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Combined effect of biochar, manure and compost on canola growth, yield parameters and soil chemical properties

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Agricultural productivity relies on several factors where soil fertility and health are some of the vital concerns that need to be addressed. Excessive use of chemical fertilizers can result in significant contamination of soil and other environmental media. The use of organic amendments, such as biochar, compost, manure and their combination, can serve as cost effective and environmentally friendly strategy for healthy soil management. The present study was designed to evaluate the impact of different organic amendments on canola growth and yield. Four soil types, namely loamy sand (LS), sandy loam (SL), silty clay (SC) and loam were used for cultivation of canola. Silty clay was found to produce the highest flower count and seeds' weight, while slightly higher number of seeds per pod were detected in loam. Biochar alone produced better results on flower count and 100 seeds weight, while among the combination treatments manure + compost + biochar, manure + biochar and compost + biochar showed positive impact on the number of pods and 100 seeds weight. Organic treatments, soil types and their interaction significantly impacted carbon, nitrogen (N), potassium (K) and phosphorus (P) concentrations in all soil types compared to controls. pH was found to be a limiting factor affecting shoot length, canola pods and 100 seeds weight.

Keywords Soil fertility, Organic amendments, Biochar, Compost, Manure, Soil types, Soil properties

Agricultural soils are impacted in many countries by salinity and drought, nutrient loss and other soil health problems. This impact is evident not only on agricultural production but also on environmental quality, food safety and food sufficiency[1,2]. The loss of soil nutrients, including carbon loss, also affects the functions of soil system on a global scale. Thus, there is a strong need to recognise and adopt smart agricultural practices that can help maintain and enhance soil health.

Biochar has been extensively researched in the past two decades for its effects on the soil[3]. The concept of organic agriculture is based on acquiring nutrients through the decomposition process. For maximising the benefits of using amendments, biochar and compost can be coupled together or with other organic materials to better meet the crop nutritional needs.

Biochar is a product of pyrolysis and its application to soil can result in improved soil productivity, less greenhouse gas (GHG) emissions and better carbon sequestration[4]. It can have contrasting effects due to the nature of feedstock used in biochar preparation, pyrolysis technique used and the type of soil which receives biochar application[5]. Biochar can reduce carbon emissions in agricultural soils[6], while other studies have reported increased emission due to use of biochar[7–9]. The better crop yield as a result of biochar application can be due to several services provided by biochar, including nitrogen (N) retention, improved water and nutrient uptake and the capacity of biochar to supply N, phosphorus (P) and potassium (K) nutrients for optimal plant growth[10,11]. Biochar can increase the availability of both macro and micronutrients[12] and it can also improve soil fertility by alleviating soil acidity[13,14].

One of the commonly applied strategies to improve soil fertility characteristics and maximise agricultural yield is using composted biomass and manure[15]. Biochar can be solely applied as a soil amendment, and it can also be combined with compost or manure to bring added benefits. The mixture of biochar and compost has been demonstrated in several studies[16,17] to improve soil properties, like nutrient retention, reducing greenhouse gas emissions by forming mineral complexes, thus making the soil carbon more stable. The biochar and compost blend can also serve as suitable substitutes to mineral fertilizer, as they provide a direct surge in supply of

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nutrients, promoting nutrient cycling and microbial activity[18]. The use of manure for increasing soil fertility is also a widely adopted practice as it tends to improve soil health and crop yield[14]. Like biochar, the addition of manure reduces the bulk density of soil and provides the soil environment conducive to plant growth[19].

The effect of wheat straw biochar in combination with compost and compost slurry on maize growth in sandy clay loam was tested showing not only enhanced soil chemical properties but also increased chlorophyll content and grains weight[20]. Biochar and compost mixture also had impact on maize yield in clayey soil where both amendments individually and in mixture showed positive effect on maize yield and soil total organic carbon, total N and P[21]. Another study in which composted manure was shown to improve canola growth and yield in sandy clay loam was conducted by Naveed et al.[22]. Douh et al.[23] also reported the beneficial effects of biochar, peat and compost in improving olive growth in clay loam. The dry weight of canola grown in clay loam, soil organic matter and N, P, K content were reported to increase in response to individual biochar, compost applications and their combination[24]. Increase in plant biomass and yield in response to different organic amendments was reported in other studies for corn[25], aubergine, fennel, lettuce, onion, tomato[26], chickpea and fenugreek[27].

Canola is the third largest crop in Australia after wheat and barley. It is an important and profitable rotation crop in a cereal dominated agricultural system. Due to highly variable climatic conditions across Australia, new canola varieties with agronomic traits have been introduced, which help growers cope with the agronomic challenges faced in the presence of climate change[28]. As such, there is a need to address the use of organic amendments to protect and foster this essential crop which is a source of canola oil with low linolenic acid.

Literature shows there are more studies conducted on maize and other crops compared to canola with respect to the use of organic amendments and their combination. Understanding of canola's response to different amendments is limited and there is lack of knowledge on nutrient cycling in canola grown in different soils treated with biochar, manure and compost. Similarly, the long-term effects of applying amendments for canola cultivation in different soil types need to be assessed, which will require more field experiments in addition to laboratory-based studies. The output of the experiments can help in making informed decisions about cultivating canola and promote sustainable production. The present study was conducted to: (1) evaluate the impact of biochar, organic compost, cow manure and their combination on the growth and yield of canola in four soil types, loamy sand, sandy loam, silty clay and loam, (2) assess the effect of different organic amendments on soil chemical properties and determine the soil type, which gives the best results for canola growth and yield either with sole application or combination of organic materials. The four soil types with contrasting textures were selected to elucidate how organic amendments act differently in sandy, clay dominated and loam soils. The following hypotheses were tested in this study: (1) biochar, compost and manure improve canola and soil parameters when applied individually, and (2) biochar, compost and manure used in different combinations can provide better results for canola growth-yield parameters and soil properties than the individual amendments.

Materials and methods

Biochar preparation

Wheat straw (WS) was used as a biomass for biochar production. Wheat straw biochar (WSB) was prepared by pyrolysing wheat straw in a fixed bed reactor. The temperature was raised continuously at the rate of 10 °C/min until it reached 500 °C. N was used as a carrier gas with the pyrolysis total retention time of 1 h. The biochar produced was further ground and passed through 2 mm mesh.

Soils and organic amendments

The river sand and topsoil were purchased from Australian Native Landscape supplies (ANLs) while clay was supplied from Blackwattle Pottery, Sydney, Australia. Commercial organic compost and blended cow manure were purchased from Bunnings, Carlingford, Australia. A soil jar test was conducted to determine the texture of topsoil which showed loam texture[29]. Then sand and clay were mixed in different proportions with the loam, according to United States Department of Agriculture (USDA) soil manual[30], to generate loamy sand (LS), sandy loam (SL) and silty clay (SC). The four soil types used in this experiment were LS, SL, SC and loam.

Determination of soil physiochemical properties

The pH and electrical conductivity (EC) of biochar, compost, manure and the soil samples were measured using portable combined meter (Hannah combo pH EC meter, HI98129, Romania). For biochar, compost and manure, water suspension with a 1:10 ratio was prepared and stirred for an hour prior to collecting the data[20]. The pH and EC of the soil samples were measured from a soil suspension prepared at 1:5 ratio using Milli Q water, stirred for a few minutes and then left for 30 min to settle[31]. All measurements were obtained in triplicates. The soil samples were ground using a ball mill for further analytical tests. Carbon and N contents were measured using Elementar vario MICRO (Elementar Analysensysteme GmbH, Germany) analyser[27]. In this procedure, 2 mg of dried soil was loaded into a tin foil crucible with 5 mg of WO_3 oxidation catalyst. The sample was combusted at 1150 °C with oxygen supplied at the flow rate of 30 mL/min after 80 s interval. Sulfanilamide was used as a standard and measurements were recorded every 15–20 min using thermal conductivity (TCD) detector. P and K were measured using Olympus VMR XRF, 50 KV workstation, Waltham, MA, USA[32]. Table 1 presents the chemical properties of the soil, wheat straw, biochar, compost and manure used in the pot experiments.

Glasshouse experiment

A pot trial was carried out in the glasshouse at Plant Growth Facility, Macquarie University, Australia (33.7738° S, 151.1126° E) to examine the effects of biochar, compost and manure on canola growth and yield parameters and soil chemical properties. For this purpose, 550 g of air-dried soil was used in each pot with the following dimensions, 19.3 cm in height and 7.6 cm in width. A slow-release N P K fertilizer with the ratio of 16:4.4:8.3 (1.3 g) was added to each pot. Different levels of compost and manure with and without biochar were homogenously

Properties	Units		Soil types			Biomass	Biochar		
		Loam	LS	SL	SC	WS	WSB	Compost	Manure
pH		8.2	6.82	7.36	8.08	7.51	10.35	5.08	8.41
EC	µS/cm	527.4	192.2	264.4	97.2	347.6	1082	563.3	861.2
Total carbon	%	3.5	1.72	1.2	1.44	46.16	73.01	44.3	29.56
Total N	%	0.16	0.13	0.1	0.12	1.49	2.73	0.44	1.45
P	ppm	3893	1143	1354	851	2836	2077	3342	13,875
K	cmol/kg	28.6	71.2	44.3	37.2	107.2	310.8	9.04	125.3
Ca	cmol/kg	48.7	41.1	36.9	27.7	59.3	46.6	87.3	117.1

Table 1. Chemical properties of soil, wheat straw, biochar, compost and manure used in pot experiments. *LS* loamy sand, *SL* sandy loam, *SC* silty clay, *WS* wheat straw, *WSB* wheat straw biochar.

mixed in the soil. Canola was used as a test crop for the experiment where six canola seeds were grown broadcasted in the pots[22]. There were 8 treatments with 4 soil types and six replicates for each type, making a total of 192 pots. The levels of organic amendments were decided based on published studies[20,24]. The different combinations of organic amendments used in the experiment were:

T1 Control soil (CS).
T2 Biochar (B) 1%, (5.5 g)
T3 Manure 0.3% + Compost 0.3% + Biochar 0.3% (M + C + B) (1.83 g each).
T4 Manure (M)1%, (5.5 g)
T5 Compost (C)1%, (5.5 g)
T6 Compost 0.5% + Biochar 0.5% (C + B), (2.75 g each)
T7 Manure 0.5% + Biochar 0.5% (M + B), (2.75 g each)
T8 Compost 0.5% + Manure 0.5% (C + M), (2.75 g each)

The day and night temperature inside the glasshouse was set at 26 °C and 18 °C, respectively. During the germination phase, plants were watered through a drip system where the flow was set for one minute every four hours. Germination was recorded for a period of 10 days and then thinning was performed to maintain two vigorous plants per pot. Thereafter, manual watering was continued according to the need until the end of the experiment.

Growth and yield measurements
Chlorophyll measurements were taken from intact plants using SPAD-502 plus chlorophyll meter (Konica Minolta Sensing, Tokyo, Japan) following the protocol by Kamran et al.[33]. Three chlorophyll readings were recorded from an expanded leaf of each treatment pot. The values were averaged, and the resulting figure was included in the analysis. Mature and intact fully expanded upper leaves from each treatment pot were used to determine the leaf area using LI-3100 Area meter (LI-COR, inc. Lincoln. Nebraska USA)[34]. The other measurements included leaf fresh weight followed by dry weight taken after drying the leaves at 60 °C for 72 h.

The number of flowers were counted during the whole flowering stage. To facilitate pollen transfer, manual pollination using thin brush was employed between vigorous flowers. The pollination was performed in four days interval considering that anthesis period for canola also has the same time interval[35]. The experiment lasted for a period of 3.5 months after which plants were harvested and different morphological and yield related parameters were studied, including shoot length, root length, shoot and root dry weight, number of pods and seeds, and the weight of 100 seeds from each canola plant[36].

Statistical analysis
Minitab software (SAS Inc, Cary, NC) was used to statistically analyse the data. General linear model and two-way ANOVA was carried out to evaluate the effects of organic amendments, soil types and interaction between soil types and organic amendment on canola growth, yield parameters and soil properties. After the analysis of each treatment significance, Tukey's test was used to assess and compare the means of the treatments. Analysis of covariance (ANCOVA) was conducted to determine the effect of pH and EC as covariates on canola growth and yield parameters.

Results
Effect of treatments on canola growth and yield parameters
The treatments, soil type and their interaction had significantly impacted germination of canola, as shown by the analysis of variance in Table 2. CS and C + B treatments in LS resulted in greater germination compared to the other treatments. The interaction of C treatment with LS was the third in order, while similar responses were recorded for B, M + C + B and C + M treatments. In SL, the most prominent interaction was observed with M + B treatment, which was 3.2% higher compared to CS and C + B. Germination was found to be the lowest in SL with M treatment, which had assigned letter "c" in Table 3. In SC, the order of the treatments with improved germination was C + M > M + B > M + C + B > C. Similar to SL, the lowest germination in SC soil occurred with M treatment. Overall, loam was the soil where application of organic amendments did not exert significant impact compared to control, as shown by the lower mean values presented in Table 3.

Parameters	Germination (%)	LA (m^2)	LFW (g)	LDW (g)	Chlorophyll	Shoot length (cm)	RL (cm)	SDW (g)	RDW (g)
Treatment (Trt)	0.000***	0.599^{ns}	0.435^{ns}	0.004**	0.002**	0.030*	0.000***	0.065^{ns}	0.054^{ns}
Soil type	0.000***	0.004**	0.402^{ns}	0.000***	0.026*	0.000***	0.003**	0.354^{ns}	0.000***
Trt*soil type	0.000***	0.113^{ns}	0.476^{ns}	0.000***	0.013*	0.003**	0.13^{ns}	0.803^{ns}	0.122^{ns}

Table 2. p-value from ANOVA test on Canola growth parameters. LA = leaf area, LFW = leaf fresh weight, LDW = leaf dry weight, SL = shoot length, RL = root length, SDW = shoot dry weight, RDW = root dry weight ns = non-significant, * = significant at α = 5%, ** = significant at α = 1%. *** = significant at α = 10%.

Treatments		Germination %	LA (m^2)	LFW (g)	LDW (g)	Shoot length (cm)	RL (cm)	SDW (g)	RDW (g)	Chlorophyll
LS	CS	91.67 ± 13.94 a	58.77 ± 7 a	0.34 ± 0.22 a	0.12 ± 0.03 cd	50.05 ± 7.57 af	19.23 ± 2.44 ac	1.62 ± 0.66 a	1.08 ± 0.48 b	42.5 ± 2.52 ac
	B	86.11 ± 12.55 a	87.41 ± 9.37 a	0.49 ± 0.26 a	0.24 ± 0.04 cd	62.15 ± 6.63 a	19.23 ± 2.69 ac	2.69 ± 1.03 a	1.72 ± 0.77ab	42.87 ± 1.04 ac
	M + C + B	86.11 ± 12.55 a	69.25 ± 14.66 a	2.19 ± 0.73 a	0.35 ± 0.13 ad	48.93 ± 14.49 af	16.81 ± 1.36 ac	2.11 ± 1.26 a	1.35 ± 0.90 b	46.8 ± 7.51 ac
	M	63.89 ± 12.55ac	79.51 ± 16.1 a	2.08 ± 0.52 a	0.17 ± 0.01 cd	43.61 ± 5.19 bf	20.86 ± 4.81 ab	1.18 ± 0.56 a	0.98 ± 0.37 b	45.6 ± 2.02 ac
	C	88.89 ± 8.61 a	77.62 ± 12.68 a	2.39 ± 0.65 a	0.28 ± 0.09 bd	45 ± 5.98 bf	17.7 ± 1.81 ac	2.53 ± 0.92 a	0.98 ± 0.64 b	44.9 ± 0.85 ac
	C + B	91.67 ± 13.94 a	88.45 ± 8.04 a	2.24 ± 0.24 a	0.27 ± 0.06 bd	47.88 ± 4.74 af	18.56 ± 3.15 ac	2.39 ± 1.77 a	1.71 ± 1.07 ab	43.7 ± 2.15 ac
	M + B	80.56 ± 12.55 ab	51.92 ± 4.41 a	1.11 ± 0.19 a	0.23 ± 0.09 cd	49.69 ± 6.01 af	18.38 ± 4.27 ac	2.58 ± 1.55 a	2.66 ± 2.18 ab	36.9 ± 4.03 c
	C + M	86.11 ± 19.48 a	65.21 ± 6.45 a	1.54 ± 0.33 a	0.24 ± 0.05 cd	48 ± 4.80 af	14.8 ± 0.47 bc	2.11 ± 0.56 a	1.43 ± 0.45 ab	43.1 ± 6.86 ac
SL	CS	86.11 ± 12.55 a	80.7 ± 261.5 a	1.12 ± 0.31 a	0.19 ± 0.04 cd	49.66 ± 6.87 af	18.35 ± 2.81 ac	1.38 ± 0.94 a	1.68 ± 1.08 ab	46.9 ± 2.88 ac
	B	75 ± 9.13 ac	42.65 ± 7.24 a	1.46 ± 0.26 a	0.29 ± 0.07 bd	50.48 ± 11.95 af	18.35 ± 2.17 ac	2.03 ± 1.77 a	0.69 ± 0.67 b	36.4 ± 2.66 c
	M + C + B	83.33 ± 10.54 ab	83.26 ± 16.9 a	2.02 ± 0.38 a	0.28 ± 0.11 bd	52.26 ± 17.33 af	18.63 ± 2.07 ac	1.89 ± 1.20 a	0.86 ± 0.62 b	48.3 ± 2.80ac
	M	41.67 ± 20.4 c	71.17 ± 16.2 a	1.85 ± 0.63 a	0.27 ± 0.04 cd	47.9 ± 10.35 af	18.01 ± 4.85 ac	1.72 ± 1.65 a	1.98 ± 1.85 ab	45.4 ± 1.73 ac
	C	83.33 ± 14.91 ab	69.45 ± 1.8 a	1.79 ± 0.03 a	0.26 ± 0.01 cd	46.51 ± 5.65 af	16.83 ± 2.89 ac	1.61 ± 1.29 a	0.78 ± 0.67 b	44.9 ± 0.85 ac
	C + B	86.11 ± 12.55 a	73.95 ± 9.49 a	2.03 ± 0.32 a	0.29 ± 0.04 bd	56.96 ± 6.94 ac	16.01 ± 1.07 ac	3.60 ± 2.63 a	1.46 ± 1.27 ab	43.6 ± 6.75 ac
	M + B	88.88 ± 8.61 a	54.71 ± 3.4 a	1.38 ± 0.16 a	0.19 ± 0.01 cd	55.23 ± 3.19 ad	13.38 ± 1.73 c	2.16 ± 1.27 a	2.08 ± 1.25 ab	50.1 ± 2.93 ab
	C + M	83.33 ± 10.54 ab	75.35 ± 7.43 a	1.64 ± 0.09 a	0.24 ± 0.01 cd	52.75 ± 3.19 ae	14.8 ± 1.54 bc	1.63 ± 0.94 a	1.43 ± 0.95 ab	47.3 ± 1.96 ac
SC	CS	72.22 ± 22.77 ac	81.53 ± 15.8 a	1.64 ± 0.13 a	0.65 ± 0.13 a	47.11 ± 6.25 af	22.38 ± 6.87 a	1.94 ± 1.29 a	1.02 ± 0.57 b	39.5 ± 3.6 bc
	B	77.78 ± 2.19 ac	81.07 ± 9.17 a	1.84 ± 0.49 a	0.39 ± 0.08 ad	52.33 ± 2.78 af	20.63 ± 6.80 a	2.31 ± 1.21 a	1.61 ± 0.93 ab	44.1 ± 1.76 ac
	M + C + B	80.56 ± 12.55 ab	66.16 ± 12.5 a	2.20 ± 0.75 a	0.28 ± 0.11 bd	40.75 ± 10.82 cf.	20.66 ± 3.31 ab	1.19 ± 1.02 a	0.54 ± 0.45 b	46.6 ± 4.01 ac
	M	66.67 ± 18.26 ac	66.46 ± 14.1 a	1.68 ± 0.29 a	0.25 ± 0.06 cd	47.9 ± 7.81 af	20.66 ± 2.89 ab	2.04 ± 0.90 a	1.09 ± 0.89 b	47.6 ± 1.74 ac
	C	83.33 ± 14.91 ab	72.99 ± 9.74 a	2.06 ± 0.40 a	0.35 ± 0.06 ad	48.46 ± 6.10 af	17.1 ± 2.93 ac	1.73 ± 1.25 a	2.07 ± 1.78 ab	47 ± 3.96 ac
	C + B	72.22 ± 13.61ac	82.76 ± 6.87a	2.23 ± 0.10 a	0.32 ± 0.03 bd	56.76 ± 7.42 ac	17.06 ± 2.62 ac	2.64 ± 1.64 a	1.15 ± 0.87 b	46.1 ± 6.63 ac
	M + B	91.67 ± 9.13 a	57.64 ± 5.42 a	1.50 ± 0.33 a	0.24 ± 0.08 cd	55.4 ± 7.60 ad	17.03 ± 1.52 ac	1.89 ± 0.99 a	1.28 ± 0.97 ab	52.1 ± 5.67 a
	C + M	94.44 ± 8.61 a	65.65 ± 4.65 a	1.86 ± 0.24 a	0.29 ± 0.06 bd	57.4 ± 3.44 ab	17.16 ± 2.67 ac	2.71 ± 0.87 a	1.87 ± 0.63 ab	47 ± 2.43 ac
Loam	CS	72.22 ± 17.21 ac	78.63 ± 9.26 a	3.29 ± 0.58 a	0.44 ± 0.19 ac	41.86 ± 2.89 bf	18.18 ± 1.99 ac	2.68 ± 1.26 a	2.22 ± 0.80 ab	37.6 ± 2.35 c
	B	63.89 ± 26.7 ac	98.43 ± 19.5 a	4.61 ± 0.72 a	0.58 ± 0.08 ab	39.11 ± 5.24 df	19.78 ± 1.79 ac	2.53 ± 1.31 a	3.96 ± 3.27 a	44.2 ± 2.86 ac
	M + C + B	47.22 ± 16.39 bc	96.63 ± 14.26a	4.23 ± 2.20 a	0.32 ± 0.11 bd	37.26 ± 3.57 ef	18.61 ± 2.36 ac	2.02 ± 0.60 a	1.76 ± 0.78 ab	43.8 ± 1.62 ac
	M	72.22 ± 31.03 ac	88.1 ± 24.17 a	0.36 ± 0.11 a	0.19 ± 0.06 ad	41.1 ± 5.77 bf	15.71 ± 0.64 ac	2.33 ± 0.88 a	1.72 ± 0.62 ab	45.9 ± 3.67 ac
	C	63.89 ± 6.8 ac	76.36 ± 18.2 a	3.51 ± 0.82 a	0.63 ± 0.20 a	40.93 ± 6.46 bf	16.28 ± 2.42 ac	2.51 ± 1.18 a	2.33 ± 0.88 ab	46.3 ± 5.10 ac
	C + B	61.11 ± 20.18 ac	72.24 ± 7.06 a	3.23 ± 0.84 a	0.48 ± 0.16 ac	40.66 ± 6.17 cf.	16.7 ± 1.71 ac	2.81 ± 1.05 a	2.93 ± 1.02 ab	45.1 ± 2.01 ac
	M + B	47.22 ± 12.55 bc	96.76 ± 22.2 a	3.67 ± 0.76 a	0.38 ± 0.10 ad	36.13 ± 0.49 f	16.83 ± 2.50 ac	1.90 ± 0.58 a	1.88 ± 0.84 ab	46.9 ± 0.49 ac
	C + M	58.33 ± 22.97 ac	84.69 ± 18.85 a	3.40 ± 0.37 a	0.33 ± 0.03 bd	36 ± 3.64 f	18.51 ± 2.67 ac	2.59 ± 1.39 a	1.83 ± 0.74 ab	45.4 ± 3.94 ac

Table 3. Effect of organic amendments on Canola growth and physiological parameters. Data presented are means + standard deviations. Pairwise differences connecting letters were generated based on p-value of the interaction between soil types and organic treatments. Means followed by different letters indicate statistically significant differences. LA = leaf area, LFW = leaf fresh weight, LDW = leaf dry weight, SL = shoot length, RL = root length, SDW = shoot dry weight, RDW = root dry weight; Means that do not share a letter are significantly different, LS = loamy sand, SL = sandy loam, SC = silty clay.

ANOVA analysis indicated that among the organic treatments, soil types and interaction between soil types and organic treatments, only the soil type had a significant impact on the leaf area, as demonstrated by its p value of less than 0.05 (Table 2). In LS, the highest leaf area was recorded with C + B treatment which was 50.5% higher compared to CS treatment. Biochar was the second dominant treatment (48.8% higher than control), while the lowest leaf area was recorded with M + B treatment. M + C + B caused maximum increase in the leaf area of canola in SL compared to the other treatments. The order for the other treatments in this soil was CS > CM > C + B > M > C > M + B > B. In SC, the highest leaf area was detected with the C + B treatment, which

Source	DF	Shoot length	RL	SDW	RDW
pH (covariate 1)	1	4.24*	0.18^{ns}	0.11^{ns}	0.01^{ns}
Trt	7	2.61*	3.67**	2.02^{ns}	1.76^{ns}
soil type	3	10.99***	4.63**	0.89^{ns}	4.48**
Error	180				
EC (covariate 2)	1	2.2^{ns}	0.93^{ns}	0.2^{ns}	5.54*
Trt	7	2.19*	4.66***	2.02^{ns}	2.29*
soil type	3	5.56**	4.95**	0.7^{ns}	7.32***
Error	180				

Table 4. F-values from ANCOVA test on growth parameters. DF = degree of freedom, Trt = treatment, ns = non-significant, * = significant at α = 5%, ** = significant at α = 1%. *** = significant at α = 10%. RL = root length, SDW = shoot dry weight, RDW = root dry weight.

Parameters	Branches	Flower count	No of pods	Seeds per pod	100 seeds weight (g)
Treatment (Trt)	0.161^{ns}	0.989^{ns}	0.006**	0.964^{ns}	0.876^{ns}
Soil type	0.000***	0.000***	0.002**	0.031*	0.000***
Trt*soil type	0.153^{ns}	0.248^{ns}	0.001**	0.334^{ns}	0.004**

Table 5. p-value from ANOVA test on Canola yield parameters. ns = non-significant, * = significant at α = 5%, ** = significant at α = 1%. *** = significant at α = 10%.

was slightly higher by 1.5% compared to CS and 2.1% higher compared to B treatment. The lowest leaf area was recorded for M + B treatment.

For the leaf fresh weight, no significant impact was observed for any of the organic amendments, soil types and their interaction, as implied by the p values, which are all non-significant. On the other hand, leaf dry weight was significantly influenced by all sources of variation (Table 2). Tukey's mean comparison test assigned the same letters to the means for leaf fresh weights, meaning no statistical difference among the means. For the leaf dry weight, the highest interaction effect was noted in SC with CS treatment and in loam with C treatment. These interactions were assigned letter "a". The second highest interaction effect for leaf dry weight was recorded in loam with B treatment. In LS, the most effective treatment was found to be M + C + B (0.35 g) while CS resulted in the lowest dry weight (0.12 g). Treatment "B" showed the highest leaf area in SL, followed by C + B (Table 3).

Chlorophyll was significantly affected by organic treatments, soil type and interaction between the soil type and organic treatment (Table 2). The highest chlorophyll value in LS was recorded with the treatment M + C + B followed by M and C treatments. Compared to control, M + C + B raised the chlorophyll content by 10.11%. The treatment with the least performance in LS was M + B. In SL, M + B was the dominating treatment, followed by C + M and CS. The lowest chlorophyll level occurred in response to SL with B treatment. In SC, M + B led to 31.8% higher chlorophyll content compared to control. This was followed by M, C and M + C + B treatments. Chlorophyll was higher in loam with M + B, C, M and C + M treatments compared to CS and B (Table 3).

Shoot length was another parameter where all sources of variation had significant impact (Table 2). Organic treatment B significantly impacted shoot length in LS, and this interaction was assigned letter "a" as the highest shoot length mean among all the interactions between soil type and organic amendments. C + B treatment had the strongest impact on shoot length in SL and SC. Comparatively, lower shoot length was recorded for all treatments in loam (Table 3). Table 2 indicates that treatment and soil type had a significant effect on root length, but the interaction between treatment and soil type was non-significant. Root length in SC soil was the highest for the CS treatment, followed by B and M + C + B. Treatment M gave better root length in LS compared to C and C + M treatments where the lowest root length was recorded. The prominent interaction effect in loam was observed with treatment B, while in SL, CS treatment dominated, and the lowest root length was recorded with C + M (Table 3).

Analysis of variance (Table 2) shows that shoot dry weight did not vary in response to treatment, soil type and interaction between treatment and soil type, thus, the same letter was assigned to all means for this variable, according to Tukey's mean comparison test. Root dry weight was significantly influenced by soil type, with the highest root dry weight recorded in loam treated with B, followed by C + B and C. LS was the second soil after loam in which M + B gave better root dry weight compared to the rest of the interactions. The lowest root dry weight was observed in response to M + C + B treatment in SC (Table 3).

The ANCOVA results presented in Table 4 showed that the covariate had significant impact on shoot length only while the rest of the growth parameters were significantly impacted either by treatment or soil type. EC did not exert a significant effect on any of the four growth parameters.

Statistical analysis revealed that for branches and flower count, only soil type caused a significant impact, while the effect of treatment and interaction between soil type and treatment was found to be non-significant (Table 5). In LS, the CS and M treatments showed almost the same number of branches, while the other treatments resulted in even fewer branches, however the differences were non-significant. The SL soil type under control conditions (CS) surpassed all soil types, followed by C treatment. In SC, B and M + C + B gave rise to more branches compared to the rest while the least number of branches were noticed in loam (CS) and its treated types (Fig. 1). For the flower count parameter, SC treated by biochar produced the maximum number of flowers, while CS and M + C + B produced almost the same results. LS showed the second performance after SC, where CS, C + B and M + B played comparatively positive roles. Fewer number of flowers were observed in SL and loam (Fig. 2, A).

The General linear model for analysis of variance showed that treatments, soil type and interaction between treatments and soil types all had significant impact on the number of pods (Table 5) The number of pods was the highest in loam under the M + C + B treatment, followed by M + B and C + M. Treatment B gave slightly more pods in LS followed by C + B and M + B treatments. In SL and SC, M + B treatment performed better than the other treatments (Fig. 2, B).

The soil type had a slightly positive significant effect on the number of seeds in a pod with the highest number recorded in loam for M + B, followed by B and C + M treatments. In SL, M + B resulted in the highest seed number, while in SC, C was the most dominating treatment. In LS, treatment B produced better seed numbers compared to the counterparts (Fig. 2, C). Table 4 shows that both soil type and treatment soil type interaction enhanced the seeds weight with SC giving the highest seed weight with treatments B and M + C + B. SL was the second with CS and B treatments showing maximum weight. In LS, the best treatment was C + B, while treatment M led to the highest seeds weight in loam compared to the rest of the treatments (Fig. 2, D). ANCOVA results implied that pH had a significant impact on the number of pods, seeds per pod and 100 seeds weight ($\alpha = 5\%$). There was no significant effect of the covariate EC on the yield parameters (Table 6).

Effect of treatments on soil parameters

As presented in Table 1, loam, SL and SC were alkaline in nature, while the pH of LS was near neutral. WSB and manure had an alkaline pH, while compost had an acidic pH. The biochar also had a higher C and K content compared to the other two organic amendments. P and Ca were the highest in manure.

Table 7 shows that the p-values resulting from ANOVA test on soil parameters were all less than 0.05 at a standard level of significance of 5%, implying that organic treatments, soil types and interaction between soil types and organic amendments had significant impact on the soil chemical properties. Tukey's test further confirmed five interactions where pH was the highest, CS in SL, CS and B in SC, B and C + M in loam. The rest of the treatments caused the pH to decline in the four soil types (Table 8). B application in SC elevated soil pH by 0.6%, while the same treatment led to 8.2% increase in pH of loam. In LS, treatment C lowered the pH value by 16.01% compared to control, while a reduction of 28.2%, 23% and 1.05% was detected with treatment C in SL, SC and loam respectively. With respect to EC, the most noticeable increase was recorded in the loam under C + M, followed by M + C + B and M treatments. EC was the lowest in loam under CS, B and M + B treatments. Biochar induced a significant increase in EC of SL, while lower differences were observed among the other treatments in SL, LS and SC (Table 8).

Total carbon level was the highest under the C + B treatment in loam, followed by C, M + B and M treatments. M + B, C + B and M + C + B were the dominant treatments in LS, while in SL and SC, the highest total carbon level was recorded in response to C + B treatment (Table 8). The highly significant impact on N level was imparted by treatment M in SL. Differences in total N levels under CS and B treatments in loam were also statistically significant. C + B was the leading treatment for N level in SC (Table 8).

Treatments, soil types and interaction between treatments and soil types all played a vital role in increasing P and K levels. The highest P level was noticed in the loam under treatment M, followed by M + C + B and C. P concentration was found to be the most elevated in SL under C + M, while in LS, M + B caused the maximum increase by 50% compared to CS. C + B was the most effective treatment in SC, which raised P level by 90.4%

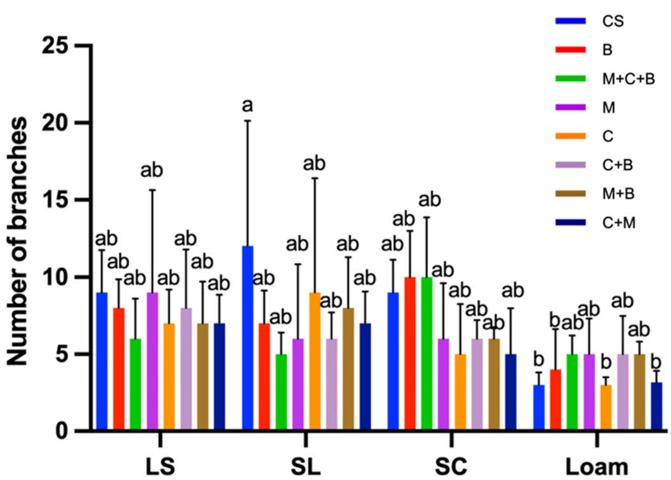

Fig. 1. Effect of organic amendments on canola germination. Data presented are the means ± standard deviation (Mean ± SD). Means that do not share a letter are statistically significant, LS = loamy sand, SL = sandy loam, SC = silty clay.

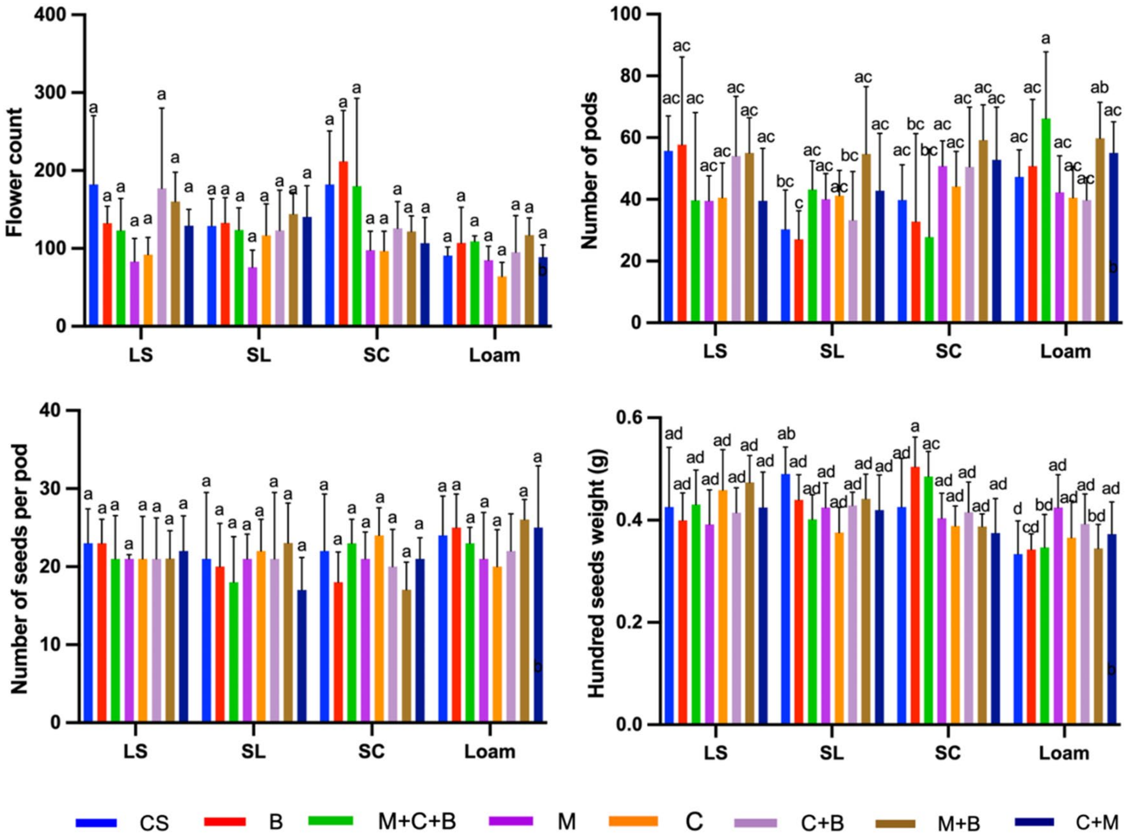

Fig. 2. Effect of soil amendments on canola yield parameters; Data presented are the means ± standard deviation (Mean ± SD). Means that do not share a letter are statistically significant. LS = loamy sand, SL = sandy loam, SC = silty clay, CS = control, B = biochar, M = Manure, C = compost, M + B = manure + biochar, C + B = compost + biochar, C + M = compost + manure, M + C + B = manure + compost + biochar.

Source	DF	Branches	Flower count	No of pods	seeds per pod	100 seeds weight (g)
pH (covariate 1)	1	2.76^{ns}	0.08^{ns}	4.48*	4.31*	12.3**
Trt	7	0.52^{ns}	0.14^{ns}	3.02**	0.75^{ns}	0.7^{ns}
soil type	3	9.97***	3.11*	6.08**	4.28**	13.16***
Error	180					
EC (covariate 2)	1	0.47^{ns}	0.27^{ns}	1.35^{ns}	2.16^{ns}	0.09^{ns}
Trt	7	1.52^{ns}	0.16^{ns}	2.19*	0.3^{ns}	0.39^{ns}
soil type	3	5.19**	3.38*	2.71*	0.51^{ns}	3.46^{ns}
Error	180					

Table 6. F-values from ANCOVA test on yield parameters. DF = degree of freedom, Trt = treatment, ns = non significant, * = significant at α = 5%,** = significant at 1%. *** = significant at α = 10%.

Parameters	pH	EC (μScm^{-1}))	Total carbon (%)	Total *N* (%)	Total *P* (%)	Total K (%)
Treatment	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
Soil type	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
Trt*soil type	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***

Table 7. p-value from ANOVA test on soil properties. trt = treatment, ns = non-significant, * = significant at α = 5%, ** = significant at α = 1%, *** = significant at α = 10%.

Treatments		pH	EC (μScm$^{-1)}$)	Total carbon (%)	Total *N* (%)	Total *P* (%)	Total K (%)
LS	CS	7.6 ± 0.01 ef	228 ± 4.24 gi	1.38 ± 0.03 pq	0.09 ± 0.00 i	0.26 ± 0.0001 t	1.37 ± 0.0003 b
	B	6.47 ± 0.02 mo	286.5 ± 0.70 dg	2.43 ± 0.12 no	0.11 ± 0.00 ij	0.35 ± 0.0002 o	1.25 ± 0.0003 h
	M + C + B	6.64 ± 0.19 km	84 ± 0.00 hi	6.55 ± 0.08 gi	0.33 ± 0.03 df	0.39 ± 0.0002 j	1.27 ± 0.0001 g
	M	6.46 ± 0.03 mo	118.5 ± 10.6 gi	6.17 ± 0.01 hj	0.34 ± 0.02 cf.	0.31 ± 0.0003 r	1.35 ± 0.0004 d
	C	6.51 ± 0.05 ln	80.5 ± 0.70 hi	1.31 ± 0.1 q	0.19 ± 0.01 h	0.25 ± 0.0009 u	1.28 ± 0.0003 f
	C + B	7.14 ± 0.06 gh	167.5 ± 0.70 gi	7.28 ± 0.69 e.g.	0.34 ± 0.01 cf.	0.36 ± 0.0002 n	1.41 ± 0.0004 a
	M + B	6.96 ± 0.01 hj	162 ± 7.07 gi	7.87 ± 0.44 df	0.38 ± 0.02 ae	0.39 ± 0.0004 i	1.24 ± 0.0003 i
	C + M	6.67 ± 0.02 km	137 ± 8.48 gi	5.02 ± 0.35 kl	0.29 ± 0.01 fg	0.24 ± 0.0002 v	1.37 ± 0.0003 c
SL	CS	8.4 ± 0.01 a	171.5 ± 3.53 gi	1.45 ± 0.00 oq	0.07 ± 0.01 j	0.22 ± 0.0002 w	0.70 ± 0.0002 ab
	B	7.73 ± 0.04 de	435.5 ± 0.70 ce	3.45 ± 0.01 m	0.18 ± 0.04 hi	0.21 ± 0.0003 x	0.73 ± 0.0001 aa
	M + C + B	6.55 ± 0.04 lm	84.5 ± 10.6 hi	8.77 ± 0.12 bd	0.4 ± 0.01 ad	0.36 ± 0.0004 m	0.93 ± 0.0003 t
	M	6.75 ± 0.01 jl	104.5 ± 9.19 gi	8.71 ± 0.02 bd	0.45 ± 0.01 a	0.37 ± 0.0002 m	0.90 ± 0.0002 v
	C	6.03 ± 0.11 p	62 ± 2.82 i	2.3 ± 0.36 oq	0.24 ± 0.04 gh	0.21 ± 0.000 y	0.62 ± 0.0004 ac
	C + B	7.11 ± 0.13 hi	251.5 ± 3.53 eh	10.35 ± 0.29 a	0.42 ± 0.03 ab	0.43 ± 0.0006 e	0.87 ± 0.0006 x
	M + B	7.14 ± 0.01 gi	200 ± 3.53 gi	8.54 ± 0.07 cd	0.41 ± 0.01 ac	0.38 ± 0.000 k	1.09 ± 0.0002 n
	C + M	6.76 ± 0.02 jl	185 ± 15.5 gi	8.25 ± 0.14 de	0.42 ± 0.00 ab	0.49 ± 0.0004 c	0.98 ± 0.0003 s
SC	CS	8.14 ± 0.05 bc	233.5 ± 0.70 fi	1.64 ± 0.01 oq	0.1 ± 0.01 j	0.21 ± 0.0002 z	1.01 ± 0.0001 r
	B	8.19 ± 0.04 ab	220 ± 5.65 gi	2.38 ± 0.06 op	0.11 ± 0.01 ij	0.14 ± 0.0002 ab	1.06 ± 0.0002 p
	M + C + B	6.67 ± 0.04 km	149 ± 9.89 gi	6.67 ± 0.06 gi	0.34 ± 0.01 cf.	0.34 ± 0.0004 p	1.09 ± 0.0007 m
	M	6.21 ± 0.12 op	259.5 ± 3.53 df	5.75 ± 0.19 ik	0.33 ± 0.01 df	0.30 ± 0.0004 s	1.12 ± 0.0002 j
	C	6.25 ± 0.06 np	198.5 ± 10.6 gi	3.44 ± 0.18 mn	0.28 ± 0.02 fg	0.33 ± 0.0005 q	0.79 ± 0.0002 z
	C + B	6.74 ± 0.01 jl	285.5 ± 4.94 dg	7.13 ± 0.31 fh	0.39 ± 0.01 ad	0.40 ± 0.0002 h	1.14 ± 0.0004 g
	M + B	7.38 ± 0.06 fg	82.5 ± 4.94 hi	4.62 ± 0.14 l	0.28 ± 0.02 fg	0.25 ± 0.0003 u	1.30 ± 0.0004 e
	C + M	6.88 ± 0.05 ik	96.5 ± 3.53 hi	5.37 ± 0.34 jl	0.31 ± 0.02 e.g.	0.2 ± 0.0002 aa	1.08 ± 0.0003 o
Loam	CS	7.6 ± 0.05 ef	489 ± 4.24 c	3.5 ± 0.03 m	0.24 ± 0.02 gh	0.53 ± 0.000 b	0.91 ± 0.000 u
	B	8.22 ± 0.04 ab	699 ± 2.82 c	9.80 ± 0.09 a	0.37 ± 0.01 ae	0.39 ± 0.000 ij	1.01 ± 0.000 r
	M + C + B	7.73 ± 0.02 de	1100 ± 12.72 b	9.65 ± 0.02 ab	0.41 ± 0.01 ac	0.53 ± 0.0001 b	1.02 ± 0.000 q
	M	7.94 ± 0.06 cd	1080.5 ± 10.6 b	9.79 ± 0.07 a	0.33 ± 0.01ac	0.6 ± 0.000 a	1.31 ± 0.000 e
	C	7.52 ± 0.01 ef	496.5 ± 26.6 cf.	9.96 ± 0.07 a	0.39 ± 0.00 ad	0.46 ± 0.000 d	0.88 ± 0.0002 w
	C + B	7.48 ± 0.02 f	439.5 ± 3.53 cd	10.2 ± 0.67 a	0.36 ± 0.00 be	0.37 ± 0.000 l	0.85 ± 0.000 y
	M + B	7.62 ± 0.02 ef	1036 ± 3.53 b	9.85 ± 0.01 a	0.38 ± 0.00 ad	0.42 ± 0.000 g	1.09 ± 0.000 n
	C + M	7.98 ± 0.10 bd	1629.5 ± 31.1 a	9.38 ± 0.03 ac	0.39 ± 0.00 ad	0.43 ± 0.000 f	1.09 ± 0.000 l

Table 8. Effect of organic amendments on soil chemical properties. Data presented are means ± standard deviations. Pairwise differences connecting letters were generated based on p-value of the interaction between soil types and organic treatments. Means followed by different letters indicate statistically significant differences among treatments. LS = loamy sand, SL = sandy loam, SC = silty clay.

compared to CS treatment. C + B led to the most significant increase in K level in LS, followed by C + M and M. The highly significant rise in K level was recorded in SL and SC under M + B treatment, while in loam treatment M caused the most significant increase in K level (Table 8).

Discussion

Effect of treatments on canola morphological and yield parameters

The present study was conducted in a completely randomised design to assess the impact of different organic amendments on canola morphology, yield parameters and soil chemical properties. The study highlighted significant improvement in chlorophyll, leaf area and leaf dry weights in response to the treatments provided in four soils, namely LS, SL, SC and loam. The increase in chlorophyll content of leaves is also supported by previous study where bagasse biochar and biochar compost mixture were applied for cultivation of maize in clayey soil[21]. Chlorophyll indicates the level of photosynthetic activity during plant growth and gives information about the reaction of a crop to N fertilizer application. Surge in chlorophyll levels of canola leaves in the present study can be related to better uptake of N in the presence of organic amendments. Khan et al.[37] also reported an increase in chlorophyll content, photosynthesis and stomatal traits, which all contributed to enhanced yield in canola. Biochar induced rise in leaf area (17%) was also reported for maize in clay loam[38] where this increase was also related with the improved level of nutrients provided by biochar. For the present trial, treatment B led to a 44.8% increase in leaf area of canola cultivated in loam.

Shoot dry weight of canola was not significantly impacted by any of the treatments, soil type and interaction between treatments and soil type. This was contrary to the result reported in another study where both shoot length and shoot dry weight of maize increased under the presence of wheat straw biochar and compost combination in sandy clay loam[20]. Abbas et al.[20] attributed this increase in the growth parameters to soil high organic carbon levels, improved porosity and high P and K levels. Similar study by Abagandura et al.[1] in which B and M used as a combination treatment did not result in increasing corn and soybean dry weight. Increased dry weights were also reported for maize grown in LS and CL treated with biochar at three different application rates (1, 2 and 4%)[39]. Increase in dry weight of beans was highlighted by Inal et al.[13] in response to B and M treatments. Besides biochar, compost also reduces bulk density while manure can increase the water use efficiency of crops.

Statistical analysis clarified that the treatments, soil type and interaction between treatments and soil types all exerted significant effects on the number of branches. This is in agreement with the results published in another study where acacia biochar increased the number of branches in canola grown in sandy loam[36]. A number of previous studies revealed the positive relation between organic materials and enhanced growth and yields for maize, wheat, basil rice and sunflower[40,41]. These organic amendments are rich in nutrients that provide N, carbon and P in bioavailable forms, making them readily available for plant uptake and hence increase production. The rise in the number of canola pods and number of grains per pods with and manure biochar mixture were also reported in another study on canola that was planted in sandy clay loam soil[22].

The increase in 100 seeds weight caused by treatment M in loam could be due to supply of organic matter which led to improved K^+, P and carbon concentrations in soil. Similar observation in rise in canola yield parameters was reported by Inal et al.[22], who applied composted manure to evaluate its effect on alleviating salt stress in canola grown in sandy clay loam and improvement in canola and growth parameters. The calcium fortified animal manure improved the canola growth and yield compared to control. Organic amendments, like animal manure, can lead to changes in biological activities in soil, which regulate cycling of nutrients and in turn can support better growth and yield[42]. Improved nutrients which contributed to increased seeds weight in the present experiment can be due to the nutrient rich environment created by the addition of organic amendments.

Effect of treatments on soil parameters

The current study revealed that soil parameters were significantly affected by the sources of variation. The increase in pH of SC and loam can occur because of the alkaline nature of biochar. The reduction in pH by C + B, M and C treatments are similar to the results reported in another study on canola[24], which revealed that organic amendments, like compost, biochar and their combination, alleviate alkalinity owing to the release of organic acids from these amendments, which cause drop in pH. In another study it was demonstrated that when organic compost and manure undergo oxidation they release acidic functional groups which can be accounted for reducing pH[43].

The high EC values under the treatment B are due to the alkaline nature of biochar, while low values under the presence of compost and manure treatments resulted due to the acidic nature of compost and manure which reduced electrical conductivity. The high EC in loam resulted in reduced number of branches, flower count and 100 seeds weight. This is also evident from the ANCOVA analysis showing significant influence of the soil type on canola yield parameters. The impact of EC on canola growth parameters was observed for root dry weight in loam where a decrease in root dry weight occurred in response to every treatment, with exception of treatment B (Table 6). ANCOVA results also showed that soil pH had a significant influence on canola pods and 100 seeds weight (Table 6). Changes in pH can affect nutrient uptake by increasing or suppressing mobility of nutrients present in the soil[20]. Increase in EC was also reported for a less productive soil where WSB and other organic amendments were utilised to test the response of maize to these soil additives[20].

The organic amendments improved carbon levels in all soil types. These substantial improvements in carbon can be attributed to the carbon rich nature of WSB[44], while rise in N levels can result from the nutrient rich nature of all these three organic amendments, where biochar has higher N levels, followed by compost and manure[21,45]. Improved uptake of N P K nutrients by the application of B, C and combinations can also result from an increase in efficiency of the canola plants to effectively use N fertilizer. Besides, this improvement can also result due to the biochar's low particle density, porous nature and nutrient retention potential. Improvement in soil properties and dry weight of crop as a result of the biochar and compost has also been reported for canola[46]. Improved soil fertility and crop production as a result of biochar and compost combination was also discussed by Sánchez-Monedero[18]. However, literature shows variations when it comes to evaluating the effect of biochar alone or biochar in combination with other organic amendments.

Conclusions

The present study was undertaken to evaluate the impact of organic amendments compost (C), manure (M), biochar (B) and their mixtures on canola growth and yield attributes cultivated in four different types of soils, loamy sand (LS), sandy loam (SL), silty clay (SC) and loam. The statistical analysis showed that the soil type, organic treatments and interaction between soil type and organic treatments had a significant impact on germination, chlorophyll, shoot length, root length and root dry weight. However, among the yield parameters, the significant effect by all sources was only noted for the number of pods, while the soil type and organic amendment interaction had impact on the 100 seeds' weight. SC under the treatment B produced the maximum seeds weight, while significant interaction effects was observed in LS for M + B treatment, in SL for CS, while for loam under M treatment. Among the different treatments, M + B, B, C and M were better promoters for soil carbon, N, K and P concentrations. The ANCOVA results with pH as a covariate clarified that pH significantly affected the number of pods and seeds weight among the yield parameters and shoot length among the growth parameters. These better yield results for SC appeared in the presence of biochar, which indicated that biochar was the most suitable amendment for promoting yield in this soil type. M + B also led to increased germination, chlorophyll and number of pods in SC. The results of this study imply that canola can improve well in SC with the application of biochar, manure or their combination at an appropriate rate which can intensify the capacity of soils to promote canola growth and yield. Further research is needed to test the impacts of organic amendments on canola in field settings to consider environmental variation and long-term effects. It is also important to explore the optimal rate when these amendments are applied in combination in field trials.

Data availability

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

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Author contributions

M.H. performed the research, conducted analysis and wrote the original manuscript.V.S. supervised the project, reviewed and contributed to writing of the final version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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