# Surface soil phosphorus and phosphatase activities affected by tillage and crop residue input amounts

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**ABSTRACT**

The effects of tillage and residue input amounts on soil phosphatase (alkaline phosphomonoesterase ALP, acid phosphomonoesterase ACP, phosphodiesterase PD, and inorganic pyrophosphatase IPP) activities and soil phosphorus (P) forms (total P, organic P, and available P) were evaluated using soils collected from a three-year experiment. The results showed that no-till increased soil total and organic P, but not available P as compared to conventional tillage treatments. Total P was increased as inputs of crop residue increased for no-till treatment. There were higher ALP and IPP activities in no-till treatments, while higher PD activity was found in tillage treatments and tillage had no significant effect on ACP activity. Overall phosphatase activities increased with an increase of crop residue amounts. Soil total P was correlated negatively with PD activity and positively with other phosphatase activities. Organic P had a positive correlation with ACP activity, but a negative correlation with PD activity. Available P had no significant correlation with phosphatase activities. Our data suggests that no-till and residue input could increase soil P contents and enhance the activities of phosphatase.

**Keywords**: straw mulching and burying; wheat-maize rotation; soil nutrient; soil biochemical activities

Phosphorus (P) is one of the limiting nutrients for plant growth (Redel et al. 2007) and organic P may constitute 20–80% of the total P in the surface soil (Turner and Haygarth 2005). Phosphorus is available to plants after it is hydrolysed into orthophosphate by phosphatases in the soils. Thus, the soil phosphatase activities greatly affect the bioavailability of organic P. The phosphatases in soils include not only phosphomonoesterases (for hydrolyzing organic phosphate monoesters) and phosphodiesterase (for hydrolyzing phosphate diesters) but also pyrophosphatase (which transfers pyrophosphate into orthophosphate) (Tabatabai 1994).

The phosphatase activities in cultivated land are highly affected by the agricultural practices, such as tillage, cropping systems and crop residues managements (Deng and Tabatabai 1997, Zhang et al. 2010). Both no-till and straw mulching leave a large amount of crop residues on the surface soils. It increases total P of surface soil and the activity of phosphatases (Redel et al. 2007). Most researchers focused on the response of phosphomonoesterase to tillage and residue managements (Turner and Haygarth 2005), while little information is available of their effects on phosphodiesterase and pyrophosphatase. Much of the organic phosphate in plant residues is in the form of phosphate diesters (Turner and Newman 2005), and the crop residues which are added into the soil by tillage practices mainly increase phosphate diesters, such as phospholipids and nucleic acids. Little is known

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about how tillage and different levels and sources of crop residues affect soil inorganic pyrophosphatase activity. Deng and Tabatabai (1997) reported that tillage could enhance inorganic pyrophosphatase activity of soil. However, more information is necessary to confirm the response of soil inorganic pyrophosphatase activity to tillage and residue managements. This study investigated the effects of tillage and residue managements on soil total P, organic P, and available P and on the activities of soil alkaline phosphomonoesterase (ALP), acid phosphomonoesterase (ACP), phosphodiesterase (PD) and inorganic pyrophosphatase (IPP) in the Huang-Huai-Hai plain, a major grain growing area in China.

## MATERIALS AND METHODS

**Site characteristics**. A field experiment was conducted in September 2007 at Fengqiu State Experimental Station for Agro-Ecology, Chinese Academy of Sciences, located in Fengqiu town (35°01'N, 114°32'E), Henan province, China. It is located in the warm, temperate semi-humid monsoon climatic zone, with mean annual precipitation of 605 mm and an average annual air temperature of 13.9°C. The experiment was established as a rotation of winter wheat (early-October to mid-May) and maize (early-June to mid-September). Initial soil chemical characteristics and further details of the site characteristics were described by Zhu et al. (2009).

**Experimental design**. The study was based on a wheat-maize rotation system which included tillage (T) and no-till (NT) as main plots and three subplots [no residue (R0), 50% residue (R50) and 100% residue (R100)]. The mean 100% residue treatment was 7.51 t/ha in wheat season and 8.12 t/ha in maize season. The chopped crop residues were ploughed into soil to a depth of 23 cm under tillage and mulched on the soil under no-till after wheat and maize harvest, respectively. Fertilisers were applied for all treatments. Two fertilisations were applied in wheat season or maize season. The first was carried out when sowing in June and October with the mount of 150 kg/ha (N:P:K = 17:9:5). The second was carried out in August in wheat season and in March in maize season with urea (120 kg N/ha). Each treatment was arranged randomly and replicated six times with plot size of 4 m × 100 m.

**Soil sampling and analysis**. Ten subsamples were collected from the surface soil layer (0–20 cm) in each plot and combined into soil samples (~500 g) after the winter wheat harvest on June 4, 2010. After plant materials and stones were removed, soil samples were sieved (2 mm). A portion of the sieved soils (~200 g) was kept at 4°C until analysis of soil phosphatase activities, while the rest was air-dried and stored at room temperature for the chemical analysis. The soil phosphatase activities and other soil properties were assayed within two weeks of sampling. Soil pH, total P (TP), organic P (OP), available P (AP), soil organic carbon (SOC) and total nitrogen (TN) were determined according to Ryan et al. (2001). Total P was determined by the molybdenum blue colorimetric method following perchloric acid ($HClO_4$) digestion. Organic P was calculated after igniting the soil at 550°C, and subtracting P in the unignited sample from P in the ignited sample. Available P was determined by molybdenum blue colorimetric method after extraction by sodium bicarbonate ($NaHCO_3$).

The activities of ALP, ACP, PD and IPP were analyzed with the field-moist soils as described by Tabatabai (1994). ALP and ACP were determined by measuring the release of *p*-nitrophenol by incubating 1 g soil at 37°C for 1 h with 0.2 ml toluene, 4 ml universal buffer (pH 6.5 for ACP and pH 11.0 for ALP), and 1 ml 50 mmol *p*-nitrophenyl phosphate. Enzyme activity was expressed as mg *p*-nitrophenol/kg soil/h. PD was assayed by a similar procedure but with *bis-p*-nitrophenyl phosphate as the substrate and the buffer was adjusted to pH 8.0. Enzyme activity was expressed as mg *p*-nitrophenol/kg soil/h. IPP was determined by measuring the concentration of $PO_4^{3-}$-P which was released by incubating 1 g soil with 3 ml 50 mmol sodium pyrophosphate at 37°C for 5 h. Enzyme activity was expressed as mg $PO_4^{3-}$-P/kg soil/5 h.

**Statistical analysis**. Soil data were calculated based on oven-dried (105°C) weight. The influence of tillage and residue managements on soil chemical properties and soil phosphatase activities were estimated with a simple two-factorial ANOVA. Multiple comparisons (Student-Newman-Keuls) were analysed by one-way ANOVA. Correlation of soil parameters was based on the Pearson correlation coefficients. All statistical analyses were conducted with the software SPSS 16.0 for Windows.

## RESULTS

**Soil chemical properties**. Total N, pH, and C/N ratio were significantly affected by tillage (Table 1). Residue input amounts and their interactions with tillage had significant effects on SOC content, soil

Table 1. *F* value of ANOVA of the effect of tillage, residue input amounts and their interaction on chemical properties of soil, soil phosphatase activities and the contents of soil P

| Factors | Tillage (T) | | Residue input amounts (R) | | T × R | |
|---|---|---|---|---|---|---|
| | *F* value | *P* value | *F* value | *P* value | *F* value | *P* value |
| ALP | 2.32 | 0.139 | 3.09 | 0.061 | 2.08 | 0.144 |
| ACP | 10.35 | 0.003 | 0.39 | 0.679 | 2.80 | 0.077 |
| PD | 37.35 | 0.000 | 1.53 | 0.236 | 5.04 | 0.014 |
| IPP | 16.66 | 0.000 | 14.69 | 0.000 | 36.00 | 0.000 |
| TP | 32.40 | 0.000 | 0.79 | 0.464 | 5.30 | 0.011 |
| OP | 98.97 | 0.000 | 8.62 | 0.001 | 2.80 | 0.078 |
| AP | 9.57 | 0.005 | 3.18 | 0.060 | 13.19 | 0.000 |
| SOC | 0.14 | 0.707 | 4.49 | 0.020 | 9.21 | 0.001 |
| TN | 27.03 | 0.000 | 1.52 | 0.238 | 1.13 | 0.338 |
| pH | 17.44 | 0.000 | 4.46 | 0.022 | 48.17 | 0.000 |
| C/N ratio | 27.72 | 0.000 | 9.75 | 0.001 | 32.28 | 0.000 |

ALP – alkaline phosphomonoesterase activity; ACP – acid phosphomonoesterase activity; PD – phosphodiesterase activity; IPP – inorganic pyrophosphatase activity; TP – total P; OP – organic P; AP – available P; SOC – soil organic carbon; TN – total N

pH and C/N ratio (Table 1). Total N content was higher in no-till treatments than in conventional tillage. With the increasing residue input amounts, SOC content was increased in the no-till treatments and similar tendencies were found for total N content, soil pH and C/N ratio (Table 2).

**Soil phosphatase activities**. Tillage significantly affected soil phosphatase activities except ALP. Residue managements also significantly affected soil IPP activity. The interaction of tillage and residue managements had significant effects on soil PD and IPP activities (Table 1).

Among treatments, ALP and IPP activities were higher in no-till treatments compared with conventional tillage. ALP and IPP activities in no-till treatments were significantly increased with an increase of residue amounts. ACP and PD activities had the same tendency but had no significant differences (Figure 1).

**Soil phosphorus**. Soil total P ranged from 597 to 745 mg/kg. Of this total, 15.0 to 22.5% was in the organic P form (71.7 to 151 mg/kg). Available P was only 11.0 to 24.8 mg/kg (Figure 2).

Tillage had significant effects on the content of total P, organic P and available P. Organic P was also significantly influenced by residue input amounts. The interaction of tillage and residue input amounts had significant effects on the contents of total P and available P (Table 1).

The total P and organic P under no-till treatments were significant higher than conventional tillage treatments. No significant difference of available

Table 2. Selected chemical properties of test soil as affected by tillage and residue input amounts treatments

| Treatments | SOC (g/kg) | Total N (g/kg) | pH | C/N ratio |
|---|---|---|---|---|
| TR0 | 12.7 (0.93)$^{a}$ | 0.88 (0.07)$^{bc}$ | 7.71 (0.08)$^{a}$ | 14.4 (0.80)$^{a}$ |
| TR50 | 8.85 (0.91)$^{c}$ | 0.83 (0.10)$^{c}$ | 7.46 (0.10)$^{c}$ | 10.7 (0.46)$^{bc}$ |
| TR100 | 10.2 (1.34)$^{bc}$ | 0.87 (0.08)$^{bc}$ | 7.33 (0.04)$^{c}$ | 11.7 (1.05)$^{b}$ |
| NTR0 | 9.71 (0.70)$^{bc}$ | 0.98 (0.09)$^{abc}$ | 7.26 (0.04)$^{c}$ | 9.92 (0.82)$^{c}$ |
| NTR50 | 10.3 (1.31)$^{bc}$ | 1.01 (0.07)$^{ab}$ | 7.30 (0.07)$^{c}$ | 10.8 (1.01)$^{bc}$ |
| NTR100 | 11.2 (2.36)$^{ab}$ | 1.10 (0.13)$^{a}$ | 7.58 (0.12)$^{b}$ | 11.7 (0.54)$^{b}$ |

Values are means with standard deviations in parenthesis. Different lower case letters indicate significant differences ($P < 0.05$). TR0, TR50, and TR100 are conventional tillage with 0, 50, and 100% residue input amounts. NTR0, NTR50, and NTR100 are no-till with 0, 50, and 100% residue input amounts, respectively

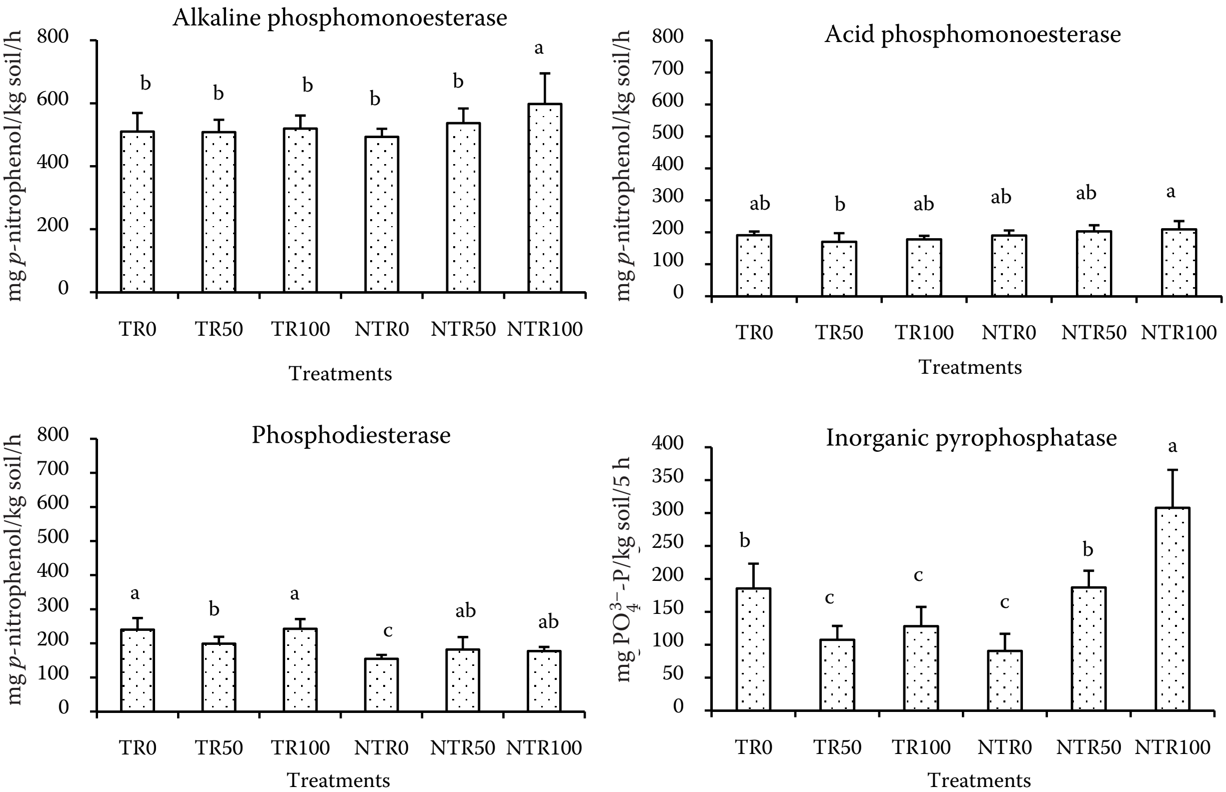


Figure 1. Soil phosphatase activities under no-tillage and tillage at different residue input amounts. Error bars represent standard deviation. TR0, TR50, and TR100 are conventional tillage with 0, 50, and 100% residue input amounts. NTR0, NTR50, and NTR100 are no-till with 0, 50, and 100% residue input amounts, respectively

P content was observed among treatments except for TR0 which was higher than for the other treatments. Along with the increasing residue input amounts, total P content under no-till treatments increased, but the opposite was found for organic P, and no significant changes were observed in available P. No consistent trends were found in total P, organic P and available P under conventional tillage as the amount of residue returned to the soil was increased (Figure 2).

**Correlations between soil chemical properties and phosphatase activities**. Phosphatase activities had significant positive correlations with total P except PD activity, which had a negative correlation with total P content (Table 3). Organic P had a significant positive correlation with ACP activity but a significant negative correlation with PD activity. No significant correlations between available P and the four phosphatase activities were found. SOC content had significant positive correlations with all properties except organic P content. Total N content had a negative correlation with PD activity and positive correlation with the other enzymes except for IPP activity and available P content. PD and IPP activities were positively correlated with soil pH. PD activity also positively correlated with C/N ratio (Table 3).

## DISCUSSIONS

Previous studies confirmed that no-till and residue application increased the accumulations of SOC and total N in surface soils (Halpern et al. 2010, Qin et al. 2010). However, our study showed that tillage systems had only a very limited influence on SOC storage after three years of the experiment. The SOC contents under no-till treatments were higher than the corresponding tillage treatments after 50–100% residue inputs, but the opposite occurred for the treatments TR0 and NTR0 treatments. There are some possible reasons: one is the sampling method, for example, Baker et al. (2007) hypothesized that sampling methodology may affect C accumulation measured for conservation tillage, due to shallow sampling introducing a bias. Pretreatments of soil samples by removing all plant materials could be another reason for the phenomena. Our study also found that residue treatments increased nutrient con-

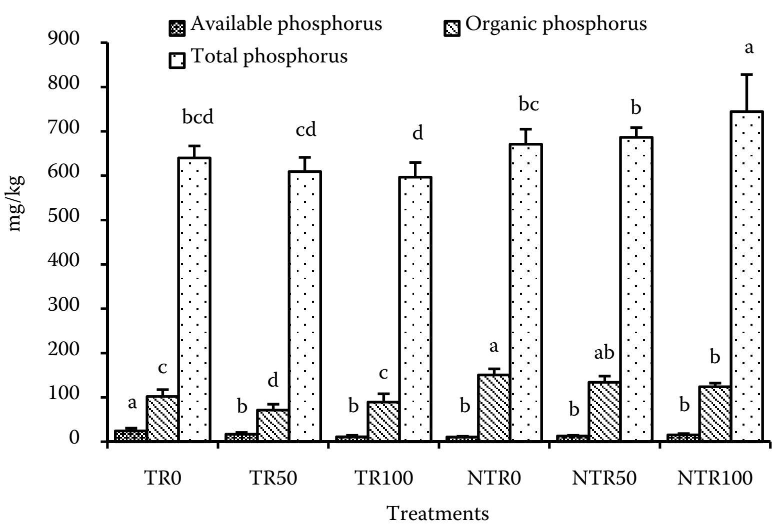


Figure 2. Soil total P, organic P and available P under no-tillage and tillage at different residue input amounts. Error bars represent standard deviation. TR0, TR50, and TR100 are conventional tillage with 0, 50, and 100% residue input amounts. NTR0, NTR50, and NTR100 are no-till with 0, 50, and 100% residue input amounts, respectively

tents. Compared to conventional treatments, total N was higher under conservation treatments, and C/N ratio, total N and SOC content increased with an increased input of residues. Halpern et al. (2010) also reported that soil C and N concentrations were higher in higher residue inputs treatments in a long-term experiment of tillage and residue management.

Compared with conventional tillage treatments, higher total P was detected in NT treatments in our study. This was attributed to minimal soil disturbance and mulching of crop residues on the surface of soil. Our study also indicated that total P content was significantly affected by tillage treatments. Some studies reported a greater total P and available P accumulation under no-till management (Redel et al. 2007, Mina et al. 2008). Available P did not increase in no-till treatments in our study. Similar results were reported by Roldán et al. (2005), who found that slightly alkaline soil pH decreased solubility of P. High soil pH (~7.44) and low extractability of P suggested more stable P forms presented in soils when crop residues were added to soil (Zibilske et al. 2002). Available P precipitated with soil Fe, Al, and Ca and became unavailable to plants. Soil organic P, however, was an important potential source of available P for plants (Linquist et al. 1997). Compared with conventional tillage, organic P was in higher concentration in no-till treatments in our study. With the increase of residue amounts, the organic P content was decreased; one reason

Table 3. Correlations between soil chemical properties and soil phosphatase activities

| | ALP | ACP | PD | IPP | TP | OP | AP | SOC | TN | pH | C/N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ALP | 1 | | | | | | | | | | |
| ACP | 0.453** | 1 | | | | | | | | | |
| PD | –0.009 | –0.047 | 1 | | | | | | | | |
| IPP | 0.412* | 0.504** | –0.072 | 1 | | | | | | | |
| TP | 0.404* | 0.639** | –0.383* | 0.712** | 1 | | | | | | |
| OP | 0.091 | 0.468** | –0.546** | 0.214 | 0.524** | 1 | | | | | |
| AP | –0.068 | –0.034 | 0.179 | 0.179 | 0.070 | –0.164 | 1 | | | | |
| SOC | 0.399** | 0.539** | 0.395* | 0.508** | 0.471** | 0.212 | 0.448** | 1 | | | |
| TN | 0.595** | 0.655** | –0.475** | 0.388 | 0.690** | 0.655** | –0.062 | 0.509** | 1 | | |
| pH | 0.158 | 0.064 | 0.374* | 0.486* | 0.182 | –0.303 | 0.618** | 0.489** | –0.075 | 1 | |
| C/N | –0.106 | 0.043 | 0.621** | 0.309 | –0.064 | –0.196 | 0.539** | 0.580** | –0.301 | 0.672** | 1 |

ALP – alkaline phosphomonoesterase activity; ACP – acid phosphomonoesterase activity; PD – phosphodiesterase activity; IPP– inorganic pyrophosphatase activity; TP – total P; OP – organic P; AP – available P; SOC – soil organic carbon; TN – total N; *, **correlations are significant at the 0.05, and 0.01 level, respectively can be ascribed to higher amount of organic P hydrolysed by soil phosphatase. Higher soil phosphatase (ALP, ACP and PD) activities was detected in no-till treatments with the increase of residue input amounts (Figure 1), and another reason could be attributed to the sampling method or pretreatment which was conducted by removing all plant material from test soils.

Phosphomonoesterase activity was higher in no-till treatments (Omidi et al. 2008), and increased along with the amount of residue application (Deng and Tabatabai 1997). Similar results were found in this study, our data also showed that ALP and ACP activities were higher in no-till treatments and increased along with the amount of residue inputs.

Soil pH can limit enzyme-mediated reaction rates by affecting the maximum activities of enzymes, and the solubility of substrates and cofactors (Dick et al. 1988). The activities of both alkaline and acid phosphatase are closely related to soil pH, with acid phosphatase dominating in acid soils, and alkaline phosphatase in alkaline soils (Eivazi and Tabatabai 1977). In our study, ALP activity was more than twice as high as ACP activity, mainly because soil pH was in the range of 7.2–7.7 (Table 2).

Sparling et al. (1986) found that PD activity was only 50–80% of the phosphomonoesterase activity. In our study, PD activity was as high as ACP activity, but was only half of the ALP activity. Turner and Haygarth (2005) report that phosphate diesters in soils are quickly hydrolysed into phosphate monoesters, which stimulates more production of phosphomonoesterases. PD activity was lower in no-till treatments, compared with conventional treatments. This result contradicted previous findings by Deng and Tabatabai (1997), who demonstrated that PD activity in no-till or mulching was generally greater than those in tillage treatments. The substrates of PD are phospholipids, nucleic acids, etc. Most of them enter the soil in the form of crop residues (Turner and Newman 2005). Conventional practice had more crop residue being buried in soil, and more phosphate diesters were brought into soil, so higher PD activity was detected in tillage treatments. The findings in different studies on the effect of tillage and residue input amounts on phosphodiesterase activity and phosphomonoesterase activities may differ due to the differences in the origin, states and/or persistence of the different groups of enzymes (Deng and Tabatabai 1997).

The activity of IPP was higher in no-till treatments except the NTR0, compared with conventional tillage, and was increased with the increase of residue amounts. This result confirmed the conclusion of Deng and Tabatabai (1997), whose results indicated that IPP activity was higher in no-till treatments compared with tillage treatments, and was increasing with the increase of mulch in no-till treatments.

Soil P was presumably related closely to soil phosphatase activity. Soil microorganisms and plants produce extracellular phosphatase which mineralises organically bound phosphorus. When available phosphorus is deficient in soil, soil biota can increase the production of extracellular phosphatases to enhance the supply of inorganic P in soil. Higher concentrations of soil available P tend to inhibit biota to produce phosphatase. Relationships between soil P supply and phosphatase activities were regulated by the negative feedback mechanism (Olander and Vitousek 2000). Gianfreda et al. (2005) reported a significant and positive correlation between phosphatase activity and total P content in agricultural soils but a negative correlation was found between phosphatase and the labile P content in non-cultivated soils. Margesin and Schinner (1994) showed that low content of available P induced high phosphomonoesterase activity. Tarafdar and Jungk (1987) found a significant correlation between phosphatase activity and organic P in wheat and clover rhizosphere soil. In our study, the activity of PD was significantly and negatively correlated with organic P, indicating that higher PD activity played a part in the process of organic P hydrolysis to some extent. ACP activity was significantly and positively correlated with soil organic P. The correlations between phosphatase activities and organic P suggest that the bioavailability of phosphate diesters was higher than phosphate monoesters in this test soil.

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