Soil Microbial Budgeting as Influenced by Contrasting Tillage and Crop Diversification Under Rice based Cropping Systems in Inseptisol of Bihar

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This research concerns the influence of contrasting tillage practices (Zero Tillage (ZT), Permanent Bed (PB) and Conventional tillage (CT)) in main plots and crop rotation (rice-wheat (R-W), rice-maize (R-M) and rice-lentil (R-L)) under rice based cropping system under split plot design with three replication, on soil microbial budgeting in terms of size and structure of microbial population, dehydrogenase activity (DHA), phosphatase activity and fluorescein diacetate (FDA) hydrolysis. Rice base cropping system is advocated as the dominant system prevailing in India due to the better suitability to its landforms and climatic conditions. Within tillage system, SOC was reported higher in zero tillage (0.67%), compared to permanent bed planting (0.66%) and CT (0.62%) at 0-15cm. At 15-30cm depth, zero tillage (0.57%) and PB (0.58%) registered significantly ($P < 0.05$) higher SOC compared to CT (0.52%). In this study, we estimated the microbial community size and structure, enzymatic activities, mychorrizal root infection and soil organic carbon (SOC). Root and rhizospheric soil samples were collected during two consecutive seasons from a 5 year old long term field experiment on conservation agriculture located at Research farm, Bihar Agricultural College, Sabour and still continuing. ZT treatment resulted higher soil organic carbon content (0.57%), viable microbial population (19.6% higher fungi, 10.63% bacteria, and 12.6% actinomycetes), dehydrogenase activity (5.3-9.11 %), phosphatase activity (9.3-10.57%) which was at par with PB and differed significantly to that of CT treatment. Thus tillage practices and crop diversifications are the important factors affecting soil microbial community size and structure.

Keywords: Zero tillage, permanent bed planting, cropping system, soil micro-flora.

Cereal–cereal systems, especially rice (*Oryza sativa* L.) –wheat (*Triticum aestivum* L.) (R-W) or rice- maize (*Zea mays*), contribute to satisfy the bulk of the food demand in South Asia. Rice followed by wheat or maize forms the predominant cropping systems in the subtropical areas of the Indo-Gangetic Plains (IGP), where rice prevails during the monsoon (*kharif*) season, while wheat, maize, and sometimes winter pulses like chickpea (*Cicer arietinum*) or lentil (*Lens culinaris* L.) are grown during the winter (*Rabi*) season. The rice-wheat system alone occupies 13.5 million ha of productive land in the IGP. Moreover, the area under rice-maize is also expanding rapidly, especially in the eastern IGP. Maize is the third most important cereal crop in India after rice and wheat. At present, maize cultivation during winter is becoming common in eastern India due to its

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higher productivity and profitability and grown in 1.2 million ha producing 5.5 mt of grain, with an average productivity of 4.0 t ha^{-1}. In recent years, cereal production growth in terms of grain and residue has slowed with annual growth rates falling below 1% and staying well below annual population growth for the past decade (DACNET, 2014).

Today, the most successful resource conserving technology in the rice–wheat systems has been zero-tillage wheat, particularly in the Indian part of IGP. But now with the introduction of different modified planters and multi crop planters, zero tillage (ZT) has started to pick up pace both in terms of area under adoption and seeding of different crops like maize or lentil. ZT allows for timelier wheat establishment as it greatly reduces the turn-around time, allowing wheat establishment in a single pass almost immediately after the rice harvest. Moreover, the reduced turn-around time is reported to have allowed wheat planting to be advanced by 8– 25 days in Bihar. To date, the most widely adopted resource conserving technology in the Indo-Gangetic Plains (IGP) of South Asia has been zero-tillage (ZT) wheat after rice, particularly in India (Erenstein *et al*., 2008). Thus, conservation tillage, along with some complimentary practices such as soil cover and crop diversity (Corsi *et al*., 2012) has emerged as a viable option to ensure sustainable food production and maintain environmental integrity. This implies that conservation tillage is a component of conservation agriculture (CA). Corsi *et al*. (2012) define CA as a method of managing agro-ecosystems for improved and sustained productivity, increased profits and food security while preserving and enhancing the resource base and the environment. Crop management practices (tillage systems or cropping sequences) can affect soil health. Karlen *et al*. (2013) observed that deep soil ploughing with mouldboard plough had significant negative impact on soil health and quality parameters. Soil with better health and quality will be able to produce higher crop yield under favourable as well as extreme climatic conditions (Congreves *et al*., 2015), and soil health acts as a critical component for adaptation and mitigation of climate change effects by the crops (Congreves *et al*., 2015). Sandy loam (Typic Haplustept) soil is the most dominant soil texture of Indo Gangetic Plains of Bihar. The main production constraints of this type of soil are higher bulk density, poor water retention capacity, higher hydraulic conductivity, lower soil organic carbon and lower biological activities (Singh *et al*., 2016). Optimization of tillage practices lead to improvement in soil quality in various dimensions, including soil structure, soil fertility, and soil biological properties. Similarly, diversification in crop rotations can also affects soil quality by affecting carbon contents and soil biological activity, due to the difference in chemical composition of different crop residues that are added to soil (Srinivasarao *et al*., 2013).

Keeping in view the above facts in mind, the investigation was carried out to determine the effects of different tillage practices and intensive rice based crop rotations on organic carbon content and various biological traits in sandy loam (Typic Haplustept) soils of Bihar.

MATERIALS AND METHODS

Experimental site

The study site was located at 25^0 23' N and 87^0 07' E, altitude of 37.19 m above mean sea level (AMSL) under Agricultural farm of Bihar Agricultural University. The field experiment has been started from 2011 till date and laid out in a split plot design of three replications with tillage being the main factor. It comes under sub – tropical climatic conditions characterized with hot desiccating summer, cold winter and moderate rainfall. May is the hottest month with an average maximum temperature of 35 – 39^0 C. January is the coldest month of the year with minimum temperature varying from 5 – 10^0 C. The annual rainfall of this region is around 1300 mm, precipitating mostly between mid June – mid October.

Crop establishment and management

There were three main plots of different contrasting rice establishment techniques viz. Zero tillage (ZT), permanent bed (PB) and conventional tillage (CT) and sub plot treatments comprising of three cropping systems viz. rice-wheat (R-W), rice-maize (R-M) and rice-lentil (R-L). In the ZT flat system and PB systems 30% anchored residues were retained at the end of each season while in CT system all the residues were removed and rice was puddle transplanted followed by seeding of rabi season crops after tillage. In CT, rice was transplanted at a spacing of 20cm × 15cm and in ZT; rice was direct seeded at an average row distance of 20cm. In CT and ZT planting of rabi crops, wheat was drilled within the rows of rice at a row distance of 20cm, maize was hand dibbled at a row to row and plant to plant distance of 60cm and 20cm while lentil was sown within the rice rows with row to row distance of 30cm. In the permanent beds (PB) with 30% residue retention, one row of rice, wheat and lentil was sown on either side of beds, 67.5 cm wide in the respective treatments while one row of maize was hand dibbled on top of each bed. In conventional tillage rice was puddle transplanted as hills (2 plants/hill) with a row to row and plant to plant distance of 20 and 15 cm while for the other crops the spacing was similar to that of ZT treatment. Rice (*Oryza sativa* L.) cv 'Susk Samrat', was grown during rainy (*kharif*) season while maize (*Zea mays*) cv 'DHM-117', wheat (*Triticum aestivum*) cv 'HD-2888' and lentil (*Lens culinaris*) cv 'HUL-57'was sown during the rabi season.

Root infection by Mychorrhizal fungi

Root infection was assessed on a representative root sample taken from each plot at harvest. At harvest roots of 15 cm were taken from plants evenly distributed in each plot. Mycorrhiza infection of each plant was determined by estimating the percent of root segments colonised with AM with the method as described by Bierman and Linderman (1981).

Percent root infection was obtained as follows:

$$\%\,Root\,infection = \frac{Number\ of\ root\ segment\ infected\ with\ AM}{Total\ number\ of\ segment} X100$$

Soil sampling and analysis

Soil sampling was performed in every season at the time of harvesting to minimize the effect of plant growth on microbial communities in order to observe the tillage treatment effect. Field-moist samples were transported to the laboratory on ice and then passed through a 2 mm sieve within 24 hours. The soil samples were in laboratory using standard procedures. For organic carbon, available nitrogen, phosphorus and potassium were determined by Walkley and Black (1934), Subbiah and Asija (1956), Olsen *et al.* (1954) and Hanway & Heidel (1952). Total bacteria, fungi and actinomycetes population were estimated by following the serial dilution and plating techniques as described by Schmidt and Caldwell (1967). Dehydrogenase activity was also determined following the standard protocol by Casida *et al.*, 1964 and Phosphomonoesterases (acid and alkaline phosphatases) activity was estimated as described by Tabatabai and Bremner (1969). All analyses were done in triplicate. The initial soil properties are depicted in Table 1.

Statistical analysis

All the data analyzed for analysis of variance (ANOVA) technique as applicable to split-plot design (Gomez and Gomez 1984). The significance of the treatment effect was determined using F -test and to determine the significance of the difference between the means of the two treatments, least significant differences (LSD) were estimated at the 5 % probability level. The differences were considered significant only when $P<0.05$.

RESULTS AND DISCUSSION

Soil organic carbon (SOC) content

The conservation agriculture (CA) practices (PB and ZT) had significant ($P < 0.05$) effect on SOC content of surface soil layers (Table 2). The SOC content in PB (56%) and ZT (56%) plots were significantly ($P < 0.05$) higher than the CT (55%) plots for the 0-15 cm layer of soil depth. However, the SOC content of PB, ZT plots were statistically at par. The soil tilling increases organic matter decomposition and decreases carbon content by increasing organic matter oxidation (Six *et al*., 1999; Balasdent *et al*., 2001; Balota *et al*., 2003; Thomas et al., 2007). Aziz et al. (2015) had observed that No Tillage enhanced the total carbon by 30% and active carbon by 10% in corn-soybean-wheat cowpea rotation over CT. The result demonstrated higher carbon content over other crop rotations; this might be due to differences in quantity and chemical composition of crop residue biomass and/or root exudates among the crop rotations (Congreves *et al*., 2015). The crop rotations had significant ($P < 0.05$) interaction effect on SOC content of 0–15 cm soil layers (Table 2). R-L cropping system registered 0.58% and 0.59% higher SOC in kharif and rabi season compared to CT for above soil layer, respectively. Thierfelder *et al*. (2012) found 31% greater soil carbon by inclusion of cowpea and sunhemp in maize based crop rotations. Saha and Ghosh (2013) also reported the positive effects of legume residue application in cereal cropping systems on soil carbon content. The study by Gosai *et al*., (2009) revealed higher concentration of soil organic matter in the no-till and shallow-tilled plots compared to other conventionally tilled plots that confirms to the findings of Robbins and Voss (1991) and Angers *et al*. (1995).Increase in soil organic matter under no-tillage may have been a result of reduced contact of crop residues with soil. Surface residues tend to decompose more slowly than soil-incorporated residues, because of greater fluctuations in surface temperature and moisture and reduced availability of nutrients to microbes colonizing the surface residue (Schomberg *et al*., 1994).

Effect on soil biological properties

Microbial population dynamics

Soil microbial density as envisaged through the population of bacteria, actinomycetes and fungi observed in the experimental plots. It has been revealed by the observation that the microbial count was found higher in the plots of ZT which was statistically at par with PB and varied significantly when compared to CT. Retention of crop residue and minimal soil disturbance resulted in increased soil micro-flora populations both under ZT and PB.

Bacterial population

Bacterial population depends upon the management of crop residues and tillage operation as well. A significant higher population of total bacteria was detected in ZT treatment followed by PB systems which was 10.63 % and 7.6 % higher than the CT treatment irrespective of all the

Table 1. Initial status (2011) of soil properties 0-15cm (soil depth) at the experimental site

Soil Properties		Value
pH(1 : 2.5)		7.35
EC(dSm^{-1})		0.301
OC (%)		0.53
Available nitrogen (kgha^{-1})		160.2
Available phosphorus (kgha^{-1})		26.7
Available potassium (kgha^{-1})		221.6
Particle size distribution (%)	Sand	47.4
	Silt	32.6
	Clay	19.6

Table 2. Soil Organic carbon content

	OC (%)	
	Kharif 14-15	Rabi 14
Initial	0.53	0.53
T_1- Zero tillage	0.56	0.57
T_2- Permanent bed	0.56	0.57
T_3-Conventional Method	0.55	0.56
CD (P=0.05)	0.008	0.008
Initial	0.53	0.53
S_1- Rice-wheat	0.55	0.56
S_2- Rice- maize	0.55	0.55
S_3- Rice- Lentil	0.58	0.59
CD (P=0.05)	0.009	0.009

Table 3. Microbial population dynamics

Treatments	Bacteria(CFU *10^{-5})		Fungi(CFU *10^{-4})		Actinomycetes (CFU *10^{-5})	
Rice establishment techniques(Main Plot)	Rabi 14-15	Kharif 15	Rabi 14-15	Kharif 15	Rabi 14-15	Kharif 15
T_1- Zero tillage	45.47	46.70	19.53	20.37	33.17	34.80
T_2- Permanent bed	44.20	45.23	17.43	18.27	31.50	33.17
T_3-Conventional Method	41.10	42.10	16.33	16.67	29.47	30.27
CD (P=0.05)	1.19	1.42	1.68	2.15	1.19	1.03
Cropping systems (Sub-plot)						
S_1- Rice-wheat	42.07	42.90	17.63	18.17	31.37	32.83
S_2- Rice- maize	42.50	43.83	16.00	16.77	28.60	30.00
S_3- Rice- Lentil	46.20	47.30	19.67	20.37	34.17	35.40
CD (P=0.05)	2.36	2.73	2.52	2.71	1.59	1.64

cropping systems in rabi season (Table 3). The similar trend was also found in kharif season with a numerical increment. R-L cropping system proved well among all the other three cropping systems with insignificant difference and accounted 46.20 CFU $*10^{-5}$ in rabi and 47.30 CFU $*10^{-5}$ in kharif season in the rhizospheric soil. Reduced tillage indirectly defines the species composition of the soil microbial community by improving retention of soil moisture and modifying soil temperature (Krupinsky *et al*., 2002). The similar findings are also resulted by Sharma *et al*., 2011 and Helgason *et al*., 2009.

Actinomycetes population

Maintaining cover crop residues on the surface or incorporation provides a stimulating substrate for microbial growth. The highest actinomycetes population was found in surface soils sampled from ZT and PB plots by 12.6 % and 6.9 % over CT in the rabi season which was relatively similar to kharif season with a slight increment (Table 3). From this table, it was also observed that actinomycetes population was at its higher site for the R-L system (34.17 CFU $*10^{-5}$ in rabi and 35.40 CFU $*10^{-5}$ in kharif) followed by R-M and R-W cropping systems. The highest microbial count has been resulted in the plots treated with substantially proved that it could be the resultant of the degradation of crop residues and slow release of nutrients with balanced C/N ratio and ensured a faster microbial proliferation. Mohammadi *et al*., 2011 and Sharma *et al*., 2011 also found the similar results to the current study.

Table 4. Colonization of roots by VAM

Treatments	VAM infection (%)	
Cropping systems (Sub-plot)		
S_1- Rice-wheat	50.67	51.00
S_2- Rice- maize	61.00	52.33
S_3- Rice- Lentil	40.33	51.00
CD (P=0.05)	4.22	NS

Fungi population

In our study we found increasing fungi population by 19.6 % and 6.73 % in ZT and PB plots over CT in rabi season and was increased following the same trend in kharif season respectively. On the other hand, highest fungi count has been resulted in the plots of R-L cropping system (19.67 CFU $*10^{-4}$ in rabi and 20.37 CFU $*10^{-4}$ kharif) as because R-L system is a legume based cropping system receiving greater organic root exudates and organic acids in the rhizosphere caused a higher build-up of fungal density. The results corroborates with the findings of Helgason *et al*, 2009 and Sharma *et al*, 2011.

Dehydrogenase activity

Dehydrogenase (DHA) activity reflects the oxidative activity or intensity of metabolism of soil microflora and can be used as an indicator of microbial activity or populations in soils. Tillage and crop rotations and their interactions had significant ($P < 0.05$) effect on soil dehydrogenase enzyme activity of top soil layer (fig. 1).Higher DHA is the sign of stable soil health and higher microbial activity. The maximum soil DHA was recorded under ZT plots, which was significantly ($P < 0.05$) higher than CT plots and was statistically at par with PB treatment. The DHA in the rhizospheric soil was 9.11 and 5.30 % higher in ZT and PB treatments compared to CT in rabi season, respectively. Similar trend was also resulted in rabi season with an increasing rate. The DHA was found maximum under R-L crop rotation (16.54 and 16.81 µg TPF/hr/g soil in rabi and kharif) when compared to R-M and R-W cropping system (fig.

Table 5. Correlation and Linear Regression equation between soil organic content and microbial population

Mocrobial Population	Correlation matrix	Linear Regression equation	R^2X 100
Bacteria population	0.842**	y = 133.3x - 30.85	70.8
Fungi population	0.815**	y = 98.94x - 37.55	66.4
Actinomycetes population	0.864**	y = 147.3x - 50.79	74.5

Where y= microbial population; x is soil organic carbon
** Significant at 1% of level of significance

1).This can be attributed to the organic carbon content of the soil. Tao *et al*. (2009) have also observed higher DHA in conservation agriculture with legume crop. Gajda *et al*. (2013) and Perez-Brandan *et al*. (2012) also reported higher soil microbial enzymatic activities due to conservation agriculture or legumes. Inclusion of legumes in the R-L system compared to other crop rotations might result in higher SOC due to faster and easier decomposition of lower C:N ratio residues and root nodules which resulted higher DHA activity (Srinivasarao *et al.,* 2013).

Phosphatase activity

Similar to the dehydrogenase activity, phosphatase activity in the crop rhizosphere were assayed during maturity stage (after harvest) in the top soil layer. Results revealed that there was significant difference in enzymatic activities between ZT and PB in comparison with CT (Fig. 2). The maximum activity of acid and alkaline phosphatise activity (ALP) was recorded under ZT and PB which was 10.57 and 9.3 % higher over CT, respectively. The minimum ALP activity was recorded under CT possibly due to lower SOC content. Amongst crop rotations; the R-L rotation demonstrated higher ALP activities (158.50 and 160.93 µg PNP released /g soil/hr in rabi and kharif) compared to R-M and R-W cropping system. The acid phosphatase activity has also followed the same trend which is clearly depicted in fig. 2. Dodor and Tabatabai (2003) observed differential activities of ALP under various crop rotations. They also found significant correlation of ALP with SOC. Zero tillage in a volcanic soil in Chile increased dehydrogenase, acid phosphomonoesterase and urease activities mainly in the 0-5 cm layer compared

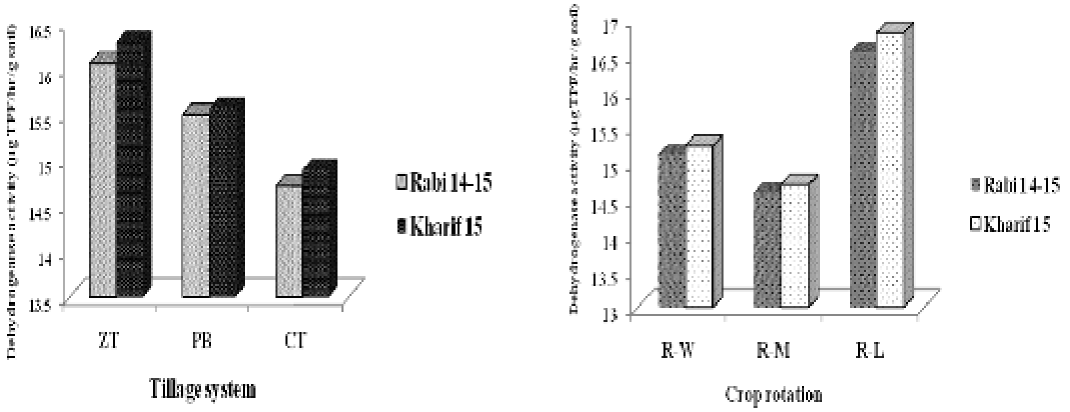

Fig. 1. Influence of tillage practices and crop rotations on dehydrogenase activity

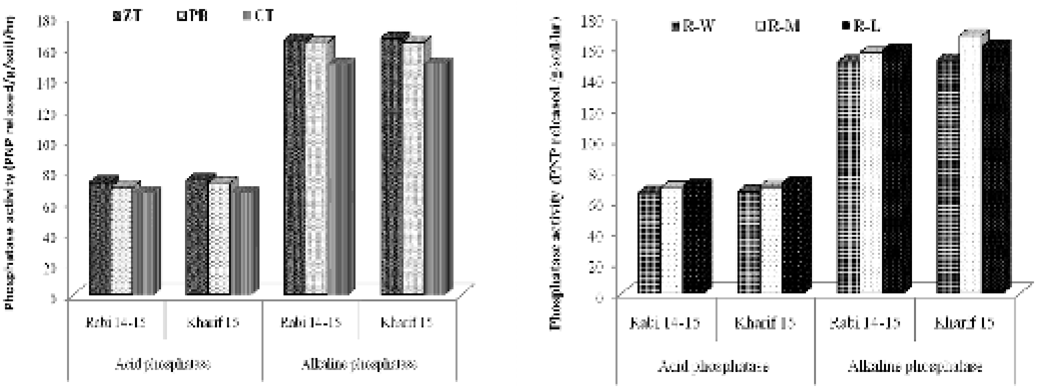

Fig. 2. Influence of tillage practices and crop rotations on phosphatase activity

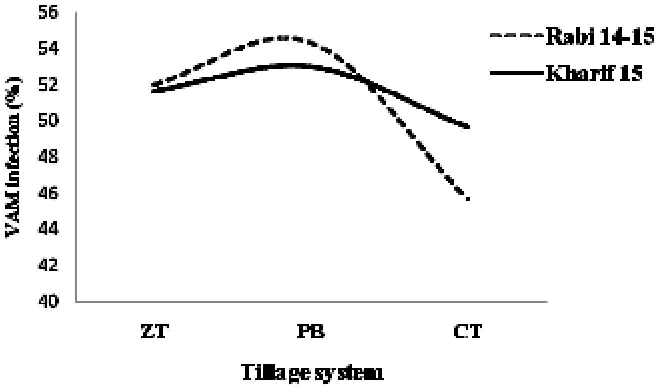

Fig. 3. Influence of tillage practices on root colonization by VAM

with a soil disk-harrowed to 20 cm (Alvear *et al*. 2005). Angers *et al*. (1993) reported 15% larger alkaline phosphatase activity in a barley-red clover rotation than in continuous barley on a clay soil in Quebec.

Mychorrhizal infection

Mycorrhiza is a mutualistic symbiosis between certain groups of soil fungi and most plant root systems (Hata *et al*., 2010). Mychorrhizal root colonization was significantly ($P < 0.05$) varied with tillage practices and crop rotations. The maximum root colonization was recorded under PB and ZT which was 18.96 % and 13.86 % higher compared to CT, respectively in rabi season (Fig. 3). The activity of Mychorrizal fungi was highest 61 % in R-M cropping system followed by the 50.67 % in R-W and was lowest in R-L system (40.33 %) during rabi season. The higher mycorrhizal colonization in maize could be due to the extensive root system of maize crop (Singh *et al*., 2015). There was any statistical difference for the root colonization in kharif season as because the crop was rice for every tillage systems.

Correlation matrix and regression between soil biological attributes and soil organic carbon

The relationships of soil microbial population and organic carbon illustrated in Table 5. Organic carbon was positively and significantly correlated with bacterial population (r=0.842**), fungal population (r= 0.815**) and actinomycetes population (r = 0.864). From the result presented in table 5, size of the co-efficient of the multiple determinations (R^2) indicated that 70.8 % of bacterial population was determined by organic carbon. Similarly, 66.4 % of fungal population was determined by organic carbon whereas 74.5 % of available lead was determined by organic carbon.

CONCLUSION

It was also observed that with ZT followed by PB led to significant improvement in soil biological health. A significant higher population of microorganisms was detected in zero tillage ZT treatment followed by PB systems irrespective of all the cropping systems. Soils managed under zero tillage contained approximately 19.6% higher fungi, 10.63% bacteria, and 12.6% actinomycetes population. Enzymatic activity viz. Dehydrogenase (5.3-9.11 %) and alkaline phosphatase (9.3-10.57%) activities also followed the same trend for the tillage systems. Among the rice based crop rotations, Rice –lentil system proved to be the best for the soil microbial activity and organic carbon status of the soil.

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