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ORIGINAL RESEARCH

Pigeon Pea Green Manuring and Nitrogen Fertilization Increase Agronomic Efficiency by Improving Yield and Ear Characteristics of Maize

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ABSTRACT: Green legume incorporation is an encouraging, at least unfinished, substitute for chemical fertilizers, particularly for nitrogen (N). The experiment was conducted in an RCB design with a split plot arrangement replicated four times. Pigeon pea green manuring (GM) of 3.4, 6.3 and 7.3 t ha^{-1} at pre flowering (GM1), at flowering (GM2) and post flowering (GM3) were assigned to the main plots, respectively, and nitrogen levels (N) (0, 70, 100 and 130 kg N ha^{-1}) were allotted to the subplots. Results showed that GM2 significantly improved plant height (183 cm) and leaf area (393.6 cm^2). Whereas, GM1 significantly enhanced biological yield (9826 kg ha^{-1}), grain yield (3500 kg ha^{-1}), thousand grain weight (203.6 g), grain ear^{-1}(319), ear length (18 cm) and ear diameter (11.4 cm) as compared to GM2. Similarly, nitrogen application at the rate of 130 kg ha^{-1} resulted in taller plants, higher leaf area, thousand grain weight, biological and grain yields, harvest index, grains ear^{-1}, ear height, length, weight and diameter than other N levels. The agronomic efficiency (AE) was significantly increased by 13.8 kg kg^{-1} and 11.8 kg kg^{-1} at GM1 and 70 kg N ha^{-1}, respectively. It was concluded from the outcomes of the study that pigeon pea GM at pre flowering stage and 130 kg N ha^{-1} improved maize crop production.

KEYWORDS: Maize, green manures, agronomic efficiency, ear characteristics, yield

1. Introduction

Maize (*Zea mays* L.) is a monoecious plant belongs to family Poacea. It is extensively grown in tropical, sub-tropical and temperate areas of the world. Among cereals crops in Pakistan, it stands third after wheat and rice. Maize is an important source of staple food for humans, feed for cattle and raw material for industry (Arif et al., 2010). It has nutritive value and contains 10% protein, 72% starch, 4.9% oil, 8.6% fibre, 1.8% ash and 3% sugar (Ali et al., 2015). In Pakistan, during the year 2018, maize crop was raised in the area of 1.22 million ha with 5701 thousand tons productivity and 3620 kg ha^{-1} average yield. In most of farming systems, it is used as a fodder crop and also a staple food in different rural areas of the country, especially at high elevations. The maize yield is still very low in Pakistan as compared to other advanced countries. The reasons for low production include poor soil preparation, weed infestation, improper fertilization and low soil organic matter.

Green manure (GM) is the practice of incorporating green and immature crops especially leguminous into the soil for the purpose of improving and fertilizing the soil as it makes available both biologically fixed and mineralized N to the soil (Adesoji et al., 2013). Incorporation of legumes as GM has been used to increase the fertility of soil by adding nutrients and building soil organic matter (Fabumni et al., 2012a). Both legumes and non-legumes have been used successfully to improve the growth and yields of tropical species, especially maize crops. In addition, the allocation of green manures also enhances the benefits of added fertilizers in terms of increased uptake due to the ability of the organic matter to retain nutrients in the rhizosphere (Sakala et al., 2003). Thus, green manures could help tropical smallholders to maintain soil fertility and use added nutrients, especially mobile elements such as N, more efficiently for successful crop production.

Green manures are either applied after being grown in situ during fallow periods, after harvest, or from external sources, when it is referred to as ex situ manuring (Aulakh and Grant, 2008). Incorporating legumes crops contribute greatly in building up of soil fertility and their ability to exploit the enduring water and nutrients in the subsoil that crops cannot utilize, withstand drought, and therefore produce higher yield. Moreover, it is a management practice that is environment friendly and capable of maintaining or building up soil fertility for sustainable maize production (Adesoji et al., 2013). Likewise, other benefits of legumes include opportunity to grow crops at the same time without degrading land and improved soil series and higher water infiltration rate because of their root movement (Rao and Mathuva, 2000). Practicing green manuring in agriculture is also the way of coping and overcoming the effect of change in climate which is presently a global and local concern. Thus, green manuring can reduce the dependence on chemical fertilizers as well as help to extend the period of soil cover (Fabunmi et al., 2012a).

Nitrogen is the most vital component that contributes greatly to the yield of crops and is the most restrictive element in crop productivity (Jin et al., 2012). N is the major yield determining factor and an important plant nutrient required for the production of maize (Adediran et al., 1995). When there is N deficiency in the soil, adding N improves the corn crop seed yield (Wienhold et al., 1995). Ideal management of N improves grain yield, farm profit and NUE while it decreases the chances of leaching N beyond the root zone of the crop (Raun and Johnson, 1999). Though the maize crop is very responsive to N fertilization; however, excessive or constant application of these chemical fertilizers decreases production, damages the quality of the soil, and some other issues of the environment, like contamination of soil water and nitrate leaching are evolved (Ali et al., 2015). Maximum efficiency is obtained when N is applied and is available for uptake by the plant as needed. This suggests that plant uptake of fertilizers N is more efficient when applied just prior to maximum plant need (Arif et al., 2010).

Residue incorporation and nitrogen application are being followed by the farmers who are well experienced in traditional agronomic practices but cannot adopt advance methods and techniques because of poor financial conditions, lack of education and technical skills. In the light of the economic and financial status of the farmers, their education and farming experience, legume incorporation and appropriate integrated nitrogen can be good options to reduce the cost of production. Researchers have experimentally tested these technologies individually; however, the collective effects of legume incorporation in combination with nitrogen have not been thoroughly explored. Therefore, the experiment was planned to investigate the effect of pigeon pea incorporation combined with N fertilizer to improve agronomic efficiency and maize production.

2. Materials and methods

2.1 Experimental Site

The field experiment was performed at the Agronomy Research Farm, The University of Agriculture, Peshawar, Pakistan (34° 1′ 2′′ N, 71° 28′ 5′′ E). The climate at the study site is subtropical and semi-arid, having a mean annual rainfall of 360 mm and mean maximum and minimum temperatures of 40 and 25 °C, respectively in summer from May to September. The soil was silty clay loam and alkaline calcareous (pH 8.23) with electrical conductivity (EC) of 0.16 dS m^{-1} (Table 1). Bulk density (BD) and cation exchanged capacity (CEC) of the soil was 1.35 Mg m^{-3} and 30.1 $cmol_c$ kg^{-1}, respectively. The soil had low native soil organic matter (total organic C 12.7 g kg^{-1} and TN 0.61 g kg^{-1}) and plant available nutrients (available N 23.7 mg kg^{-1} and P 3.20 mg kg^{-1}), and had adequate available K contents (85.8 mg kg^{-1}). Mean monthly rainfall and air temperature data were taken from the meteorological office of Peshawar and is presented in Figure 1.

2.2 Experimental design and treatments

The experiment was conducted in RCB design with a split plot arrangement having four replications. Pigeon pea incorporation (Pre flowering (50 DAS) GM1, at flowering (65 DAS) GM2 and post flowering (80 DAS) GM3 was allotted to the main plots while N levels (0, 70, 100 and 130 kg ha^{-1}) were allocated to subplots.

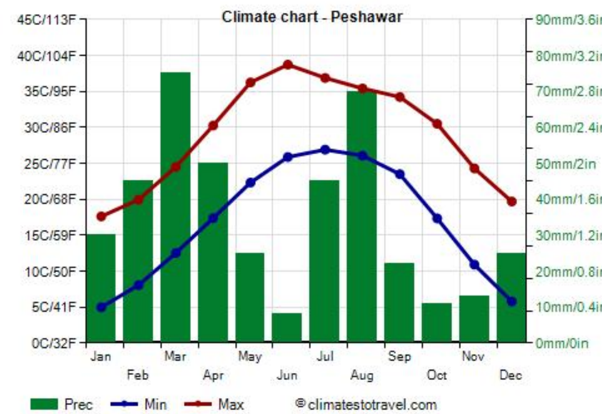

Figure 1. Mean monthly rainfall and air temperature of the experimental period

Pigeon pea crop was sown during the month of April (26th April) and hereafter fresh biomass of (3.4, 6.3 and 7.3 tons ha^{-1}) was incorporated at pre flowering, flowering and post flowering stage of crop, respectively. Azam variety was sown on 14th July after the treatment's application to experimental units. The size of the experimental units was 3 m × 3.5 m (10.5 m^2). Each experimental unit was comprised of five rows, maintained at a distance of 75 cm. A basal dose of 30 (kg ha^{-1}) P at sowing time was supplied from DAP. The amount of nitrogen received through the application of DAP was deducted from urea. Other agronomic practices like weeding, hoeing, thinning and irrigation were performed as required. The crop was harvested on October 20 at proper maturity.

2.3. Measurements

2.3.1. Yield attributes and root dry biomass

To determine leaf area (LA), ten randomly selected leaves were collected from each experimental unit, and their average length and width were measured, with width measurements taken near the stem's base, in the middle of the leaf, and near the tip. The average of these three values was calculated, and the mean leaf area per leaf (leaf^{-1}) was determined using the formula: leaf length × leaf width × CF (0.75). For plant height, data were recorded by measuring the height of five random plants in each plot, from the base to the tip, and these measurements were then averaged. Root biomass was quantified by randomly selecting and digging up five plants from the border rows of plots during the grain filling stage. Subsequently, the collected root samples were dried and weighed, and data were obtained from the three central rows at the time of harvesting. Plant numbers were calculated within each plot and then converted to plants per hectare (plants ha^{-1}) using the formula: counted plants per unit area × 10,000 m^{-2}. Thousand grains weight was determined by collecting 1000 grains from the seed lot of each plot and measuring their weight using a digital balance. For recording grain yield, the middle rows of the plots were harvested, and these harvested rows were dried in the sun and threshed separately. Grain yield was measured in the plots and converted into kilograms per hectare (kg ha^{-1}), with data collected from three harvested middle rows of subplots at maturity. Subsequently, the harvested rows were bundled and allowed to dry in the sun, and these bundles were then weighed to calculate the biological yield, which was also converted to kilograms per hectare (kg ha^{-1}). Finally, the harvest index was derived by dividing seed yield by the biological yield.

2.3.2. Ear characteristics

To record ear height, we randomly selected five plants in each plot and, measured the distance from the base to the ear, then calculated the averages. To determine the ear nodal position of maize, we selected five plants in each plot and counted their nodal positions from the base to the ear-bearing node. To assess productive plants, we recorded data by selecting three central rows, each one meter in length, in every plot. We calculated the total number of plants and then counted the eared plants among them. Ear length was measured by selecting five ears from each subplot and determining their length using a ruler; averages were then calculated. Maize ear weight was determined by collecting five ears from each sub-plot, weighing them, and calculating the averages. To calculate grain numbers, we randomly selected five ears from every experimental unit and counted the grains in each ear, averaging the results. Ear diameter was noted by selecting five ears from each experimental unit and measuring their diameter using a measuring scale. The data were averaged, and the total diameter, along with their circumference, was calculated using the expression: Diameter = $2\pi r$."

2.4. Statistical analysis

The recorded data were statistically analyzed using an analysis of variance procedure following a randomized complete block (RCB) design. Means of the data were compared using the least significant differences (LSD) test at a significance level of P ≤ 0.05 when a significant F-test was observed (Steel and Torrie, 1997). Figures were generated by using Prism 8.0 software.

3. Results

3.1. Yield traits

Table 1 exhibited the data of maize plant height as affected by green manuring and N. Both GM and N significantly influenced plant height. The interactive impact of GM × N was found non-significant. A plant height of 183 cm was noted in experimental plots incorporated at the flowering stage followed by plant heights of 175 cm and 173 cm from the plots incorporated at post flowering and pre flowering, respectively. Amongst nitrogen levels, taller plants (192 cm) were recorded from plots supplied with 130 kg N ha^{-1}. Likewise, plants with a height of 178 cm were noted from plots supplied with 100 kg N ha^{-1}. Short stature plants (165 cm) were noted in control plots.

GM and N also significantly influenced leaf area $leaf^{-1}$ (LAL) of maize (Table 1). The interaction of GM × N was not significant. Maximum LAL (393.6 cm^2) was observed from the treatment of green manure incorporated at the flowering stage followed by LAL of 376.7 cm^2 in plots where incorporation at pre flowering stage was done. This was statistically similar to LAL of 375.7 cm^2 in plots green manure was incorporated at post flowering stage. Among nitrogen levels the maximum LAL (412.5 cm^2) was recorded from 130 kg N ha^{-1} followed by LAL (382.9 cm^2) recorded in plots supplied with 100 kg N ha^{-1} in comparison with the lowest LAL (353.2 cm^2) from the control plots.

Data analysis indicated no impact of GM and N rates on plants at maize harvest (Table 1). The GM × N interaction was also not significant. Although the effects of GM and N rates were not significant on plants at harvest however, green manuring at post flowering stage and nitrogen fertilization by 130 kg ha^{-1} showed relatively more plants (67963 ha^{-1}) and (69012 ha^{-1}), respectively. The impact of incorporated legumes (pigeon pea) and nitrogen on grain ear^{-1} of maize was significant. However, the interactive effect of (GM × N) was non-significant (Table 1). Plots incorporated with GM at pre and at flowering stages showed relatively more grains ear^{-1} (319, 311, respectively) followed by 295 grains ear^{-1} from the plots incorporated with green manure at post flowering stage. Plots added with 130 kg ha^{-1} nitrogen produced maximum grains ear^{-1} (336), while the lowest grains ear^{-1} (274) were documented in plots where no N was used (control).

Effects of preceding legume crop incorporation and N rates were significant on 1000 seed weights of maize. However, the GM × N interaction was found to be non-significant (Table 1). Heavier grains (203.6 g) were noted in plots with the incorporation of GM at pre flowering stage. The minimum weight of grains (195.3 g) was noted in plots incorporated with green manure at post flowering stage. Among nitrogen levels, plots applied with 130 kg N ha^{-1} had higher seed weight (214.7 g) which was statistically at par with thousand grains weight (202.6 g) from the plots added with 100 kg ha^{-1} N. Lower thousand seed weight (185 g) was observed in control plots.

Table 1. Response of green manuring and nitrogen fertilizer on root biomass, yield and yield components of maize

Treatments	Plant height (cm)	Plants at harvest (ha^{-1})	Root biomass (g)	Leaf area leaf^{-1} (cm^2)	1000 grain weight (g)	Biological yield (kg ha^{-1})	Grain yield (kg ha^{1})	Harvest index (%)
Green manuring (GM)								
GM1	173b	67500	4.5a	376.7b	203.6a	9826a	3500a	35
GM2	183a	66574	4.2b	393.6a	197.3ab	9588b	3258b	35
GM3	175b	67963	4.0c	375.7b	195.3b	9568b	3235b	34
LSD	6.40	ns	0.20	7.50	6.60	219.5	196.14	ns
F-value	7.88	1.32	9.57	21.36	5.22	5.46	6.74	2.55
Nitrogen levels (N kg ha^{-1})								
N1	165c	66419	4.1	353.2c	18.50c	8228d	2669d	33b
N2	174bc	66296	4.3	379.3b	192.7bc	9221c	3201c	35ab
N3	178b	67654	4.2	382.9b	202.6ab	10272b	3588b	35ab
N4	192a	69012	4.3	412.5a	214.7a	10921a	3867a	36a
LSD	10.23	ns	ns	15.48	12.8	522.9	191.94	2.20
F-value	10.65	1.93	0.67	20.75	8.44	61.55	61.57	5.42
Interaction								
GM x N	ns	ns	ns	ns	ns	ns	347.3	ns

Note: Nitrogen levels represents N1=0, N2=70, N3; 100; and N4=130 kg N ha^{-1}, and pigeon pea green manuring represented by GM1 (3.4 t ha^{-1} at 50 days of pre flowering); GM2 (6.3 t ha^{-1} at 65 day of flowering); GM3 (7.3 t ha^{-1} at 80 days of post flowering). Means of the same category followed by different letters are significantly different at 5 % level of probability.

Table 2. Variations in the ear characteristics of maize with the application of green manuring and N fertilization

Treatments	Productive ear plant^{-1}	Grains ear^{-1}	Ear height (cm)	Ear nodary position	Ear length (cm)	Ear weight (g)	Ear diameter (cm)
Green manuring (GM)							
GM1	6.5	319a	77	7	18a	225a	11.4a
GM2	6.4	311a	72	7	17a	212b	12.0a
GM3	6.6	295b	72	7	16b	209b	11.7ab
LSD	ns	14.10	ns	ns	0.90	10.90	0.30
F-value	2.79	9.05	3.42	0.26	7.66	7.74	7.68
Nitrogen levels (kg ha^{-1})							
N1	6.4	274b	64c	6	16c	194c	11.3c
N2	6.3	294b	71b	7	17b	202c	11.5bc
N3	6.5	329b	77ab	7	17b	223b	11.7b
N4	6.7	336a	83a	7	18a	243a	12.2a
LSD	ns	25.7	6.26	ns	0.74	18.55	0.38
F-value	2.65	11.00	13.25	2.69	8.64	11.72	7.64
Interaction							
GM x N	ns	ns	ns	ns	ns	ns	ns

Note: Nitrogen levels represents N1=0, N2=70, N3; 100; and N4=130 kg N ha^{-1}, and pigeon pea green manuring represented by GM1 (3.4 t ha^{-1} at 50 days of pre flowering); GM2 (6.3 t ha^{-1} at 65 day of flowering); GM3 (7.3 t ha^{-1} at 80 days of post flowering). Means of the same category followed by different letters are significantly different at 5 % level of probability

3.2. Crop yield

Data analysis revealed that green manure and N levels significantly affected grain yield of maize. A higher grain yield (3500 kg ha^{-1}) was noted in plots incorporated with GM at pre flowering stage. Similarly, plots incorporated with GM at the flowering stage showed grain yield of 3258 kg ha^{-1} compared with a grain yield of 3235 kg ha^{-1} from the post flowering stage. Among N levels, grain yield of 3867 kg ha^{-1} was noted in plots supplied with 130 kg N ha^{-1} followed by seed yield of 3588 kg ha^{-1} from the plots applied with N at the rate of 100 kg ha^{-1}. Lower grain yield (2669 kg ha^{-1}) was recorded in control plots. The interactive effect (GM × N) was also significant. Figure 2 showed that increment in N from 0 to 130 kg ha^{-1} raised grain yield linearly for green manure incorporation stages. Moreover, once N was raised from 100 to 130 kg ha^{-1} grain yield declined for green manures incorporated at post flowering stage. The highest grain yield (4081 kg ha^{-1}) was observed with 130 kg ha^{-1} N and incorporation at pre flowering stage. Increasing N dose from 100 to 130 with legumes incorporated at flowering and post flowering stage indicated no rise in grain yield. Data analysis indicated that both the treatments (GM and N) had significant impact on maize biological yield (Table 1). More biological yield (9826 kg ha^{-1}) was recorded from green manure incorporated at pre flowering stage. Likewise, experimental units incorporated at flowering stage resulted in biological yield of 9588 kg ha^{-1}. Lower biomass (9568 kg ha^{-1}) was recorded in plots incorporated with green manure at post flowering stage. The interactive effect of GM × N was non-significant. Comparing different N levels, plots supplied with 130 kg ha^{-1} N resulted in more grain yield (10921 kg ha^{-1}) followed by biological yield (10272 kg ha^{-1}) from 100 kg ha^{-1} N. The lowest biological yield of 8228 kg ha^{-1} was observed in control plots. N levels significantly affected the harvest index. Impact of GM and association of GM × N was found non-significant. Plots applied with 130 kg ha^{-1} N resulted in more harvest index (36%). Similarly, experimental units supplied with 100 kg ha^{-1} N revealed harvest index of 35% while minimum harvest index (33 %) was documented in control.

3.3. Ear characteristics of maize

Maize productive ear plants as impacted by GM and N are exhibited in Table 2. Analysis of the data showed a significant effect of N and GM on productive ear plants. Interactive impact of (GM × N) was also found non-significant. Incorporation at post flowering stage and 100 kg ha^{-1} nitrogen indicated relatively more productive plants. Likewise, data analysis showed a significant impact of N on corn ear height but the effect of (GM × N) was non-significant. Plants with ear height of 83 cm were measured in plots with 130 kg ha^{-1} nitrogen. Likewise, 100 kg ha^{-1} N showed ear height of 77 cm. The minimum ear height (64 cm) were reported in control plots.

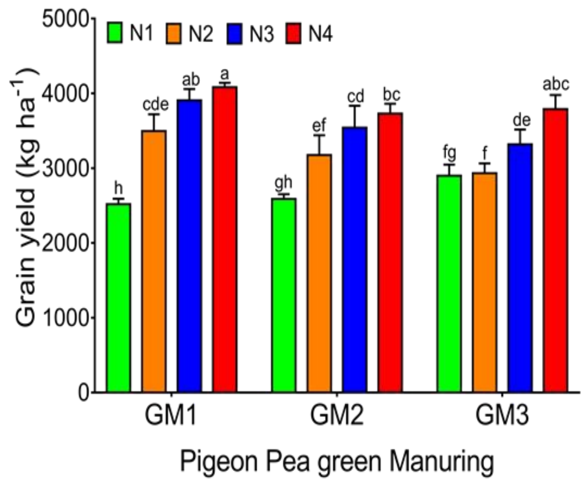

Figure 2. Variation in grain yield with respect to green manuring at different crop stages with nitrogen fertilization.

Data analysis showed no significant effects of green manuring and N on ear nodary position of maize (Table 2). The interactive effect of GM × N was also non-significant. Significant effects of both N and green manuring were found on ear length of maize (Table 2). Interactive effect of GM × N was found non-significant. Experimental units incorporated with green manure at pre flowering stage indicated higher ear length (18 cm). Similarly, ear length of 17 cm was recorded at flowering stage. The lowest ear length (16 cm) was recorded at post flowering stage. Comparing different levels of nitrogen,

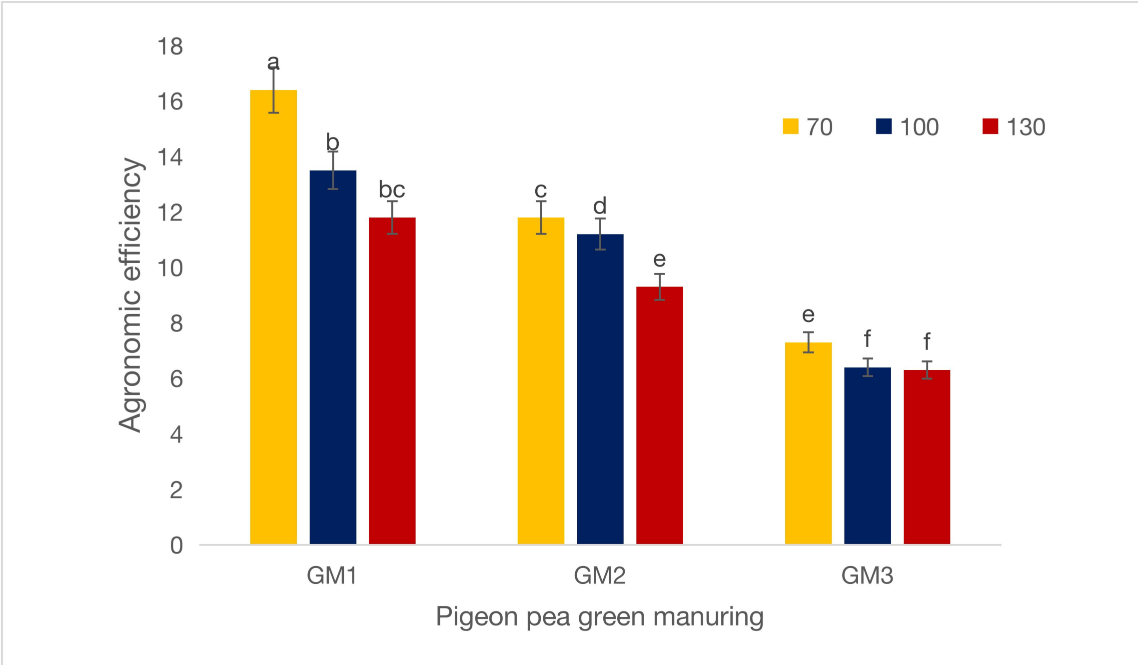

Figure 3. Variation in Agronomic efficiency with respect to green manuring at different crop stages with nitrogen fertilization.

Note: Nitrogen levels represents N1=0, N2=70, N3; 100; and N4=130 kg N ha^{-1}, and pigeon pea green manuring represented by GM1 (3.4 t ha^{-1} at 50 days of pre flowering); GM2 (6.3 t ha^{-1} at 65 day of flowering); GM3 (7.3 t ha^{-1} at 80 days of post flowering). Means of the same category followed by different letters are significantly different at 5 % level of probability.

maximum ear length (18 cm) was noted in plots with the application of 130 kg N ha^{-1}. Likewise, ear length of 17 cm was noted in plots with 70 and 100 kg N ha^{-1}. The lowest ear length of 16 cm was recorded in control.

Analysis showed that GM and N significantly affected the ear weight of maize (Table 2). Maximum ear weight (225 g) was noted with green manure at pre flowering stage followed by 212 g in plots with GM at flowering stage though it was not statistically different from ear weight of 209 g in plots incorporated with GM at post flowering stage. Among nitrogen levels, application of 130 kg ha^{-1} N indicated ear weight of 243 g followed by 223 g ear weight from 100 kg N ha^{-1}. Lower ear weight (194 g) was noted in control. Data analysis indicated that both GM and N had significantly influenced ear diameter (Table 2). Maximum ear diameter (12 cm) was recorded in plots with GM at flowering stage followed by ear diameter (11.7 cm) in plots with GM at post flowering stage while least ear girth (11.4 cm) was measured in plots with GM at pre flowering stage. Comparing levels of nitrogen, maximum ear diameter (12.2 cm) was measured in plots with application of 130 kg N ha^{-1}. Likewise, plots with 100 kg N ha^{-1} had ear diameter of 11.7 cm. The lowest ear diameter (11.3 cm) was recorded in control.

Table 3. Agronomic efficiency (kg kg^{-1}) as affected by green manuring and N fertilizer levels.

Treatments	Agronomic efficiency (kg kg^{-1})
Green manuring	
GM1	13.8
GM2	10.8
GM2	6.6
LSD	2.4
Nitrogen Levels	
N1	0
N2	11.8
N3	10.3
N4	9.1
LSD	1.9
Interaction	
GM*N	3.3

3.4 Agronomic efficiency

Green manuring and N levels significantly affected agronomic efficiency (Table 3). Plots with GM at pre flowering stage had higher agronomic efficiency (AE) of 13.8 kg kg^{-1} followed by green manuring at flowering stage (10.8 kg kg^{-1}). Green manuring at post flowering stage resulted in the lowest AE (6.6 kg kg^{-1}). Among N levels, the N application at the rates of 70 and 100 kg ha^{-1} had higher and statistically similar AE (11.8 and 10.3 kg kg^{-1}, respectively) as compared to 130 kg N ha^{-1} (9.1 kg kg^{-1}).

4. Discussion

Green manure had significantly affected the root biomass (g $plant^{-1}$) of maize. The impact of N and interactive effect of (GM× N) was found non-significant. Green manure at pre flowering stage resulted in more root biomass followed by root biomass of plots incorporated at flowering stage. The lowest root biomass was recorded at post flowering stage. The increase in root biomass with green manuring may be due to application of legumes, has improved soil properties which tend to produce more biomass as a result of porous soil. These finding are in line with Sangakkara et al. (2004) who specified development in growth and root weight with green manure. The Pigeon pea green manured at flowering stage showed maximum leaf area compared with incorporation at pre and post flowering stage. Likewise, plots fertilized with nitrogen produced maximum leaf area. Interaction of (GM × N) for leaf area was found non-significant. Increase in leaf area with incorporated legumes and fertilization of N could be due to the accessibility of N in soil. The documented outcomes are in similarity with Ali et al. (2015). Likewise, Onasanya et al. (2009b) stated, plots treated with N indicated greater plant LA and also leaf area $plant^{-1}$ in comparison of plots in which no N was supplied. These conclusions are also in line with Cox et al. (1993). Maximum height was measured by 130 kg ha^{-1} N, which could be because of more vegetative crop growth and development triggered by N. These outcomes are in accord with those of Fabunmi et al. (2012b) who noted improvement in height of plant with green manuring. Akmal et al. (2010) also reported increased in height of plant with N. The effect of legume green manure and N was significant for yield components (grain ear^{-1} and 1000 seed weight) of maize crop. Green manure at pre flowering stage showed more grains ear^{-1} and thousand grain weight followed with green manure at flowering stage. Lowest grains ear^{-1} and 1000 seed weight was noted at post flowering stage. The obtained outcomes are in similarity with those of Fabunmi et al. (2012b). Likewise, Zakikhani et al. (2016) reported increased in grains number and seed weight with green manure incorporation. Among N levels, application of 130 kg ha^{-1} N indicated more grains number and higher seed weight. The increased in grains numbers and grain weight could be due to the possibility that, N improve yield and yield traits. Nitrogen also increases the availability of nutrients as a result more grains ear^{-1}. The obtained outcomes are in similarity with Amanullah et al. (2009) who specified enhancement in grain ear^{-1} with increment in N. Arif et al. (2010) stated that, nitrogenous fertilizers to maize improved yield traits and yield. The higher seed yield, more grain numbers and more biomass accumulation is because of more exploitation of solar energy, greater production of assimilate and its conversion to starches as a result more grain numbers its weight and greater biomass and grain yield Derby et al. (2004). Nitrogen rates can enhance yield and attributes of yield as reported by El-sheikh et al. (1998). Documented outcomes are in promise with those of Mahmood et al. (2001).

Growing legumes as green manures and nitrogen application had significantly higher grain yield. Interaction of (GM × N) was also found significant. Green manuring at pre flowering stage showed higher grain yield followed by flowering stage. The significant response of grain yield on green manured plots could be ascribed to the nutrients released from the incorporated biomass of legume. The obtained outcomes are in similarity with those of Fabunmi et al. (2012b) and Rao et al. (1983) who documented greater maize seed yield with green manure. N dose of 130 kg ha^{-1} indicated greater seed yield followed with 100 kg N ha^{-1}. The obtained outcomes are in line with Azeem et al. (2014) who obtained more seed yield with 200 kg N ha^{-1}. Productivity of more assimilates depend on more exploitation of solar radiations and its transformation to starches occasioned more seed weight and number that resulted in higher seed yield and biomass Derby et al. (2004). N can increase yield traits and maize yield El-shekh et al. (1998). Significant impact of incorporated green legumes and nitrogen was reported for maize biological yield. Highest maize biological yield was noted from green manure at pre flowering stage followed by flowering stage. The favorable growth and rise in biological yield by legume green manured plots could be due to the increase in the quantity of N fixed by legumes and total of N derived from the incorporated green manure by decomposition. Significant maize growth as a result of green manure was observed by Tanimu et al. (1999). Likewise, William et al. (1992) described increased biomass yield when legumes were incorporated into the soil. Interaction of (GM × N) was noted non-significant. Application of 130 kg N ha^{-1} indicated higher biomass yield followed by 100 kg ha^{-1} N. The more biological yield is possibly because of greater crop vegetative. Imran et al. (2015) documented highest biological yield by 150 kg N ha^{-1}. The obtained outcomes are also in line with those of Akmal et al. (2010). Harvest index which is the competence and capability of crop for transforming the whole dry matters into economic yield was significantly affected by N. Experimental fertilized with 130 kg ha^{-1} nitrogen indicated higher harvest index followed by 100 kg N ha^{-1}. Lowermost harvest index was calculated in the control plot. The differences in harvest index with N due to the vital role of N in plant vegetative growth. Mahmood et al. (2001) obtained maximum harvest index with 180 kg ha^{-1} N. Likewise, differences in harvest index with N were obtained by Sharifi et al. (2016). Lawrence et al. (2008) specified that, the harvest index in corn increases when N rates increases.

Minimum ear length and ear weight were documented in plots incorporated at post flowering stage. ear length and weight generally declined with decreasing nitrogen rates, higher ear length and ear weight resulted from the plots with 130 kg ha^{-1} N. Likewise, with application of 100 kg N ha^{-1}. Lowest ear length and ear weight were noted in control. These findings are in line to Fabunmi et al. (2012). Bakht et al. (2007) who described that ear length improved with increasing N rates. These outcomes are also in promise with Imran et al. (2015) who recorded rise in ear weight with N. further, more ear diameter was observed with green manure at flowering stage followed with post flowering stage. Lowest ear diameter circumference was recorded with incorporation at pre flowering stage. The obtained findings are in accordance with those of Fabunmi and Balogun, (2015) who documented significant influence of green manuring on ear diameter of maize crop. Comparing different levels of nitrogen, maximum ear diameter was noted with 130 kg ha^{-1} N followed with 100 kg N ha^{-1}. The documented outcomes are in agreement with Ogunlela et al. (1998) and Onasanya et al. (2009a) who specified substantial influence of nitrogen on ear diameter.

Agronomic efficiency (AE) calculated in units of yield increase per unit of nutrient applied. Green manuring at pre flowering stage with 130 kg ha^{-1} resulted in more agronomic efficiency. It might be due to the availability of nutrients from the green manuring which was done early and the nitrogen applied. Vanlauwe et al. (2011) stated that application of organic resources in combination with N fertilizer improving agronomic efficiency. These results are also in accordance with those of Fixen et al. (2015). They reported that efficiency measures are greatly influenced by nutrient rate applied, residues, crop management, and by soil fertility.

5. Conclusion

Preceding legume (Pigeon pea) as a green manure had enhanced yield traits and yield of maize crop. Pigeon pea green manure incorporated at pre flowering stage enhanced root biomass, grain number ear^{-1}, thousand seeds weight, grain and biological yield. The application of 130 kg N ha^{-1} showed highest grains numbers, thousand seeds weight, seed yield, biological yield and maize harvest index. Further, Pigeon pea green manuring at pre flowering stage with 130 kg N ha^{-1} showed higher grain yield. Thus ccultivating preceding legume (Pigeon pea) as green manured at pre flowering stage integrated with 130 kg N ha^{-1} fertilization are endorsed for greater grain yield and productivity of maize.

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ORIGINAL RESEARCH

Identification of Heat Stress Tolerant Wheat Genotype Using Stress Tolerance Indices

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ABSTRACT: This experiment was conducted to identify heat stress tolerant wheat genotypes using stress tolerance indices. A total of twenty wheat genotypes, provided by the National Wheat Research Program (NWRP) in Bhairahawa, were evaluated in both irrigated and heat stress environments. These genotypes comprised three Bhairahawa Lines (BL), fifteen Nepal Lines (NL), and two commercial checks—Bhrikuti and Gautam. The research was conducted at the Institute of Agriculture and Animal Science (IAAS) in Paklihawa, using alpha lattice design. Results showed that the mean grain yield of wheat was reduced by 24.82% under heat stress conditions as compared to irrigated conditions. Notably, mean productivity (MP), geometric mean productivity (GMP), stress tolerance index (STI), and yield index (YI) exhibited strong and highly significant positive correlations with yield under both irrigated and heat stress conditions. In contrast, tolerance index (TOL) and stress susceptibility index (SSI) displayed negative correlations under heat stress conditions. Genotype NL 1384 exhibited the highest MP, GMP, and STI, closely followed by NL 1417, establishing them as the most stable and productive genotypes. These findings suggest that these genotypes have the potential to be selected for high yields under both irrigated and heat stress conditions. The biplot analysis showed a positive correlation of MP, STI, GMP, YI, and yield stability index (YSI) with yield in the irrigated environment (Ys) and yield in the heat stress environment (Yp), and a negative correlation of stress susceptibility index (SSI), TOL, and reduction (Red). Hence, these indices could potentially be used for the evaluation of wheat genotypes under both irrigated and heat stress conditions.

KEYWORDS: *Triticum aestivum* L., abiotic stress, heat stress, tolerant, yield, stability

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1. Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop that supplies substantial quantities of protein and calories worldwide (Chand et al., 2022; Fu et al., 2023). It contributes to around 30% of world grain production and 20% of grain production in Nepal (Akter & Rafiqul Islam, 2017; Timsina et al., 2018). Wheat ranks first in world grain production, cultivated across approximately 217 million hectares with a productivity of 3460 kg ha-1 as of 2018 (Erenstein et al., 2022). In Nepal, wheat is the third most important crop, grown on around 711,067 hectares with a productivity of 2990 kg ha^{-1} (MoALD, 2021). Its contribution to Nepal's GDP and AGDP is

2.30 percent and 6.98 percent (Gairhe et al., 2017). In the year 2020-2021, wheat production in Nepal reached 2.13 million metric tons, showing a growth of only 15.22% from 2011 to 2020 (MoALD, 2021).

Temperature range for the cultivation of wheat is relatively narrower whose suitable range during sowing is 10°C-15°C and during ripening period is 21°C-26°C (Poudel et al., 2020). Increase in temperature above the specified range for a significant period damages the plant growth and development (Iqbal et al., 2017). Most of the wheat growing areas of South Asia are affected by heat stress. Anthesis in wheat occurs in mid-march and at this time, western hot wind blows with sudden increase in temperature in Terai area of Nepal (Poudel et al., 2021). Constant high temperature or transition of temperature cause change in morphology, physiology and biochemistry of a plant. These effect plant growth and cause heavy reduction in economic yield (Hossain et al., 2012). The early flowering or anthesis stage is regarded to be most sensitive to heat stress (Riaz et al., 2021). High temperature during development of wheat pollen inhibits translocation of nutrient and decrease pollen viability (Kumar & Nagora, 2023). Complete sterility may occur when temperature is greater than 30°C during floret formation. Grain yield is reduced when ambient temperature exceeds 22°C during the period between anthesis to grain maturity (Kamrani et al., 2018). It is estimated that for each degree rise in temperature, 3-17% yield loss occurs (Pokhrel et al., 2019).

There is an urgent need to enhance crop yield to fulfill demand due to the rapid increase in population (Poudel et al., 2020). Additional 198 million tons of wheat would be required for world by 2050 as per FAO (Singh et al., 2021). Breeders are trying hard to develop heat and water tolerant wheat varieties (Poudel et al., 2020). Increasing heat stress tolerance in wheat has been felt as great challenge by wheat breeders. The step required to increase heat stress tolerance in wheat is to screen wheat genotypes by breeders to recognize germplasm having better heat tolerance (Kamrani et al., 2018).

Effect of heat stress can be evaluated by the use of various indices and some of these are Tolerance index (TOL), Stress Susceptibility Index (SSI), Yield Stability Index (YSI), Mean Productivity (MP), Geometric Mean Productivity (GMP), Yield Index (YI), and Stress Tolerance Index (STI) (Fernandez et al., 1992). Stress Tolerance (TOL) is defined as the difference between yield in stress environment (Ys) and yield in non-stress environment (Yp) and mean productivity as average of Ys and Yp (Rosielle & Hamblin, 1981). (Fischer & Maurer, 1978) proposed Stress Susceptibility Index (SSI). (Fernandez et al., 1992) defined STI to identify high yielding genotypes under stress condition. This experiment was conducted to evaluate heat stress tolerance in wheat genotypes using various stress tolerance indices and disclose heat tolerant genotype in Terai region of Nepal among the tested twenty wheat genotypes.

2. Materials and methods

2.1 Experimental site

The field experiment was carried out at Institute of Agriculture and Animal Science (IAAS), Paklihawa, Nepal. Research site is located at 27°30′N, 83°27′E and 79 meter above sea level. The experimental materials consisted of twenty wheat genotypes provided by National Wheat Research Program (NWRP), Bhairahawa. There were three Bhairahawa Lines (BL), fifteen Nepal Lines (NL), and two commercial check Viz., Bhrikuti and Gautam. The list of all the genotypes used in the experiment are presented in Table 1.

2.2. Experimental design

The field experiment was conducted using alpha lattice design consisting of two replications and five blocks. The size of the experimental unit was 4 m^2 (2m × 2m) and each genotype was planted at a row to row spacing of twenty-five cm. The inter-plot space was kept fifty cm and inter block spacing was kept one m. Spacing between two replications was one m. Experiment was conducted in two environments: irrigated as normal season and heat stress as late season.

Land preparation was done by tractor and final levelling was done manually. Wheat genotypes were sown in their respective plot by line sowing method. Eight rows were made in each plot leaving 12.5 cm border and inter-row spacing 25 cm. Sowing was done on November 25, 2022 in irrigated environment and on December 25, 2022 in heat stress environment.

The seed rate was maintained at 100 kg per hectare. The recommended dose of 120:50:50 kg NPK per hectare was applied in both conditions (MoALD, 2021). Full dose of DAP and MOP and half dose of nitrogen was applied as basal dose at the time of sowing. The remaining dose of nitrogen was applied in two splits: one at 30 DAS another at 70 DAS. Pre-sowing irrigation was done and remaining irrigation was done at crown initiation stage, booting stage, flowering stage, heading stage, milking stage and soft dough stage One weeding was done at 45 DAS.

2.3. Measurement and analysis

The grain yield was taken by harvesting wheat from 2 m^2 area using sickle except from the border lines and threshing was done manually. Grain were weighed and converted to kg per hectare (kg ha^{-1}). Mean daily maximum and minimum along with the precipitation during wheat growing season at the experimental site is shown in Figure 1.

Eight stress tolerance indices were used in the evaluation of the genotypes. These were calculated by using following relationships:

1. Tolerance Index (TOL) = $Ys - Yp$ (Hossain et al., 1990)
2. Stress Susceptibility Index (SSI) = $(1 - (Ys/Yp))/SI$ (Fischer & Maurer, 1978)

   Where, Stress Intensity (SI)= $1-(\frac{\bar{Ys}}{\bar{Yp}})$ (Fischer & Maurer, 1978)
3. Mean Productivity (MP) = $(Ys + Yp)/2$ (Hossain et al., 1990)
4. Geometric Mean Productivity (GMP) = $\sqrt{(Yp \times Ys)}$ (Fernandez et al., 1992)
5. Stress Tolerance Index (STI) = $(Ys \times Yp)/\bar{Yp}^2$ (Fernandez et al., 1992)
6. Yield Index (YI) = $Ys/\bar{Ys}$ (Khan & Kabir, 2015)
7. Yield Stability Index (YSI) = Ys/Yp (Bouslama & Schapaugh Jr., 1984)
8. Reduction (Red) = $[(Yp - Ys)/100] * 100$ (Bennani et al., 2017)

Table 1: List of all genotypes used in experiment.

S. N	Genotypes	Origin
1	Bhrikuti	CIMMYT, Mexico
2	BL 4407	Nepal
3	BL 4669	Nepal
4	BL 4949	Nepal
5	Gautam	Nepal
6	NL 1179	CIMMYT, Mexico
7	NL 1346	CIMMYT, Mexico
8	NL 1350	CIMMYT, Mexico
9	NL 1368	CIMMYT, Mexico
10	NL 1369	CIMMYT, Mexico
11	NL 1376	CIMMYT, Mexico
12	NL 1381	CIMMYT, Mexico
13	NL 1384	CIMMYT, Mexico
14	NL 1386	CIMMYT, Mexico
15	NL 1387	CIMMYT, Mexico
16	NL 1404	CIMMYT, Mexico
17	NL 1412	CIMMYT, Mexico
18	NL 1413	CIMMYT, Mexico
19	NL 1417	CIMMYT, Mexico
20	NL 1420	CIMMYT, Mexico

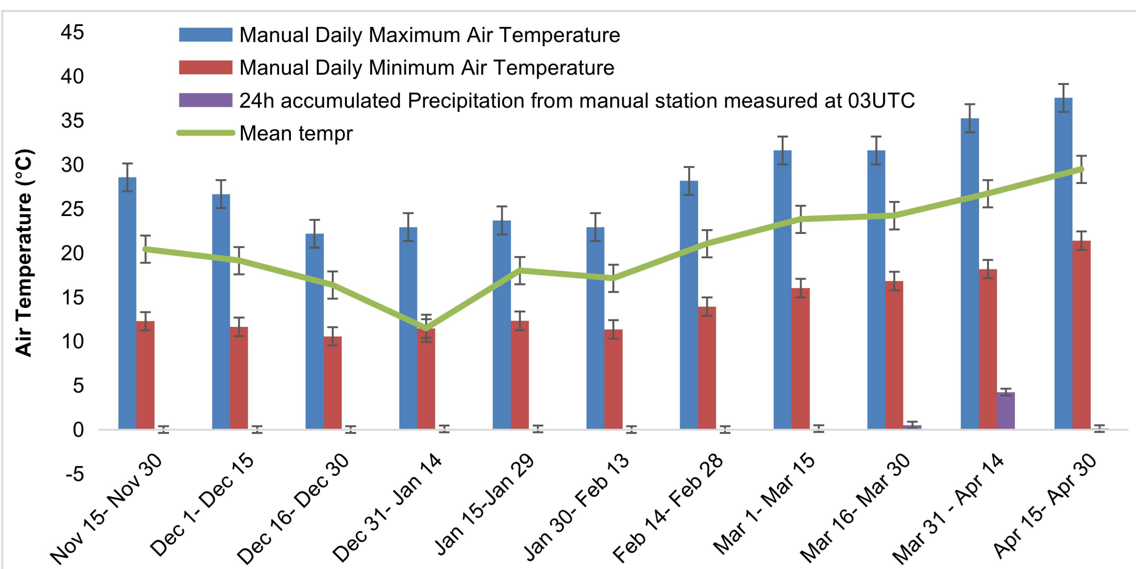

Figure 1. Manual daily maximum and minimum temperature along with precipitation from November 15 to April 30.

Whereas Ys indicates yield under heat stress condition, YP indicates yield under irrigated condition, $\bar{Ys}$ indicates mean of grain yield under heat stress and $\bar{Yp}$ indicates mean of grain yield under irrigated condition.

2.4. Statistical Analysis

Data entry and processing was done on Microsoft Excel office 2019. Stress tolerance indices were also calculated for evaluation of genotypes using Microsoft Excel office 2019. Analysis of variance for mean comparison, correlation among stress tolerance indices, principal component analysis and biplot analysis were performed using IBM SPSS statistics V. 25.

3. Results and Discussions

3.1. Yield performance

Under heat stress environment, days to booting (DTB), plant height (Ph), number of spikes per meter square (NSPMS), and thousand kernel weight (TKW) has shown significant difference among different genotypes as shown in Table 2 while DTB, days to heading (DTH), days to anthesis (DTA) and NSPMS has shown significant difference among wheat genotypes under irrigated environment as shown in Table 3. NL 1384 (3755 kg ha^{-1}), NL 1413 (3210 kg ha^{-1}), NL 1417 (3185 kg ha^{-1}) and NL 1420 (3010 kg ha^{-1}) yielded highest under irrigated condition. While NL 1384 (2473.33 kg ha^{-1}), NL 1412 (2320 kg ha^{-1}), Gautam (2293.33 kg ha^{-1}) and BL 4919 (2286.67 kg ha^{-1}) had shown highest yield under heat stress condition. Whereas, NL 1369 (1795 kg ha^{-1}) and NL 1368 (2015 kg ha^{-1}) had yielded lowest under irrigated condition. NL 1387 (1386.67 kg ha^{-1}) and NL 1369 (1406.67 kg ha^{-1}) had yielded lowest under heat stress condition as shown in Table 4. Similar result was reported by (Poudel et al., 2021), where maximum grain yield was observed in NL 1179 and Bhrikuti under normal and heat stress condition. Mean grain yield was found to be reduced by 24.82% under heat stress as shown in Table 4. Heat stress decreases growth cycle, number of tillers, photosynthetic area, chlorophyll content and increases photorespiration (Aberkane et al., 2021). This forces premature ripening which shortens number of grains/spikes and finally results in low grain yield (Jatoi et al., 2021). Yield reduction of wheat varies according to the severity of heat stress. Puri et al., 2015 reported 27.45 % reduction on yield. Higher temperature being linked with limitation of water is observed which causes rapid shrinkage of grain volume (Jatoi et al., 2021).

3.3. Stress tolerance indices

The highest TOL was recorded in NL 1413 followed by NL 1384 and NL 1386. TOL has negative correlation with yield under stress condition as shown in Table 5. So, these genotypes had high grain yield under non-stress condition while low yield under stress condition. So, they could be considered stress susceptible genotypes. Lowest TOL was recorded in NL 1179 and NL 1404. Both of these genotyp es yielded low in both irrigated and heat stress condition. Low TOL was due to low difference among yield in two conditions. NL 1413 had highest SSI while NL 1179 had lowest SSI which means NL 1413 is most susceptible genotype and NL 1179 is least susceptible genotype to heat stress. SSI value higher than one indicates above-average susceptibility, while SSI less than one indicates below-average susceptibility.

Table 2. Yield attributing characteristics of different wheat genotypes under heat stress environment.

Genotypes	DTB	DTH	DTA	Ph	SL	NSPMS	NSPS	NGPS	TSW	TKW	GY
1	65	68	71	81.2	9.7	205.0	14.8	33.1	22.3	41.4	1760.0
2	63	68	70	77.1	9.6	175.5	15.0	39.8	24.3	39.4	2240.0
3	66	69	71	75.7	8.6	199.5	17.2	34.1	18.8	36.2	1720.0
4	63	68	71	84.4	9.1	163.5	16.6	35.5	21.9	36.9	2286.7
5	67	68	70	82.9	8.7	258.5	16.4	40.7	20.2	32.7	2293.3
6	66	68	69	72.4	8.3	216.5	15.2	31.4	20.0	36.9	2260.0
7	64	68	69	75.9	9.2	223.0	14.5	33.0	20.2	38.4	2180.0
8	62	66	68	84.8	9.9	183.0	12.1	30.9	23.2	47.1	1633.3
9	65	68	70	73.3	8.6	220.5	15.0	33.3	18.8	36.7	1753.3
10	63	67	69	66.3	8.5	200.0	13.7	25.0	18.7	43.2	1406.7
11	66	68	71	79.4	9.2	127.5	16.7	31.9	21.5	38.4	1446.7
12	64	68	70	77.1	8.3	171.0	18.6	31.9	17.8	33.9	1673.3
13	68	71	73	82.1	9.7	273.5	17.1	41.6	21.5	32.9	2473.3
14	68	71	74	71.0	8.8	140.0	16.3	34.7	23.6	39.4	1480.0
15	63	67	72	65.6	8.1	186.5	13.4	27.4	17.6	39.0	1386.7
16	64	67	71	72.6	8.4	208.0	14.0	30.3	19.5	39.3	1946.7
17	66	68	69	84.4	8.9	232.0	16.0	32.4	20.0	39.5	2320.0
18	64	68	69	75.1	9.2	231.5	15.5	34.4	21.9	34.4	1766.7
19	66	69	71	80.1	9.5	215.5	17.8	38.4	21.4	32.9	2186.7
20	65	68	69	83.2	9.7	225.0	17.0	38.6	21.8	33.2	2206.7
Mean	65	68	70	77.2	9.0	202.8	15.6	33.9	20.8	37.6	1921.0
F value	**	ns	ns	**	ns	**	ns	ns	ns	**	ns

Note: DTB: Days to booting, DTH: Days to heading, DTA: Days to anthesis, Ph: Plant height, SL: Spike length, NSPMS: Number of spikes per meter square, NSPS: Number of spikelets per spike, NGPS: Number of grains per spike, TSW: Ten spike weight, TKW: Thousand kernel weight, GY: Grain Yield. For Genotypes see table 1.

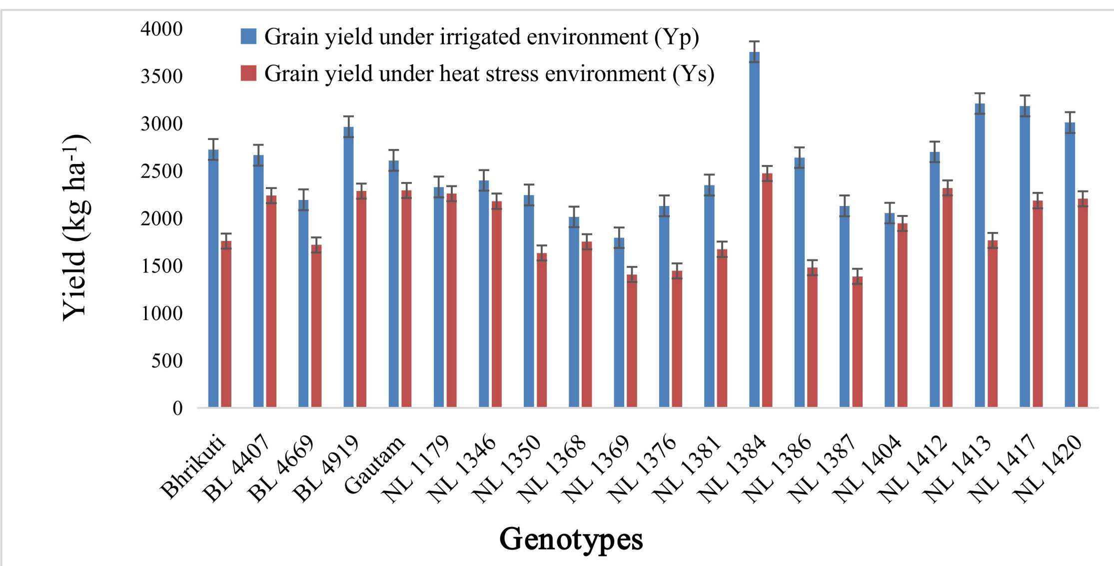

Figure 3. Yield of twenty wheat genotypes under irrigated (Yp) and heat stress condition (Ys).

Low TOL and SSI does not mean the genotype is high yielding. Grain yield must also be taken under consideration while selecting stress tolerant genotype. (Thapa et al., 2022) also stated to take grain yield into consideration. Highest MP, GMP and STI was obtained in genotype NL 1384 followed by NL 1417. Hence, they were concluded as most stable and most productive genotypes among the cultivated 20 genotypes. (Kamrani et al., 2018) also concluded genotypes having highest MP, GMP and STI as highest producing genotypes. According to YSI, NL 1179, NL 1404 and Gautam were identified as more stable and heat tolerant genotypes under heat stress condition.

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Table 3: Yield attributing characteristics of different wheat genotypes under irrigated environment

Genotypes	DTB	DTH	DTA	Ph	SL	NSPMS	NSPS	NGPS	TSW	TKW	GY
1	75	80	82	92.4	9.8	272.0	17.3	695.0	38.2	45.8	2725.0
2	71	74	79	86.2	9.6	256.5	15.1	601.5	36.7	43.4	2665.0
3	73	77	80	85.9	9.6	247.5	17.4	569.0	37.4	42.6	2195.0
4	68	73	78	88.4	9.5	239.5	15.9	595.0	39.4	46.9	2965.0
5	77	82	84	90.5	9.4	314.5	17.5	585.0	37.7	38.1	2610.0
6	75	80	82	82.8	9.3	264.0	18.4	586.5	36.3	38.6	2330.0
7	69	75	78	74.6	9.5	201.0	16.8	590.0	32.5	38.2	2400.0
8	68	74	78	89.6	10.3	209.5	14.3	518.0	37.0	46.7	2245.0
9	70	75	80	78.3	9.9	259.5	18.0	629.0	36.0	37.2	2015.0
10	70	75	80	76.6	9.3	179.5	16.2	486.0	34.3	43.0	1795.0
11	72	78	81	79.4	8.9	203.5	15.0	536.5	31.0	39.2	2130.0
12	72	79	81	84.3	9.9	266.0	18.1	814.5	40.5	34.8	2350.0
13	77	82	84	93.8	10.6	379.0	19.3	674.0	36.3	35.8	3755.0
14	76	81	85	87.2	10.8	209.5	18.9	701.0	44.6	44.2	2640.0
15	72	80	81	80.0	10.1	225.5	17.1	501.5	37.8	42.2	2130.0
16	70	75	79	76.8	9.2	282.0	15.7	538.0	32.8	38.6	2055.0
17	69	79	81	91.6	9.0	350.5	15.5	386.0	28.7	46.8	2700.0
18	73	79	82	91.5	9.9	268.5	18.0	720.5	43.4	40.2	3210.0
19	75	80	81	94.2	10.2	273.5	18.2	645.5	40.2	44.1	3185.0
20	76	81	82	89.2	10.5	339.0	18.4	687.5	41.7	33.9	3010.0
Mean	72	78	81	85.7	9.8	262.0	17.1	603.0	37.1	41.0	2555.5
F value	**	**	**	ns	ns	**	ns	ns	ns	ns	ns

Note: DTB: Days to booting, DTH: Days to heading, DTA: Days to anthesis, Ph: Plant height, SL: Spike length, NSPMS: Number of spikes per meter square, NSPS: Number of spikelets per spike, NGPS: Number of grains per spike, TSW: Ten spike weight, TKW: Thousand kernel weight, GY: Grain Yield.

Table 4. Yield under irrigated and heat stress environment (kg ha^{-1}) with stress tolerance indices.

S. N	Genotype	Yp	Ys	TOL	SSI	MP	GMP	STI	YSI	YI	Red (%)
1	Bhrikuti	2725	1760.00	965.00	1.43	2242.50	2189.98	0.73	0.65	0.92	35.4
2	BL 4407	2665	2240.00	425.00	0.64	2452.50	2443.28	0.91	0.84	1.17	15.9
3	BL 4669	2195	1720.00	475.00	0.87	1957.50	1943.04	0.58	0.78	0.90	21.6
4	BL 4919	2965	2286.67	678.33	0.92	2625.83	2603.84	1.04	0.77	1.19	22.8
5	Gautam	2610	2293.33	316.67	0.49	2451.67	2446.55	0.92	0.88	1.19	12.1
6	NL 1179	2330	2260.00	70.00	0.12	2295.00	2294.73	0.81	0.97	1.18	3.0
7	NL 1346	2400	2180.00	220.00	0.37	2290.00	2287.36	0.80	0.91	1.13	9.1
8	NL 1350	2245	1633.33	611.67	1.10	1939.17	1914.90	0.56	0.73	0.85	27.2
9	NL 1368	2015	1753.33	261.67	0.52	1884.17	1879.62	0.54	0.87	0.91	12.9
10	NL 1369	1795	1406.67	388.33	0.87	1600.83	1589.01	0.39	0.78	0.73	21.6
11	NL 1376	2130	1446.67	683.33	1.29	1788.33	1755.39	0.47	0.68	0.75	32.0
12	NL 1381	2350	1673.33	676.67	1.16	2011.67	1983.01	0.60	0.71	0.87	28.7
13	NL 1384	3755	2473.33	1281.67	1.37	3114.17	3047.52	1.42	0.66	1.29	34.1
14	NL 1386	2640	1480.00	1160.00	1.77	2060.00	1976.66	0.60	0.56	0.77	43.9
15	NL 1387	2130	1386.67	743.33	1.41	1758.33	1718.60	0.45	0.65	0.72	34.9
16	NL 1404	2055	1946.67	108.33	0.21	2000.83	2000.10	0.61	0.95	1.01	5.2
17	NL 1412	2700	2320.00	380.00	0.57	2510.00	2502.80	0.96	0.86	1.21	14.0
18	NL 1413	3210	1766.67	1443.33	1.81	2488.33	2381.39	0.87	0.55	0.92	44.9
19	NL 1417	3185	2186.67	998.33	1.26	2685.83	2639.04	1.07	0.69	1.14	31.3
20	NL 1420	3010	2206.67	803.33	1.07	2608.33	2577.22	1.02	0.73	1.15	26.6
Mean		2555.5	1921.0	634.5	0.9	2238.2	2208.7	0.7	0.7	1.0	23.9

Note: YP; yield under irrigated condition, Ys-yield in the irrigated environment, TOL; tolerance index , SSI; stress susceptibility index ; GMP: geometric mean productivity, STI: stress tolerance index, YSI: yield stability index , YI: Yield index, Red: Yield Index.

Table 5. Correlation among yield under irrigated and heat stress condition and stress tolerance indices.

	Yp	Ys	TOL	SSI	MP	GMP	STI	YSI	YI	Red
Yp	1									
Ys	0.621**	1								
TOL	0.692**	-0.136	1							
SSI	0.416	-0.446*	0.936**	1						
MP	0.931**	0.865**	0.381	0.058	1					
GMP	0.901**	0.899**	0.312	-0.012	0.997**	1				
STI	0.909**	0.882**	0.337	0.014	0.994**	0.996**	1			
YSI	-0.416	0.446*	-0.936**	-1.000**	-0.058	0.012	-0.014	1		
YI	0.621**	1.000**	-0.136	-0.446*	0.865**	0.899**	0.882**	0.446*	1	
Red	0.416	-0.446*	0.936**	1.000**	0.058	-0.012	0.014	-1.000**	-0.446*	1

Note: * and ** denotes level of significance at 5 and 1%, respectively. Check table 4 for other abbreviation.

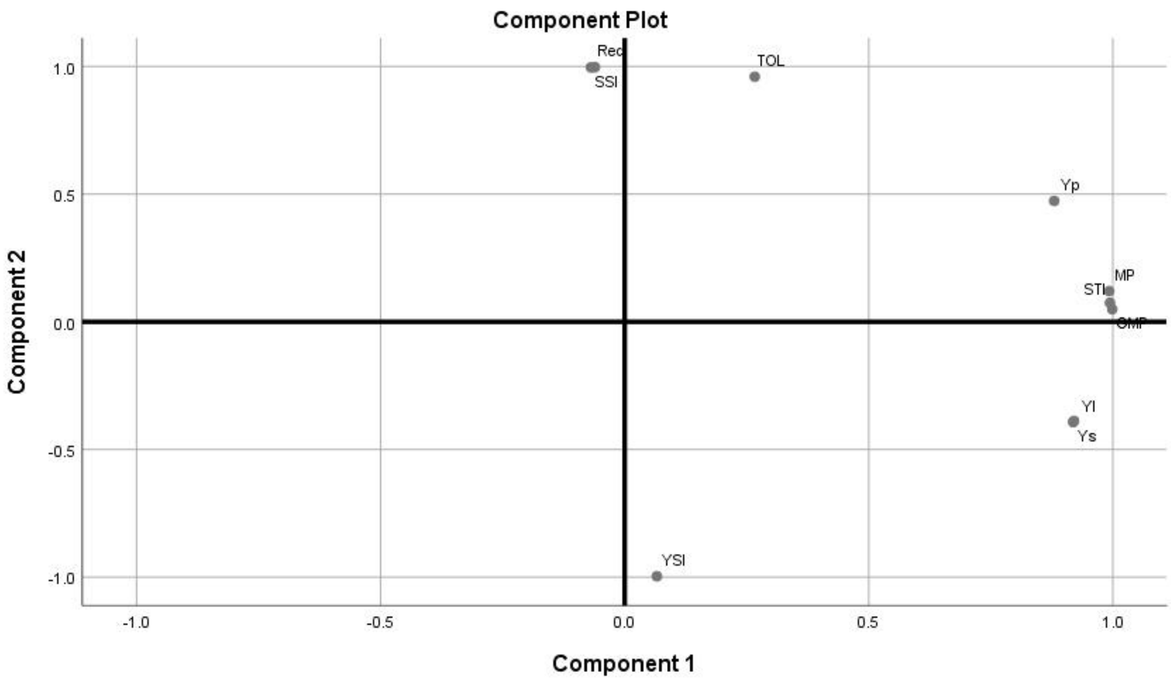

Figure 5. Biplot based on PC1 and PC2 using result of principal component analysis.

3.4. Correlation

To determine the most suitable heat stress tolerance selection criterion, we calculated the correlation between Yp, Ys, and other stress tolerance indices (Table 5). The analysis revealed a positive and significant relationship between grain yield under irrigated and heat stress conditions. This indicates that genotypes with high grain yield in normal irrigated conditions are likely to exhibit higher grain yields under heat stress conditions. A similar result was reported by Thapa et al. (2022), who found a positive and significant relationship between grain yield in irrigated and heat stress conditions.

On the other hand, SSI displayed a negative and significant correlation with yield under heat stress conditions, suggesting that an increase in SSI will lead to a significant decrease in yield. YSI exhibited a negative but non-significant correlation with yield under irrigated conditions, while it displayed a positive and significant correlation with yield under heat stress conditions. Therefore, selecting genotypes based on higher YSI and lower SSI values will help identify heat stress-tolerant genotypes. Poudel et al. (2021) also identified genotypes with higher YSI and lower SSI as heat-tolerant.

Additionally, MP, GMP, STI, and YI demonstrated positive and highly significant correlations with yield under both irrigated and heat stress conditions. As a result, these parameters MP, GMP, STI, and YI should be taken into consideration when selecting high-yielding genotypes under both conditions. These findings align with the results reported by Chand et al. (2022), Poudel et al. (2021), and Thapa et al. (2022).

4. Conclusion

Under heat stress condition, tested genotypes showed significant reduction in yield. So, it can be considered as one of the major causes of low wheat production. Grain yield had shown positive and highly significant correlation with MP, GMP, STI and YI in both environments. As a result, these are considered appropriate indices for the selection of high yielding genotypes under both irrigated and heat stress environment. NL 1384, NL 1412, Gautam and BL 4919 had shown higher production under heat stress condition with grain yield of 2473.33 kg ha^{-1}, 2320 kg ha^{-1}, 2293.33 kg ha^{-1} and 2286.67 kg ha^{-1} respectively. Hence, these genotypes can further be used for breeding program to cultivate in heat prone areas.

Conflicts of Interest: The authors declare no conflict of interest.

Availability of Data and Materials: Data will be available on formal request from the corresponding authors.

Authors Contributions: S Sharma, E Chaudhary, P Gautam, R Poudel, S Sapkota, S Ghimire, B Timalsina, P Roka, K Bhattarai, M Pariyar, K Neupane, A Aryal, and G G.C conducted field trial and collected data. S, Sharma analyzed the data and wrote the manuscript. MR Poudel and R Bhandari revised the final version of the manuscript.

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Review

Sources, Persistence, Ecotoxicology and Transformations of Anticancer Pharmaceutical Drug Residues in the Soil Environment: A Review

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ABSTRACT: Release and environmental consequences of drug residues pose a major challenge for soil quality management. This review aims to synthesis the literature related to the transformations of anticancer drugs at the soil-water interphase and their ecological effects. Pharmaceutical drugs including anticancer drugs originate form point and non-point sources of human and animal background. While detrimental effects of anticancer drug residues on human health are widely reported, a relatively little body of knowledge focus on their persistence, decomposition and interaction with soil biological health and quality. Assessment of potential ecotoxicological effects of the residues of anti-cancer drugs is far less frequent compared to other xenobiotics. However, a substantial concern is growing to understand the fate of these drug residues in the environment, particularly, under high environmental risk scenarios. Sewage sludge and hospital wastewaters are the primary sources of anticancer drug residues into the soil and their effects and transformations in soil depend on nature and persistence of drug residues. Depending upon their structure, anticancer drug residues can undergo biodegradation and biochemical transformations to form highly mobile molecules, which move into surface and ground waters, ultimately end up in the soil to alter microbial communities and their functions associated with flow of energy, nutrient cycling and ecosystem functions. This manuscript reviews the behavior of anticancer pharmaceutical residue in the soil environment in terms of effects on soil functions and quality by summarizing the limited available data.

KEYWORDS: Anticancer drugs, Microbial transformation, Drug degradation, Soil biological health, Soil quality

1. Introduction

The presence of pharmaceutical residues and their possible negative effects on non-target organisms have become an area of emerging concern in basic and applied research in environmental sciences over the last decade (Vazquez-Roig et al., 2010; Negreira et al., 2014). A global review has suggested the presence of 631 out of 713 pharmaceuticals and their metabolic products above detection limits in the environment (IWW, 2014). Cancer has become the second most dangerous and death-causing disease, and this has led to an enormous increase in the development and use of anticancer drugs and, consequently, their release into environment on global scale (Besse et al., 2012; Booker et al., 2014). Presence of anticancer drugs, also considered as emerging contaminants, is fetching a global concern due to their consistent release into the environment and potential adverse effects on ecosystems (Yadav et al., 2021). Emerging contaminants are defined as any synthetic or naturally occurring chemical that is not commonly monitored in the environment, although it has the potential to enter soil and aquatic ecosystems, causing known or suspected adverse ecological and/or human health effects (USGS, 2009). Unlike the pharmaceutical used in other therapeutic fields, anticancer drugs have quite different toxicological properties (Seira et al., 2013). Most of these drugs interfere with genetic material and consequently have carcinogenic, mutagenic and teratogenic potential and their residues represent hazardous contaminants that may enter water cycle and biosphere (Kümmerer and Al-Ahmad, 2010). Considering the environmental perspectives of these contaminants, the key steps in the life cycle of drug residues involve manufacturing, consumption and waste management. According to the European Environment Agency, the anticancer drug residues are identified from diffuse sources, through the discharge of human and animal excretion (EEA, 2010). In soils, residues of these drugs interact with clay minerals and organic matter following sorption and fixation processes, and these interactions are controlled by both environmental, soil and drug-based characteristics (Kumar et al., 2005). Many drug residues in the soil are directly ingested due their application via manures or sludge which increase human exposure to such drug residues and their metabolites.

1.1 Anticancer drugs in environment

The anticancer pharmaceuticals are released into the environment, mainly through municipal wastewater effluents, hospitals and live-stock activities (Kosjek et al., 2013; Isidori et al., 2016). Discharge of wastewater effluents into rivers and application of sludge amendments on the soil results in cascading drug residues through the environmental compartments (Fig. 1). Physicochemical analyses have indicated the presence of anticancer drug residues and their metabolites in aquatic environments such as wastewater, groundwater, surface water, and drinking water (Rowney et al., 2009; Besse et al., 2012). The sewage systems and wastewater from hospitals contains high concentration of drug residues because they neither undergo complete degradation during treatment process (Schuster et al., 2008; Loos et al., 2013; Zhang et al., 2013; Cesen et al., 2015). Landfills also receive pharmaceuticals from municipal waste disposal and, after the processes of biodegradation and adsorption, the pharmaceutics reach the groundwater and surface water resources (Musson et al., 2009). Extent of decomposition and biodegradation of these compounds depend on their physicochemical properties, especially during the sewage treatment processes, and when sewage sludge is applied to increase soil fertility, the residues contaminate soil and crops (Kumar et al., 2005; Gielen et al., 2009; Baresel et al., 2015; Haiba et al., 2016; Magnér et al., 2016). In addition, veterinary drugs from livestock farming also contaminate soil directly through manure and slurry (Song and Guo, 2014). The soil contamination, then, affects surface water, groundwater and the water intended for human consumption (Magnér et al., 2016). Although the drug residues occur as micropollutants and in low concentrations does not reduce their toxicological concerns because they consist of biological active mol-

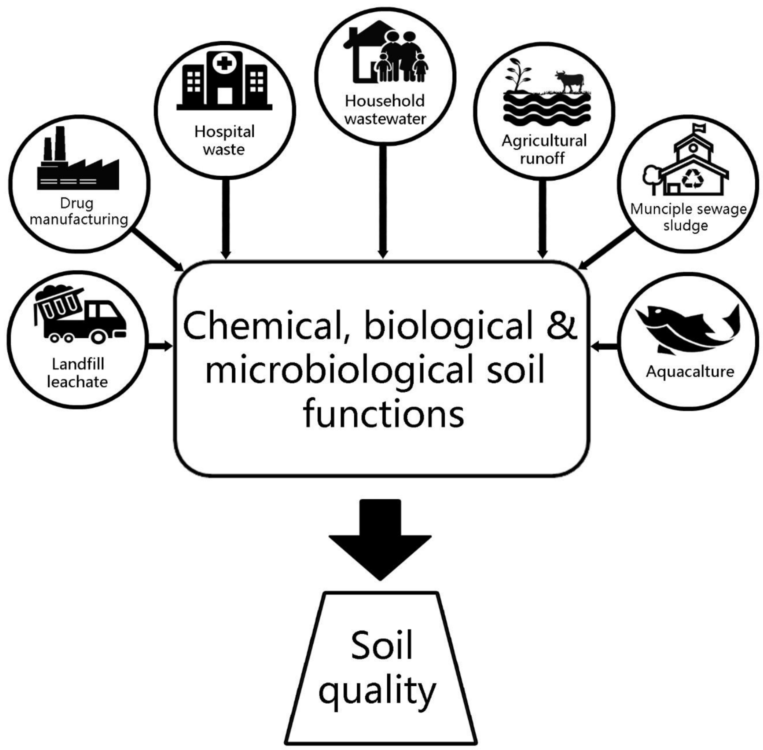

Figure 1. Schematic diagram of anti-cancer drug residue cycling in the environment.

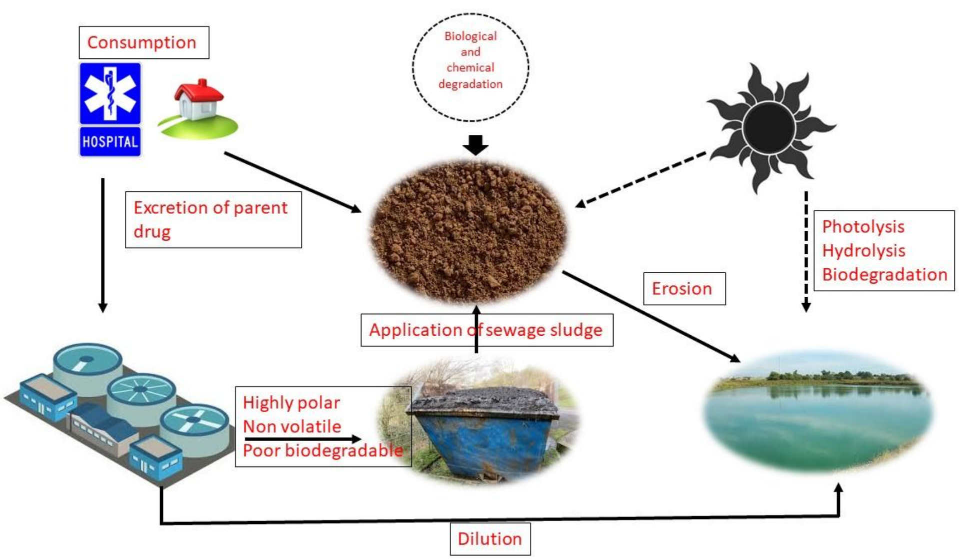

Figure 2. Conceptual diagram of anticancer drug transformation in the environment (Adapted from Booker et al. 2014).

ecules intrinsically (Allen et al., 2010). These drug residues are also considered as pseudo-persistent due to their constant discharge and accumulation into the environment (Daughton, 2003). As a consequence, uptake of pharmaceutical residues by plants from water and/or soils under sewage sludge recycling, demonstrated by soil column studies, is demonstrated an important pathway of drug residue movement into the environment (Hillis et al., 2011; Tanoue et al., 2012).

1.2 Emissions of anticancer drugs into environment

Anticancer drugs have been in extensive use for chemotheraphic treatment for many decades (Mioduszewska et al., 2016; Novak et al., 2017). However, presence of carcinogenic, mutagenic and teratogenic compounds in these drugs have fueled widespread concerns of their ecotoxicological effects and risks to the environment, especially when their potential behavoiur and associated risks are still not clear (Allwood et al., 2002; Toolaram et al., 2014). Anticancer drugs and their metabolites are released into the environment through effluents (Larsson, 2014; Ebele et al., 2017). The presence of cytostatic drugs such as oxazaphosphorine, cyclophosphamide and ifosfamide in surface and groundwater has been confirmed recently (Isidori et al., 2016). The anticancer drugs such as CP and IF generally do not undergo biodegradation during municipal sewage treatment processes. Fates and effects of such drugs in hospital wastewater has been reported recently (Prasanna et al., 2015).

The anticancer drug residues can also originate during the sewage and solid-waste treatment from the manufacturing units at industrial level to the consumption levels (e.g. excretions) (Yin et al., 2010; Xie, 2012; Baresel et al., 2015). The sources such as households, hospitals, health care centers, manufacturing facilities, and waste treatment plants contribute to the occurrence of these residues in waste streams (Ebele et al., 2017). However, a little systematic information exists about the relativeness of these resources for the emissions of drug residues into environment and the information available deals with only a small part of the actual process and/or specific substance. In addition, to a lesser level, release of such drug residues can also come from their volatilization and/or the aerial transport of dust from animal rearing units (GACE, 2007). However, the significance of such releases into the enviroment is still largely remain unknown (BIO-IS, 2013), as discussed above.

1.3 Behavior of anticancer drugs in environment

The residues of anticancer drug of various therapeutic categories e.g. hormones, cytostatics, antidepressants and antibiotics have been observed in the environment at the soil-biota-water-air interphase; although the data on the presence of these drug residues in soil, air and biota are still scarce. These drug residues can generally degrade following both biotic and abiotic paths in soils and water (BIO-IS, 2013). Transformations of anticancer drugs and their metabolites can lead to their movement within different parts of the environment such as from wastewater to sludge/sediments to soils to water bodies (Table 1). This movement, however, depends on various factors including molecular characteristics of drugs, retention behavior (a-

Table 1 Summary of major transformation processes of drug residues in environment **(**Modified from Haddad et al. 2015).

Mechanisms	Transformation products	Activity hotspots
Biodegradation	Microbial metabolites, Biodegradation products, Biotransformation residues, Complex metabolites	Water and wastewater treatment plants, Surface water systems, Anaerobic digesters, Bacterial and fungal dominated hot-spheres in soil
Photolysis, Photocatalysis	Photo-degradation products, Photoproducts	Surface water bodies, Water and wastewater treatment
Chlorination, Ozonation, Advanced oxidation	Metabolites from chlorination, Products of oxidation and photo-oxidation,	Water and wastewater treatment
By products of xenobiotic nature	Biotransformation products, Metabolites, Recalcitrant products	Occur in majority of transformation products

bsorption/adsorption), properties of soils and sediments, pH, quantity of organic molecules, water saturation and aerobic properties (Wang and Wang, 2015). The sorption rate of drug residues is a fundamental factor which influences their transportation rates and, as a result, the products with non-sorptive behavior are rapidly transported to the surface and groundwater whereas sorptive substances follow a much slower transportation mode (Holten-Lützhøf, 1999; Doretto and Rath, 2013; Wegst-Uhrich et al., 2014). Nevertheless, these properties of drugs and soils control the leaching of drug residues into subsurface soil and groundwater (Dolliver and Gupta, 2008; Kwon, 2011). Higher polarity and lower volatilization potential of the most of pharmaceuticals also make them more susceptible to be leached down with water (Breton and Boxall, 2003). Both abiotic and biotic pathways are responsible for degradation of drug residues and converting them into less potent yet hazardous byproducts (Halling-Sørensen, 2002). The degradation rates of these drug residues depend largely on environmental factors including temperature, pH, soil type, and the nature of the pharmaceutical under consideration (BIO-IS, 2013). Interaction of pharmaceuticals with clay minerals and soil organic matter through sorption, binding and fixation determine their persistence and decomposition in the soil matrix (Avisar et al., 2010; Liu et al., 2011). The strength of the interactions also depends on the chemical species and the soil characteristics (Kumar et al., 2005). Other factors regulating environmental fate of anticancer drugs include carbon and energy sources, mineral nutrients, growth factors, ionic composition, water availability, pressure, air composition, electromagnetic radiation, pH, oxidation–reduction potential, spatial relationships, and genetics and interaction of the microorganisms which can alter the microbial diversity and activity.

1.4 Transformations and persistence of anticancer drugs

Since anticancer drugs are excreted with faeces and urine, and composed of xenobiotic-nature parent compounds and metabolites, they enter into the soil by means of aquatic environment through hospital and wastewater treatment plant wastes, landfill leachates and, to a minor amount, in the discharge from the pharmaceutical industry. For example, the platinum-based anticancer drugs including cisplatin, carboplatin and oxaliplatin, and their residues enter into the soil mainly through the municipal wastes containing excretions from patients undergoing chemotherapy (Ferrando-Climent et al., 2014; Petrie et al., 2015). Transformations of anticancer drugs are directly linked to the fate of parent compounds (Haddad et al., 2015). Different environmental processes are linked with wastewater and potable water treatment plants (Zwiener, 2007). During aerobic wastewater treatment or anaerobic digestion of sludge, transformation of these drugs and their metabolites may take place, and, as a result, bacterial metabolite-based biotransformation products are formed (Längin et al., 2009). The formation of several biotransformation products, having genotoxicity and mutagenic potential, during these processes are related to the anticancer drugs (Table 2). It must be noticed these anti-cancer drugs have significant potential to cause cytotoxic, genotoxic, mutagenic and teratogenic effects, however, studies on such effects are confined to aquatic environments (Touraud et al., 2011; Turner and Mascorda, 2015; Heath et al., 2016; Novak et al., 2017). Booker et al. (2014) summarized the discharge of some anticancer drugs including capecitabine, imatinib, sorafenib, lapatinib, and mitotane to the soil via sewage sludge and showed that sorption potential of these drugs ranged from 6 (imatinib) to 92% (lapatinib) whereas some of them have very high bioaccumulation potential such as lapatinib and mitotane. The pharmaceutical residues with neutral to alkaline characteristics are retained more strongly by soil compared to the those more mobile in soil with acidic properties because:

- Pharmaceuticals having neutral chemistry are more hydrophobic and partition to soil organic matter (Schwarzenbach et al., 2003);
- Basic chemical nature pharmaceuticals are dominated by cationic groups with positive charges and are held strongly by negatively-charged soil particles (Magnér et al., 2009); and,
- Pharmaceuticals with acidic functional groups are anionic having negative

charge and tendency to be repelled by soil (Magnér et al., 2016).

Some of these drugs undergo fractional elimination during activated sludge treatment which is the most common wastewater treatment system (Lutterbeck et al., 2015; Kosjek et al., 2016). Hydraulic retention time and age of sewage sludge are important factors for biological transformations of pharmaceuticals during sewage treatment (Kreuzinger et al., 2004). During this treatment process, trace toxins are generally affected by three mechanisms of volatilization, biodegradation or sorption onto sludge, however, relative strength of these pathways depend on the physicochemical properties of compound and sludge (Seira et al., 2013). Due to the direct and indirect interactions of these highly active compounds, unsafe levels of the drug residues often occur in the environment (Kummerer et al., 2016).

2. Experimental parameters

Several experimental parameters have been used to define distribution and the fate of anticancer drugs in the environment. These parameters predict the behavior drug residues based on their chemical structures and physicochemical properties such as dissociation constant (pK_a), octanol-water partition coefficient (K_{ow}), bioconcentration factor (BCF), atmospheric OH rate, organic carbon partition coefficient (K_{oc}), solid water distribution coefficient (K_d), n-octanol or water distribution coefficient (D_{ow}), vapor pressure (P), degradation half-life (DT_{50}) and Henry's coefficient (KH). A number of studies have used these parameters to describe the physicochemical nature, occurrence and fate of various anticancer compounds. For examples, comparison of dissociation constant of five anticancer drugs e.g. 5-Fluorouracil (5-FU), Gemcitabine (GEMc), IF, CPA and Methotrexate (MTX) showed that MTX had low pKa value and higher polarity than others (Besse et al., 2012; Xie, 2012; Zhang et al., 2013). According to the Guideline of Medicinal Products on the environmental risks associated with anticancer drugs, European Medicines Agency (EMA) requires K_{ow} to be greater than 4.5 as a pre-requisite for further screening of drugs for their toxicity, persistence and bioaccumulation in environment (European Commission, 2011; Vestel et al., 2016).

2.1 Dissociation and sorption mechanisms

For dissociation of drugs, the constant pK_a is used as equilibrium constant which defines the degree of dissociation at a specific pH of compounds. Dissociation increases the polarity and mobility of drug residues and affect their environmental fate at a broader pH range of 5–9 (Kosjek and Heath, 2011).

The sorption of drug residue is one the fundamental factor affecting transformation of anticancer drugs in the environment. Anticancer drugs can be degraded both abiotically or biotically at the soil-water interphase and these transformations generally reduce their harmful effects by converting them into less hazardous products (BIO-IS, 2013). The sorption rate on organic matter is determined by using two types of coefficients i.e. K_{ow} and K_{oa} which are derived from the D_{ow} and K_d coefficients. D_{ow} coefficient specifies the affinity of an organic substance to allocate between lipids and fats while sorbing to particulate matter (Kosjek and

Heath, 2011). For example, aromatic amines bind strongly to soil organic matter or humic substance because of higher reactivity of their aromatic amino groups (Richnow et al., 1997). This mechanism results in lowering mobility of these compounds than predicted from the physicochemical parameters. However, in contrast, anthracyclines, vinca alkaloids and their correspondent mitoxantrone adsorb freely to steel, glass, and plastics, and also show their potential for sorption by the sludge and sediments (Kümmerer, 2008).

Interaction of the drug residues with soil organic matter and clay particles take place through processes such as binding, sorption and fixation of these substances within the soil matrix (Thiele-Bruhn et al., 2004). Sorption of the anticancer drugs residues to the soil matrix depend greatly on the properties of soil and chemical species along with the temperature, moisture and the soil solution chemistry (Xu et al., 2021). For sorption and/or interaction of these drug residues with soil, the distribution coefficient (K_d) is used which measures sorption of a solute in soil medium. K_d indicates the ratio between the quantity of an adsorbate per unit mass of sorbent to the concentration of the adsorbate in solution at equilibrium. Soil organic matter (SOM) being the key determinant of the fate of organic pollutants in soil (Nowara e al., 1997), K_d is modified as K_{OC} which takes into consideration the role of soil organic carbon (SOC) for pollutant sorption (Song and Guo 2014). If the $K_{OC} > 5$, the drug residues have high bioaccumulation potential e.g. lapatinib and mitotane has high bioaccumulation potential with $K_{OC} > 5$ (Booker et al., 2014). In addition, sorption potential of anticancer drugs increases linearly with the increase in K_{OC} values. Sorption of the drug residues to soil has been shown to be governed by SOM quantity and quality (Gruber et al., 1990; Chefetz et al., 2008).

2.2 Biodegradation and decomposition

Anticancer drugs and their metabolites are released into rivers and pose a serious risk of contaminating aquatic and terrestrial ecosystems (Fig. 2). Mostly diverse classes of anticancer drugs have low biodegradability but varied and wide range of persistence patterns through (Toolaram et al., 2014; Kosjek et al., 2016). Booker et al. (2014) summarized a number of studies indicating relatively low biodegradation of the majority of anticancer. The biodegradability of anticancer drugs has been shown to be lower in soils compared to water. For example, Zahn-Wellens/EMPA test along other studies suggested that there was little CP degradation in swage water treatment plants and, also, when it enters into water cycle (OECD, 1992; Steger-Hartmann et al., 1997; Kiffmeyer et al., 1998). Similar degradation behavior was observed for IF in both wastewater treatment and Zahn-Wellens experiments (Steger-Hartmann et al., 1996). The data showed that etoposide biodegrade slowly in the environment whereas vincristine, vinca alkaloids, vinblastine and vindesine lack inherent biodegradability (Al-Ahmad and Kümmerer, 2001). Despite most of the anticancer drugs exert low biodegradability (Table 1), some of them show substantial biodegradation e.g. cytarabine decomposed up to 70% after 10 days in activated sludge and similarly, 5-FU was completely eliminated from a spiked influent under laboratory conditions within days (Kiffmeyer et al., 1998). These differences in biodegradation suggested that 5-FU was resistant to degradation both in the closed-bottle and Zahn–Wellens tests. A general trend in biodegradability of 5-FU, cytarabine and gemcitabine is related to their chemical structures. For example, molecules of 5-FU contain no easily biodegradable sugar while cytarabine consists of a pyrimidine with arabinose and gemcitabine groups, and arabinose being fluorinated shows resistant to biodegradation due to high redox potential (Kümmerer and Al-Ahmad, 1997). CPA and IF are other widespread anticancer drugs in the environment with little tendency for biodegradation. For example, Buerge et al. (2006) conducted laboratory simulation tests using lake water under dark conditions to investigated degradation of CPA and IF, and found a half-life of 80 days for CPA whereas IF followed incomplete degradation pathway. However, under irradiated conditions of lake water, degradation of CPA and IF proceeded at half-life of 44 and 144 days respectively. MET is another anticancer drug which show little biodegradation and 7-hydroxymethotrexate is the major byproduct resulting from its degradation (Kiffmeyer et al., 1998). Johnson et al. (2008) suggested that these compounds at higher concentrations results in cytotoxic effects microbial populations. In addition, following conditions limit removal of anticancer drugs during wastewater treatment processes:

- Hydrophilic nature of the drugs does not allow sorption to the sludge;
- Presence of halogen atoms within molecules of some compounds which hinder biodegradation; and,
- Intrinsic toxicity of compounds to bacteria.

Chee-Sanford et al. (2009) suggested hydrolysis as an important phenomenon of pharmaceutical transformation in the environment as water is always an integral part of animal manures and sludges which are the major source of the drug residues. Hydrolysis of various veterinary drugs have already been reported under acidic and alkaline environments (e.g. Doi and Stoskopf, 2000; Huang et al., 2001). Nevertheless, biodegradation represents the major mechanism of drug transformation in soil. Biodegradation pathway is controlled by enzymatic degradation and addition of microbial inoculants with wastewater, sewage sludge and sediments enhanced microbial degradation of drug residues (Al-Ahmad et al., 1999; Gartiser et al., 2007). However, abiotic degradation of the drug residues is more dominant in soils compared to the biodegradation processes (Clarke and Smith, 2011). The persistence and biodegradability of the drugs in soils depends on number of soil and environmental factors, as discussed above. While many drugs are degradable in soils with a half-life <30 days under controlled experimental conditions, a few such as sarafloxacin, roxithromycin, and virginiamycin exhibit higher persistency and stay in the soils unchanged over the scale of months (Song and Guo, 2014). These drug residues have been shown to be taken up by plants such as corn, onion and cabbage, and can also by other organisms such as earthworms (Kumar et al., 2005; Carter et al., 2016).

2.3 Stability towards photolysis

Photolysis is considered a major pathway for abiotic transformations of anticancer drugs in the environment (Calza et al., 2014). Photolysis can be both direct and indirect i.e. direct photolysis results from the direct absorption by solar light through the substrates whereas indirect photolysis occurs due to natural photosensitizers such as dissolved organic matter (DOM) which can produce species with strong oxidation potential including hydroxyl radicals (HO) upon irradiations (Nikolaou et al., 2007; Michael et al., 2014). For example, functional groups on molecules can absorb light in the range of 200–800 nm region having pi electron functionalities and hetero atoms containing nonbonding valence shell electron pairs. Other light absorbing groups may include chromophores with C=C, C=O, N=O and C–X (X = I, Br) functional groups. Indirect photolysis depends on the physicochemical characteristics of organic compounds determined from a rate coefficient called ''atmospheric OH rate''. For example, atmospheric OH rates of vincristine and vinblastine are 200 times higher than that of carmustine which suggest that vinblastine and vincristine possess more potential for advanced oxidation processes than the compounds having lower atmospheric OH rate constants (Shi et al., 2013). MET is susceptible to photolysis because of its potential to absorb ultraviolet (UV) light of wavelengths greater than 290 nm compared to 5-FU which do not absorb light of wavelength greater than 290 nm and resist direct photolysis, however, it can be degraded by ozonation process. (Pérez Rey et al., 1999). In contrast, 5-FU was sensitive to light at remained 266 nm and followed photodegradation under Hg medium pressure lamp in solution (Straub, 2010). Capecitarabine (CAP) showed slow abiotic degradation in solution at low wavelengths (<190 nm) indicating the needs to analyze stability of drug compounds exposed to low wavelength light (Baumann and Preiss, 2001). CP can also degrade via hydrolysis at temperature above 30 °C due to presence of chlorine atoms and slow dark chemical degradation whereas IF did not follow such degradation mechanism (Bicer et al., 2013). The indirect photochemical degradation due to OH radicals resulted in relatively faster degradation rates in treated lake water samples which highlighted the significance of transitory photo oxidants responsible for the degradation processes (Buerge et al., 2006). However, photodegradation of drug residues could be limited under field conditions due to restricted exposure to light (Beausse, 2004). Nevertheless, biodegradation and photolysis are the most important primary pathways of degradation (Booker et al., 2014).

3. Effect of anticancer drugs on soil quality indicators

Since the soil quality is of significant importance, researchers have proposed a large number of soil quality indicators and indices since soil quality cannot be estimated directly. Majority of these soil quality indicators integrate changes in soil physical, chemical and biological properties over time in response to natural and anthropogenic factors. Use of such soil quality indicators has generally been applied at pilot, field and global scales (Karlen et al., 2001). However, recently, the concept of soil quality index has been suggested as a more comprehensive tool to describe soil quality that integrates soil physical, chemical, microbiological and biochemical properties (Halvorson et al., 1996; Torres et al., 2015). Soil biological and microbiological parameters are considered sensitive and relatively quick response soil quality indicators as they represent microbially mediated soil processes. Soil microorganisms are directly related to soil quality as they are responsible for organic matter turnover, biogeochemical C, N and P cycling, soil structural stability and fate of xenobiotics applied to the soils (Turco et al., 1994; Wardle and Giller, 1996).

4. Effects on soil microbial activity and microbial communities

Microorganisms are an extremely diverse group of organisms constituting about 60% of the total Earth's biomass . According to an estimate, about 1.2×10^{29} and $4\text{-}5 \times 10^{30}$ microorganisms are inhabitant to aquatic and terrestrial environments respectively (Singh et al. 2009). Microorganisms play an integral part in biogeochemical nutrient cycles, flow of energy and matter, plant biomass production and environmental health in majority of ecosystems (Desai et al., 2009; Grenni et al., 2018). Therefore, biological and biochemically processes in soil and water mediate ecosystem functions (Zabaloy et al., 2008). As a result, microbes are critical for carbon and nutrient transformations, and any change in their community structure may alter the cycling and recycling of nutrients, and thus affect soil and water functions indirectly (Wang et al., 2008). The soils, the ultimate sink of pollutants, are generally contaminated with pharmaceuticals drugs through the following channels (Oppel et al., 2004):

a) Using of activated sewage sludge as organic amendment and fertilizer on agricultural fields;
b) Irrigation of agricultural fields with wastewater containing drug residues;
c) Contaminating groundwater from wastewater drainage; and
d) Leakage from drains and sewage treatment works.

Soils are the ultimate sink of drug residues where they can cause strong effects on soil such as inducing antibiotic resistance in soils (Kemper, 2008; Marti et al., 2013). Environmental factors, microbial communities and interactions between microorganisms can alter microbial diversity, activity and community composition in soil which regulate soil functions. The soil solid surfaces containing 80–90% of the microorganisms are hotspots of positive (symbiosis and metabiosis) and negative (competition, parasitism, and predation) microbial interactions which control the secretion of the bioactive compounds. As a result, some microbes secret compound that affect their competitors negatively under conditions of limited resources. Antibiotic resistance genes (ARGs) have also been reported in soil receiving antibiotic rich wastewater which have potential to affect human health (Amarasiri et al., 2020). There is evidence that antibiotic resistance bacteria could alternative microbial community structure and composition (Negreanu et al., 2012; Meena et al., 2015), however, very little is known on how antibiotics and antibiotic resistance bacteria may affect the soil processes and nutrient cycling. For example, oxytetracycline decreased activities of soil enzymes including urease, sucrase and phosphatase but increased microbial biomass N (Yao et al., 2010). Kotzerke et al. (2008) found significant effect of sulfadiazine on N cycling. Similarly, negative effects of sulfadiazine on soil bacteria and their diversity have been reported (Hammesfahr et al., 2008). However, such type of studies is few and far between especially with reference to anticancer drugs.

Environmental pollution has the substantial potential to adversely affect and/or alter the microbial communities playing a vital role in provision of important ecosystem processes such as biomass decomposition and nutrient cycling (Fig. 3). Microbes are the most important biological agents responsible for degradation and recycling of waste materials in the environment. They colonize the polluted sites and enable biodegradation of recalcitrant xenobiotics (Galvao et al., 2005). Application microbial ecology approaches help in environmental risk assessment of soil and water contamination from pharmaceuticals pollution. Grenni (2011) investigated the effects of anticancer drugs from waste disposal/manufacturing on bacterial populations and linked the change in microbial community to soil and groundwater quality. The bacterial community was analyzed using fluorescence in situ hybridization (FISH) and their abundance was measured by using the epifluorescence direct count method. The results demonstrated negative effects of trace pollution from antibiotics and chlorinated volatile organics as indicated by the change in microbial communities. A recent review by Grenni et al. (2018) has highlighted direct and indirect the effects of various antibiotics including anticancer drugs on structure and functioning of microbial communities. Such changes in microbial diversity and structure hinder ecosystem processes including nitrogen cycling, sulphur transformations and organic matter decomposition (Laveman et al., 2015; Roose-Amsaleg and Laveman, 2016).

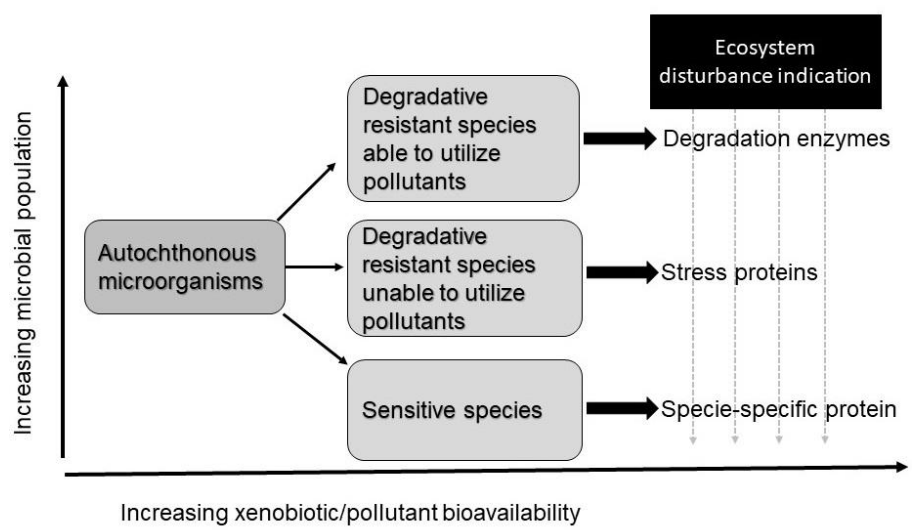

Figure 3. Schematic diagram of microbial response to environmental xenobiotics/pollutants (Modified from Ogunseitan 2000).

Table 2. Summary of degradation and transformation of some anticancer drugs in environment.

Anticancer drug	Elemental formula/Group	Environmental fate			
		Biodegradability	Adsorption onto sludge/sediments	Direct photolysis	Indirect photolysis
Cyclophosphamide (CP)[2,3,5,11,13]	$C_7H_{15}C_{12}N_2O_2P$/Alkylating agent, nitrogen-mustard analogue	No	No	No	Yes
Ifosfamide (IF)[2,3,4,7,11,13]	$C_7H_{15}C_{12}N_2O_2P$/ Alkylating agent, nitrogen-mustard analogue	No	No	No	Yes
Cytarabine[5,7]	$C_9H_{13}N_3O_5$/Antimetabolic agent, pyrimidine analogue	Yes	--	--	--
Gemcitabine[7]	$C_9H_{11}F_2N_3O_4$/Nucleoside analogue	Yes	--	--	--
5-fluorouracil (5-FU)[5,9,10]	$C_4H_3FN_2O_2$/Antimetabolic agent, pyrimidine analogue	Yes	No	No	Yes
Capecitarabine (CAP)[12]	$C_{15}H_{22}FN_3O_6$/Antimetabolic agent, pyrimidine analogue	Yes	No	--	--
Methotrexate (MET)[5]	$C_{20}H_{22}N_8O_5$/Antimetabolic agent, folicacid analogue	Yes	--	Yes	--
Vinblastine[1]	$C_{46}H_{58}N_4O_9$/Plant alkaloids and other natural products, vinca alkaloid	No	Yes	Yes	Yes
Vincristine[1]	$C_{46}H_{56}N_4O_{10}$/Plant alkaloids and other natural products, vinca alkaloid	No	Yes	--	--
Etoposide[9]	$C_{29}H_{32}O_{13}$/Plant alkaloids and other natural products, podophyllotoxin derivative	No	--	Yes	Yes
Doxorubicin[8,9]	$C_{27}H_{29}NO_{11}$/Cytotoxic antibiotics, anthracycline	No	Yes	--	--
Epirubicin[6,8,9]	$C_{27}H_{29}NO_{11}$/Cytotoxic antibiotics, anthracycline	No	Yes	--	--
Daunorubicin[8,9]	$C_{27}H_{29}NO_{10}$/Cytotoxic antibiotics, anthracycline	No	Yes	--	--
Mitoxantrone[6]	$C_{22}H_{28}N_4O_6$/An anthracenedione-derived antineoplastic agent	No	Yes	--	--
Cisplatin[5]	$C_{12}H_{19}N_3O$/ Other antineoplastic agents, methylhydrazine	No	--	--	--

[1]Al-Ahmad and Kümmerer (2001), [2]Baumann and Preiss (2001), [3]Buerge et al. (2006), [4]Halling-Sørensen et al. (1998), [5]Kiffmeyer et al. (1998), [6]Kümmerer (2008), [7]Kümmerer and Al-Ahmad (1997), [8]Mahnik et al. (2006), [9]Mahnik et al. (2007), [10]Pérez Rey et al. (1999), [11]Steger-Hartmann et al. (1996), [12]Straub (2010), [13]Ternes et al. (2005).

A significant body of knowledge shows change in microbial community structure and function as a result of exposure to antibiotics designed with selective mode of operation (Mohamed et al., 2005; Yang et al., 2009; Ding and He, 2010). These drugs change the microbial community abundance and their interactions with other microbial species, however, the effects depend on soil characteristics, drug dose and native microbial populations (Zielezny et al., 2006). Effects of such anticancer drug pollution to soils are largely unknown (Boxall, 2004; Bérdy, 2012; Larsson, 2014). For example, drugs induce a significant threat to the edaphic and aquatic organisms because of their significant bioavailability.

However, such effects are overshadowed due to occurrence of the co-contaminants e.g. metoprolol strongly sorbs to soil particles and reduces bioavailability but not the persistence levels of CP and IF in the environment (Turner and Mascorda, 2015). In another study, transport and mobility of CP and IF with MET anticancer drugs was negatively correlated with the turbidity of the solution in a soil column (Mioduszewska et al., 2016). Some previous studies have indicated higher persistence levels of the two oxazaphosphorines, however, these studies were performed at relatively higher concentrations which affected the microbial activities negatively leading to increased persistency of these compounds (Steger-Hartmann et al., 1996, 1997; Kiffmeyer et al., 1998). In contrast, a number of studies have reported the effects of veterinary drugs residues on soil biodiversity e.g. Thiele-Bruhn (2003) found noticeable effects of veterinary drug monensin on soil respiration. Similarly, Patten et al. (1980) observed an increase in soil respiration after application of beef cattle feces on a sandy loam soil. However, Bauger et al. (2000) did not find any negative effects of antibiotics on soil fauna even at concentrations higher than 100 mg kg^{-1}. Data on tetracyclines toxicity to soil fauna/flora and plants showed non-substantial environmental risk whereas the drug has noticeable effects on soil microorganisms and enzymatic activities at realistic concentrations (BIO-IS, 2013). However, association of such observations with soil and ecosystem functions are still not clear.

The effects of drug residue on microbial communities generally involve changes in phylogenetic structure, resistance and ecological functions at micro-ecosystem level (Ding and He, 2010). However, our understanding of the direct and indirect effects of drug residues on ecosystem functioning is very limited whereas it has been established since long that such disturbance could significantly alter microbial and enzymatic activities to modify the ecosystem functioning and stability on long-term basis because of changes in biomass synthesis and nutrient transformations (Perry et al., 1989; Koike et al., 2007; Martinez et al., 2009).

5. Ecological and toxicological effects of drug residues

Pharmaceuticals cascading through the ecosystem behaves as an “ecological factor” which generally change the community structure of the ecosystem and alter ecological functions of water and soil at ecosystem levels (Aminov and Mackie, 2007; Kotzerke et al., 2008). Despite of the clear evidences of persistence and stability of the anticancer drugs in the environment and their potential ecological effects on soil and water, studies investigating their chronic and acute ecological effects are not very common. Consequently, both short and long-term ecological effects of pharmaceuticals in soil, water and plants are largely unknown (Brain et al., 2006; Song and Gao, 2014). However, an escalating trend in research advocating effects of these drug residues on terrestrial and aquatic environment has been observed (Isidori et al., 2016). For example, the US FDA guidelines for drugs safety include both the toxicity at environmental biodiversity and ecological community and ecosystem level (FDA 1998). In another study by Lutterbeck et al., (2015), the author found significant inhibition of lettuce seed germination when exposed to anticancer drugs (CP, MTX, 5-FU and IM). Their study also indicated mutagenic and cytotoxic potential of these anticancer pharmaceuticals. A limited number of studies have evaluated the long-term ecological risks of pharmaceutical drug residues; however, little focus was given to the potential effects of the metabolites and intermediate products of these drug residues (e.g. Cleuvers, 2003; Bound and Voulvoulis, 2004; Fatta-Kassinos et al., 2011). Since the pharmaceutical residues are generally present in the environment as mixture, therefore, despite of the sub-optimal concentrations of individual compound, the so-called “cocktail effect” might pose a significant ecological and ecotoxicological concern (Heath et al., 2016). A few recent studies have reported ecotoxic effects of anticancer drugs on zebrafish (Kovacs et al., 2016), fertility in higher plants (Misik et al., 2016) and green alga and cyanobacterium (Elersek et al., 2016).

6. Conclusions and limitations

Pharmaceuticals including anticancer drugs are being recognized a significant environmental concern because of their increasingly widespread use and potential ecological effects on terrestrial and aquatic biodiversity. Hospital, household and sewage treatment plants are the major point sources of anticancer drug residue discharge into the environment. Once these pharmaceuticals enter the environment, their fate depend on the physical, chemical, biological and biochemical processes such as photolysis/photodegradation, biodegradation/biotransformation in soil and water, sorption to soil particles and sediments and direct uptake by flora and fauna. However, a little knowledge exists on the behavior of these drugs residues to processes occurring in the soil and water. There are limited studies describing effects of these drugs on microbial communities inhabiting in soil and water at micro-ecosystem scale. There is literally very little information available on the behavior these drug residues in soils, especially soil function and, hence, the ecosystem response. As a result, it is apparently difficult to apply mitigative measure for restricting their emissions into water and soil. Poor removal of some anticancer during the treatment process and their high resistance to biodegradation suggest the need for other methods to eliminate these compounds from wastewater. There are no research studies that would clearly indicate the effects of the prolonged exposure of organisms to anti-cancer drugs. Therefore, it is difficult to introduce measures restricting their emissions into surface waters. The appearance of some high-profile publications over the recent years has started to fill in existing knowledge gaps and provide a more reliable information about the environmental and human health risk assessment associated with the use of anticancer drugs and their metabolites and transformation products (TPs). However, effects of these drug residues on soil processes and functions, soil quality and, hence, the ecological role of ecosystem remain largely unknown.

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ORIGINAL RESEARCH

Residual Effect of Biochar and Legumes on Soil Fertility, Yield and Yield Components of Wheat

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ABSTRACT: Biochar and the use of legumes in cropping systems are considered sustainable approaches to boost crop yield and preserve soil fertility. In the current study, the effects of leftover biochar and previously planted legumes on wheat yield and soil N status were examined at various nitrogen (N) levels. The experiment included testing two levels of previously applied biochar (0 and 50 tons ha^{-1}), three legumes under four levels of N (0, 60, 90, and 120 kg ha^{-1}), cowpea (*Vigna unguiculata*) for fodder, Sesbenia (*Sesbenia grandaflora*) for green manuring, and mung bean (*Vigna radiata*) for grain. Results showed that biochar application enhanced wheat tiller m^{-2}, spikes m^{-2}, grains per spike, thousand grain weight, grain yield, biological yield, and soil total N status by 3%, 6.5%, 3.7%, 1.8%, 7.8%, 9.5%, and 11%, respectively. Moreover, applying nitrogen at a rate of 90 kg ha^{-1} increased the amount of wheat spike m^{-2} by 20%, grain spike^{-1} by 10%, grain yield by 70%, biological yield by 48%, harvest index by 27%, and the N content of the grain, straw, and soil by 13%, 14%, and 36% respectively. Meanwhile, 1000 grain weight resulted higher by 6.17%. Legumes that had been previously seeded outperformed fallow and increased spikes m^{-2}, grain yield, biological yield, grain N content, and soil total N content by 8.2%, 11%, 6.78%, 25%, and 42%, respectively. It is determined that applying biochar to the summer gap left by legumes can increase soil fertility and wheat output.

KEYWORDS: Biochar, legumes, nitrogen, wheat yield, soil fertility.

1. Introduction

The various challenges faced by agricultural development such, as the reduction in land, climate change, scarcity of water unpredictable temperature changes, shifts in rainfall patterns increasing input costs and significant migration of people from rural to urban areas highlight the urgent need to improve agricultural productivity through innovative crop production strategies. Wheat (*Triticum aestivum* L.) a crop that plays a major role in meeting a large portion of global human dietary energy requirements has experienced a rise in demand recently due to its product's availability at more affordable prices compared to other cereal crops. According to the Food and Agriculture Organization (FAO) it is projected that by 2050 the world will require 840 million tons of wheat, excluding the demand for animal feed and considering the effects of climate change on wheat production. This means developing nations would need to increase their wheat output by 77% with development methods contributing over 80% of the supply (FAO, 2009).

The most significant cereal crop in Pakistan is wheat (Ali et al., 2019a). However, due to its continuous cereal cropping method and minimal or nonexistent application of organic matter, deficits in nitrogen and phosphorus severely restrict wheat productivity (Ali et al., 2019a). Apart from deflation of nutrients, this practice also leads to the development of a hard pan that might cause surface runoff and negatively impact on crop productivity (Yasnolo et al., 2018). Cereal mono-cropping, especially maize-wheat-maize, causes fast nutrient loss and erosion from crop harvest, therefore reducing soil fertility. In Pakistan, cropping techniques based on cereals are widely utilized because they provide food security, high productivity, and profitability. According to Laye et al. (2018), cereal crops are extremely demanding and need a large amount of nutrients to produce more products. In Pakistan, small-scale farmers typically work with substandard alkaline or saline soils, which leads to sterility and deficiencies in nitrates, phosphates, and other micronutrients. Frequently, ineffective solutions are implemented to address this issue (Burt et al., 2001). Legumes can help with this condition. Green manuring, including legumes in crop rotation, are thought to be the key ways to keep soil fertility and high crop yield (Meena et al., 2018; Yang et al., 2023).

However, Gogoi et al. (2018) have demonstrated a positive impact of legumes on the structure and function of the agroecosystem. Several studies have revealed improved crop quality and yield when planted legumes (Jalal et al., 2020). Legume farming guarantees the replenishment of nutrient-deficient soils and supplies animals and humans with necessary protein, mineral, and vitamin intake. Because of legumes fix nitrogen from the atmosphere, they may maintain the fertility of the soil. Although, it is necessary to step up efforts to use land wisely by applying fertilizers in a balanced manner with a focus on nitrogenous fertilizers. The bright use of nitrogen fertilizer increases crop yield and improves soil fertility. The addition of nitrogenous fertilizer raised the grain yield of maize from 43-68% and the biomass yield from 25-42% (Ogola et al., 2002). One of the most important variables limiting agricultural output and productivity is adequate nutrient management (Zhu et al., 2023, Ali et al., 2019b). Crop productivity has recently dropped as a result of decreasing soil fertility. Due to delays in the timely delivery of fertilizer producers are finding it difficult to maintain the soil's fertility. Confirming that a given soil has a tolerable supply of nutrients for optimal plant development is now the largest challenge, as soil types differ in their potential for production (Zhu et al., 2023).

Moreover, In Pakistan low soil fertility and increasing costs of artificial fertilizers are two of the main problems to a high grain yield. Reducing production costs, maintaining soil health and fertility, and raising crop productivity are all significant challenges facing agricultural scientists. Many solutions, including the use of organic materials (biochar), integrated nutrient management, and organic farming, are being explored globally to address these issues (Ali et al., 2011; Ullah et al., 2020). The addition of organic amendments is the simplest approach to boost soil productivity and stabilize crop yield, given the extremely low soil organic matter in degraded land (Amanullah et al., 2007; Ismail et al., 2011). However, the primary drawback of adding organic matter, particularly in moist tropical conditions, is its quick decomposition, which necessitates regular application during each planting season and is illogical given how difficult it is to get enough organic manure. Thus, refractory organic elements, such as "biochars," have been assessed by certain researchers for their potential to enhance soil qualities and carbon sequestration (Glaser et al., 2002; Liang et al., 2006; Ullah et al., 2021), and boost crop yields (Yamato et al., 2006; Chan et al., 2008). Through enhanced soil carbon (Lehmann et al., 2006), decreased greenhouse gas emissions, improved soil fertility, and greater agricultural production (Major et al., 2010), the effective use of biochar can help moderate climate change. Because of the high porosity of the biochar, the physical features of the soil, such as structure and water-holding capacity, are enhanced (Song et al., 2022; Ali et al., 2022; Karhu et al., 2011; Ullah et al., 2023; Vaccari et al., 2011), which lessens the drought stress on dry land that is increased by climate change. Biochar-amended soil improved crop nitrogen usage efficiency and decreased nitrogen demand, which may have a knock-on impact on lowering greenhouse gas emissions from the N fertilizer industry (Gaunt and Lehmann, 2008; Zheng et al., 2010).

The beneficial effect of biochar on crop yield and soil fertility has been reported by many scientists throughout the world (Ali et al., 2020a; Ali et al., 2020b; Ahmad et al., 2022, Ahmad et al., 2023) but yet need more study particularly in Pakistan. The main objectives of the current study are given (1) To evaluate the potential of biochar for soil management in cereal-cereal based cropping system with adjustment of legumes in the summer gap. (2) To determine the impact of biochar on yield and yield components and residual soil fertility. (3) To find out the beneficial effects of biochar as organic amendments in different cropping patterns would last longer compared to that of conventional organic manures such as farm yard manure or not.

2. Materials and methods

2.1 Experimental site

To study the residual impacts of biochar and legumes on wheat crop under different nitrogen rates, a field trial was conducted in the winter season of 2013-2014 at the Agronomy Research Farm, The University of Agriculture Peshawar. The experimental site is 340m above sea level. The soil type of the experimental site is considered as clay loam having a soil pH of 7.8, EC 1.2 and found to be deficient in Nitrogen (N), Phosphorous (P) and Potassium (K) contents i.e., N 16 mg kg^{-1}, P 8.0 mg kg^{-1} and K 50 mg kg^{-1}, respectively.

2.2 Experimental materials

The experiment was arranged in a

Table 1. Represent treatments of preceding legumes with and without biochar.

Codes	Treatments
T1	Mungbean (grain purpose) + 0 t ha^{-1}Biochar
T2	Mungbean (grain purpose) + 50 t ha^{-1}Biochar
T3	Cowpea (fodder purpose) + 0 t ha^{-1}
T4	Cowpea (fodder purpose) + 50 t ha^{-1}
T5	Sesbania (green manure) + 0 t ha^{-1} Biochar
T6	Sesbania (green manure) + 50 t ha^{-1} Biochar
T7	Fallow + 0 t ha^{-1} Biochar
T8	Fallow + 50 t ha^{-1} Biochar

Table 2. The treatments for the current wheat experiment were as follow along with the study of residual effects of the above mentioned legumes and biochar.

Wheat Experiment (Nitrogen Levels)	Treatment
N1	0 kg ha^{-1}
N2	60 kg ha^{-1}
N3	90 kg ha^{-1}
N4	120 kg ha^{-1}

Randomize Complete Block (RCB) design with four replications. The land was properly prepared by using a cultivator twice, followed by a rotavator for a smooth seedbed. The residual effect of summer legumes grown for grain, fodder and green manure purposes was studied on the subsequent wheat crop (Table 1). Mung-bean was used for grain purposes and cowpea was used for fodder purposes. Likewise, Sesbania were purely used for green manure purpose. A fallow treatment was included in the experiment as a control. Two levels of biochar (0 and 50-ton ha^{-1}) for legumes and four different N rates (0, 60, 90 and 120 kg ha^{-1}) were used for wheat crop (Table 2). N fertilizer was applied in two split doses i.e. half at sowing and half at booting. The subplot size for wheat was 5m by 4m.

Summer legumes, i.e. mung bean, cowpea and Sesbania with and without biochar, were sown in the first week of May following recommended agronomic practices. The biomass of Sesbania were incorporated into the field in the month of early July.

2.3 Data collection and measurements

Data on emergence m^{-2} was recorded by counting a total number of plants that emerged in one-meter row length at three randomly selected rows in each subplot. The data were converted to emergence m^{-2}. The plant height was measured as the distance from the base to the tip of the plant of five randomly selected plants in each sub-plot and was averaged. Grains from five randomly selected ears were obtained by hand threshing and were counted and converted into an average number of grains $year^{-1}$. Fifty grains were counted at random from the grain sample of each sub-plot of wheat and were weighed with an electronic balance and then converted into thousand grain weight. For recording grain yield data, three central three rows were harvested in each sub-plot with the help of a sickle. Samples were sun dried, threshed and grains were weighed with the help of an electronic balance and the data were converted into kg $ha^{-1.}$ Three central rows were harvested at maturity from each subplot, tied into bundles separately and were sun dried and weighed by spring balance for calculating biological yield. The data were converted into kg $ha^{-1.}$ Furthermore, harvest index was calculated and expressed in percentage for each plot using the following formula for each crop:

$$\text{Harvest index (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \text{x}100$$

Wheat grain, straw and soil samples were analyzed for total N content. Wheat grain and straw samples were oven dried at 80 °C to a constant mass, weighed, then finely ground (< 0.1 mm) and analyzed for total N (Bremner and Mulvaney, 1982). The soil samples were air dried for one day, ground and then sieved (< 2 mm) and analyzed for total N following the Kjeldahl method of Bremner and Mulvaney (1982). Soil samples were collected at a depth of 0-15 cm from each sub plot.

2.4. Statistical analysis

The research data were statistically analyzed by using the statistical software Statistix version 8.1 and the hypothesis were tested via the statistical technique ANOVA for RCB design with split plot arrangement. The treatment means were compared and calculated at P< 0.05 level of probability by using the LSD test (Jan et al., 2009). Correlation analysis was done by using the R-studio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA (URL; http://www.rstudio.com/.)

3. Results

3.1. Emergence m^{-2}

Analysis of the data indicated that emergence m^{-2} of maize were not significantly varied due to previously applied biochar, legumes or nitrogen applied to the current crop (Table 3). Though the effect of all treatments was found non-significant, higher emergence was recorded in plots previously treated with 50-ton ha^{-1} biochar as compared to no biochar treated plots. Similarly, more seedlings were counted in plots where sesbania was incorporated as green manuring, followed by ^{mung} bean, while lower emergence was recorded in plots previously sown with cowpea.

Table 3. Effects of biochar, nitrogen and legumes on wheat growth yield and yield components.

B rates	Legumes	N rates	Emergence m^{-2}	tiller m^{-2}	Spike m^{-2}	Grains $Spike^{-2}$	Thousand grain weight (g)	Grain yield (kg ha^{-2})
0	Cowpea	0	148	295	282	49	48.0	2210
0	Mungbean	0	141	357	344	50	47.0	2580
0	Sesbania	0	131	416	410	48	51.0	2508
0	Fallow	0	142	378	365	48	48.3	1892
50	Cowpea	0	126	373	360	48	50.3	2213
50	Mungbean	0	148	376	363	50	48.7	2432
50	Sesbania	0	121	360	355	51	46.7	2409
50	Fallow	0	123	364	351	50	48.7	2020
0	Cowpea	60	127	435	424	49	51.7	2810
0	Mungbean	60	130	446	435	47	52.0	3063
0	Sesbania	60	153	392	386	50	53.7	3000
0	Fallow	60	135	339	328	52	51.3	2494
50	Cowpea	60	139	395	384	52	52.7	2985
50	Mungbean	60	140	407	396	51	51.3	2971
50	Sesbania	60	154	439	428	52	50.0	2930
50	Fallow	60	140	392	381	53	49.3	2918
0	Cowpea	90	129	413	406	51	50.3	3481
0	Mungbean	90	143	422	415	51	51.3	3305
0	Sesbania	90	144	427	420	46	51.3	3142
0	Fallow	90	124	394	387	48	48.7	3293
50	Cowpea	90	111	443	432	59	54.0	4209
50	Mungbean	90	112	475	456	58	53.7	4225
50	Sesbania	90	119	459	452	61	54.3	3874
50	Fallow	90	122	459	452	53	49.0	3175
0	Cowpea	120	120	415	408	58	49.3	3846
0	Mungbean	120	123	393	386	57	49.0	3660
0	Sesbania	120	134	393	357	55	47.0	3298
0	Fallow	120	142	360	353	56	49.7	3979
50	Cowpea	120	157	417	407	56	50.0	4151
50	Mungbean	120	156	444	434	53	52.0	4052
50	Sesbania	120	157	458	455	49	51.7	4037
50	Fallow	120	154	406	399	47	51.7	3865
Source of variation			Emergence	Tiller	Spikes	Grains	TGW	Grain yield
Biochar (B)			ns	*	*	*	*	*
Legumes (L)			ns	ns	ns	ns	ns	ns
Nitrogen (N)			ns	ns	ns	ns	ns	*
B×L			ns	ns	ns	ns	ns	ns
B×N			ns	ns	ns	*	*	ns
L×N			ns	ns	ns	*	ns	*
B×L×N			ns	**	ns	ns	ns	*

Note: Values followed by the same letters, within column, are not significantly different at $P \leq 0.05$. SOV- source of variation, ** indicate the significant difference $P \leq 0.01$ and * indicate $P = 0.01 - 0.05$. ns-non-significant.

3.2 Yield and yield components

The application of biochar significantly affected tillers m^{-2}, spikes m^{-2}, grains $spike^{-1}$, thousand grain weight (g), and yield (kg ha^{-1}). However, the impact of legumes, nitrogen (N), and the interaction between biochar and legumes (B×L) was not significant, except for grain yield. Furthermore, B×N considerably affected grains $spike^{-1}$ and thousand grain weight (g), while B × L× N significantly influenced tillers m^{-2} and grain yield (Table 3). Plots treated with 90 kg ha^{-1} and 120 kg N ha^{-1} exhibited higher tillers m^{-2}, while control plots with N application had lower tillers m^{-2}. Biochar at 50-ton ha^{-1} increased tillers m^{-2} compared to non-biochar plots. The effect of legumes was not significant, but sesbania incorporation led to higher tillers m^{-2}, while cowpea resulted in lower tillers m^{-2}. Data on spike m^{-2} showed that biochar-treated plots had higher spike m^{-2} than non-biochar plots. Spike m^{-2} increased with N application up to 90 kg ha^{-1}, and plots with 90 kg N ha^{-1} had similar spike m^{-2} to 120 kg N ha^{-1}. Sesbania incorporation, similar to mung bean, resulted in more spikes. Fallow plots had a lower number of spike m^{-2}.

Biochar (50-ton ha^{-1}) application resulted in a higher number of grains $spike^{-1}$ compared to plots without biochar. Similarly, a higher number of grains $spike^{-1}$ was observed with 120 kg N ha^{-1}, which was statistically similar to N application at 90 kg ha^{-1}. Control plots (0 kg N ha^{-1}) exhibited lower grains per spike. In terms of thousand grain weight, plots with previous biochar application at 50-ton ha^{-1} recorded higher weights, while no biochar plots had lighter grains (Table 3). Nitrogen application at 90 kg ha^{-1} produced heavier grains, similar to 60 kg ha^{-1}, followed by 120 kg ha^{-1}. Control plots had lower thousand grain weights. Plots treated previously with 50-ton ha^{-1} biochar yielded higher wheat grain compared to no biochar plots (Table 3). Additionally, 90 kg N ha^{-1} resulted in a greater grain yield, statistically similar to the yield obtained with 120 kg N ha^{-1}) (Table 3). Grain yield was lower in control plots. Regarding legumes, including (cowpea, sesbania, and mung bean) produced higher grain yields compared to fallow plots.

The plots treated with 50-ton ha^{-1} biochar outperformed those without biochar, displaying a higher biological yield. A consistent linear increase in biological yield was observed with rising nitrogen levels. Specifically, plots treated with 120 kg ha^{-1} N showed the highest biological yield, followed closely by those with 90 kg N ha^{-1}. Contrastingly, control plots exhibited a lower biological yield (Figure 1). Although the impact of legumes was statistically non-significant, plots previously sown with legumes exhibited a notably higher harvest index compared to fallow plots. Moreover, a significant difference emerged with N applications: a superior harvest index resulted from the application of 120 kg ha^{-1} N, while a slightly lower harvest index was observed with 90 kg ha^{-1} N. Control plots, as demonstrated the lowest harvest index (Figure 1).

3.3 N contents in soil, grain and straw of different legumes

Application of biochar at a rate of 50 tons ha^{-1} led to a higher grain N content (2.25%) compared to plots without biochar amendment (2.05%) (Table 4).

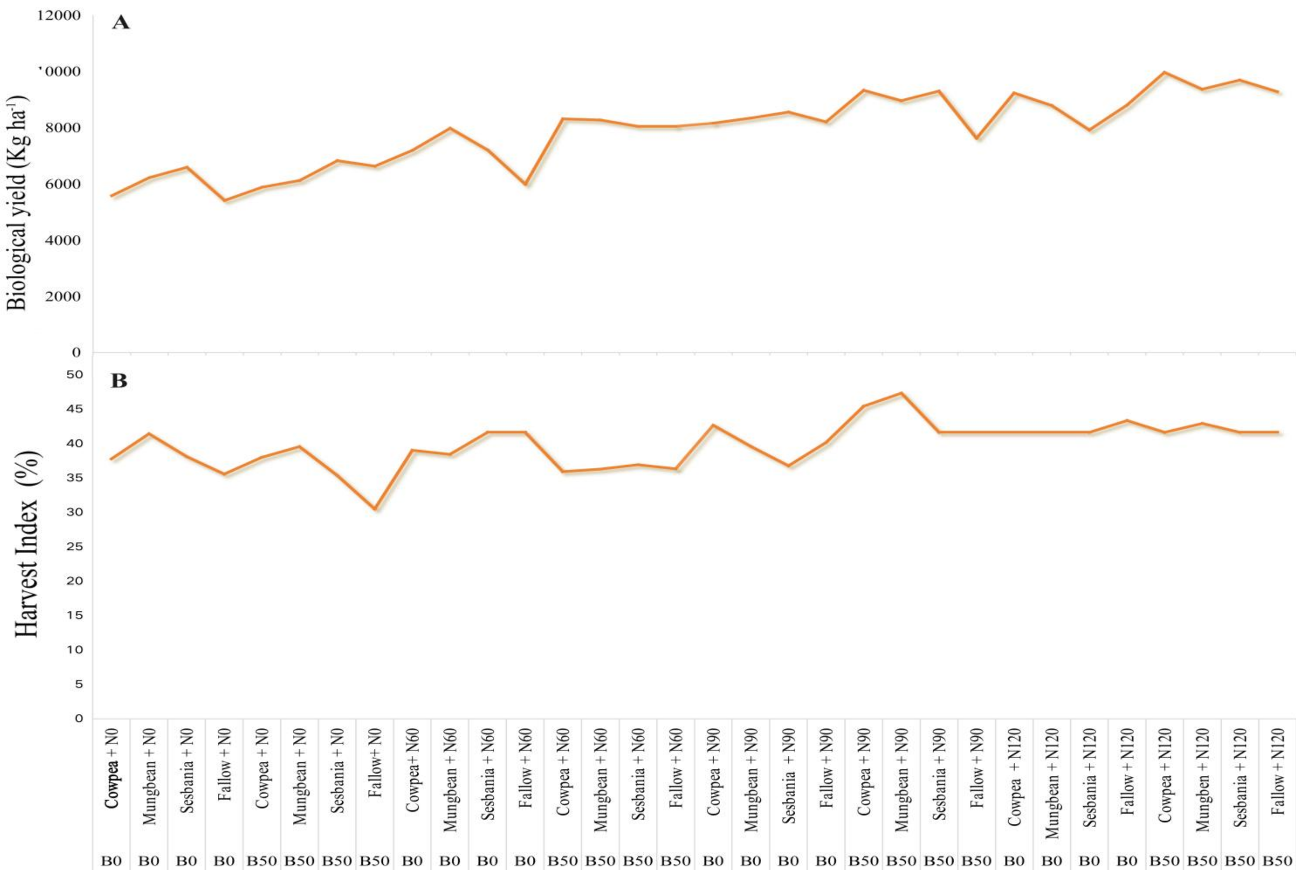

Figure 1. Impact of various biochar and nitrogen levels on biological yield and harvest index of different legumes. Note: B0 and B50 indicated biochar 0 and 50 tin ha^{-1}, while N0, N60 and N120 indicate nitrogen rates of 0, 60 and 120 kg ha^{-1}

The grain N content exhibited a linear increase with the N application rate, reaching its peak (2.23%) in grains collected from plots treated with 120 kg ha^{-1} N, followed closely by 90 kg N ha^{-1} (2.14%). Control plots exhibited a lower grain N content (1.97%). Incorporating sesbania resulted in a higher grain N content (2.19%), comparable to cowpea and mung bean sown plots, while fallow plots showed a lower grain N content (1.75%) (Table 4).

For straw N content, plots previously sown with mung bean displayed a higher value (0.47%), equivalent to sesbania incorporated plots. Fallow plots exhibited a lower wheat straw N content (0.37%). Additionally, N application at a rate of 120 kg ha^{-1} resulted in higher straw N content (0.47%), followed by 60 kg ha^{-1} N (0.43%), whereas control plots displayed a lower wheat straw N content (0.42%). In terms of soil N content, plots treated with 50 tons ha-1 biochar exhibited higher levels (0.07%) compared to plots without biochar (0.06%).

Furthermore, N application at a rate of 120 kg ha^{-1} resulted in elevated soil N content

Table 4. Nitrogen contents in grain, straw and soil after biochar and N application of different legumes.

Biochar rates (ton ha^{-1})	Legumes	N rates (kg ha^{-1}	Grain N content (%)	Straw N content (%)	Soil N content (%)
0	Cowpea	0	1.88	0.4	0.05
0	Mungbean	0	1.85	0.39	0.05
0	Sesbania	0	1.93	0.41	0.05
0	Fallow	0	1.79	0.38	0.04
50	Cowpea	0	1.94	0.41	0.06
50	Mungbean	0	2.17	0.46	0.06
50	Sesbania	0	2.38	0.51	0.06
50	Fallow	0	1.82	0.39	0.05
0	Cowpea	60	2.44	0.52	0.07
0	Mungbean	60	2.2	0.47	0.07
0	Sesbania	60	2.43	0.52	0.06
0	Fallow	60	1.79	0.38	0.04
50	Cowpea	60	2.58	0.55	0.06
50	Mungbean	60	1.81	0.39	0.06
50	Sesbania	60	1.85	0.39	0.06
50	Fallow	60	1.93	0.41	0.06
0	Cowpea	90	1.85	0.39	0.06
0	Mungbean	90	1.94	0.41	0.06
0	Sesbania	90	2.17	0.46	0.07
0	Fallow	90	1.78	0.38	0.04
50	Cowpea	90	2.27	0.48	0.08
50	Mungbean	90	2.44	0.52	0.08
50	Sesbania	90	2.2	0.47	0.07
50	Fallow	90	1.38	0.29	0.05
0	Cowpea	120	2.4	0.51	0.08
0	Mungbean	120	2.58	0.55	0.09
0	Sesbania	120	2.27	0.48	0.08
0	Fallow	120	1.84	0.39	0.04
50	Cowpea	120	2.2	0.47	0.07
50	Mungbean	120	2.43	0.52	0.08
50	Sesbania	120	2.4	0.51	0.08
50	Fallow	120	1.69	0.36	0.06

Source of variation	Grain N	Straw N	Soil N
Biochar (B)	*	ns	*
Legumes (L)	ns	ns	ns
Nitrogen (N)	ns	ns	ns
B×L	ns	ns	*
B×N	*	ns	*
L×N	*	*	*
B×L×N	*	*	*

Note: N-nitrogen, Values followed by the same letters, within column, are not significantly different at P ≤ 0.05. SOV- source of variation, ** indicate the significant difference P ≤ 0.01 and * indicate P = 0.01 − 0.05. ns-non-significant.

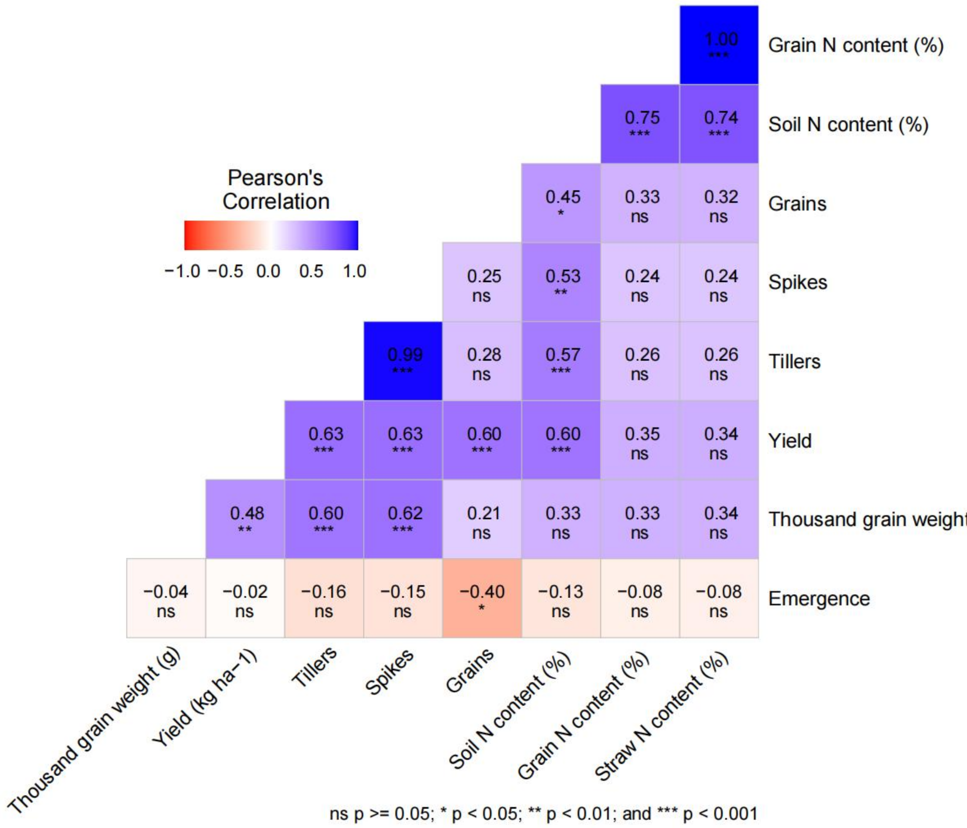

Figure 2. Correlation analysis among all traits across the biochar and N treatments.

(0.07%), followed by 90 kg N ha^{-1} (0.06%), while control plots displayed lower soil N content.

3.4 Relationship of growth, N contents and grain yield

The correlation analysis was performed by using R Studio, utilizing the "metan" package (Figure 2). The findings revealed significant correlations between straw nitrogen (N) content (R=0.74) and grain N content (R=0.75) with soil N content, as illustrated in Figure 2. Moreover, the grain yield of wheat exhibited noteworthy correlations with various factors: tillers (R=0.63), grains (R=0.60), soil N content (R=0.60), grain N content (R=0.35), and straw N content (R=0.34). These results emphasize the intricate interplay between soil and plant components, shedding light on key relationships that influence wheat productivity.

4. Discussion

The terminal objective of various organic and inorganic amendments is to improve crop yield due to different processes and biochemical changes in the soil as well as due to various anthropogenic activities. Crop yield is the ultimate task of various nutrient management practices aiming to increase income. Various yield components and growth parameters (grains $spike^{-1}$, 1000 grains weight, grain yield and total biomass) of wheat significantly enhanced with the use of biochar, mineral nitrogen and previously sown legume as compared to no biochar, control and fallow, respectively. Mineral N application resulted in 51%, 65%, 12%, 12%, 12%, 12% and 12% in tiller m^{-2}, spikes m^{-2}, grains $spike^{-1}$, thousand grain weight, grain yield, biological yield and grain and stover N contents respectively.

However, nitrogen application significantly enhanced tillers m^{-2} as compared to control. The minimum tillers in control plots probably may be due to the exhaustive effect of wheat in terms of nutrient absorption that led to nutrient deficiency and poor crop performance (Salim et al., 2020). The fact that tillers m^{-2} of wheat varied significantly with the application rate of nitrogen strongly underscores the necessity of an accurate N application rate to match nutrient supply to crop demand. Nitrogen application resulted in 27% increase, while previously applied biochar caused a 30 % increase in wheat spikes m^{-2} over control. Wheat spikes m^{-2} positively responded to the N sources, either organic or inorganic, which might be the reason that biochar has a carry-over effect. The lower number of spikes m^{-2} due to lower N level was may be due to the lower availability of nitrogen during plant growth (Ciampitti et al., 2012). Rehman et al. (2008) also reported that a combination of NPK and FYM gave a higher number of spikes m^{-2}. Biochar ensures greater nutrient retention and water holding capacity of the soil (Lehman et al., 2003) might have produced more tiller and spike m^{-2} in biochar treated plots over control. Higher tiller and spike m^{-2} were counted in plots sown after legume crops (i.e., Cowpea, sesbania and mungbean).

Moreover, Nitrogen application significantly improved number of grains $spike^{-1}$ of wheat over control and this increase could be accredited to higher levels of available N for plant uptake (Ullah et al., 2013). Our results are confirmed by the findings of Costa et al. (2002) who stated an increase in spike length and diameter via the addition of N up to an optimum level and higher level did not increase in both of the parameters considerably. Biochar application convincingly improved the number of grains $spike^{-1}$ as compared to the control. This increase in grains $spike^{-1}$ may be attributed to the slow release of N from biochar in these plots. Our results are similar to those reported earlier by Lehmann et al. (2003). This increase could be accredited to the positive effect of biochar on soil organic matter nutrient holding capacity as well as available N during the growth period and the improvement in moisture content of the soil (Brar et al., 2001). Thus, biochar amended plots had more grains $spike^{-1}$ as compared to control plots. Likewise, other yield components of thousands of grain weight of wheat were synergistically improved by nitrogen application rate. The increase in thousand grain weight has been attributed to the increased application rate of nitrogen fertilizer (Ullah et al., 2013). Similar results were reported by Makowska et al. (2008), who found an increase in thousand grain weight of wheat by increasing the level of nitrogen. Significantly the greater 1000-grain weight of wheat in biochar amended plots over control might be due to higher uptake of P because of its involvement in grain development as biochar application improved soil P content in the experimental fields.

Further, the Biological yield of wheat increased in N applied plots over control. It could be attributed to higher plant height in N treated plots and positive impact of N on vegetative growth. More leaves plant^{-1} and leaf area could be noted in nitrogen fertilized plots (Shafi et al., 2012) which ultimately improved biological yield of wheat in fertilized plots. These findings are in full agreement with that of Muhammad and Hassan (2011) who reported that the increase in leaf to stem ratio with nitrogen application is probably due to the increase in number of leaves and leaf area under nitrogen treatments, producing more and heavy leaves in result biological yield is increased.

Additionally, Biochar application increased grain yield of wheat as compared to control. This increase in biochar amended plots could be attributed to nutritional value of biochar. Biochar increase crop productivity by applying nutrient directly to the crop or by improving soil fertility and productivity and enhance fertilizer use efficiency especially nitrogenous fertilizer by reducing leaching of N (Ullah et al., 2021). Other reasons for the increase in grain yield due to biochar application could be its ability to enhance organic matter mineralization (Wardle et al.,1998) and improved crop yield and growth (Chan et al., 2007). Technically, biochar acts as a buffer and contains some essential plant nutrients which influentially increase crop yield. Therefore, grain yield was considerably enhanced with higher rates of biochar. Being a comprehensive and multifunctional entity, biochar increases soil fertility, organic matter, porosity, and improves nutrients availability and nutrients use efficiency in crops. Uzoma et al. (2011) achieved similar results and also stated that biochar incorporation in soil at the rate of 30 and 20-ton ha^{-1} would significantly increase maize grain yields by 150% and 98% as compared with the control, respectively. Moreover, the increase in soil organic matter content improved the physical properties of the soil and would have caused increased root development that acted positively in more uptakes of water and nutrients and caused increase in wheat grain yield (Khan et al., 2008; Ali et al., 2012).

Combined application of organic and inorganic fertilizers affirmatively affects wheat grain yield owing to incorporation of sesbania which improves soil physical properties. Use of mineral fertilizers increases mineralization and makes the soil more productive (Ali et al., 2011). These results are in line with Negassa et al. (2001) who found that maize yield was 35% increased by integrated N management. The significant correlations between soil and plant variables was noticed in the study. Notably, the strong correlations of straw nitrogen (N) content (R=0.74) and grain N content (R=0.75) with soil N content highlight the intricate interplay within the soil-plant system. Additionally, the noteworthy associations observed between grain yield of wheat and key factors such as tillers (R=0.63), grains (R=0.60), and soil N content (R=0.60) further elucidate the complex dynamics influencing wheat productivity. These findings contribute valuable insights to our understanding of the nuanced connections shaping agricultural outcomes.

5. Conclusion

Our results concluded that previously incorporated biochar at the rate of 50-ton ha^{-1} improved yield and yield components of wheat and enhanced nitrogen (N) content of soil. In addition, previously sown legumes i.e., mungbean, cowpea and Sesbania for grain, fodder and green manuring purposes, respectively had positive effects on wheat yield and yield components and soil N status. Higher grain yield of wheat was recorded with 120 kg N ha^{-1} but it was at par with 90 kg N ha^{-1} when sown after legumes. Therefore, it is recommended that 50 t B ha^{-1} is suitable for soil health and sowing of legumes like cowpea, mungbean and Sesbania in summer gap are recommended for getting fodder, grain or biomass for green manure, respectively. Furthermore, nitrogen level of 90 kg ha^{-1} instead of 120 kg ha^{-1} is recommended for having higher grain yield of wheat if sown after legumes.

Green manuring at post flowering stage resulted in the lowest AE (6.6 kg kg^{-1}). Among N levels, the N application at the rates of 70 and 100 kg ha^{-1} had higher and statistically similar AE (11.8 and 10.3 kg kg^{-1}, respectively) as compared to 130 kg N ha^{-1} (9.1 kg kg^{-1}).

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Availability of Data and Materials: Data will be available on formal request from the corresponding authors.

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ORIGINAL RESEARCH

Impact of Long-Term Organic Manure Application on Yield, Zinc, and Copper Uptake in Maize, Peas, and Mungbean (*Vigna radiata* L.) Cropping System

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ABSTRACT: To evaluate the impact of the long-term application of organic manures on yield, uptake of zinc and copper in maize, peas and summer mungbean cropping systems, a field study was conducted at the integrated farming system of Punjab Agricultural University, Ludhiana. The treatment combinations were; T_1: 50% N through recommended NPK + 50% N was substituted through FYM, T_2: 100% N through FYM, T_3: T_2 + intercropping (marigold in pea, cowpea in maize), T_4: T_2 + agronomic practices for weed and. pest control, T_5: 50% N as FYM + rock phosphate to substitute the P requirement of crops + phosphate solubilizing bacterial. cultures (PSB), T_6: T_2 + biofertilizer (consortium) containing N and P carriers and T_7: 100% Recommended NPK through chemical fertilizers. Significant increases in the yield, micronutrient content and uptake were recorded due to the application of 50% nitrogen through farmyard manure (FYM) and 50% of the recommended dose of fertilizers (T_1) followed by 100% N through FYM + biofertilizer containing. N and P, carriers (T_6). The highest grain yield of maize (5.72 t ha^{-1}), pea (16.2 t ha^{-1}) and summer mungbean (11.6 t ha^{-1}) were recorded in treatment T_1, surpassing the 100% recommended dose of fertilizer (T7) by 13.7%, 20% and 10.4 %, respectively. The concentration of copper (Cu) and zinc (Zn) in the grains of maize, pea and summer mung bean was 38.3%, 14.1%, 29.6% and 53.4%, 22.8 % and 19.8% higher in treatment T_1 as compared to treatment T_7. Moreover, the concentration of copper and zinc in the grains of maize, pea and summer mung bean was 32.1%, 24.2% and 29.5 % and 21.7%, 17.6% and 11.6% higher in treatment T_1, respectively, compared to treatment T_7. Similarly, the increase in the uptake of Cu and Zn was observed in both grain and straw of maize, pea and summer mung bean. The study concluded that the integrated nutrient management (INM) treatment is to substitute a portion of chemical fertilizers with a more sustainable and environmentally safe organic compost in order to mitigate soil degradation, improve crop production, and protect the environment.

KEYWORDS: Maize, peas; mungbean, micronutrients, cropping system, organic manure, integrated farming

1. Introduction

Organic matter plays a crucial role in increasing micronutrients availability and mitigating the adverse effects of free cations (Rani et al., 2023). Due to their limited mobility, plants face challenges in obtaining micronutrients from the solid phase of the soil to their roots (Dhaliwal et al., 2019;

Bhatla et al., 2018). The addition of organic matter to the soil enhances its physical, chemical, and biological properties, resulting in increased DTPA-extractable (diethylenetriaminepentaacetic acid) content of iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) in the soil (Dhaliwal et al., 2013; Ali et al., 2020). This increase occurs through various processes, such as chelation (Sharma et al., 2014), helping overcome micronutrients deficiencies.

Chelating agents form soluble complexes with metallic micronutrients, increasing the carrying capacity of soil solutions, and are being developed, potentially accounting for the positive effects of organic manures (Sinegani et al., 2015; Sharma et al., 2014). Biofertilizers, living microorganisms applied to soil, seeds, or plant surfaces, colonize the rhizosphere or the interior of the plant (Hernández et al., 2023), promoting growth by enhancing the supply or availability of primary nutrients to the host plant (Daniel et al., 2022). The activity of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizal fungi also increases with organic matter addition, further enhancing zinc uptake (Masrahi et al., 2023; Lehmann et al., 2014). These activities support biochemical processes in the soil, such as nitrogen fixation, phosphorus mobilization, solubilization, zinc solubilization, and overall plant growth (Silva et al., 2023).

By mobilizing micronutrients, biofertilizers not only accelerate plant development but also reduce micronutrients deficiency (Mandal et al., 2023). The availability of nutrients in the soil depends on the chemical equilibrium between nutrient ions in the soil solution and solid phases. Several variables, including soil type, crop species, fertilizer supplier, and the yield potential of the variety, affect how well various crops absorb secondary nutrients (Aulakh et al., 2022). According to Choudhary et al. (2018), different cropping systems that received combined applications of organic manures and chemical fertilizers showed better micronutrients uptake. Furthermore, Rutkowska et al. (2014) reported that the integrated use of organic manures and chemical fertilizers improved the availability of micronutrients to plants.

Despite the positive effects of predominant cropping systems like rice-wheat, cotton-wheat, and maize-wheat on building soil organic matter and nutrient status (Sharman et al., 2023), the rice-wheat system is also associated with the appearance of iron deficiency in rice and manganese and zinc deficiency in the subsequent wheat crop (Yadav et al., 2023). The inclusion of deep-rooted crops and pulses in the cropping system helps overcome nutrient deficiencies by mobilizing zinc, copper, iron, and manganese, thus reducing micronutrients deficiencies (Kumar et al., 2020). Pulses contribute to organic matter through litter fall and have higher root biomass, serving as a crucial source in redistributing soil micronutrients and secondary plant nutrients (Edwards et al., 2022).

However, certain cropping systems like moongbean-wheat, soyabean-wheat, and moongbean-raya play a pivotal role in building nutrient status and ameliorating deficiencies. In intensive agricultural systems where, high nitrogen levels are applied without organic additions, such as in rice-wheat systems, micronutrients depletion occurs (Ali et al., 2019; Gupta et al., 2000). Organic manures, containing both macro and micronutrients, contribute to soil improvement by significantly enhancing nitrogen fixation. They establish a positive nutritional balance and improve soil physical qualities by providing an excellent substrate for microorganism growth (Kumar et al., 2011). The incorporation of organic manures into the soil supplies valuable nutrients to plants and the soil, contributing to the maintenance of soil fertility (Prasad et al., 2002). Farmyard manure, acting as a reservoir of nutrients, is known to improve soil productivity on a sustainable basis (Chaudhary and Narwal, 2005). Long-term application of farmyard manure has been shown to increase DTPA-extractable micronutrients in the soil (Richards et al., 2011; Wang et al., 2016).

Given the pivotal role of organic manures, this experiment was conducted to assess the long-term impact of organic manure application on the yield, micronutrients content, and uptake in a cropping system involving maize, peas, and summer mung beans. The investigation aims to provide valuable insights into the sustained effects of organic manures on overall productivity and nutrient dynamics within this agricultural context

2. Materials and methods

2.1 Experimental location and design

The field experiment was carried out at School of Organic Farming, PAU, Ludhiana by choosing maize - pea - summer mungbean as the testing cropping system, comprised of seven treatments, replicated thrice in a randomized block design. In each treatment, different organic and integrated nutrient sources were applied. The various organic and inorganic combination treatments were; T_1:50% N through recommended NPK + 50% N was substituted through FYM, T_2: 100% N through FYM, T_3: T_2 + intercropping (marigold in pea, cowpea in maize), T_4: T_2 + agronomic practices for weed and pest control, T_5: 50% N as FYM + rock phosphate to substitute the P requirement of crops + phosphate solubilizing bacterial cultures (PSB), T_6: T_2 + biofertilizer (consortium) containing N and P carriers and T_7: 100% Recommended NPK through chemical fertilizers.

2.2 Measurement and analysis

The basic soil sample was collected before the start and harvest of crops by giving a V-shaped cut. The samples were collected from 3-4 places and thereafter, soil samples were mixed together to obtain a representative sample for analysis. The chemical properties of surface soil were determined using the standard analytical procedures (Jackson 1973). Plant samples were collected after harvesting of maize, pea and summer mungbean. Grain and straw samples of cropping system were collected, dried in the sun, and then oven-dried. Grain and straw samples of maize, pea and summer moong were digested in a di-acid mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in the ratio of 3:1 for the analysis of Fe, Mn, Zn and Cu. The concentration (ppm) of Fe, Mn, Zn and Cu were determined by using the Atomic Absorption Spectrophotometer method (Lindsey and Norvell 1978). The grain and straw yield of maize, pea, and summer mung bean was reported in ton hectare.

2.3. Statistical analysis

The effects of several treatments on yield and micro nutrients concentration were assessed using the ANOVA technique in Statistics 8.1 (Analytical Software Tallahassee, FL, USA). First, the data were subjected to routine testing to meet the normality assumptions. Furthermore, before analyzing the data, the percentages were arcsine transferred to normalize the variables. Tukey's post hoc test was used to compare means for parameters with significant treatment effects

3. Results

The results of this study showed that the various organic and INM treatments played an important role in regulating several soil chemical properties. The impact of different types and rates of organic compost application, either solely or in combination with chemical fertilizers on crop yield, micronutrients content, and uptake by plant were presented in different sections.

3.1 Grain and straw yield of maize

Results showed that maize grain yield was significantly affected by different treatments (Figure 1A). Among the treatments, T_1 treatment increased the grain yield of maize (5.72 t ha^{-1}) compared to all other treatments followed by T_6. In contrast, the lowest grain yield (4.17 t ha^{-1})of maize was recorded in treatment T_5(50% N as FYM + Rock phosphate + PSB), Whereas the treatment T_7 with 100% recommended NPK recorded 5.03 t ha^{-1} of grain yield .

Maize residue has economic significance since it is given to the animals as feed. The maize stover yield was significantly influenced by different treatments (Figure 1A). The highest stover yield of maize (9.78 t ha^{-1}) was observed in treatment T_6 followed by treatment T_1 (9.48 t ha^{-1}). Whereas the lowest stover yield (7.03 t ha^{-1}) was observed in the treatment $T_{5,}$ having 50% N applied through FYM, rock phosphate and PSB.

3.2 Pea pod and stover yield

Pea pod yield varied from 10.6 to 16.2 t ha^{-1}under various organic and integrated treatments, significantly increased the pea pod yield (Fig. 1B). Among the different treatments, the highest pod yield (16.2 t ha^{-1}) of peas was observed under the treatment T_1 where 50% N was substituted through FYM and 50% recommended NPK were applied followed by treatment T_6 (15.8 t ha^{-1}) which include 100% nitrogen through FYM along with biofertilizer. The lowest pod yield (10.6 t ha^{-1}) was observed under the treatment T_5 having 50% N was substituted through FYM and rock phosphate and PSB was applied.

The pea stover yield varied from 0.152 to 0.196 t ha^{-1} (Fig. 1C). The results showed that different organic and integrated treatments significantly improved the pea stover yield. The highest stover yield of pea (19.6 t ha^{-1}) was observed in treatment T_1 where 50% N was substituted through FYM and 50% recommended NPK were applied, followed by treatment T_6 (19.0 t ha^{-1}), which included 100% nitrogen through FYM along with biofertilizer. Thereafter, the lowest stover yield (15.2 t ha^{-1}) was observed in the treatment T_5 with 50% N as FYM and rock phosphate and PSB.

3.3 Grain and straw yield of summer mung bean

Grain yield of summer mung bean varied from 0.88 to 11.6 t ha^{-1}(Figure 1C). Among the different treatments, the highest grain yield of mung bean (1.16 t ha^{-1}) was observed in the treatment T_1 where 50% N was

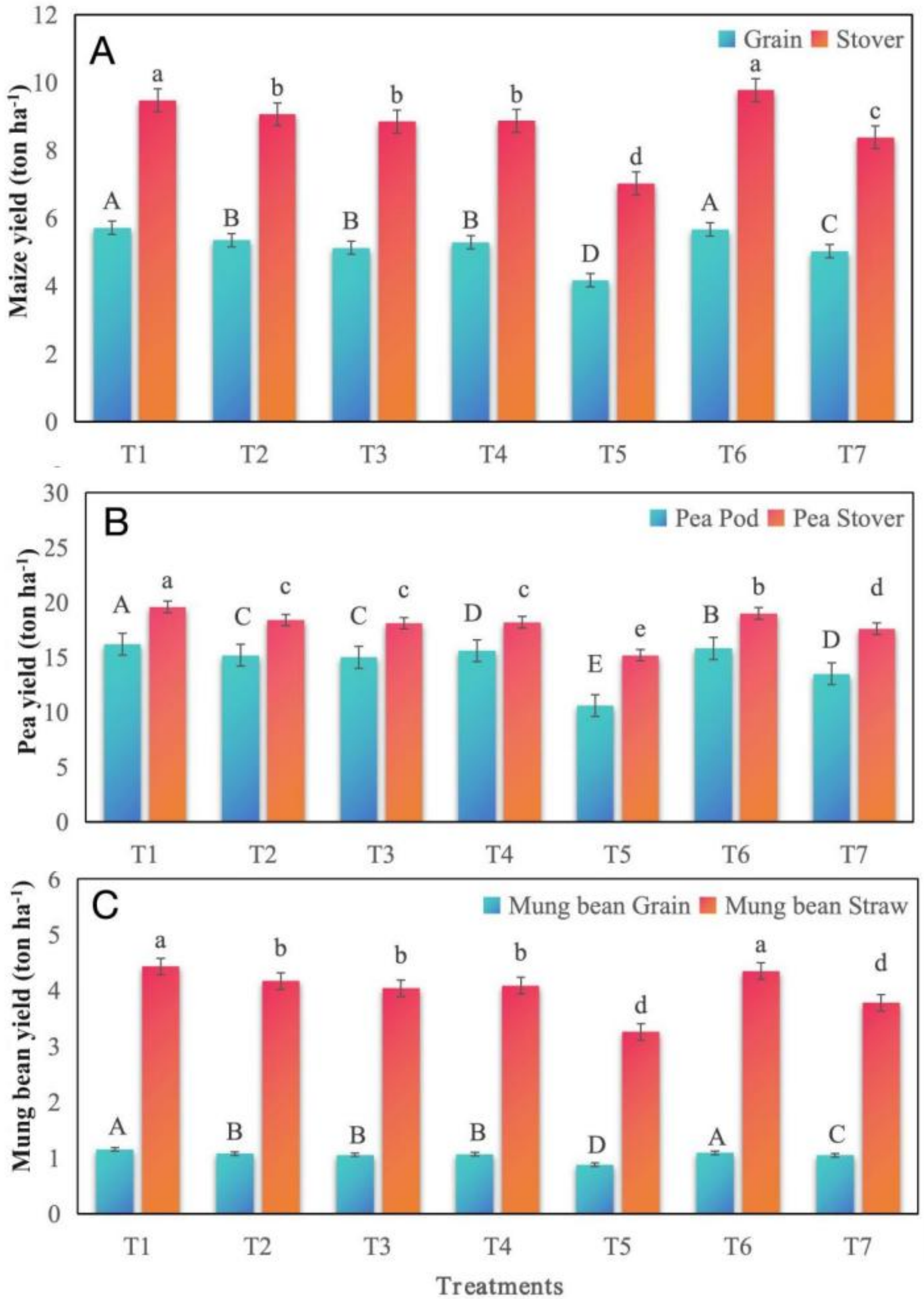

Figure 1. Effect of long-term application of organic manures on the (A) maize, (B) mung bean and (C) pea grain and straw yield (ton ha^{-1}). Note-lowercase and upper-case letters indicate significant differences (*$P < 0.05$*) among the treatments of grain yield and straw yield respectively. T_1- 50% of the recommended, NPK + 50% N through. FYM; T_2 - 100% N through FYM; T_3 - 100% N through FYM + intercropping; T_4 - 100% N through FYM+ agronomic measures for weed and pest management; T_5 - 50% N as FYM + Rock phosphate + PSB; T_6 - 100% N through FYM + biofertilizer, containing. N and P, carriers; T_7 - 100% recommended NPK

substituted through FYM and 50% recommended NPK were applied, followed by treatment T_6 (1.09 t ha^{-1}), which included 100% nitrogen through FYM along with biofertilizer. The lowest grain yield (0.88 t ha^{-1}) was observed in the treatment T_5 with 50% N substituted through FYM, rock phosphate, and PSB. The straw yield of the summer mung bean among different treatments varied from 3.26 to 4.43 t ha^{-1}(Figure 1C). The highest straw yield of mung bean (4.43 t ha^{-1}) was observed in treatment T_1, where 50% N was substituted through FYM and 50% recommended NPK was applied followed by treatment T_6 (4.35 t ha^{-1}), which included 100% nitrogen through FYM along with biofertilizer and the lowest stover yield (3.26 t ha^{-1}) was observed under the treatment T_5 having 50% N as FYM with rock phosphate and PSB.

3.4 Copper and zinc content of maize grain

The effect of the long term application of organic manures on the micronutrients concentration of maize grain is given in Table 1. The Cu concentration of maize grain varied from 1.98 to 2.74 mg kg^{-1}. Among all the treatments highest Cu content (2.74 mg kg^{-1}) was observed under treatment T_1, where 50% N was substituted through FYM and 50% recommended NPK was applied, followed by T_6 (2.55 mg kg $^{-1}$) where 100% nitrogen through FYM along with biofertilizer was applied. The lowest content of Cu (1.98 mg kg^{-1}) was observed in the treatment T_7 having 100% recommended NPK. The integrated treatment significantly increased the Cu content of maize grain as compared to the recommended dose of fertilizer. The Zn concentration in maize grain under different treatments varied from 14.6 to 22.4 mg kg^{-1}. The highest Zn content (22.4 mg kg^{-1}) was recorded in the T_1 where 50% N through FYM and 50% recommended NPK were applied followed by T_6 (20.0 mg kg^{-1}) which included 100% nitrogen through FYM along with biofertilizer and the lowest content of Zn (14.6 mg kg^{-1}) was observed in the treatment T_7. The integrated treatment significantly increased the Zn content of maize grain as compared to the treatment with 100% recommended dose of fertilizer.

3.5 Copper and zinc content of pea grain

The copper concentration of pea grain varied from 5.40 to 6.16 mg kg^{-1}. Among all the treatments, the highest Cu content (6.16 mg kg^{-1}) was observed under treatment T_1 where 50% N was substituted through FYM and 50% N through recommended NPK, followed by T_6 (6.10 mg kg $^{-1}$) where 100% nitrogen was substituted through FYM along with biofertilizer was applied which was higher than the treatment T_4 (5.75 mg kg^{-1}) where 100% N through FYM and agronomic measures for weed and pest management were adopted. The lowest content of Cu (5.40 mg kg^{-1}) was observed in the treatment T_7 with 100% recommended NPK. The integrated treatment significantly increased the Cu content of pea grain as compared to the 100% recommended dose of fertilizers. The Zn concentration in pea grain varied from 29.7 to 36.5 mg kg^{-1}. Highest Zn content (36.5 mg kg^{-1}) was recorded in the T_1 where 50% N was substituted through FYM and 50% recommended NPK were applied followed by T_6 (34.4 mg kg^{-1}). Lowest content of Zn (29.7 mg kg^{-1}) was observed in the treatment T_7. The integrated treatment management treatment significantly increased the Zn content of pea grain as--

Table 1. Effect of long term application of organic manures on micronutrients concentration (mg kg^{-1}) of maize, peas and summer mung bean.

Treatment	Maize				Pea				Summer Mung bean			
	Cu		Zn		Cu		Zn		Cu		Zn	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
T_1	2.74 a	5.43 a	22.4 a	30.2 a	6.16 a	8.96 a	36.5 a	41.9 a	3.28 a	6.75 a	39.8 a	50.9 a
T_2	2.32 d	4.98 b	17.8 c	25.6 c	5.69 d	7.9 d	32.4 c	38.9 c	2.98 c	6.14 c	34.4 c	49.9 b
T_3	2.25 d	4.23 c	16.1 d	25.2 c	5.63 e	7.48 e	30.9 d	38.2 c	2.76 d	5.68 d	34.4 c	47 c
T_4	2.43 c	5.17 b	19.9 b	27.2 b	5.75 c	8.53 c	32 c	41.1 b	3.05 c	6.28 c	36.8 b	50.2 a
T_5	2.14 e	4.2 c	14.7 e	24.9 c	5.53 f	7.47 e	29.8 e	37.7 d	2.64 e	5.44 e	34.3 e	46.8 c
T_6	2.55 b	5.36 a	20 b	27.6 b	6.1 b	8.75 b	34 b	41.5 d	3.17 b	6.53 b	38.9 a	50.9 a
T_7	1.98 f	4.11 c	14.6 e	24.8 d	5.4 g	7.21 f	29.7 e	35.6 e	2.53 f	5.21 f	33.2 d	45.4 d
LSD (0.05)	0.09	0.21	1.12	0.71	0.01	0.03	0.9	0.8	0.1	0.21	0.93	0.81

Note-lowercase letters indicate significant ($P < 0.05$) difference among the treatments. T_1- 50% of the recommended, NPK + 50% N through. FYM; T_2 - 100% N through FYM; T_3 - 100% N through FYM + intercropping; T_4 - 100% N through FYM+ agronomic measures for weed and pest management; T5 - 50% N as FYM + Rock phosphate + PSB; T_6 - 100% N through FYM + biofertilizer, containing. N and P, carriers; T_7 - 100% recommended NPK compared the treatment where 100% recommended dose of fertilizer was applied.

3.6 Copper and zinc content of summer mung bean grain

Copper concentration of mung bean grain was varied from 2.53 to 3.28 mg kg^{-1}. Among all the treatments highest Cu content (3.28 mg kg^{-1}) was observed in the treatment T_1 where 50% N through FYM and 50% recommended NPK were applied, followed by T_6 (3.17 mg kg $^{-1}$) where 100% nitrogen through FYM applied with biofertilizer. The lowest content of Cu (2.53 mg kg^{-1}) was observed in the treatment T_7 having 100% recommended NPK. The Zn concentration of mung bean varied from 33.1 to 39.5 mg kg^{-1}. Among different treatments, highest Zn content (39.5 mg kg^{-1}) was observed under the treatment T_1 where 50% N through FYM and 50% recommended NPK were applied followed by T_6 (38.9 mg kg^{-1}) which include 100% nitrogen through FYM along with biofertilizer. The content was 36.8 mg kg $^{-1}$ in treatment T_4 where 100% NPK was applied through FYM along with agronomic measures for weed and pest management was followed and the lowest content of Zn (33.2 mg kg^{-1}) was observed in the treatment T7. The integrated treatment significantly increased the Zn content of mung bean grain as compared to the treatment with 100% recommended dose of fertilizer.

3.7 Copper and zinc content of maize straw

Micronutrients concentration of maize stover is presented in the table 1. The Cu concentration in maize varied from 4.11 to 5.43 mg kg^{-1}. Highest Cu content (5.43 mg kg^{-1}) was observed in the treatment T_1 where 50% N through FYM and 50% recommended NPK were applied followed by T_6 (5.36 mg kg^{-1}) where 100% nitrogen was applied through FYM along with biofertilizer was applied. The lowest content of Cu (4.11 mg kg^{-1}) was observed in the treatment T_7 where 100% recommended NPK was applied through chemical fertilzers. Zn content varied from 24.8 to 30.2 mg kg^{-1}. The highest stover Zn concentration (30.15 mg kg^{-1}) was observed under the treatment T_1 where 50% N through FYM and 50% recommended NPK were applied followed by T_6 (27.6 mg kg^{-1}) which included 100% nitrogen through FYM along with biofertilizer was applied. The lowest content of Zn (24.8 mg kg $^{-1}$) was observed in the treatment T_7 having 100% recommended NPK through chemical fertilizers were applied. The integrated nutrient management treatments (T_1, T_4, T_6) increased the maize stover zinc concentration as compared to the chemical fertilizer alone.

3.8 Copper and zinc content of pea straw

Copper concentration in pea straw showed variation among different treatments from 7.21 to 8.96 mg kg^{-1}(Table 1). Highest Cu content (8.96 mg kg^{-1}) was observed in the treatment T_1 where 50% N through FYM and 50% recommended NPK were applied followed by T_6 (8.75 mg kg^{-1}) where 100% nitrogen was applied through FYM along with bio fertilizer. The lowest content of Cu (7.21 mg kg^{-1}) was observed in the treatment T_7 where 100% recommended NPK was applied through chemical fertilizers. The Zn content of pea straw varied from 35.6 to 41.9 mg kg^{-1}. The highest straw Zn concentration (41.9 mg kg^{-1}) was observed under the treatment T_1 where 50% N through FYM and 50% recommended NPK were applied followed by T_6 (41.5 mg kg^{-1}) which included 100% nitrogen through FYM along with biofertilizer was applied. The lowest content of Zn (35.5 mg kg $^{-1}$) was observed in the treatment T_7 where 100% recommended NPK through chemical fertilizers were applied. The integrated nutrient management treatments increased the pea stover zinc concentration as compared to the chemical fertilizer alone.

3.9 Copper and zinc content of mung bean straw

Micronutrients concentration of mung bean straw showed significant variation among different treatments ranging from 5.21 to 6.75 mg kg^{-1} (Table 1). Higher Cu content (6.75 mg kg^{-1}) was observed in the treatment T_1 where 50% N through FYM and 50% recommended NPK were applied, followed by T_6 (6.53 mg kg^{-1}) where 100% nitrogen was applied through FYM along with biofertilizer. The lowest content of Cu (5.21 mg kg^{-1}) was observed in the treatment T_7 where 100% recommended NPK was applied through chemical fertilizers. The Zn concentration of mung bean straw varied from 45.4 to 50.9 mg kg^{-1}. The highest straw Zn concentration (50.9 mg kg^{-1}) was observed in the treatment T_1 where 50% N through FYM and 50% recommended NPK were applied followed by T_6 (46.8 mg kg^{-1}) which included 100% nitrogen through FYM along with biofertilizer. The lowest content of Zn (45.4 mg kg $^{-1}$) was observed in the treatment T_7 where 100% recommended NPK through chemical fertilizers alone.

3.10 Micronutrients uptake of maize grain and straw

Micronutrient uptake of maize grain and straw are presented in the table 2. Variation in Cu uptake by maize grain varied from 8.7 to 15.4 g ha^{-1}. Among all the treatments, highest Cu uptake (15.4 g ha^{-1}) was observed in the treatment T_1 where 50% N was substituted through FYM and 50% N through recommended NPK were applied followed by 14.2 g ha^{-1} in treatment T_6 where 100% nitrogen through FYM along with biofertilizer was applied. The lowest uptake of Cu (8.7 g ha^{-1}) was observed in the treatment T_5 where 50% N was applied through FYM along with rock phosphate and PSB. The integrated treatment significantly increased the Cu uptake of maize grain as compared to the recommended dose of fertilizers. Zn uptake by maize grain varied from 60.1 to 125.7 g ha^{-1}. The highest Zn uptake (125.7 g ha^{-1}) was recorded in T_1 where 50% N through FYM and 50% recommended NPK were applied followed by T_6 which included 100% nitrogen through FYM along with biofertilizer was applied. The lowest uptake of Zn (60.1 g ha^{-1}) was observed in the treatment T_5 where 100% N through FYM and rock phosphate and PSB were applied. The integrated treatment except T_5 significantly increased the Zn uptake by maize grain as compared to the application of 100% recommended dose of fertilizer.

3.11 Micronutrient uptake of pea grain and Straw

Data on effect of long term application of organic manures on the micronutrient uptake of pea grain and straw is presented in the table 2. Variation in the Cu uptake by pea grain was observed in the different treatments from 60.1 to 100 g ha^{-1}. Among all the treatments, highest Cu uptake (100 g ha^{-1}) was observed in treatment T_1 where 50% N through FYM and 50% recommended NPK were applied followed by 93.2 g ha^{-1} in treatment T_2 where 100% nitrogen through

FYM was applied. The lowest uptake of Cu (60.1 g ha^{-1}) was observed in the treatment T_5 where 50% N was applied through FYM along with rock phosphate and PSB. The integrated treatments except T_5 significantly increased the Cu uptake of pea grain as compared to the recommended dose of fertilizer alone. The Zn uptake by pea grain varied from 332 to 598 g ha^{-1}. The highest Zn uptake (598 g ha^{-1}) was recorded in T_1 where 50% N was substituted through FYM and 50% N was substituted through recommended NPK followed by (523 g ha^{-1}) in T_2 which received 100% nitrogen through FYM. The lowest uptake of Zn (332 g ha^{-1}) was observed in the treatment T_5 where 100% N through FYM, rock phosphate and PSB was applied. The integrated treatment significantly increased the Zn uptake by pea grain as compared the treatment with 100% recommended dose of fertilizer. The increase in micronutrients uptake might be due to the fact that application of organic manures decreases the soil pH and increases the availability of the plant available forms of micronutrients.

3.12 Micronutrient uptake of summer mung bean grain

Copper uptake of mungbean grain was observed in the different treatments varied from 2.56 to 3.68 g ha^{-1} (Table 2). Among all the treatments, highest Cu uptake (3.68 g ha^{-1}) was observed in treatment T_1 where 50% N was applied through FYM and 50% recommended NPK were applied The lowest uptake of Cu (2.56 g ha^{-1}) was observed in the treatment T_5 where 50% N was applied through FYM along with rock phosphate and PSB. The integrated treatment significantly increased the Cu uptake of mungbean grain as compared to the recommended dose of fertilizers. The Zn uptake by mungbean grain varied from 28.9 to 44.6 g ha^{-1}. The highest Zn uptake by moonbean grain (44.6 g ha^{-1}) was observed in T_1 where 50% N was substituted through FYM and 50% N was substituted through recommended NPK followed by 40.9 g ha^{-1} in T_6 which received 100% nitrogen through FYM along with biofertilizer containing N and P carriers was applied. The lowest uptake of Zn (29.0 g ha^{-1}) was observed in the treatment T_5 where 100% N through FYM and rock phosphate and PSB was applied. The integrated treatment except T_5 significantly increased the Zn uptake by mungbean grain as compared to the treatment with 100% recommended dose of fertilizers.

3.13 Micronutrient uptake of maize straw

Variation in the Cu uptake by maize straw observed in different treatments varied from 29.9 to 53.2 g ha^{-1} (Table 2). Among all the treatments, highest Cu uptake (53.2 g ha^{-1}) was recorded in T_6 where 100% nitrogen was applied through FYM along with biofertilizer followed by treatment T_1 (52.1 g ha^{-1}) where 50% N through FYM and 50% recommended NPK were applied followed by 46.5 g ha^{-1} in T_4 where 100% nitrogen through FYM and agronomic measures for weed and pest management were adopted. The lowest uptake of Cu (29.9 g ha^{-1}) was observed in the treatment T_5 where 50% N was applied through FYM along with rock phosphate and PSB. The integrated treatment significantly increased the Cu uptake of maize stover as compared the recommended dose of fertilizer.

The Zn uptake by maize straw varied from 177.1 to 289.7 g ha^{-1}. The highest Zn uptake

Table 2. Effect of long term application of organic manures on micronutrients uptake (g ha^{-1}) of maize, peas and summer mung bean.

Treatments	Maize				Pea				Summer Mung bean			
	Cu		Zn		Cu		Zn		Cu		Zn	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
T_1	15.4 a	52.2 a	125 a	289 a	100 a	176 a	598 a	836 a	3.68 a	30.8 a	44.6 a	232 a
T_2	12.2 c	45.8 b	93.2 d	235 b	93.3 b	146 d	523 b	726 b	3.09 c	26.3 b	35.7 d	214 a
T_3	11.3 d	38 c	80.9 e	226 c	86.5 d	135 e	485 b	700 c	2.82 d	23.6 b	35.2 d	195 b
T_4	12.6 c	46.5 b	103 c	245 b	89.3 c	156 c	512 b	761 b	3.14 c	26.4 b	37.9 c	211 b
T_5	8.7 f	29.9 d	60.1 g	177 e	60.1 f	114 f	332 e	584 e	2.23 f	18.2 d	29 f	157 c
T_6	14.2 b	53.2 a	111 b	273 a	87.8 c	167 b	475 c	801 a	3.32 b	29.2 a	40.9 b	228 a
T_7 -	9.81 e	35 c	72 f	210 d	73 e	127 e	404 d	635 d	2.56 e	20.2 c	33.5 e	176 c
LSD (0.05)	0.91	4.72	8.5	20.1	2.7	8.3	42.5	58.5	0.09	3.2	1.01	19.3

Note-lowercase letters indicate significant ($P < 0.05$) difference among the treatments. T_1- 50% of the recommended, NPK + 50% N through. FYM; T_2 - 100% N through FYM; T_3 - 100% N through FYM + intercropping; T_4 - 100% N through FYM+ agronomic measures for weed and pest management; T5 - 50% N as FYM + Rock phosphate + PSB; T_6 - 100% N through FYM + biofertilizer, containing. N and P, carriers; T_7 - 100% recommended NPK

(289.7 g ha^{-1}) was recorded in T_1 where 50% N through FYM and 50% recommended NPK were applied followed by (273 g ha^{-1}) in T_6 which included 100% nitrogen through FYM along with biofertilizer was applied.The lowest uptake of Zn (177 g ha^{-1}) was observed in the treatment T_5 where 100% N through FYM, rock phosphate and PSB were applied. The integrated treatments except T_5 significantly increased the Zn uptake of maize stover as compared to the treatment with 100% recommended dose of fertilizer.

3.14 Micronutrient uptake of pea straw

The Cu uptake by pea grain was varied in the different treatments from 60.1 to 100 g ha^{-1} (Table 2). Among all the treatments, highest Cu uptake (100 g ha^{-1}) was observed in treatment T_1 where 50% N through FYM and 50% recommended NPK were applied followed by 93.2 g ha^{-1} in treatment T_2 where 100% nitrogen through FYM was applied. The lowest uptake of Cu (60.1 g ha^{-1}) was observed in the treatment T_5 where 50% N was applied through FYM along with rock phosphate and PSB. The integrated treatments except T_5 significantly increased the Cu uptake of pea grain as compared to the recommended dose of fertilizer. The Zn uptake by pea grain varied from 332 to 598 g ha^{-1}. The highest Zn uptake (598 g ha^{-1}) was recorded in T_1 where 50% N through FYM and 50% recommended NPK were applied. The lowest uptake of Zn (332 g ha^{-1}) was observed in the treatment T_5 where 100% N through FYM, rock phosphate and PSB was applied.

3.15 Micronutrient uptake of mungbean straw

The Cu uptake by mung bean straw in different treatments varied from 18.2 to 30.7 g ha^{-1}. Highest Cu uptake (30.7 g ha^{-1}) was in T_1 where 50% N through FYM and 50% recommended NPK were applied followed by (29.2 g ha^{-1}) T_6 in which 100% N through FYM along with biofertilizer containing N and P carriers was applied. The lowest uptake of Cu (18.2 g ha^{-1}) was observed in the treatment T_5 where 50% N was applied through FYM along with rock phosphate and PSB. The integrated treatment significantly increased the Cu uptake of mungbean straw as compared to the recommended dose of fertilizers. The Zn uptake by mung bean straw varied from 157 to 232 g ha^{-1}. The highest Zn uptake (232 g ha^{-1}) was in T_1 where 50% N through FYM and 50% recommended NPK were applied followed by 228 g ha^{-1} in T_6 which included 100% nitrogen through FYM along with biofertilizer followed by (214 g ha^{-1}) in treatment T_2 where 100% NPK was applied through FYM and the lowest uptake of Zn (157 g ha^{-1}) was observed in the treatment T_5 where 100% N through FYM, rock phosphate and PSB was applied. The integrated treatment significantly increased the Zn uptake of mungbean straw as compared to the treatment with 100% recommended dose of fertilizer.

4. Discussion

The application of an integrated nutrient dose, combining organic and inorganic fertilizers, not only boosts nutritional supply for higher grain yield but also induces changes in the physical and chemical properties of the soil, thereby promoting improved crop growth and yield (Urmi et al., 2022). Gao et al.'s (2020) documented that an increase in maize grain yield was reported with integrated nutrient management.

Similarly, Geng et al. (2019) and Elduma et al. (2020) also observed heightened maize grain yield with the application of organic manures.

The present study revealed significant effects of various organic and integrated nutrient management (INM) treatments on maize, pea and mung bean stover yields and the increase in stover yield can be attributed to the addition of organic matter to the soil, potentially leading to increased nutrient solubilization and availability. This, in turn, contributes to an amplified stover yield. Furthermore, the combination of organic manures with inorganic fertilizers enhances the vegetative growth of the plant, as noted by Elduma et al. (2020) regarding an increase in maize stover yield with the application of organic manures.

This enhancement in yield might be attributed to various factors, such as the addition of organic matter in a legume-based system, root activity, and nutrient mobilization influencing the soil microenvironment (Kumar et al., 2018). Consequently, the crop may extract a higher amount of nutrients from the soil, leading to increased grain yield. Kishore et al. (2021) observed an increase in mung bean grain yield with 100% RDF + FYM at the rate 5 t ha^{-1} + Rhizobium. Similarly, Isha et al. (2021) also reported an increase in mung bean yield was observed with the application of FYM applied at the rate 5 t ha^{-1}.

Essential micronutrients, such as copper (Cu) and zinc (Zn), play vital roles in enzymatic activities, photosynthesis, cell wall formation, and overall plant growth and development (Norouzi et al., 2014).

Our study highlighted substantial variations in micronutrient concentrations, particularly copper (Cu) and zinc (Zn), across diverse treatments. Treatment T1, integrating 50% nitrogen through farmyard manure (FYM) and 50% recommended NPK, consistently demonstrated elevated Cu and Zn levels in maize grain. For peas, T1 displayed the highest Cu and Zn content in grain, with integrated treatment T6 also exhibiting significant improvement over 100% recommended NPK. Similarly, in summer mung beans, treatments T1 and T6 consistently revealed superior Cu and Zn concentrations in both grain and straw, outperforming the treatment relying solely on 100% recommended NPK. Maize stover and pea straw exhibited varying Cu and Zn concentrations, with T1 and T6 consistently leading in content. These findings underscore the positive influence of integrated nutrient management on micronutrient concentrations in crop residues, emphasising its potential for sustainable agricultural practices. This enhancement can be attributed to the application of organic manures, which lowers soil pH and increases the availability of plant-accessible forms of micronutrients. The use of FYM and the consortium further increased micronutrients mobility, thereby raising their concentration. In legume-based systems, the addition of organic matter and nutrient mobilization in the soil contributed to higher nutrient acquisition by plants (Norouzi et al., 2014).

The variation in Cu and Zn uptake across treatments underscores the impact of different nutrient management strategies. Treatment T1, involving a combination of 50% N through FYM and 50% recommended

NPK, consistently exhibited superior micronutrients uptake in pea grain and mungbean straw. In contrast, T5, which relied on 50% N through FYM with rock phosphate and PSB, demonstrated lower uptake. Notably, the integrated treatments outperformed the sole application of 100% recommended NPK, emphasizing the efficacy of combining organic and inorganic approaches in enhancing micronutrients uptake. These findings suggest the potential for optimizing nutrient management practices to promote sustainable and efficient crop production. The observed increase in micronutrients uptake can be linked to the application of organic manures, which not only decreases soil pH but also enhances the availability of plant-accessible forms of micronutrients. The application of organic manures increased plant biomass and micronutrients concentrations, resulting in elevated micronutrients uptake in both organic and integrated treatments. The integrated use of organic manures and inorganic fertilizers significantly boosted grain and straw micronutrients uptake, attributed to the release of micronutrients during the decomposition of organic matter (Dhaliwal et al., 2023).

5. Conclusion

The various organic and integrated ferilizer treatments significantly influenced crop yield and micronutrients uptake by grain and straw. Treatment T1 consistently yielded the highest maize grain, pea pod, and mung bean grain, while T5 exhibited the lowest yields. Micronutrients concentrations in grain and straw increased with organic and integrated nutrient management, with T1 and T6 displaying the highest Zn and Cu concentrations, and T7 the lowest. This pattern was consistent for all crops, with micronutrients concentration following the order Zn > Cu in both straw and grains. The assimilation of micronutrients in grain and straw indicate concentration trends, with T1 and T6 leading in uptake, and T5 showing the lowest values. This pattern was observed across all crops. Organic manure application enhanced plant biomass, micronutrients concentration, and subsequent micronutrients uptake in the organic and integrated treatments. Overall, the application of organic manures not only boosted plant biomass but also elevated micronutrients concentrations, resulting in enhanced micronutrients uptake in the organic and integrated treatments. These findings emphasize the potential of sustainable agricultural practices in optimizing crop performance and nutrient dynamics.

Author Contribution

NR, SSW, conceptualized and designed the research work. NR, SSW, SR: Execution of field/lab experiments and data collection, analysis, interpretation of results and preparation of rough draft of manuscript was prepared by all authors edited the manuscript, read and approved the final version.

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ORIGINAL RESEARCH

Enhancing Soil Fertility of Apple Orchard through Biochar and Fertilizer Amendments: A Soil Aggregation Study

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ABSTRACT: The declining productivity of apple trees can be attributed to the adverse effects of unbalanced climatic conditions and dynamic soil properties. Addressing these challenges through sustainable agricultural practices is crucial to improving apple orchard productivity and ensuring a resilient agricultural system. To enhance the function of fragile ecosystem services, the addition of biochar at an appropriate rate along with chemical fertilizers (NPK) is considered an efficient approach for improving apple trees productivity. The treatments combinations were 0 t ha^{-1} (CK), 4 t ha^{-1} (T1), 8 t ha^{-1} (T2), 12 t ha^{-1} (T3), 16 t ha^{-1} (T4), and 20 t ha^{-1} (T5). Our results demonstrated that, biochar addition rate in the T5 significantly increased macro-aggregates (WSAs > 0.25 mm), mean weight diameter (MWD) and therefore decreased micro-aggregates (WSAs < 0.25 mm) compare to the control. Soil organic carbon (SOC) and total nitrogen (T.N) in both the bulk soil and water stable aggregates (WSAs) showed similar and an increased trend with biochar addition rate. However, the trend of C:N ratio was in opposition with biochar addition rate for both the bulk soil and WSAs. Additionally, biochar addition rate (T5) significantly intensified partitioning proportion (%) of the SOC, and T.N in WSAs > 0.25 mm, and WSAs < 0.25 mm and therefore showed non significance differences for the others treatments. Such a partitioning proportion of the WSAs 0.5-0.25 mm were lower than the WSAs > 0.5 mm and WSAs < 0.25 mm. These results suggested that biochar addition rate (T5) with chemical fertilizer had a significant effect on the stability of aggregates associated SOC, T.N, and C:N ratio and it may also have a capability in optimizing partitioning proportion (%) of the SOC and T.N in WSAs > 0.25 mm. Thus, it is therefore suggested that biochar addition rate (T5) with chemical fertilizers is the best preference for the stability and optimization of the aggregate associated SOC and T.N which may enhance partitioning proportion (%) of the SOC and T.N in an apple growing soil.

KEYWORDS: Biochar, Apple orchards, Water stable aggregates, Soil organic carbon, Total nitrogen

1. Introduction

Apple (*Malus domestica* borkh) is one of the most essential temperate crops in Asia and Europe. Apple belongs to the *Rosaceae* family, and it is the most used and widely cultivated fruit (Ullah et al., 2021; Zhang et al., 2023). Pakistan grows a variety of apples including Mashaday, Kashmiri, Amri, Sky Spur, Kala Kulu, Red Delicious and Golden Delicious (Mukhtar et al., 2010). Notably, the region boasts a rich tradition of cultivating globally significant fruits, particularly apples fourth in number in Pakistan after citrus, mango and banana, originating from

Southwestern Asia and flourishing in the hilly terrains of Punjab, Khyber Pakhtunkhwa, and Balochistan within Pakistan (Shah et al., 2016). Agriculture 2014-2015, Apple fruits were yielded on 100246 hectares of land and its production in Pakistan was 616748 tons, while in KP, apple has been cultivating under in an area of 7983 hectares yielding a production of 97619 tons. Specially in Swat, the reported production stands at 32,000 tons within a cultivated area of 3,750 hectares (AMIS, 2015), is the most important of all the apple-growing districts in KP, followed by Dir, Mansehra, Chitral and Abbottabad districts (Bokhari, 2002). The Swat district comprises Lower Swat and Upper Swat; the climate in Upper Swat experiences more severe winters compared to the Lower Swat region. This valley is renowned for producing high-quality fruits, including peaches, apricots, apples, walnuts, and plums (Ullah et al., 2021; Ali .S, 2023). However, a concerning trend looms over the Swat district the dwindling productivity of apple orchards. Soil fertility issues and the impact of shifting climatic conditions have been identified as primary culprits (Shah et al., 2016). However, a limited number study has been conducted on apple in Pakistan, which is limited to northern hilly areas of Punjab, KP and Baluchistan (Mukhtar et al., 2010). In response to this challenge, our study takes center stage, aiming to reverse the declining trend by investigating the potential benefits of biochar and fertilizer applications in enhancing apple orchard productivity. As we navigate through the intricacies of this research, we aspire to shed light on viable solutions that not only address the current challenges faced by apple orchards in Swat but also pave the way for a more resilient and productive future. The promising prospect of biochar and fertilizer applications holds the key to unlocking new possibilities for apple production in the Swat district, ensuring sustainable agricultural practices in the face of evolving environmental dynamics.

Soil aggregates is regarded as one of the key component in soil system influence biogeochemical processes of the soil (Mueller et al., 2007). Several studies reveal that stability of aggregates enhance water availability in alleviating soil carbon, and nitrogen losses (Shaver et al., 2002). However, external environment is the basic factor influence both the distribution, and development of soil aggregates (Mueller et al., 2007). Among the external environment, mechanical forces and rainfall are the two basic factor that disrupts aggregate stability in changing soil properties alter decomposition of the soil organic matter, carbon sequestration, nitrogen mineralization, and nutrient cycling (Rampazzo et al., 1995), (Six et al., 2000). These soil properties act as binding agents for the soil aggregation (Ali et al., 2022c; Ali et al., 2022a; Ullah et al., 2021). However, such a lower soil organic matter, and dense soil have stronger aggregates that is resistance to disruptions under dry conditions and such a resistance are weaken in wet conditions. Therefore, aggregates stability is one of the basic indexes for the field soil to avoid the risks of soil erosion with improved nutrients availability. Previous studies revealed that organic amendment is an important management practices in enhancing aggregates stability (Doan et al., 2014),.

Among the organic amendment, biochar is one of the carbonaceous material produced under anaerobic condition at high pyrolysis temperature (Ali et al., 2020b, Laird et al., 2009). Biochar has gained wide acknowledge for more than a decade (Ali et al., 2021; Song et al., 2022; Arthur et al., 2015) in its usage for carbon sequestration and improving physicochemical properties of soil (Alghamdi, 2018), optimize soil quality (Woods et al., 2006), in reducing bulk density with enhancing soil porosity and aggregation (Ali et al., 2022a; Blanco-Canqui, 2017). Biochar modify soil acidity (Saha et al., 2020), and cation exchange capacity (Ghezzehei et al., 2014) may results in enhancing the abundance and diversity of microbes in the rhizosphere (Saha et al., 2020; Ali et al., 2022b). Due to considerable and valuable role of biochar on soil properties, the prospect of enhancing and stabilizing aggregates lower soil erosion therefore gained the interests of researchers. Researchers have extensively studied the effects of biochar on soil fertility, but its specific impact on ecosystem services related to soil aggregate size, organic carbon, and nitrogen remains unclear. Thus, the objective of this study was: (1) to assess the influence of biochar on the distribution and stability of aggregates associated with soil organic carbon (SOC), total nitrogen (T.N), and carbon-to-nitrogen (C:N) ratio, and (2) to investigate the proportion (%) of SOC and T.N partitioned within the water-stable aggregates (WSAs). The findings from this study have the potential to enhance the quality and productivity of apple orchards.

2. Materials and methods

2.1 Study site

Our research was carried out in the upper district of Swat, situated in Khyber Pakhthunkhwa (KP), Pakistan during 2022. In this region, the soil exhibits a slightly acidic to alkaline pH range, spanning from 7.21 to 8.27 . Furthermore, the electrical conductivity (EC) values, ranging from 0.06 to 0.620 dSm-1, indicate that the soil is non-saline, with values well below the critical threshold of 4 dSm^{-1}. As for the soil texture, it predominantly falls into the categories of silt loam and loamy sand. Notably, the soil in our study area exhibits a moderate level of calcareous content, which is considered advantageous for apple production due to its positive influence on nutrient availability and root health (Salam, et al. 2022). Biochar consisted of wood biomass that were subjected to pyrolysis at a temperature of 750 ^{0}C under anaerobic condition. The basic physiochemical properties of experimental field and biochar is given in Table 1

Table 1. Basic physico-chemical properties of experimental materials.

Items	Soil	Biochar
Sand (%)	26.28	–
Silt (%)	58.1	–
Clay (%)	15.24	–
Soil texture	Silt loam	–
Surface area (m^2 g^{-1})	–	1.15
SOC (g kg^{-1})	4.35	–
pH	7.36	8.5
TN (g kg^{-1})	0.55	5.42
TP (g kg^{-1})	0.97	45.22
TK(g kg^{-1})	–	46.35

Note: SOC–soil organic carbon, TN–total nitrogen, TP–total phosphorous, TK–total potassium.

2.2 Experimental set up and design

The experiment was conducted on randomized complete block design. The plots size was 6x4 = 24 m^2. All plots received an equal amount of chemical fertilizer. Biochar addition rate were 0 t ha^{-1} (CK), 4 t ha^{-1} (T1), 8 t ha^{-1} (T2), 12 t ha^{-1} (T3), 16 t ha^{-1} (T4), and 20 t ha^{-1} (T5).

2.3 Soil sampling and analysis

During each treatment, top soil layer (0-20 cm) soil was taken for the analysis of physico-chemical properties of soil. Meanwhile, undisturbed soil samples for the analysis of aggregate stability were taken in triplicates in stainless steel boxes (20 cm x 12.5 cm x 6 cm). The stainless-steel boxes were sealed and kept in polyethylene bags, and were brought into the laboratory for the analysis of water stable aggregates.

2.3.1 Soil physiochemical properties

The methodology, and instrumentations used for physicochemical properties of soil is shown in table 2.

Table 2. Testing items, and methodology for physicochemical properties of soil

Items	Methodology	Instruments
Soil texture	Laser particle analyzer	APA2000, Marvin company, England
SOC (g kg^{-1})	$K_2Cr_2O_7$ outside heating	Semi-micro titrator
pH	1:5 soil suspension	pH meter (Inolab WTW series pH 720)
T.N (g kg^{-1})	NaOH alkaline hydrolysis with reducing agent-diffusion process	Automatic kjeldahl determination method
TP (g kg^{-1})	NaOH liquation	Spectrophotometer

2.3.2 Separation of aggregates and mean weight diameter

The collected undisturbed soil samples were carefully crushed by hand and then passed through 5 mm mesh in the laboratory. Processed air-dried soils of 100 g were kept in the top sieve size of > 2 mm followed by 2-1 mm, 1-0.5 mm, 0.5 -0.25 mm, and < 0.25 mm. All the sieves were immersed in distilled water where it was mechanically shaken with up and down movement for two minutes at 30 cycles per minute. The aggregates obtained from each sieve were oven dried, weighed, and then classified into different size fractions such as > 2 mm, 2-1 mm, 1-0.5 mm, 0.5-0.25 mm, and < 0.25 mm. These collected air dried WSAs were then analyzed for the SOC, and T.N (Table 2). The isolated fractions of WSAs > 0.25 mm were designated as macro-aggregates and the WSAs <0.25 mm were considered as micro-aggregates. Calculation for the stability of WSAs was determined by the following equation (1).

$$MWD = \sum_{i=1}^{n} Xi * WSAi \quad \text{.................} (1)$$

$$WSAi\ (\%) = \frac{Wi}{\text{weight of soil}} * 100 \quad \text{..........} (2)$$

Where, *Xi* is the mean diameter of aggregates remaining on the respective sieves, WSAi represent percent mass of aggregates with respect to total weight of soil sample on the *i*-th sieve and n is the sieves number used for the separation of aggregates.

2.3.3 Partitioning proportion of the soil organic carbon, and soil total nitrogen

The partitioning proportion of the SOC, and T.N with given aggregate size was computed by the following equation (3).

$$OC \text{ in aggregates } (\%) = OCF_n \times MF_n / SM \times 100 \quad \ldots\ldots (3)$$

Where, OCF_n is the concentration of the SOC in aggregate size fraction, MF_n, and SM indicates fraction, and unfractionated mass of soil.

2.4 Statistical analysis

The statistical analyses of the data were analyzed with SPSS computer software version 20.0. Significant differences for the treatments mean data were evaluated by $P < 0.05$ through the least significance difference (LSD) method. Sigma plot 12.5 was used for figures.

3. Results

3.1 Distribution and stability of water stable aggregates

The distribution of the aggregate sizes of all the treatments showed largest proportion in WSAs < 0.25 mm (Table 3). The proportion of WSAs > 2 mm in the CK was 3.84 % to 6.89% in the T5 treatment, and is significantly higher than the other treatments ($P < 0.05$). However, no significant differences among Ck, T1, and T2, and between T3, and T4 treatments were found for the WSAs > 2 mm. The WSAs 2-1 mm showed highest proportion of 7.35 % in the T5 followed by T4 treatment in comparison to the other treatments ($P<0.05$). The range of WSAs 1-0.5 mm in the CK was 4.73 % to 7.61 % in the T5 with highest significant differences were found in comparison to the other treatments ($P<0.05$). The aggregate size fractions of WSAs 0.5-0.25 mm indicated highest proportion in the T5 treatment than the CK, T1, T2, T3, and T4 treatments ($P<0.05$). The proportion of WSAs < 0.25 mm were highest in the CK treatment ($P<0.05$) and showed significantly a decreasing trend with biochar addition rate specifically in the T5 treatment ($P<0.05$).

The increasing proportion of the macro aggregates (WSAs > 0.25 mm) and a decreasing proportion of the micro aggregates (WSAs < 0.25 mm) by biochar addition rate (table 3) significantly optimized MWD of the soil aggregates (Figure. 1A). The highest MWD in the T5 indicated highest differences in comparison to the control however, such a difference were lower for the lower rate of biochar addition rate. Therefore suggested that, biochar addition rate in the T5 highly influences the stability of WSAs (MWD).

3.2 Distribution of soil organic carbon

The highest concentration of the SOC (g kg^{-1}) in bulk soil were observed in the in the T5 with a lowest in the control (Figure 1B). Compared to the control, biochar addition rates from T1 to T5 significantly increased SOC concentration. This revealed that biochar addition rate in every treatment significantly influence variations in the SOC concentrations.

The distribution of SOC concentrations in WSAs highly depends on biochar addition rate (Table 4). The concentration of the SOC in the control of WSAs > 2 mm were 4.32 g kg^{-1} to 5.91 g kg^{-1} in the T5 treatment ($P < 0.05$). The concentration of the SOC in the T5 was significantly higher than the other treatments ($P < 0.05$) with no significant differences was observed between T3, and T4 treatment. The highest and lowest concentration of SOC in WSAs 2-1 mm was influenced by T5 and CK treatment where T1 and T2 treatment showed no significant differences.

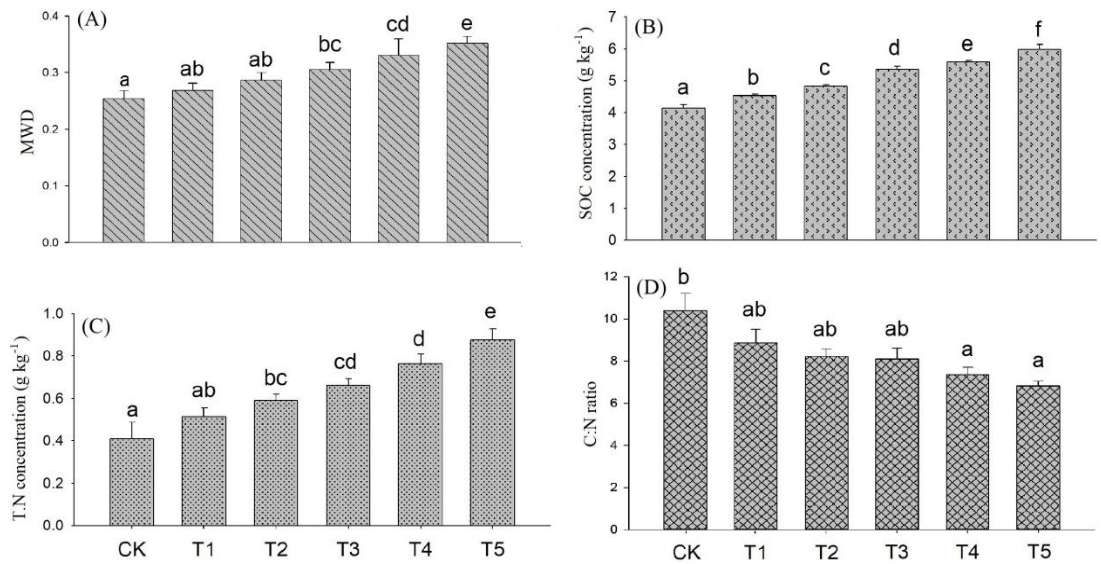

Figure 1. Impact of biochar on soil properties of bulk soil: (A) Mean weight diameter (MWD), (B) Soil organic carbon concentration (g kg^{-1}), (C) Soil total nitrogen concentration (g kg^{-1}), (D) C:N ratio. Note: CK: control; T1: 4 t ha^{-1}, T2: 8t ha^{-1} , T3: 12 t ha^{-1}, T4: 16 t ha^{-1}, T5: 20 t ha^{-1}. Different small letters indicate significant differences as determined by the LSD test ($p \leq 0.05$).

Table 3 Distribution of water stable aggregates (%) under different biochar addition rates.

Soil aggregates	CK	T1	T2	T3	T4	T5
> 2 mm	4.84±0.27a	5.15±0.62ab	5.79±0.29ab	5.91±0.62abc	5.95±0.30bc	6.89±0.24c
2-1 mm	4.12±0.49a	4.73±0.23ab	5.40±0.66abc	5.93±0.12abc	7.02±0.88bc	7.35±0.54c
1-0.5 mm	4.73±0.19a	5.57±0.58ab	5.73±0.60ab	6.01±0.42ab	6.48±0.57ab	7.61±0.25b
0.5-0.25 mm	3.76±0.56a	3.20±0.46a	3.80±0.40a	5.35±0.19ab	6.61±0.60b	7.20±0.77b
<0.25 mm	82.53±0.83a	81.34±1.41ab	81.26±0.66bc	77.16±0.88cd	73.93±1.64d	70.93±1.13d

Note: CK: control; T1: 4 t ha^{-1}, T2: 8t ha^{-1} , T3: 12 t ha^{-1}, T4: 16 t ha^{-1}, T5: 20 t ha^{-1}. Mean value ± S.E in the same row followed by the same letter are not significantly different using L.S.D test at $P< 0.05$ level.

Similarly, the remaining WSAs 1-0.5 mm, 0.5-0.25 mm and < 025 mm showed highest SOC concentration with increasing biochar addition rate while no significant difference was found among CK, T1, T2 for WSAs 1-0.5 mm, T1, and T2 for WSAs 0.5-0.25 mm and T1, and T2 as well as T3, T4, and T5 for the WSAs < 0.25 mm treatments respectively.

Partitioning proportion (%) of SOC in WSAs was affected by biochar addition rates (Figure 2). Such a partitioning of SOC with biochar addition rates in WSAs > 0.25 mm showed increasing trend than the control with most of the treatment showed slightly non-significant differences among the T1, T2, T3, and T4 treatment respectively. However, the partitioning proportion (%) of the SOC in WSAs 0.5-0.25 mm was consistently lower than the WSAs > 0.5 mm and WSAs < 0.25mm.

3.3 Distribution of total nitrogen

Among all the treatments, biochar addition rate significantly maximized concentration of the total nitrogen in the bulk soil (Figure 1C). The maximum concentration of the T.N was noted in the T5 whereas lowest in the control with no significant difference was observed between CK, and T1, and between T2, and T3 treatments ($P < 0.05$) respectively.

The concentration of the T.N showed increased trend with increasing biochar addition rate (Table 5). Significantly, biochar addition rate in the T5 showed highest T.N concentrations in WSAs > 2 mm than the other treatments ($P < 0.05$). Similarly an increasing trend of T.N was observed for WSAs 2-1 mm with no significant difference was found for CK, T1, and for T3, and T4 with slight significant differences were noted in the T5 treatment. The WSAs 1-0.5 mm showed no significant difference in CK, and T1, slight difference with in the T2 and T3 and for the T4 and T5 treatment ($P<0.05$).

Table 4. Distribution of the soil organic carbon (g kg^{-1}) under different biochar addition rate.

Soil aggregates	CK	T1	T2	T3	T4	T5
> 2 mm	4.32±0.08a	4.51±0.01b	4.81±0.03c	5.38±0.03d	5.46±0.02d	5.91±0.02e
2-1 mm	4.17±0.06a	4.47±0.01b	4.55±0.02b	5.30±0.03c	5.45±0.02d	5.82±0.02e
1-0.5 mm	4.40±0.27a	4.48±0.02a	4.56±0.03a	5.23±0.01b	5.42±0.03bc	5.84±0.02c
0.5- 0.25 mm	4.12±0.09a	4.45±0.02b	4.49±0.02b	5.27±0.02c	5.40±0.02cd	5.52±0.03d
< 0.25 mm	4.01±0.05a	4.36±0.03b	4.50±0.01b	5.16±0.08c	5.43±0.01d	5.48±0.02d

Note: CK: control; T1: 4 t ha^{-1}, T2: 8t ha^{-1}, T3: 12 t ha^{-1}, T4: 16 t ha^{-1}, T5: 20 t ha^{-1}. Mean value ± S.E in the same row followed by the same letter are not significantly different using L.S.D test at $P< 0.05$ level.

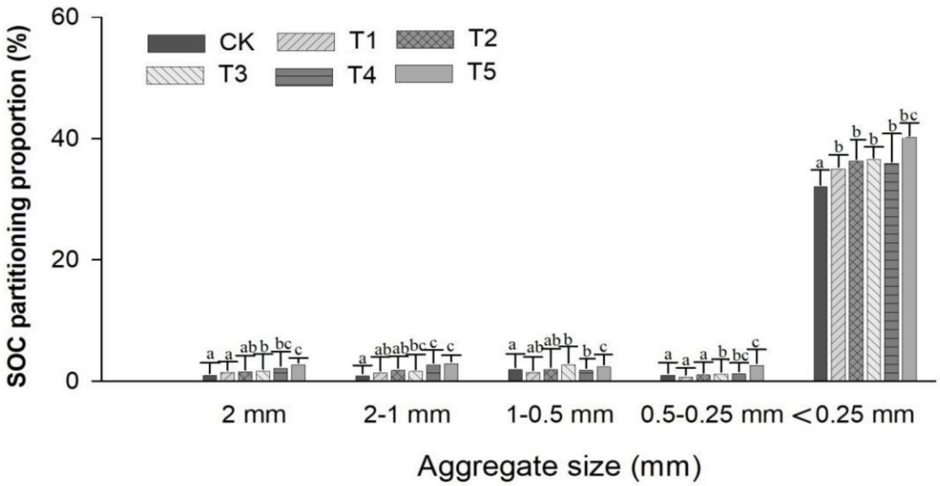

Figure 2. Partitioning proportions (%) of soil organic carbon (SOC) in water stable aggregates as influenced by biochar addition rates. Note: CK: control; T1: 4 t ha^{-1}, T2: 8t ha^{-1} , T3: 12 t ha^{-1}, T4: 16 t ha^{-1}, T5: 20 t ha^{-1}. Different small letters indicate significant differences as determined by the LSD test (*p ≤ 0.05*).

The highest concentration of the total nitrogen in WSAs 0.5-0.25 and <0.25 mm were 0.66 g kg^{-1}, and 0.59 g kg^{-1} with a lowest in the control however no significant difference was found among CK, T1, T2 and T3, and with a slight significant differences were observed for the T4, and T5 of WSAs 0.5-0.25 mm, and for CK, T1, and for the T2, T3, and T4 with a slightly significant difference were observed in between the T4, and T5 treatment in the WSAs < 0.25 mm respectively.The partitioning proportion (%) of total nitrogen under different biochar addition rates are presented in Figure 3. The maximum proportions (%) of T.N were observed in WSAs > 0.5 mm under the biochar addition rates except for the T4 in WSAs 1-0.5 mm. The partitioning proportions (%) of T.N in WSAs 0.5-0.25 mm were less than the WSAs > 0.5 mm and < 0.25 mm. The WSAs < 0.25 mm was affected by biochar addition rate with high partitioning proportion (%) were noted in the T5, however no significant differences were observed among the other treatments

3.4 C. N ratio

Biochar addition rate significantly decreased C:N ratio compared to the control of the bulk soil (Figure 1D). Increasing biochar addition rate slightly decrease C:N ratio with no significance differences were noted for the CK, T1,T2, T3, and for the T4, and T5. The lower C:N ratio in the T5 suggest the rapid released of nitrogen into the soil.

The distribution of the C:N ratio within the WSAs was significantly affected by biochar addition rate as shown in Table 6. The highest C:N ratio in the WSAs > 2 mm were observed in the control (9.84 g kg^{-1})

Table 5. Distribution of the soil TN (g kg^{-1}) under different rates of biochar.

Soil aggregates	CK	T1	T2	T3	T4	T5
> 2 mm	0.56±0.01a	0.60±0.04ab	0.66±0.04abc	0.69±0.01abc	0.71±0.04bc	0.75±0.02c
2-1 mm	0.51±0.02a	0.54±0.01a	0.59±0.03ab	0.65±0.03bc	0.67±0.01bc	0.73±0.02c
1-0.5 mm	0.48±0.02a	0.50±0.01ab	0.56±0.02abc	0.61±0.02bcd	0.64±0.01cd	0.68±0.04d
0.5- 0.25 mm	0.45±0.02a	0.49±0.01ab	0.52±0.05ab	0.56±0.03abc	0.61±0.02bc	0.66±0.02c
< 0.25 mm	0.41±0.01a	0.44±0.04a	0.48±0.03ab	0.51±0.03ab	0.54±0.01ab	0.59±0.04b

CK: control; T1: 4 t ha^{-1}, T2: 8t ha^{-1} , T3: 12 t ha^{-1}, T4: 16 t ha^{-1}, T5: 20 t ha^{-1}, Mean value ± S.E in the same row followed by the same letter are not significantly different using L.S.D test at $P< 0.05$ level.

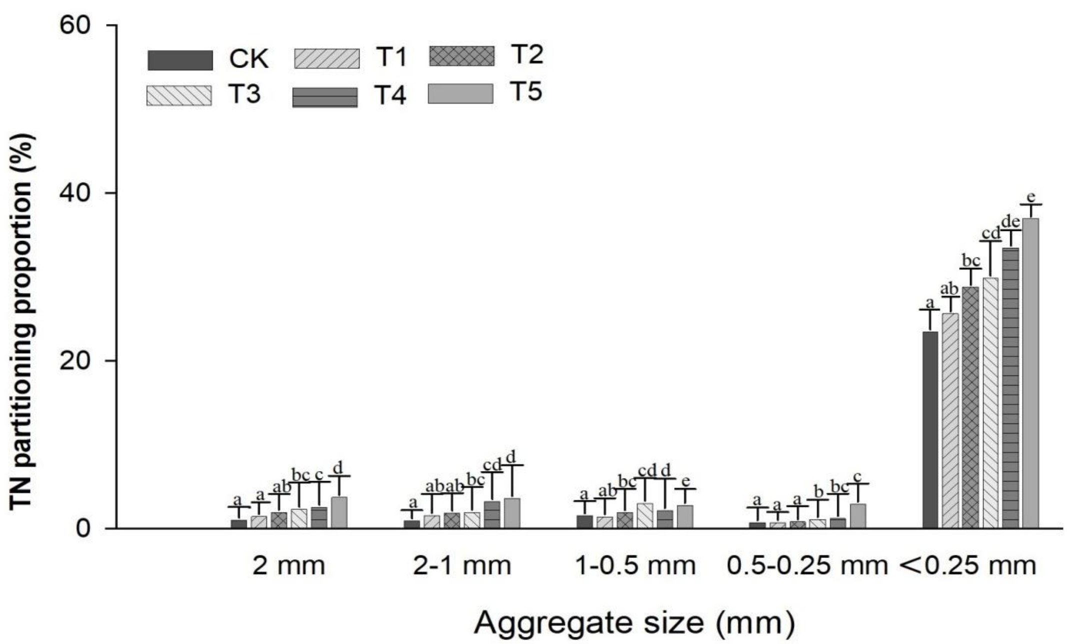

Figure 3. Partitioning proportions (%) of total nitrogen (T.N) in water stable aggregates as influenced by biochar addition rates. Note: CK: control; T1: 4 t ha^{-1}, T2: 8t ha^{-1} , T3: 12 t ha^{-1}, T4: 16 t ha^{-1}, T5: 20 t ha^{-1}, Different small letters indicate significant differences as determined by the LSD test ($p \leq 0.05$).

Table 6. Distribution of the C:N ratio (g kg^{-1}) under different rates of biochar.

Soil aggregates	CK	T1	T2	T3	T4	T5
> 2 mm	9.84±0.44c	9.12±0.45bc	8.31±0.31ab	8.09±0.29ab	7.42±0.25a	6.99±0.23a
2-1 mm	10.19±0.52d	9.80±0.27cd	8.75±0.08bc	8.47±0.42ab	8.06±0.14ab	7.22±0.15a
1-0.5 mm	12.81±1.34c	11.33±0.35bc	9.26±0.25ab	8.98±0.26ab	8.05±0.35a	7.83±0.18a
0.5- 0.25 mm	11.84±0.73c	11.83±0.30c	10.53±0.24bc	9.07±0.57ab	8.83±0.36ab	8.37±0.19a
< 0.25 mm	13.42±1.20b	13.10±0.27b	11.13±0.56ab	11.03±0.48ab	9.46±0.46a	8.78±0.45a

Note: CK: control; T1: 4 t ha^{-1}, T2: 8t ha^{-1} , T3: 12 t ha^{-1}, T4: 16 t ha^{-1}, T5: 20 t ha^{-1}. Mean value ± S.E in the same row followed by the same letter are not significantly different using L.S.D test at $P < 0.05$ level

with a lowest in T5 (6.99 g kg^{-1}), such a ratio relatively showed non-significant differences among the other treatments. The trend of C:N ratio is similar for the other remaining WSAs. Under the different treatments, biochar addition rate especially T5 significantly decreased C:N ratio ranged from 28 %, 29%, 38%, 29%, and 34% for WSAs > 2 mm, 2-1 mm, 1-0.5 mm, 0.5- 0.25 mm, and < 0.25 mm in comparison to the control. However, under the same treatment, C:N ratio showed an increasing trend with decreasing WSAs sizes, such a trend of C:N ratio indicated similar but a decreasing trend with biochar addition rate.

4. Discussion

Although it is considered that biochar addition rates with chemical fertilizers considerably altered the distribution of the soil aggregation. However, such aggregation in the control of our study are similar to the previous finding of who revealed that WSAs are influenced by the dynamics soil properties. Changes in the dynamic soil properties are limited by the inherent soil or by another dynamic's property. Among the dynamic soil properties, soil aggregation play a key role and showed positive correlation with biochar addition rate (Table 3), which is mainly influenced by the distribution, and availability of organic carbon. Such a soil aggregation acts as a physical barrier for the protection of the organic carbon (Wang et al., 2017), and their stability play a vital role to prevent rapid decomposition (Pulleman & Marinissen, 2004),. In this study, biochar addition rate significantly enhanced soil aggregations in comparison to the control (Table 3) therefore optimized stabilization of the aggregates (Figure 1A). The stability of WSAs in our study could be the hydrophobic bonding of SOC contained in soil aggregates (Piccolo et al., 1996) which therefore acts as a binding agent for soil aggregation and agglomeration of the soil mineral particles in creating aggregates hierarchy (Rabbi et al., 2020), (Juriga & Šimanský, 2018). Such a stability of the aggregation reduce the disruption of soil structure in sustaining soil cohesion and resistivity to different external environmental disturbances (Rampazzo et al., 1995). The contents of SOC in macro aggregates (Table 4) showed positive correlation with the stability of aggregates (Figure 1. B) in indicating similarity with the previous agreements of (Tisdall & OADES, 1982).

In our study, it has also been found that the highest stability of aggregates with biochar addition rate (T5) might be due to the enhanced fine roots and microbial activities, which assist in binding micro-aggregates, and therefore supporting the formation of soil aggregation as reported by (Deurer et al., 2009),. However, the decline MWD of aggregates in the control of our study are similar with the who revealed that fine soil particles could be the dominant attribute in declining the stability, and strength of aggregate. Similarly, (Munkholm et al., 2002) explained that such a soil strength of aggregates showed fragile resistance to destruction under wet condition, consistent to the control of our results. To enhance aggregates resistance to destruction under wet condition, biochar addition rate significantly maximized the proportion of the WSAs > 0.25 mm size fractions, and minimized WSAs <0.25 mm fraction (Table 3). Our result are similar with (Liu et al., 2014) who reported that water stable aggregates highly depend on biochar addition rate that acts as a conservative role in the protection of the SOC within the macro aggregates. In the same study, biochar addition rate significantly affected the distribution of SOC within the WSAs, and this possibly might be due to lower rate of SOC in the control which therefore showing similarity with the finding of. However, higher content of soil organic carbon in WSAs, and bulk of the soil (Table 4: Figure 1. B) was attributed to the preferential sequestration of the SOC. Such a sequestration of SOC with biochar addition rate was higher in the WSAs > 0.25 mm than the WSAs < 0.25 mm. Nevertheless, biochar addition rates in the T5 slightly exhibited significant impact on the partitioning proportion of SOC in both the WSAs > 0.25 mm and < 0.25 mm (Figure 2) by changing the proportion of the SOC within aggregates. However, our finding are in opposition with the study of (Tiancong et al., 2005), and (Sodhi et al., 2009) who revealed that long term effect of the organic material enhance partitioning proportion of the SOC in macroaggregates. This might be due to the differences in the inherent and dynamic soil properties such as climatic condition, elevation, organic materials, and soil texture between this and our study.

Aggregate associated T.N followed a similar trend as observed for aggregate associated organic carbon. The combined application of biochar (T5) plus chemical fertilizer resulted in highest proportion of T.N in aggregates compared to the control with prominent effect was observed in WSAs > 0.25 mm size fraction (Table 5). Such a prominent effect of T.N content in WSAs > 0.25 mm might be the microbial activity which are N rich compounds provide binding agents for the aggregation of soil aggregates (Six et al., 2000) therefore, highest N in the macro aggregates rather than the micro aggregates (Table 4) showing consistency with the previous study of (Onweremadu et al., 2007) which therefore support the theory of hierarchical aggregation (Elliott, 1986). Partitioning of the T.N in aggregates and soil T.N within aggregates followed the same pattern as for the soil organic carbon with biochar addition rates. These finding depicts that biochar addition rates had relatively similar effect on the sequestration and partitioning of the T.N and SOC within the aggregates.

5. Conclusion

This study investigated the impact of different rates of biochar (0, 4, 8, 12, 16, and 20 t ha^{-1}) in combination with chemical fertilizers (NPK) on water-stable aggregates (WSAs) and their association with soil organic carbon (SOC), total nitrogen (T.N), mean weight diameter (MWD), carbon-to-nitrogen (C: N) ratio, and partitioning proportion (%) of SOC and T.N in an apple orchard soil. The results revealed that the rate of biochar addition played a crucial role in influencing all the studied parameters. Among the different biochar rates, the addition of 20 t ha^{-1} (T5) significantly improved the distribution of WSAs, MWD, SOC, and T.N within the aggregates, showing a notable response. However, the C:N ratio exhibited an opposite trend compared to the control. Furthermore, the addition of biochar at the T5 rate significantly enhanced the partitioning proportions (%) of SOC and T.N in the macro-aggregates, although these proportions were lower than those in the micro-aggregates. Overall, this study highlights the potential of using biochar at the T5 rate, along with chemical fertilizers, to improve the distribution and stability of WSAs, as well as the associated SOC and T.N. However, further research is required to assess the impact on apple quality and productivity, promoting a sustainable farming system.

Author Contribution

AS and JB, conceptualized and designed the research work. AS conducted the experiment, data analysis and wrote the first draft: JB revised the manuscript and give suggestions. Both authors authors edited the manuscript, read and approved the final version.

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Conflicts of Interest

The authors declare no conflict of interest.

Availability of Data and Materials

Data will be available on formal request from the corresponding authors.

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