CHANGES IN SOIL ENZYMATIC ACTIVITY ASSOCIATED WITH CONSERVATION AGRICULTURE IN THE RAINFED POTHWAR PLATEAU OF PAKISTAN

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Abstract. Soil enzymes are considered sensitive indicators of soil management practices. Changes in the soil enzymatic activity in response to conservation agriculture in smallholder rainfed farming systems have not been extensively reported. Therefore, different tillage practices *viz.* zero tillage (ZT), reduced tillage (RT), and minimum tillage (MT) under fallow-wheat and mungbean-wheat rotations were compared with conventional practices in rainfed Pothwar plateau, Pakistan for C, N, P cycling enzymes. Zero tillage and reduced tillage produced urease activity 312 and 298 µg N g^{-1} soil 2 h^{-1}, alkaline phosphatise activity 40 and 39 µg p-NP g^{-1} soil h^{-1}, and dehydrogenase activity 34 and 31 TPF µg g^{-1} soil 24 h^{-1} respectively that were higher than minimum tillage and conventional tillage. ZT and RT tillage systems also had higher concentrations of soil organic C, nitrate-N and available-P. Among rotations, mungbean-wheat showed higher activities of the studied enzymes than fallow-wheat. Use of conservation tillage practices especially zero tillage and reduced tillage combined with legume-cereal rotation improves C, N, P cycling enzymes and soil biochemical properties under rainfed conditions of Pakistan.

Keywords: *tillage, urease, alkaline phosphatase, dehydrogenase, loess dryland, crop rotation*

Introduction

Soil enzymes are considered good indicators of the impact of soil management practices (Hok et al., 2018), microbial activity and nutrient cycling (Sinsabaugh et al., 2008). All biochemical processes occurring in soil for soil health improvement depend on soil enzymes (Lino et al., 2016). The enzymes which are most commonly studied under alkaline soil conditions include dehydrogenase (Dha), urease (UR), andalkaline phosphatase (ALP). Soil dehydrogenase is an intracellular enzyme that is present in living cells of microbes. It is involved in organic matter oxidation via transferring electrons and protons from substrates to acceptors (Kumar et al., 2013). Soil urease facilitates hydrolysis and transformation of urea fertilizer into CO_2 and NH_3 (Mohammadi, 2011) and is thus, involved in mineralization of organic N. Alkaline phosphatase facilitates phosphorous cycling and its activity mainly depends on inorganic phosphorous and organic carbon concentrations (Adamczyk et al., 2014).

Conventional tillage (CT) such as removal of crop residues after harvest and frequent mouldboard plowing causes soil organic carbon (SOC) loss (Zhang et al., 2015), increases emission of greenhouse gases (Pratibha et al., 2015), deterioration of soil structure (Willekens et al., 2014) and ultimately, deterioration of soil quality (Abdullah, 2014). Conservation agriculture (a combination of less tillage, crop diversification and residue retention) is considered to be effective in increasing SOC and decreasing soil degradation (Zhang et al., 2015). However, reports on the effects of conservation tillage practices on

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soil enzymes have been conflicting. Mbuthia et al. (2015) reported that no-tillage had significantly greater C, N, and P cycling enzymes, in comparison to CT practices. Similarly, Pandey et al. (2014) reported an increase in activities of polyphenol oxidase, b-D-glucosidase, UR and ALP with reduction in tillage frequency. A global meta-analysis of 62 studies also concluded that higher Dha and UR activities were frequently observed with no tillage and reduced tillage than conventional tillage (Zuber and Villamil, 2016). However, some authors contradicted this general finding and reported that ploughing resulted in higher soil enzymatic activity due to contact with new surfaces after breakdown of soil aggregates (Khan, 1996; Seifert et al., 2001). Others noted that, enzyme activity was not affected by tillage (Acosta-Martinez et al., 2011) but the changes with tillage system occurred due to changes in environmental conditions (for instance, temperature, diverse geographical locations, water content and pH) leading to spatial and temporal variations (Baldrian et al., 2013).

The rainfed Pothwar plateau of Pakistan is located in the northern Punjab province, where soils are constrained by limited moisture, low soil fertility, weak structure, and less SOC stocks (Sharif et al., 2017). Eighty percent of these rainfed smallholder farmers employ a six-month summer fallow followed by wheat (*Triticum aestivum* L.) (Arif and Malik, 2009). The soils usually receive intensive cultivation with mouldboard plough and tine cultivator during fallow period (Hassan et al., 2015). Conservation agriculture has been reported as a better alternative to improve soil health in these areas (Sharif et al., 2018; Rehman et al., 2020) however information regarding relationship between conservation agriculture and enzymatic activities is not reported so far. In view of this scenario, our research was carried out to assess the effect of conservation tillage with double cropping mungbean (*Vigna radiate* L.) - wheat on soil enzyme activity, and corresponding SOC, nitrogen and phosphorous concentrations. It was hypothesized that soil enzyme activity and nitrogen, phosphorous and SOC cycling will improve with conservation agriculture in rainfed agro ecological conditions of Pothwar plateau, Pakistan.

Materials and methods

Study location

A two-year field study was carried out from 2016 through 2018 in a long-term conservation tillage field experiment initiated in 2011 at University Koont Research Farm, Rawalpindi, Pakistan (longitude 73°02’0”E, latitude 33°36’0”N). The experimental site is located in semiarid dryland Pothwar plateau, northern Punjab province. The area of Pothwar plateau is 28488.9 sq km with an average elevation of 517 m from sea level. In this area, dryland farming (94% rainfed) is common. The major crops in dryland Pothwar include wheat, gram (*Cicer arietinum*), millet (*Pennisetum glaucum*), barley (*Hordeum vulgare*), groundnut (*Arachis hypogaea*) and maize (*Zea mays*). Fallow-wheat rotation and intensive moldboard ploughing is a common practice in this area. Summer temperatures are very hot (average from 36ºC to 42ºC) and increase to 48 ºC in extreme cases (Nizami et al., 2004). In winter (December to February) temperatures are low with a few days when temperatures go below 0 ºC. About 70% of total annual precipitation occurs during monsoon (July- August) and these heavy rains cause soil erosion (Shaheen et al., 2010). The soil of the experimental field was classified as Udic Haplustalfs, Kahuta soil series with sandy clay loam texture (55.2% sand, 23.4%

silt, 21.4% clay), pH 7.89, EC 0.60 dS^{-m}, SOC 5.5 g kg^{-1}, nitrate-N 4.18 mg kg^{-1}, available-P 3.07 mg kg^{-1} and extratable-K 124 mg kg^{-1}.

Experimental design

The total experimental area was 100 m × 60 m (6000 m^2) where thirty-two experimental plots 29 m long and 11 m wide were prepared to accommodate four tillage and two cropping system treatments in a split-plot design with four replications. The main plot treatments were tillage systems: a) Conventional Tillage (CT, control), ploughing with a moldboard plow followed by 8 cultivations with a tine cultivator; b) Minimum Tillage (MT) ploughing with a chisel plow to depth of 0.25 m followed by 4 cultivations with a tine cultivator; c) Reduced Tillage, (RT) a single cultivation with a chisel plough to a depth of 0.45 m, with roundup (Glyphosate @ 1 L acre^{-1}) used for subsequent weed control; and d) Zero Tillage (ZT) the soil remained undisturbed for the whole fallow period and weed control was done with roundup throughout the year. The residues of crops were retained only in ZT and RT. Sub-plot treatments were crop rotations; a) fallow-wheat (*Triticum aestivum* L.) (FW) and mungbean (*Vigna radiate* L.)-wheat (MW).

Wheat sowing in CT and MT was done with a seed-cum-fertilizer drill and in RT and ZT with a zero-tillage drill. The wheat variety was Chakwal-97 and mungbean MN-11. The fertilizer NPK for wheat crop was used at dose of 90-60-60 kg ha^{-1} as a DAP (18% N, 46% P_2O_5), urea (46% N), and sulfate of potash (50% K_2O). The seed rate for mungbean was 20 kg ha^{-1} (666680 no. of kernels ha^{-1}) and for wheat 100 kg ha^{-1} (2857100 no. of kernels ha^{-1}).

Soil was sampled on a bimonthly basis from each experimental plot for analysis of enzyme activities. Soil sampling was done with the help of augur from the topsoil layer at 0-15 cm depth. The soil sampling date in 2016 was December 26th. In 2017 and 2018, soil samples were collected on 26 February, 26 April, 26 June, 26 August, 26 October, and 25 December 2017. Samples were divided into two halves. One half was used for analysis of enzymatic activity (UR, ALP and Dha) and the other for chemical analysis. The soil samples destined for enzymatic analysis were immediately stored in plastic bags in a deep freezer and then analyzed within 10-15 days. The remaining soil was air-dried, ground and sieved with a 2.0 mm sieve and stored in plastic bags for analysis of properties such as available phosphorus, nitrate nitrogen, and soil organic carbon. Soil nitrate nitrogen was estimated colorimetrically and absorbance was recorded with a spectrophotometer (Model UV-1700, Shimadzu Japan) at 410 nm (Anderson and Ingram, 1993). Soil available phosphorous was analysed by sodium bicarbonate (0.5 M) method and the reading of samples was recorded on a Spectrophotometer (Model UV-1700, Shimadzu Japan) at 880 nm after 15 min of color development as described by Kuo (1996). SOC was determined using the wet oxidation method (Walkley, 1947).

Soil Dha enzyme activities was determined with the production rate of triphenyl formazan (TPF) from triphenyl tetrazolium chloride (TTC). Five-gram soil was added in test tube with TTC (5 ml) and incubated 30 ºC for 24 hours. Then, acetone (40 ml) was also added in the same test tube and incubated for 2 hrs. at room temperature in the dark. Then, filtration was done, and the optical density of soil filtrate was determined on a spectrophotometer (Model UV-1700, Shimadzu Japan) at wavelength of 546 nm. For determination of soil ALP activity, 1 g soil was mixed with Toluene (0.25 ml), 4 ml of Modified Universal Buffer (pH 11) and 1 ml of PNP (p-nitrophenyl phosphate) solution in the flask. Then, soil samples were incubated at 37 ^{o}C for one hour and 1 ml of $CaCl_2$

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(0.5 M) as well as 4 ml of NaOH (0.5 M) were also added. Filtration was done to measure the optical density on spectrophotometer (Model UV-1700, Shimadzu Japan) at 400 nm wavelength. Soil urease activity was also assayed by using an appropriate substrate (urea) as described in Alef and Nannipieri (1995). Colorimetric determination of ammonia released after incubating soil samples was done with urea solution for at 37 ^{o}C for 2 hours.

The monthly rainfall and mean minimum and maximum temperatures during the exeprimental period were collected from the nearby agro-met observatory (*Fig. 1*). For statistical analysis, split plot design was used to determine the effect of treatments on soil enzyme activity and nutrients. The analysis of variance (ANOVA) was applied with Statistics® 8.1 software to analyze the collected data with different characteristics while Tukey's HSD test at 5% level of significance was used for comparison of means (Steel et al., 1997). The correlations were drawn using MS-Excel software.

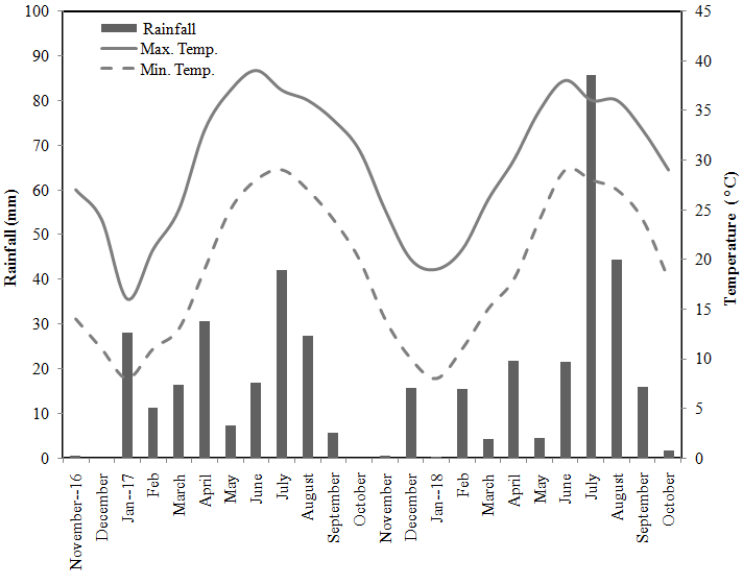

Figure 1. *Monthly rainfall and mean maximum and minimum temperatures during the experimental period*

Results

Soil organic C, nitrate-N and available-P

The tillage and crop rotations had significant effect on SOC (*Fig. 2*). The statistically highest SOC was found in ZT with both mungbean-wheat (7.66 g kg^{-1}) and fallow-wheat (7.21 g kg^{-1}) rotations, followed by RT with both mungbean-wheat (7.18 g kg^{-1}) and fallow-wheat (6.79 g kg^{-1}) rotations in the second year. The same trend was also observed in the first year. Between the crop rotations, in both the years, munbean-wheat resulted in statistically significantly higher SOC values (6.27 and 6.8 g kg^{-1}) than fallow-wheat (6.08 and 6.5 g kg^{-1}) in the first and second years, respectively.

The conservation tillage also exerted highly significant positive effects on soil nitrate-N (*Table 1*). In first experimental year, it was statistically highest in RT (5.30 mg kg^{-1}) followed by ZT (5.11 mg kg^{-1}) while CT showed the statistically significant lowest amount of nitrogen (2.08 mg kg^{-1}). In the second year, the nitrate nitrogen was also significantly highest in ZT with fallow-wheat and mungbean-wheat crop rotations

(5.27 and 4. 92 mg kg^{-1}, respectively) than CT. Between the crop rotations, mungbean-wheat had significantly higher average nitrogen (4.36 mg kg^{-1}) than fallow-wheat (3.44 mg kg^{-1}). Overall ZT and RT with mungbean-wheat rotation had higher values than all other combinations.

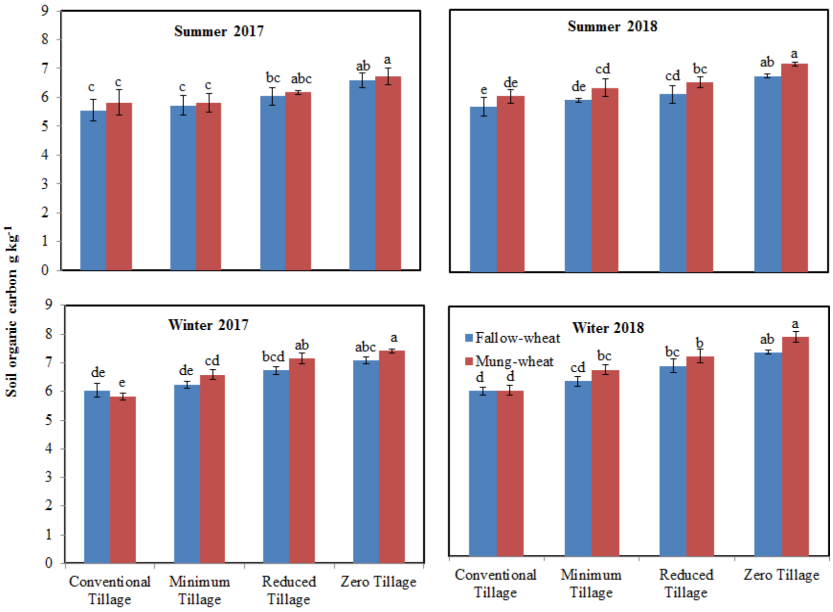

***Figure 2.** Effect of tillage systems and crop rotations on soil organic carbon during two experimental years. Error bars represent the standard error of means as determined by Turkey's test with P < 0.05*

***Table 1.** Soil nitrate nitrogen affected by tillage systems and crop rotations during two experimental years*

		Nitrate-Nitrogen (mg kg^{-1})					
Treatments		**2016-17**			**2017-18**		
Tillage	**Crop**	**Summer**	**Winter**	**Average**	**Summer**	**Winter**	**Average**
CT	MW	2.98 bc	3.39 bcd	3.19 cde	3.81 abc	3.34 bc	3.57 cd
	FW	2.14 c	2.03 d	2.08 e	2.52 c	2.64 c	2.58 d
MT	MW	3.93 ab	3.64 bcd	3.79 bcd	4.51 ab	4.52 ab	4.52 abc
	FW	2.23 c	2.96 cd	2.59 de	3.73 abc	3.76 bc	3.74 c
RT	MW	4.85 a	5.75 a	5.30 a	3.14 bc	5.81 a	4.47 abc
	FW	3.50 abc	2.61 cd	3.06 cde	3.92 abc	4.01 b	3.96 bc
ZT	MW	4.78 a	5.44 ab	5.11 ab	4.37 ab	5.47 a	4.92 ab
	FW	4.17 ab	4.38 abc	4.28 abc	4.94 a	5.59 a	5.26 a

CT, conventional tillage; MT, minimum tillage; RT, reduced tillage; ZT, zero tillage; MW, mungbean-wheat; FW, fallow-wheat. Dissimilar letters show significant differences among means as determined by Turkey's test with P < 0.05

Available-P increased in ZT and RT with mungbean-wheat crop rotation over the two experimental years (*Table 2*). In summer 2017, phosphorous concentration was significantly higher in ZT and RT with mungbean-wheat rotation (3.33 and 3.23 mg kg^{-1}

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respectively) while all other treatments were non-significant with each other. After summer 2018, ZT and RT systems showed higher values of available-P both with mungbean-wheat and fallow-wheat (3.67, 3.59 and 3.33, 3.08 mg kg^{-1} respectively), the significantly lowest was in CT (FW). After winter 2017, the concentration of phosphorous for ZT and RT in mungbean-wheat plots was 3.47 and 3.20 mg kg^{-1} and in fallow-wheat plots was 2.73 and 2.81 mg kg^{-1} and significantly minimum was obtained in CT. After winter of 2018, amount of phosphorous in ZT and RT tillage treatments with the mungbean-wheat and fallow-wheat crop rotations was 3.90 and 3.60 mg kg^{-1} and 3.48 and 3.25 mg kg^{-1} respectively. The tested crop rotations were non-significantly different in first year but in second year, mungbean-wheat had significantly higher value (3.33 mg kg^{-1}) than fallow-wheat (2.93 mg kg^{-1}).

Table 2. *Available soil phosphorous affected by tillage systems and crop rotations during two experimental years*

Available Phosphorous (mg kg^{-1})							
Treatments		**2016-17**			**2017-18**		
Tillage	**Crop**	**Summer**	**Winter**	**Average**	**Summer**	**Winter**	**Average**
CT	MW	1.90 b	2.61 bc	2.33 b	2.58 bc	2.37 cd	2.47 d
	FW	2.38 ab	2.28 c	2.25 b	2.32 c	2.30 d	2.31 d
MT	MW	2.19 ab	3.00 ab	2.59 b	3.41 ab	3.50 ab	3.46 acd
	FW	2.57 ab	2.56 bc	2.56 b	2.83 abc	2.85 bcd	2.84 bcd
RT	MW	3.33 a	3.20 ab	3.26 a	3.59 a	3.60 ab	3.59 a
	FW	2.19 ab	2.81 abc	2.50 b	3.08 abc	3.25 abc	3.16 abc
ZT	MW	3.23 a	3.47 a	3.35 a	3.67 a	3.90 a	3.78 a
	FW	2.76 ab	2.73 abc	2.75 ab	3.33 ab	3.48 ab	3.40 ab

CT, conventional tillage; MT, minimum tillage; RT, reduced tillage; ZT, zero tillage; MW, mungbean-wheat; FW, fallow-wheat. Dissimilar letters show significant differences among means as determined by Turkey's test with $P < 0.05$

Dehydrogenase activity

Conservation tillage systems had statistically significantly higher soil Dha activity than conventional tillage, whereas between the crop rotations, mungbean-wheat rotation had significantly higher enzymatic activity compared to fallow-wheat rotation. Among tillage treatments, the highest Dha activity was achieved in ZT followed by RT with values of 28.83 and 26.21 TPF μg g^{-1} soil 24 h^{-1} during first experimental year and 34.12 and 31.17 TPF μg g^{-1} soil 24 h^{-1} during second year. The significantly lowest activity was observed in CT with 22.10 and 25.27 TPF μg g^{-1} soil 24 h^{-1} in the first and second years respectively. Between the crop rotations, differences were statistically non-significant during the first year of experiment but in the second year, mungbean-wheat had statistically significant higher Dha activity than fallow-wheat rotation with values of 32.19 TPF μg g^{-1} soil 24 h^{-1} and 28.65 TPF μg g^{-1} soil 24h^{-1} respectively.

Dehydrogenase activity showed temporal variations throughout the year (*Fig. 3*). The highest enzymatic activity was observed in October closely followed by June with values of 30.66 and 28.86 TPF μg g^{-1} soil 24h^{-1} during the first year, and 37.25 and 33.96 TPF μg g^{-1} soil 24 h^{-1} during second year. The enzyme activity was statistically lowest in February (20.89 TPF μg g^{-1} soil 24 h^{-1}) in the first year while in the second year it was statistically lowest in months of February and August with values of 23.65 and 27.07 TPF µg g^{-1} soil 24 h^{-1} respectively.

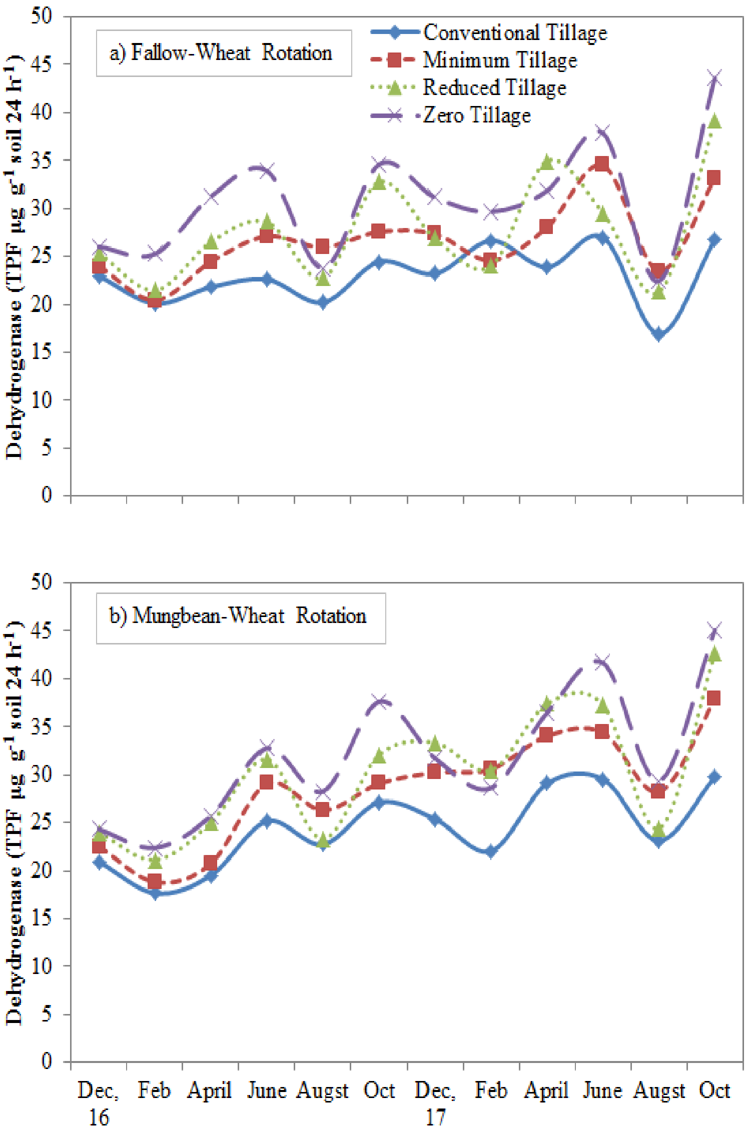

Figure 3. *Temporal variations in soil dehydrogenase enzyme activity in response to different tillage systems and crop rotations during two experimental years determined by Turkey's test with P < 0.05*

Urease activity

Soil UR activity was significantly higher under ZT followed by RT in the first year (298.22 and 287.35 µg N g^{-1} soil 2 h^{-1} respectively) as well as the second year (312.57 and 298.21 µg N g^{-1} soil 2 h^{-1} respectively). The statistically significant lowest activity was observed in CT during both experimental years (250.44 and 245.36 µg N g^{-1} soil 2 h^{-1} respectively). Between the crop rotations, no statistically significant changes were observed in the first year of experiment whereas in the second year, statistically significant results were found where mungbean-wheat rotation had higher value (298.49 µg N g^{-1} soil 2 h^{-1}) than fallow-wheat rotation (275.43 µg N g^{-1} soil 2 h^{-1}).

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Among temporal periods, the maximum urease activity was observed in August followed by April in both experimental years (*Fig. 4*). The values were 289.27 and 286.36 µg N g^{-1} soil $2h^{-1}$ respectively in first year and 313.92 and 303.32 µg N g^{-1} soil $2h^{-1}$ respectively in the second year. The activity was statistically lowest in February (259.02 and 256.47 µg N g^{-1} soil dwt $2h^{-1}$ in first and the second year respectively.

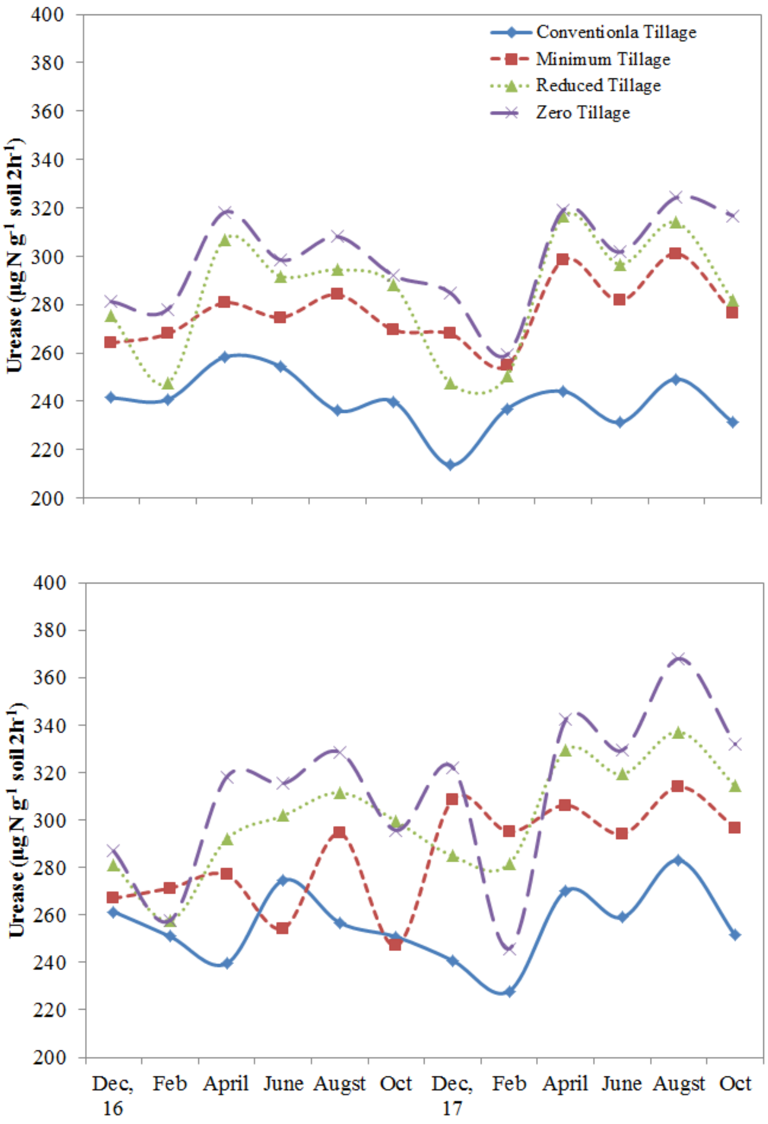

Figure 4. *Temporal variations on soil urease enzyme activity in response to tillage systems and crop rotations during two experimental years determined by Turkey's test with* $P < 0.05$

Alkaline phosphatase activity

The average soil ALP activity was also significantly improved with conservation agriculture. Among tillage treatments, soil ALP enzyme activity was higher under ZT followed by RT having average values of 33.69 and 32.54 µg p-NP g^{-1} soil h^{-1} by the end of first experimental year and 40.15 and 39.06 µg p-NP g^{-1} soil h^{-1} in the second experimental year. The statistically lowest activity was observed in CT with values of 25.18 and 29.93 µg p-NP g^{-1} soil h^{-1} in the first and second years respectively. Between

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the crop rotations, statistically non-significant difference was recorded during the first year of experimentation. However, statistically significantly higher values were obtained by mungbean-wheat rotation than fallow-wheat rotation in second year of experimentation with the values of 37.78 and 34.57 µg p-NP g^{-1} soil h^{-1} respectively.

ALP activity also showed variations around the year (*Fig. 5*). The maximum activity was observed in the months of August (35.95 µg p-NP g^{-1} soil h^{-1}) in the first year as well as in the second year of experiment (41.08 µg p-NP g^{-1} soil h^{-1}). This period was closely followed by April with values of 34.39 µg p-NP g^{-1} soil h^{-1} in first year and 40.52 µg p-NP g^{-1} soil h^{-1} during the second year. Enzyme activity was statistically lowest during February in both years (20.02 and 26.36 µg p-NP g^{-1} soil h^{-1}) when compared with other seasons of the year.

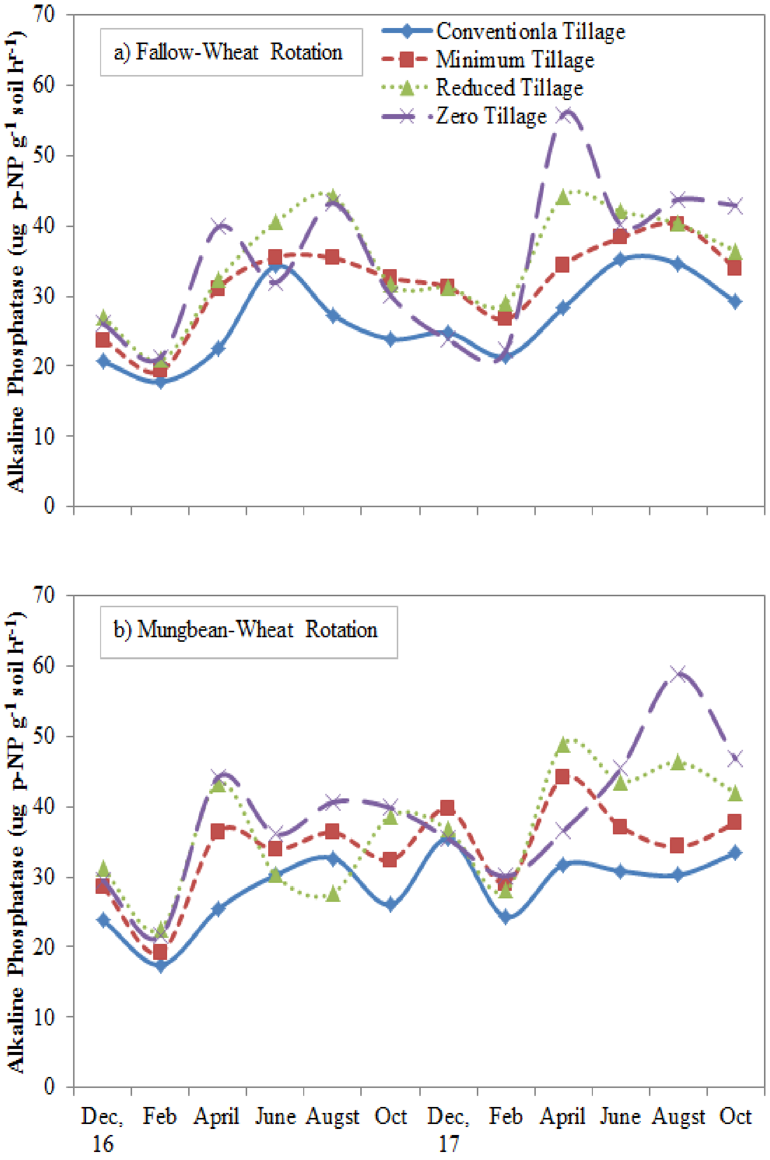

Figure 5. *Temporal variations in soil alkaline phosphatase enzyme activity in response to tillage systems and crop rotations during two experimental years determined by Turkey's test with P < 0.05*

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Correlation analysis of soil enzymes with nutrients and soil organic carbon (*Table 3*) showed that SOC had positive relation with soil Dha (r = 0.77), ALP (r = 0.56), UR (r = 0.63) and with nutrients availability i.e., N (r = 0.68) and P (r = 0.70).

Table 3. *Relationship of soil enzymes with different soil attributes*

	SOC	**Dha**	**ALP**	**UR**	**N**	**P**
SOC	1.00					
Dha	0.77	1.00				
ALP	0.56	0.41	1.00			
UR	0.63	0.57	0.85	1.00		
N	0.68	0.73	0.47	0.56	1.00	
P	0.70	0.64	0.57	0.68	0.57	1.00

SOC: soil organic carbon; UR: urease activity; Dha: dehydrogenase activity; ALP: alkaline phosphatase activity; N: nitrogen; P: phosphorous

Discussion

In our study, the highest SOC content was recorded in ZT with retained crop residues. The high amount of organic content was accumulated within soil due to slow decomposition of residues in ZT that increased SOC (Jat et al., 2019a). The increase in SOC concentration might be due to some interacting factors such as minimum soil disturbance, high moisture content, higher crop residue addition or retention, lower soil surface temperature and low risk of erosion (Ismail et al., 1994). Our results are similar with findings of Sharif et al. (2015) who found statistically higher SOC in ZT than CT under similar semiarid conditions, and reported by other researchers elsewhere (Kumar et al., 2017; Zhang et al., 2018). The higher SOC in mungbean-wheat rotation than fallow-wheat rotation might be due to improved soil biological properties and high carbon mineralization with mungbean residues that stimulated microbial activity and growth (Naeem et al., 2009). The positive relation of SOC with activities of Dha, UR and ALP was also observed in some previous studies (Parihar et al., 2016; Dixit et al., 2019).

The higher nitrogen in ZT and RT was related with soil crop residues retention that improved soil organic content (Turmel et al., 2015) and could be attributed due to lesser nitrogen loss by volatilization, denitrification, immobilization, and leaching (Malhi et al., 2001). Mungbean-wheat rotation had higher nitrate-N, as the leguminous crop residues increase mineral nitrogen than cereal residues (Ranaivoson et al., 2017). The C:N ratio of legumes residues is relatively low that lead to N mineralization while cereal residues have high C:N ratio and cause temporary immobilization of N (Govaerts et al., 2006). Soil available phosphorous was also higher with ZT and residue retention than CT. The available phosphorous was strongly correlated with organic matter content that releases carbonic acid during crop residue decomposition. Tang et al. (2015) also found higher soil nutrient availability including phosphorous due to release of inorganic phosphorous from residues decaying residues in no tillage treatment.

Increase in ALP activity with reduction in tillage intensity along with residue retention, as it increased readily available substrates (carbohydrates) for microorganisms. The positive effect of reduced or no tillage on soil enzymes had been repeatedly reported (Nivelle et al., 2016; Chen et al., 2019; Piazza et al., 2020). Saikia (2017) also found the same results in his review analysis of 68 studies that residue management and reduced tillage intensity caused significant improvement in soil ALP, UR and Dha enzymes activity. Soil ALP, UR and Dha enzymatic activities statistically increased in conservation tillage i.e., ZT and RT as compared to CT practices due to residue recycling. Microbial activities affected to a wider extent from previous residues of crop on soil surface in undisturbed soil (Choudhary et al., 2018). The reason could be that under no tillage, substrate availability, microbial biomass and microbial demand is high (Shi et al., 2013). The higher UR and ALP in the period of August might be due to rainfall and temperature variations. These results are similar with the Shao et al. (2015) who found higher enzymes activity of UR, invertase and ALP in summer and lower in winter.

Our results showed that inclusion of legume crop in the rotation also increased soil ALP, UR and Dha enzymatic activities in mungbean-wheat than fallow-wheat rotation. This trend in legume-based crop rotation was due to higher SOC (Roa-Fuentes et al., 2015; Nawaz et al., 2017), greater nutrient retention (Singh et al., 2018) as well as more biomass and more nitrogen fixation that favours microbial growth and development (Parit et al., 2019). Leguminous crop also stimulated the soil enzymatic activity due to biological nitrogen fixation process (Roldán, 2003). Some previous studies had found that leguminous crops (such as aspalathus, cyclopia, cowpea and chickpea) also released more phosphatase enzymes as compared to non-leguminous (Makoi et al., 2010; Maseko and Dakora, 2013). The reason is that leguminous crops require more phosphorus for nitrogen fixation process than cereals.

The dehydrogenase activity improved in the month of October that could be attributed to favorable soil moisture and temperatures. The month of October represents autumn season that comes after monsoon season therefore the moisture is plenty, and temperature is mild during October that is favorable for microbial growth and organic matter decomposition (Garcia et al., 2002). The extracellular enzymes activities are higher at mild moisture and temperature as compared to other seasons (Fioretto et al., 2018). The increased Dha in conservation tillage ZT and RT systems was due to more availability of soil carbon to the microbes compared with other treatments (Jat et al., 2019b).

Conclusions

From the two-year field investigation, it is clearly indicated that UR, ALP and Dha activities and C, N, P status can be increased by conservation tillage practices and legume-based crop rotations such as zero tillage and reduced tillage with mungbean-wheat crop rotation. It is also perceived that variation in temperature and rainfall considerably affects soil enzymatic activity. The information generated from this study can be utilized for improvement of soil chemical, biochemical properties, and crop management practices in rainfed farming systems.

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APPENDIX

Figure 2(a). *Analysis of Variance (ANOVA) for soil organic carbon in summer 2016-2017*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	$F_{calculated}$
Replicate	2	0.124	0.062	
tillage	3	3.521	1.174	13.15*
Error Replicate*tillage	6	0.535	0.089	
crop	1	0.156	0.156	1.13
tillage*crop	3	0.028	0.010	0.07*
Error	8	1.110	0.139	
Total	23	5.474		

Figure 2(b). *Analysis of Variance (ANOVA) for soil organic carbon in winter 2016-2017*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	$F_{calculated}$
Replicate	2	0.094	0.047	
tillage	3	3.864	1.288	53.48 *
Error I	6	0.145	0.024	
crop	1	0.291	0.291	5.45*
tillage*crop	3	0.675	0.225	4.22*
Error II	8	0.427	0.053	
Total	23	5.496		

Figure 2(c). *Analysis of Variance (ANOVA) for soil organic carbon in summer 2017-2018*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	$F_{calculated}$
Replicate	2	0.043	0.022	
Tillage	3	6.068	2.023	35.03*
Error I	6	0.346	0.058	
Crop	1	0.305	0.305	3.19
Tillage*crop	3	0.381	0.127	1.33*
Error II	8	0.765	0.096	
Total	23	7.909		

Figure 2(d). *Analysis of Variance (ANOVA) for soil organic carbon in winter 2017-2018*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	$F_{calculated}$
Replicate	2	0.037	0.019	
Tillage	3	9.026	3.009	27.06*
Error I	6	0.667	0.111	
Crop	1	0.672	0.672	5.17
Tillage*crop	3	0.231	0.077	0.59*
Error II	8	1.039	0.130	
Total	23	11.673		

Table 1(a). *Analysis of Variance (ANOVA) for soil Nitrate Nitrogen (mg kg^{-1}) in summer 2016-2017*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	$F_{calculated}$
Replicate	2	1.59	0.79	
tillage	3	14.65	4.88	6.32*
Error I	6	4.64	0.77	
crop	1	7.56	7.56	10.00*
tillage*crop	3	1.09	0.36	0.48
Error II	8	6.05	0.75	
Total	23	35.59		

Table 1(b). *Analysis of Variance (ANOVA) for soil Nitrate Nitrogen (mg kg^{-1}) in winter 2016-2017*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	$F_{calculated}$
Replicate	2	14.77	7.38	
tillage	3	16.99	5.66	8.26 *
Error I	6	4.11	0.68	
crop	1	14.68	14.68	6.33*
tillage*crop	3	5.29	1.76	0.76
Error II	8	18.55	2.31	
Total	23	74.42		

Table 1(c). *Analysis of Variance (ANOVA) for soil Nitrate Nitrogen (mg kg^{-1}) in summer 2017-2018*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	$F_{calculated}$
Replicate	2	2.41	1.20	
Tillage	3	7.75	2.58	5.78*
Error I	6	2.68	0.44	
Crop	1	0.19	0.19	0.29
Tillage*crop	3	4.58	1.52	2.28*
Error II	8	5.34	0.66	
Total	23	22.97		

Table 1(d). *Analysis of Variance (ANOVA) for soil Nitrate Nitrogen (mg kg^{-1}) in winter 2017-2018*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	$F_{calculated}$
Replicate	2	0.32	0.16	
Tillage	3	21.55	7.18	10.18**
Error I	6	4.23	0.70	
Crop	1	3.70	3.70	15.96**
Tillage*crop	3	2.79	0.93	4.01*
Error II	8	1.85	0.23	
Total	23	34.47		

Table 2(a). *Analysis of Variance (ANOVA) for soil Available Phosphorous (mg kg^{-1}) in summer 2016-2017*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	$F_{calculated}$
Replicate	2	1.38	0.69	
tillage	3	2.63	0.87	5.17*
Error I	6	1.02	0.17	
crop	1	0.21	0.21	0.31
tillage*crop	3	2.63	0.87	1.25 *
Error II	8	5.64	0.70	
Total	23	13.54		

Table 2(b). *Analysis of Variance (ANOVA) for soil Available Phosphorous (mg kg^{-1}) in winter 2016-2017*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	$F_{calculated}$
Replicate	2	1.53	0.76	
tillage	3	1.52	0.50	4.96*
Error I	6	0.61	0.10	
crop	1	1.35	1.35	7.93 *
tillage*crop	3	0.14	0.04	0.84
Error II	8	1.36	0.17	
Total	23	6.53		

Table 2(c). *Analysis of Variance (ANOVA) for soil Available Phosphorous (mg kg^{-1}) in summer 2017-2018*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	$F_{calculated}$
Replicate	2	0.26	0.13	
tillage	3	3.82	1.27	5.12*
Error I	6	1.49	0.24	
crop	1	1.07	1.07	6.40*
tillage*crop	3	0.10	0.03	0.20
Error II	8	1.34	0.16	
Total	23	8.10		

Table 2(d). *Analysis of Variance (ANOVA) for soil Available Phosphorous (mg kg^{-1}) in winter 2017-2018*

Source of variation	Degree of freedom	Sum of squares	Mean of squares	$F_{calculated}$
Replicate	2	0.09	0.04	
tillage	3	6.13	2.04	5.41*
Error I	6	2.26	0.37	
crop	1	0.83	0.83	11.62**
tillage*crop	3	0.26	0.08	1.24 *
Error II	8	0.57	0.07	
Total	23	10.17		

Figure 3(a). *Analysis of Variance (ANOVA) for soil Dehydrogenase enzyme activity in 2016-2017*

Source of variation	**Degree of freedom**	**Sum of squares**	**Mean of squares**	**$F_{calculated}$**
Replicate	2	52.91	26.45	
tillage	3	858.08	286.025	23.83**
Error I	6	72.01	12.00	
crop	1	2.33	2.32	0.31
tillage*crop	3	3.70	1.23	0.17
Error II	8	59.09	7.38	
Month	5	1564.48	312.89	47.33***
tillage*month	15	232.97	15.53	2.35**
crop*month	5	151.91	30.38	4.60**
tillage* crop*month	15	61.63	4.10	0.62
Error III	80	528.89	6.611	
Total	143			

Figure 3(b). *Analysis of Variance (ANOVA) for soil Dehydrogenase enzyme activity in 2017-2018*

Source of variation	**Degree of freedom**	**Sum of squares**	**Mean of squares**	**$F_{calculated}$**
Replicate	2	7.14	3.57	
tillage	3	1512.60	504.19	44.91**
Error I	6	67.37	11.22	
crop	1	450.75	450.74	21.83**
tillage*crop	3	37.54	12.51	0.61*
Error II	8	165.16	20.64	
Month	5	2922.21	584.44	85.44***
tillage*month	15	481.39	32.09	4.69***
crop*month	5	46.30	9.26	1.35*
tillage* crop*month	15	202.60	13.50	1.97*
Error III	80	547.24	6.84	
Total	143	6440.30		

Figure 4(a). *Analysis of Variance (ANOVA) for soil Urease enzyme activity in 2016-2017*

Source of variation	**Degree of freedom**	**Sum of squares**	**Mean of squares**	**$F_{calculated}$**
Replicate	2	2036	1017.9	
tillage	3	46748	15582.7	16.70**
Error I	6	5600	933.3	
crop	1	613	612.8	1.06
tillage*crop	3	1173	390.9	0.68
Error II	8	4621	577.6	
Month	5	16014	3202.7	7.34***
tillage*month	15	12276	818.4	1.88*
crop*month	5	2391	478.2	1.10
tillage* crop*month	15	3193	212.9	0.49
Error III	80	34900	436.3	
Total	143	129565		

Figure 4(b). *Analysis of Variance (ANOVA) for soil Urease enzyme activity in 2017-2018*

Source of variation	**Degree of freedom**	**Sum of squares**	**Mean of squares**	**$F_{calculated}$**
Replicate	2	4129	2064.4	
tillage	3	91284	30427.9	58.17**
Error I	6	3139	523.1	
crop	1	19150	19150.5	61.48**
tillage*crop	3	166	55.2	0.18*
Error II	8	2492	311.5	
Month	5	52183	10436.3	18.58***
tillage*month	15	14529	968.6	1.72*
crop*month	5	2009	40.18	0.72*
tillage* crop*month	15	4984	332.3	0.59*
Error III	80	44928	56.16	
Total	143	238993		

Figure 5(a). *Analysis of Variance (ANOVA) for soil Alkaline Phosphatase enzyme activity in 2016-2017*

Source of variation	**Degree of freedom**	**Sum of squares**	**Mean of squares**	**$F_{calculated}$**
Replicate	2	20.2	10.07	
tillage	3	1535.6	511.85	36.53**
Error I	6	84.1	14.013	
crop	1	74.6	74.6	5.48*
tillage*crop	3	63.2	21.07	1.55*
Error II	8	109.0	13.63	
Month	5	4481.2	896.23	33.07***
tillage*month	15	637.2	42.48	1.57*
crop*month	5	469.0	93.79	3.46*
tillage* crop*month	15	653.5	43.57	1.61*
Error III	80	2168.0	27.10	
Total	143	10295.6		

Figure 5(b). *Analysis of Variance (ANOVA) for soil Alkaline Phosphatase enzyme activity in 2017-2018*

Source of variation	**Degree of freedom**	**Sum of squares**	**Mean of squares**	**$F_{calculated}$**
Replicate	2	142.6	71.27	
tillage	3	2285.0	761.67	12.68**
Error I	6	360.5	60.08	
crop	1	369.6	369.6	11.20*
tillage*crop	3	20.5	6.84	0.21
Error II	8	264.0	33.00	
Month	5	3964.6	792.92	29.96***
tillage*month	15	1253.6	83.57	3.16**
crop*month	5	339.6	67.91	2.57*
tillage* crop*month	15	1337.3	89.15	3.37**
Error III	80	2117.5	26.46	
Total	143	12454.9		