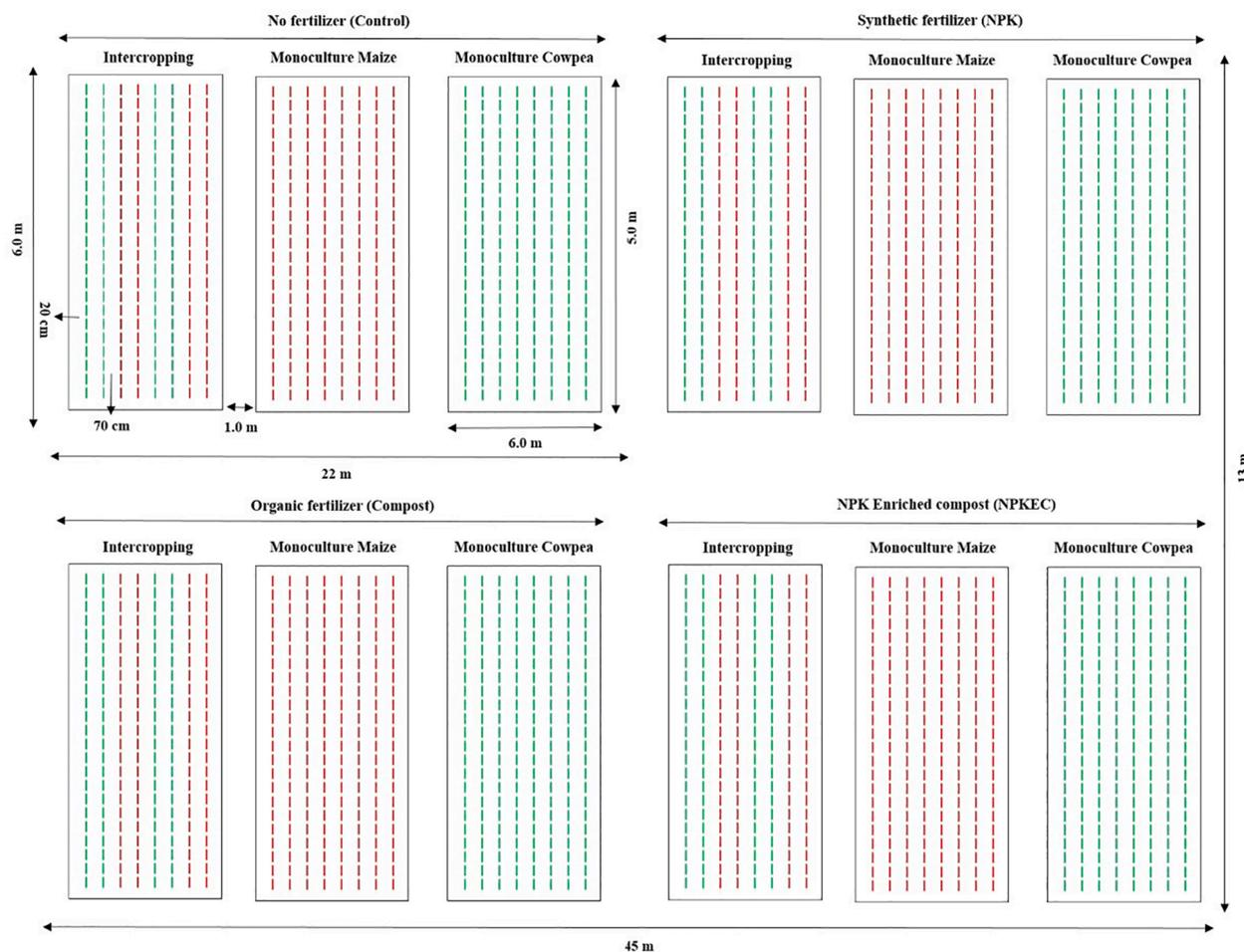


Fig. 1. Schematic representation of the layout of the experiment involving different fertilization regimes and cropping systems.

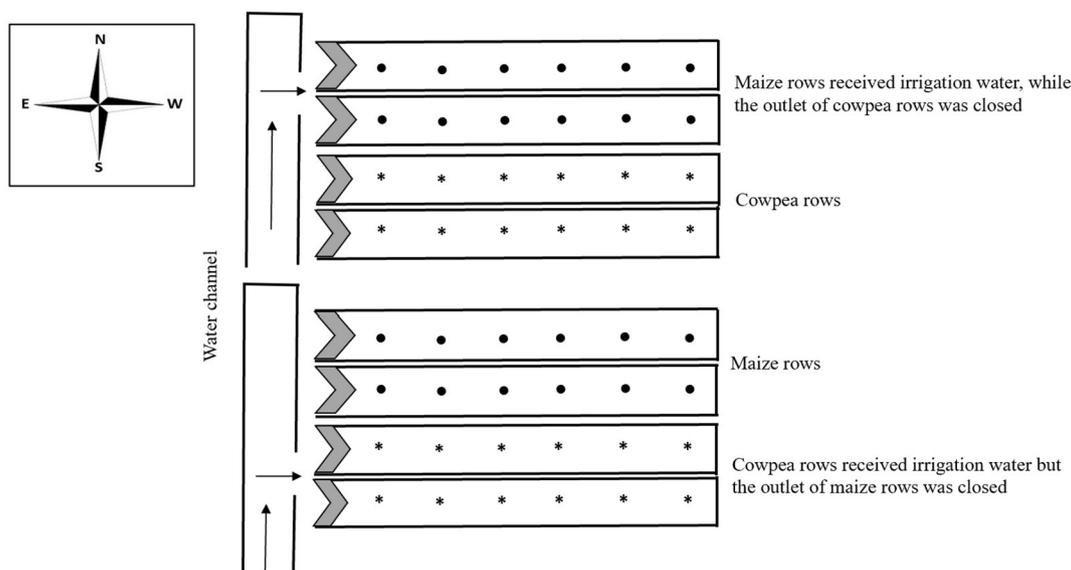


Fig. 2. Illustration of irrigation scheme for cowpea and maize based monoculture and intercropping system.

year and expressed as  $\text{Mg/ha yr}^{-1}$  at standard plant moisture. At the time of yield calculation, average moisture content of maize and cowpea samples was  $8.5 \pm 0.47\%$  and  $6.4 \pm 0.36\%$ , respectively.

#### 2.4. Soil sampling

At the end of the experiment, soil samples were collected from each treatment as well as from the control plot. In each plot, soil samples from 0 to 20 cm depth (4 random cores per plot) were taken with an Eijkelkamp auger and then pooled to obtain a representative composite sample sufficient for the targeted analysis. On the same day, collected samples were rifled through to pick out any visible coarse material and root segments before being passed through a 2-mm screen. After sieving, the samples were placed in labelled zip-lock plastic bags and immediately taken to the laboratory.

#### 2.5. Soil structural stability indicators

Whole soil bulk density (BD) was determined on undisturbed soil samples using a standard core sampler (Schipper and Sparling, 2000). For estimation of BD, ratio of soil mass to core volume was determined after oven drying at  $105^\circ\text{C}$  for constant weight. The wet sieving method was used for physical fractionation of the different soil aggregate sizes (Six et al., 1998). Briefly, a 200 g subsample of air-dried soil was placed on a nest with two sieves representing macro aggregates (greater than  $250\ \mu\text{m}$ ) and micro aggregates (greater than  $53\ \mu\text{m}$ ). Both sieves were then slowly immersed in 5 mL of deionized water for 7-mins and gently shaken vertically by hand until a cycle of 50 repetitions was achieved in 2 min. After sieving, the aggregate fractions remaining on each sieve were precipitated with 1 M  $\text{CaCl}_2$  solution for 24 h, while the sieved material ( $<53\ \mu\text{m}$ ) was considered as finer silt/clay fractions. After separation of aggregates, the samples were dried at  $60^\circ\text{C}$  and weighed according to aggregate size classes, mean weight diameter (MWD), and water stable aggregates (WSA) as described by Zhang et al. (2014).

#### 2.6. Soil carbon pools and sequestration

Soil organic carbon (SOC) was determined following the chromic acid wet-oxidation method of Walkley and Black (1934). We used a fixed depth approach for the estimation of SOC stock (SOCs,  $\text{Mg C/ha}$ ) by summing up the SOC content of the top-soil layer (0–20 cm) using the following equation (Qiu et al., 2015).

$$\text{SOCs} = \frac{CC_t \times SD \times BD}{100} \quad (1)$$

where:  $CC_t$  is the carbon content of top layer ( $\text{g kg}^{-1}$  soil),  $SD$  is the soil depth (cm), and  $BD_t$  is bulk density of top layer soil ( $\text{Mg/m}^{-3}$ ).

The C sequestration rate ( $\text{Mg C/ha yr}^{-1}$ ) under different fertilization regime as well as cropping systems was calculated as indicated in equation (2):

$$\text{C sequestration rate} = \frac{C_f - C_b}{t} \quad (2)$$

where  $C_f$  and  $C_b$  are SOC ( $\text{Mg C/ha}$ ) at the end and at the beginning of the experiment, respectively, and  $t$  is year of the experimentation.

For functionally distinct SOC fractions, mineral and particulate associated organic carbon (MAOC and PAOC, respectively) were separated based on the modified wet-sieving method of Marriott and Wander (2006). Briefly, 20 g of air-dried soil was homogenized with 50 mL of 5% sodium hexametaphosphate solution. To ramp up the dispersion process, the soil mixture was transferred to a horizontal shaker at 180 rpm for 15 h. Afterwards, the dispersed soil in the form of slurry was screened using a  $53\ \mu\text{m}$  sieve and rinsed with a continuous and steady jet of deionized water. Both sieve retained (PAOC) and passing down (MAOC) fractions were oven dried at  $60^\circ\text{C}$  for 24 h, weighed separately, and assayed for their respective SOC fractions.

#### 2.7. Statistical analysis

All statistical analyses were performed using R software 3.6.1 (R Core Team, 2020). The effects of fertilization regime and cropping system on soil and yield data, as well as the effect of years on yield data were assessed by ANOVA (function *aov*), and results are presented in boxplots (function *boxplot*). Interactions between factors were removed from the model if they were not significant. ANOVA model assumptions were tested by the Shapiro and Bartlett tests, and data were log-transformed if necessary. For C:N ratio, data were not normally distributed even after log-transformation, and treatment effects were tested separately using Dunn tests (function *dunnTest* in FSA package). Because of the consistency of the year effect across treatments, biomass and grain data in Fig. 3 and in the text are presented as the average of both years even though year was kept in the models. A redundancy analysis (RDA) was performed with the *rda* function to address the relationship between soil structural stability and SOC pool variables

constrained by cropping system and fertilization regime factors. Significance of axes and constraining factors was assessed by Monte Carlo permutation test (1000 permutations) using the *anova* function. Variation partitioning between treatments as performed with the *varpart* function. If not specified, all discussed differences are significant, at least at  $p$ -value < 0.05, and values are presented as mean  $\pm$  standard error (SE).

### 3. Results

#### 3.1. Effects of fertilization regime and cropping system on crop productivity

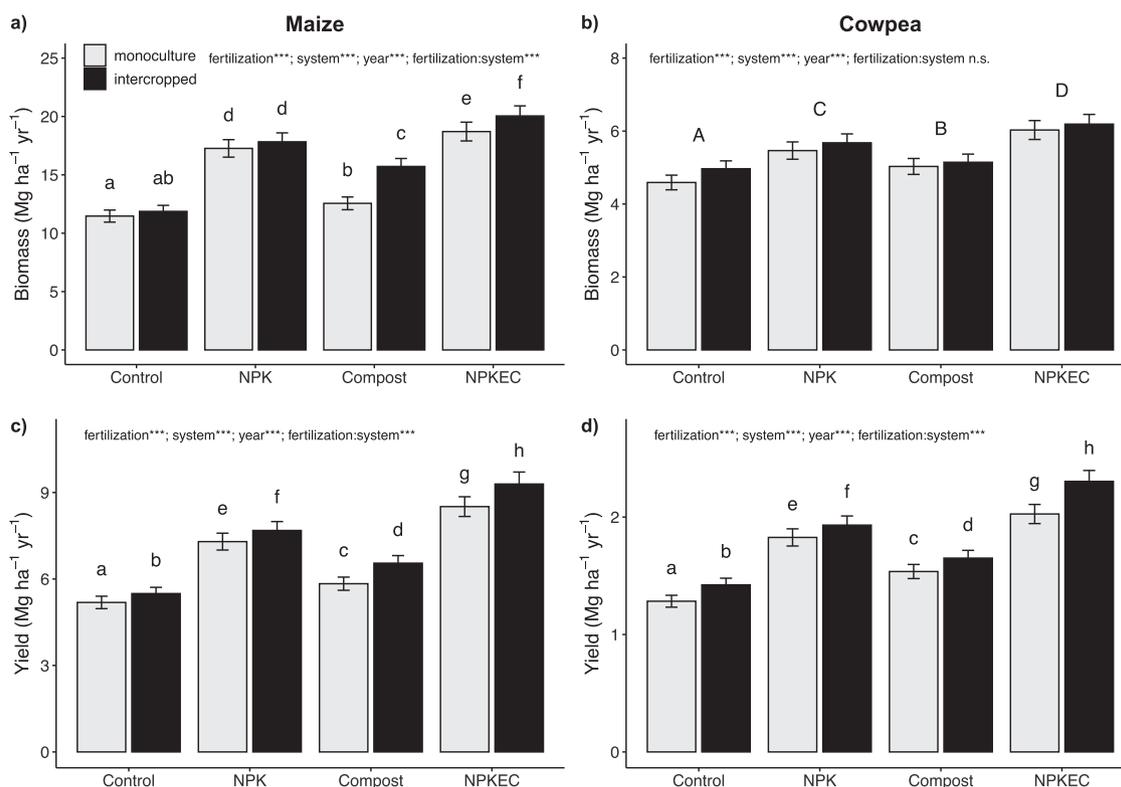
In 2017, the average biomass ( $16.1 \text{ Mg ha}^{-1}$ ) and grain yield ( $7.2 \text{ Mg ha}^{-1}$ ) of maize across all treatments were 6 and 7 % higher than in 2016. The difference was similar for cowpea biomass ( $5.6 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) and grain yield ( $1.8 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ). The effect of years was constant for all treatments, as indicated by the absence of interactions with the fertilization regime and with the cropping system (Fig. 3).

For maize, biomass yield was comparable in monoculture and intercropped plots receiving NPK fertilizer (Fig. 3a); however, relative yield differences were significant compared to the control (Fig. 3c). Compost application significantly reduced biomass yield across both cropping systems compared to NPK fertilizer, although yield recovery in intercropped plots was significantly higher than in monoculture plots. Importantly, NPKEC application improved biomass yield in monoculture plots, while yield increase was further expanded in intercropped plots ( $20.1 \pm 0.4 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ), producing the highest biomass yield regardless of fertilization regime or cropping system.

For cowpea, biomass yield in intercropped plots was, on average,  $4.3 \pm 0.3 \%$  higher than in monoculture plots, and this difference did not depend on the fertilization regime (Fig. 3b). The NPK fertilizer always had a significantly higher biomass yield than the control. Moreover, in response to compost application, a significant decline in biomass yield was observed compared to NPK fertilizer. Overall, NPKEC application accounted for the highest increase in biomass yield when compared to all the other fertilization regimes.

In intercropped maize, grain yield was significantly higher regardless of fertilization regime compared to monoculture (Fig. 3c). When comparing the effects of different fertilizers, maize grain yield was significantly higher in plots that were fertilized with NPK than in those that received no fertilizer; however, compost alone yielded less than NPK fertilizer. Overall, NPKEC application had the most significant influence on grain yield, increasing grain yield by  $64 \pm 4 \%$  and  $69 \pm 4 \%$  across monoculture and intercropped plots, respectively, compared to control.

Similarly, when cowpea was intercropped under different fertilization regimes, the yield advantage was significant compared to monoculture, with the highest increase in NPKEC (Fig. 3d). Further, NPK application significantly contributed to higher grain yields in both cropping systems compared to control. Compost alone, on the other hand, significantly reduced cowpea grain yield when compared to NPK application. Nevertheless, cowpea grain productivity responded significantly to NPKEC, resulting in the highest grain yield (monoculture  $2.03 \pm 0.04$  vs intercropped  $2.30 \pm 0.05 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) among the different fertilization regimes.



**Fig. 3.** Effects of fertilization regime (Control: no fertilization, Compost: organic fertilization, NPK: mineral nitrogen, phosphorus, potassium fertilization, NPKEC: multi-nutrient enriched compost fertilization) and cropping system (MM: maize monoculture, CC: cowpea monoculture, MC: maize intercropped with cowpea) on biomass and yield of maize (a and c) and cowpea (b and d). Histogram bars are the mean ( $\pm$ standard deviation) of years 2016 and 2017 ( $n = 8$ ) as the factor year did not interact with the other factors. Lower-case letters represent significant differences between bars when the interaction between fertilization and system was significant and kept in the ANOVA model (a, c and d). Upper-case letters represent differences between fertilization treatments in the absence of significant interaction (b).

### 3.2. Effects of fertilization regime and cropping system on soil C pools and C sequestration

When comparing the effects of fertilization regime, soil receiving compost alone had a significantly higher C sequestration compared to both control and NPK application, with the last two having comparable C sequestration (Fig. 4). However, NPKEC application strongly affected soil C sequestration, resulting in the highest C sequestration rate among all fertilization regimes, with a gain of  $0.28 \pm 0.05 \text{ Mg C/ha yr}^{-1}$  compared to control (Fig. 4). Similarly, in the cropping system, intercropped plots accounted for a small but significantly higher soil C sequestration (confidence interval:  $0.03\text{--}0.07 \text{ Mg C/ha yr}^{-1}$ ) than either of the monoculture plots. In contrast, differences in SOC sequestration between monocultures were statistically insignificant. Regarding soil C pools, PAOC and MAOC remained unresponsive to NPK application compared to control (Fig. 4). Differently, in compost amended plots, significantly higher PAOC ( $3.20 \pm 0.09 \text{ g C kg}^{-1} \text{ soil}$ ), as well as MAOC ( $4.31 \pm 0.27 \text{ g C kg}^{-1} \text{ soil}$ ), were found compared to both control and NPK fertilizer (Fig. 4; Table 1). However, NPKEC application showed the highest PAOC ( $4.36 \pm 0.21 \text{ g C kg}^{-1} \text{ soil}$ ) and MAOC ( $5.43 \pm 0.30 \text{ g C kg}^{-1} \text{ soil}$ ) accumulation in the soil among all the fertilization regimes. Most importantly, intercropped plots contributed the most to PAOC and MAOC, while cowpea and maize monoculture plots did not show significant differences in either C pool. However, the interaction between the nutrient regime and the cropping system was significant for PAOC (Table 1; Fig. 4). It was mainly due to a very high amount of PAOC in intercropped plots as compared to monoculture plots in the NPKEC fertilization regime, whereas no significant differences between

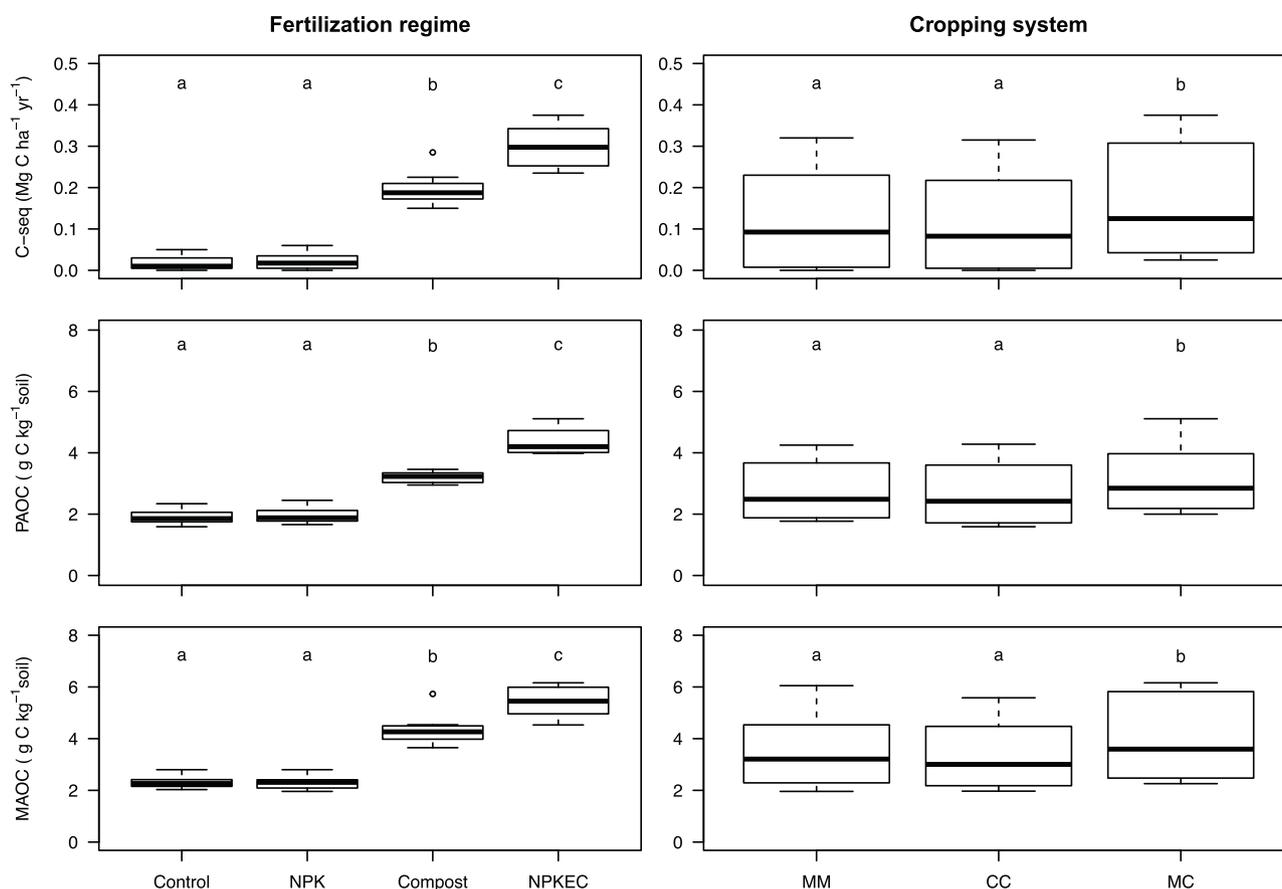
cropping systems were observed for the compost treatment.

### 3.3. Contribution of fertilization regime and cropping system to soil structural stability and C sequestration

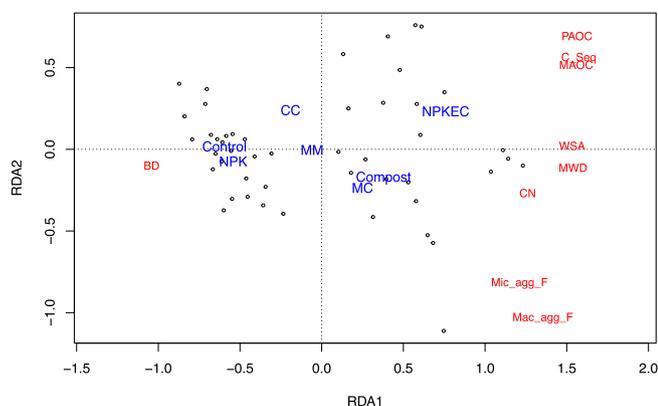
We used the RDA model to distinguish the relationship between soil structural stability and SOC pool variables constrained by cropping systems and fertilization regimes (Fig. 5). According to permutable tests, the RDA results were significant ( $F = 33.12$ ,  $df = 5$ ,  $p < 0.001$ ) with adjusted multivariate redundancy statistics model ( $R_{\text{adj}}^2 = 0.77$ ). Our RDA analyses identified the fertilization regime as the most important factor in explaining variation across the soil functional attributes ( $R_{\text{adj}}^2 = 0.73$ ). However, the cropping system also appeared to play a significant but less important role in the variability pattern ( $R_{\text{adj}}^2 = 0.08$ ). Specifically, NPKEC fertilization showed a positive correlation with MAOC, PAOC, and C-sequestration within the upper right quadrant of the triplot. Moreover, soil structure variables, including WSA, MWD, micro, and macro-aggregates fractions, were better correlated under compost and intercropping combination (Fig. 5; Table 1). More importantly, in the triplot's left quadrant, control and NPK fertilization were closely associated with soil BD, particularly in mono-cropping systems (Fig. 5).

## 4. Discussion

Low soil fertility, limited moisture and a declining organic matter content are the main reasons for low crop productivity in semi-arid agroecosystems. In addition, traditional rotations of mono-cropping with intensive farming regimes lead to greater unsustainability in crop



**Fig. 4.** Effects of fertilization regime (Control: no fertilization, Compost: organic fertilization, NPK: mineral nitrogen, phosphorus, potassium fertilization, NPKEC: multi-nutrient enriched compost fertilization) and cropping system (MM: maize monoculture, CC: cowpea monoculture, MC: maize intercropped with cowpea) on soil C storage rate (C-seq), particulate-associated organic C (PAOC) and mineral-associated organic C (MAOC) during the two-year long experiment. Lower-case letters represent significant differences between fertilization regime or cropping system levels from two-way ANOVA model. Interaction terms were removed for C-seq and MAOC as they were not significant.



**Fig. 5.** Redundancy analysis triplot with fertilization regime (Control: no fertilization, Compost: organic fertilization, NPK: mineral nitrogen, phosphorus, potassium fertilization, NPKEC: multi-nutrient enriched compost fertilization) and cropping system (MM: maize monoculture, CC: cowpea monoculture, MC: maize intercropped with cowpea) as explanatory variables (blue). Response variables (red) are bulk density (BD), C:N ratio (CN), C storage rate (C<sub>Seq</sub>), particulate-associated organic C (PAOC) and mineral-associated organic C (MAOC), water stable aggregates (WSA), mean weight diameter (MWD), the amount of micro- (Mic<sub>agg\_F</sub>) and macro- (Mac<sub>agg\_F</sub>) aggregate fraction. Empty dots represent observations ( $n = 48$ ). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

production, resulting in growing yield disparities among different regions. Our study extended the knowledge of the relationship between soil structural stability and SOC sequestration when different organic and inorganic fertilization regimes were applied to an intercropped soil under semi-arid climatic conditions. Here, we have demonstrated that switching from conventional monocultures to intercropping (e.g., a diverse mix of legumes and cereals) and applying fertilizers that have been enriched with organic additives (e.g., NPKEC) can improve soil structural stability, promote SOC sequestration, and ensure optimum crop yield. From the perspective of future food-security, an adequate supply of mineral nutrients is one of the most critical factors in all agroecosystems to achieve higher crop productivity (Achari and Kowshik, 2018). Therefore, a positive effect of NPK fertilization on biomass and grain yield of maize-cowpea compared to the unfertilized control was expected, as the use of chemical fertilizers is still the preferred option to meet the immediate nutritional needs of crops in degraded agricultural soils, despite the high production cost and potential negative environmental impact (McLeod et al., 2020; Ye et al., 2020). However, when biomass and grain yields were split for NPK fertilizer, grain yield was significantly higher in intercropping than in monocropping, which was not the case for biomass yield in either cropping system. These results point to an early competition between cowpea and maize for biomass accumulation, while the productive stage seemed to exert a complementary positive effect that increased grain yield in the intercropping system (Xiao et al., 2018). Furthermore, improved grain yield in an intercropping system may also be explained by the facilitative interactions between intercrops that significantly improve nutrient availability, ultimately resulting in a higher grain yield compared to monoculture (Zhao et al., 2019; Hu et al., 2020). While NPKEC fertilization showed a significant overall increase in biomass and grain yields in monoculture and intercropping systems, these gains did not translate into higher crop productivity when only compost was added. There is sufficient evidence that compost application improves soil properties such as porosity, water-holding capacity, nutrient replenishment, and organic matter status (Chen et al., 2019; Somerville et al., 2020; Teixeira et al., 2021). However, adding compost alone as a substitute for inorganic fertilizers is not sustainable for meeting plant nutritional needs and optimizing crop yields in degraded soils (Maucieri et al., 2019;

Salehin et al., 2020; Ejigu et al., 2021). Given the poor status of soil organic matter and low fertility conditions, farmers are now increasingly encouraged to combine chemical fertilizers with organic amendments to improve soil fertility and achieve better yields in semi-arid agroecosystems (Arif et al., 2016; Iqbal et al., 2019). Similarly, in the present study, the significantly higher yield with NPKEC application, especially in the intercropping regime, could be explained by the accumulated nutrient advantage (Fan et al., 2020), as the slow-release pattern of the applied NPKEC fertilizer could promote adequate nutrient supply (i.e., NPK) to the crop and, ultimately, a higher yield (Arif et al., 2017; Shahzad et al., 2017; Imran et al., 2020). The yield increase of intercropping over mono-cropping under different fertilization regimes confirms the importance of intercropping for sustainable agriculture. A previous meta-analysis on intercropping found that intercropping cereals and legumes not only increases the yields but also provides yield stability compared to continuous mono-cropping (Raseduzzaman and Jensen, 2017). Notably, low moisture availability and poor fertility are the most important yield determining factors in semi-arid agroecosystems (Li et al., 2020). Regardless of the cropping system and the fertilization regime, the higher yield indices (i.e., biomass and grain) of maize measured in the second year were more likely due to the well-watered condition than those measured in the first year of cultivation (Das et al., 2018; Mbava et al., 2020). Indeed, such positive effects of ecological intensification practices, including organic slow-release fertilization and intercropping, are expected to be enhanced under improved water conditions.

As an important indicator of soil quality and health, soil organic C (SOC) is the most active biochemical parameter (Lorenz et al., 2019), which is not only significant due to the diverse ecosystem services it provides but is now also considered a critical element for the sustainability of global agricultural systems (Rumpel et al., 2020; Kumar and Kalambukattu, 2021). However, the organic C content of agricultural soils is mainly determined by the type of cropping system and management practices. Our study shows that in degraded semi-arid soils, it is possible to increase SOC stocks with greater potential for C sequestration using compost alone or stoichiometrically balanced NPK fertilizer (i.e., NPKEC), although their relative effectiveness depends on whether the cropping system is monocropped or intercropped (Fig. 4; Table 1). The effects on SOC and C sequestration may support the view that the diverse root biomass of the intercropped mixture may contain an increased supply of chemically complex rhizodeposits that promote the establishment of new SOC (Villarino et al., 2021; Zhang et al., 2021). On the other hand, compost application could promote soil aggregation through its conditioning effect and possibly provide a C stabilization and storage mechanism (Das et al., 2017; Zhao et al., 2020). Despite being one of the most important sustainability factors in degraded agroecosystems, SOC storage, sequestration, and stabilization pathways, especially in intercropping systems, are poorly understood. Given the enormous size of the soil C pool, understanding its sensitivity to different land management practices, such as fertilization, is critical to accurately assessing the SOC's stability. Specifically, the significant interaction between the fertilization regime and the cropping system for PAOC possibly implies an important role of fresh rhizodeposits in the PAOC (Angst et al., 2018; Dijkstra et al., 2021). On a relative basis, the MAOC pool was slightly higher than the PAOC pool, which could be related to the different origins of the two C pools (Chen et al., 2020; Samson et al., 2020). The apparent increase in PAOC and MAOC under NPKEC fertilization was associated with C sequestration, suggesting that these two fractions are the primary reservoir of SOC and the main drivers of C storage in soil (Cotrufo et al., 2019; Lavalée et al., 2020). Indeed, the importance of these two distinct C pools was further supported by the positive correlation in the RDA analyses (Fig. 5). Based on the strong positive effect of intercropping on C sequestration, we can hypothesize that the combination of various root biochemical secretions in close and direct proximity to the rhizosphere microbiota and mineral surfaces may promote efficient C storage compared to litter input by roots and shoots

(Jackson et al., 2017; Sokol et al., 2019).

Based on the RDA results, compost alone and intercropping were associated with a lower C-sequestration rate but with better soil structure. It is an interesting finding, especially in long-term C-sequestration targets, because it implies that it is more important to prioritize the recovery of key soil structural attributes before obtaining any C storage gain in these degraded soils. Mechanistically, in low organic matter soils receiving stable organic amendments (i.e., compost), greater aggregate stability (e.g., WSA, MDA) may result from developing a predominantly macro-aggregates fraction. This hypothesis was also supported by the significant additive effect of the fertilization regime on C:N ratio (Table 1), and its negative association with soil BD (Fig. 5). In line with this observation, Fang et al. (2021) attributed the modifications in soil C:N ratio to the additive effect of organic-inorganic inputs, which are a likely reliable predictor of soil aggregation in degraded soils with low fertility. Overall, our work provides field-based empirical evidence that suitable land management practices, such as compost-enriched fertilization with intercropping, can increase soil structural stability and, in parallel, are more efficient in promoting C sequestration than other conventional practices in degraded soils (Zhao et al., 2018; Parihar et al., 2020; Cao et al., 2021).

At the study site, an intensive agricultural system of continuous mono-cropping combined with only chemical fertilizers has been practiced for more than 20 years, resulting in progressive soil degradation. This conclusion is well supported by the positive relationship between BD and chemical fertilization or no fertilization in monocultures (Fig. 5). Indeed, this is consistent with previous studies that showed a significant decline in crop yield potential and soil C stocks due to unsustainable land use management with monocultures (Shah et al., 2017; Terefe and Kim, 2020). Despite increasing awareness of the benefits of adopting other suitable crop rotations and intercropping for better soil quality and higher crop productivity on these degraded soils, farming communities continue to prefer mono-cropping due to limited arable land holdings, economic interest in cash crops and climatic constraints. During this two-year experiment, we paid landowners an estimated amount equivalent to cash crop income for participatory intercropping, assuming that this could be the most appropriate land use management to overcome the compelling problem of soil degradation. We discovered that farmers' willingness to replace traditional mono-cropping with intercropping to improve C content and soil health is still less attractive unless their economic concerns about undermining smallholder sustainability are addressed.

## 5. Conclusions

Rapid SOC depletion and low soil fertility are the leading causes of land degradation, contributing to poor crop yield returns in semi-arid climates. The results of our study suggest that replacing traditional monocultures with legume-cereal intercropping provides a higher yield (biomass and grain) when slow-release compost enriched with NPK is used. Further empirical measurement indicated that stoichiometrically balanced fertilization regimes increase the SOC stock and sequestration rates, although the effectiveness depends on the cropping system. Similarly, MAOC and PAOC pools were strongly associated with SOC-sequestration rate, implying the importance of both pools in SOC storage. On the other hand, compost and intercropping alone had a lower C-sequestration. Still, they improved the indicators related to soil structural stability, i.e., BD, micro-macro aggregation, WSA, and MDA. We conclude that the observed increase in SOC stock and C sequestration rate following the adoption of an intercropping system with a suitable fertilization regime can improve soil quality and crop productivity. Notwithstanding this field study's short duration (2 years), we provided promising empirical evidence for adopting sustainable land management practices on these degraded soils.

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## CRediT authorship contribution statement

**Mahnaz Roohi:** Writing – original draft. **Muhammad Saleem Arif:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Thomas Guillaume:** Software, Formal analysis, Writing – review & editing. **Tahira Yasmeen:** Methodology, Resources, Writing – review & editing. **Muhammad Riaz:** Visualization, Methodology, Resources, Formal analysis. **Awais Shakoor:** Formal analysis, Validation. **Taimoor Hassan Farooq:** Writing – review & editing. **Sher Muhammad Shahzad:** Conceptualization, Validation. **Luca Bragazza:** Visualization, Supervision, Writing – review & editing.

## Declaration of Competing Interest

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

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2022.116152>.

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