Soil phosphorus changes in a grey desert soil in Xinjiang, China, using $^{31}$P nuclear magnetic resonance spectroscopy

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Abstract. This study investigated the effects of different long-term fertilization regimes on the total phosphorus (TP) content and the phosphorus (P) composition of grey desert soil in Xinjiang, China, and clarified the variation in P content under different long-term fertilization regimes. Solution $^{31}$P nuclear magnetic resonance spectroscopy ($^{31}$P NMR) was used to analyze the variation in TP and P composition under different long-term fertilization regimes. The results showed that phosphate monoester was the major form of P and that no other forms of soil organic P (OP) were detected in grey desert soil, Xinjiang, China.

1. Introduction

$^{31}$P NMR ($^{31}$P nuclear magnetic resonance spectroscopy) has been widely used to determine the phosphorus (P) forms in soil samples since the early 1980s [1-5]. Compared with traditional sequential extractions [6,7], $^{31}$P NMR is able to determine the P forms in soil. Due to this advantage, the technique is widely used. A long-term experiment was carried out in a grey desert soil at the monitoring station in Xinjiang, China. The soil samples in the experiments were used to study organic P (OP) composition in a grey desert soil under different long-term fertilization regimes by solution $^{31}$P-NMR. This study evaluated the effects of long-term fertilization (including organic and inorganic fertilizers) on total OP
and its composition content, and provides the basis for a reasonable fertilization regime combined with P application in grey desert soil in China.

2. Materials and methods

2.1. Experimental site conditions

The experiment site of the grey desert soil is a part of “the National Long-term Monitoring Network of Soil Fertility and Fertilizer Effects Program”, which was established in 1989 to investigate the effects of soil fertility, crop rotation, climate, and the application of organic and inorganic fertilizers on crop yield in the major agricultural areas of China. The experiment site (43°58′23″N, 87°25′58″E) is located on the outskirts of Urumqi, Xinjiang, China, which has a typical continental arid climate. Its climate characteristics are as follows: the effective accumulated temperature, altitude, frost-free period, precipitation, average annual temperature and sunshine hours of the location are 1734°C, 600 m, 156 days, 242 mm, 7.6°C and 2454 hours, respectively. Grey desert soil is Chinese classification system (Calcaric Cambisol by FAO) [8,9]. The experimental plot covers 468 m² and all the experimental plots were separated by 1.2 m deep cement slabs. The main soil properties of the 0–20 cm soil layer prior to the experiment were soil organic matter 8.8 g·kg⁻¹, pH (soil : water = 1 : 2.5) 8.1, total N (Nitrogen) 0.87 g·kg⁻¹, total P (Phosphorus) 0.67 g·kg⁻¹, alkaline hydrolysable N (available N) 55.2 mg·kg⁻¹, Olsen-P (available P) 3.9 mg·kg⁻¹, total K (Potassium) 23.0 g·kg⁻¹ and NH₄OAc–K (available K) 288.0 mg·kg⁻¹. For decrease the experimental field soil variability prior to the long-term fertilization experiments in 1990, a local wheat was grown for two years (1988–1989 year) without fertilizer. From 1990, wheat maize or cotton in rotation was planted each year at the local experimental site.

2.2. Experimental design

This experiment uses a randomized complete block design. The treatments were: (1) CK (unfertilized), (2) NPK (nitrogen, phosphorus and potassium), (3) NPKM (NPK and manure) and (4) NPKS (NPK and crop straw returned to the field). The NPK fertilizer application rates are listed in Table 1. The 60% of the N, all the P and K fertilizers as basal fertilizers were applied prior to seeding. In crop growing season, the remaining N fertilizer was applied as a top dressing by drip irrigation.

Table 1. Nutrient input between 1990 and 2012.

| Treatments | Inorganic fertilizer (kg·ha⁻¹) | Organic fertilizer (kg·ha⁻¹) |
|------------|-------------------------------|-----------------------------|
|            | N    | P      | K      | Manure (M) | Straw (S)   |
| CK         | 0.00 | 0.00   | 0.00   | 0.00       | 0.00        |
| NPK        | 99.40–241.50 | 29.20–60.20 | 18.80–50.40 | 0.00       | 0.00        |
| NPKM       | 29.80–84.90 | 8.70–22.40 | 6.70–10.10 | 30 000     | 0.00        |
| NPKS       | 89.40–216.70 | 24.50–50.80 | 16.90–42.30 | 0.00       | 4 500–9 000 |

Notes: CK means no fertilizer applied to the crops. The application rates for N, P and K were 99.40 kg·ha⁻¹, 29.20 kg·ha⁻¹ and 18.80 kg·ha⁻¹, respectively, for 1990–1994, and 241.50 kg·ha⁻¹, 60.20 kg·ha⁻¹ and 50.40 kg·ha⁻¹ between 1995 and 2011 in NPK treatment. In NPKS treatment, the contribution of straw to soil nutrients was calculated by using 10% of the current year's utilization rate. In NPKM treatment, organic manure is sheep
manure, and its nutrient contribution is calculated according to the 15% utilization rate of organic manure in that year. The nutrient utilization rate of the two treatments was the same as that of NPK treatment.

Except in NPKS, all harvested crop biomass was removed from the plots, no crop or with little residues returned to the field. The long-term location monitoring experiment was conducted from 1990 to 2012. Maize, wheat and cotton were be planted in long-term monitoring experimental plot, but only one crop was grown per year. Spring wheat and cotton were planted in 2002 and 2012 year, respectively.

2.3. Test items and methods

2.3.1. NMR analysis. Pretreatment method for the soil samples: A 3.00 g soil sample was filtered through a 2 mm sieve and then placed in a 100 mL centrifuge tube. The sample was extracted with 0.05 mol·L$^{-1}$ Na$_2$EDTA (sodium ethylene diamine tetra acetic acid) and 0.25 mol·L$^{-1}$ NaOH using a sediment: solution ratio of 1:20 (w: v). The extraction procedure involved shaking the sample for 16 h at 20°C [1,10], and then centrifuging (20°C, 10 000 g, 20 min) and filtering the supernatant with a 0.45 μm sieve before removing the supernatant. The 15 mL supernatant was immediately frozen and dried. The lyophilized extract was re-dissolved in 1 mL of 0.25 mol·L$^{-1}$ NaOH and centrifuged (4 °C, 5 min, 10 000 g). The 0.6 mL supernatant was then mixed with 0.05 mL D$_2$O (heavy water) and transferred to a 5 mm NMR tube for $^{31}$P NMR analysis. The TP (total soil P) content of the soil was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) after 5 mL of the sieved supernatants were digested in a H$_2$SO$_4$–HClO$_4$ mixture. The chemical shifts were recorded relative to an 85% H$_3$PO$_4$ standard (δ = 0 ppm) [4, 11-13]. $^{31}$P-NMR analysis was undertaken by the Analytical Instrumentation Center, Peking University, Beijing, China, which used a Bruker AVANCE III 500 MHz NMR (Switzerland).

2.3.2. Conventional analysis. Total NPK, available NPK, soil organic matter, pH, soil bulk density and cation exchange capacity (CEC) were determined and analyzed using conventional agricultural analysis methods [14].

2.4. Data analysis

The test data were analyzed by SAS 9.1 and Excel 2010 software, and the LSD (least significant difference) method was used to compare the data.

3. Results and Analysis

3.1. NMR characteristics of organic phosphorus (OP) in grey desert soil

The $^{31}$P NMR spectra show the effects of different fertilization times and fertilizer treatments on the OP of the grey desert soil under the long-term fertilization experiment (Figure 1) in Xinjiang, China. Compared to the results reported by Andrea (2012) [15], Margarita (2004) [16] and Turner (2003) [11,12], the chemical shifts (X axis) at around 6.44 and 6.38 PPM were the characteristic peaks for inorganic orthophosphate and the phosphate monoesters, respectively. The spectra showed in Figure 1
indicate that the phosphate monoesters were the main form of soil OP and other forms of OP were not found in CK, NPKS, NPKM and NPK treatments.

![NMR characteristics of soil OP in different treatments and years](image)

**Figure 1.** NMR characteristics of soil OP in different treatments and years.

Note: The X-axis is the chemical shift (PPM). The size of the area enclosed by the NMR spectra and the X-axis indicates the content of the corresponding P molecules. The dotted line is the boundary between inorganic orthophosphate and the phosphate monoester. The numbers on the right side of the dotted line represent the ratio of the phosphate monoester to inorganic orthophosphate.

The relative contents of the phosphate monoester varied across different treatments and years (Figure 1). The relative content of the phosphate monoester increased, but then decreased in CK treatment. It gradually reduced in NPK, whereas it decreased first and then increased in NPKS and NPKM treatments. To a certain extent, the change trends for phosphate monoester relative contents indicated that it gradually decreased in CK and NPK because there was no external supplementation with organic materials. In contrast, the relative contents of the phosphate monoester decreased at first, but then increased again in NPKM and NPKS when organic matter was gradually added.

The absolute contents of soil OP and inorganic phosphorus (IOP) can be obtained by calculating the ratio of the P compositions in NMR spectra. The soil P compositions after different fertilization times (1989, 2002 and 2012 years) and treatments were quantified and analyzed in order to further study the impact of different long-term fertilization regimes on the accumulation of various P compositions in grey desert soil across Xinjiang, China.
3.2 P content variations in grey desert soil

The results of a 23-year monitoring experiment showed that TP, OP and IOP contents in different treatments (CK, NPK, NPKM, NPKS) all changed significantly in grey desert soil under long-term fertilization (Figure 2 and Table 2).

![Figure 2. Total soil phosphorus (TP) and its fractions changed with time in different treatments.](image)

Note: The upper and lower case letters above the column show significant differences at $P < 0.01$ and $P < 0.05$, respectively. Soil IOP and OP were inorganic orthophosphate and orthophosphate monoesters, respectively, which were measured by $^{31}$P-NMR.

| Treatments | Total phosphorus (g kg$^{-1}$) | Inorganic orthophosphate (mg kg$^{-1}$) | Orthophosphate monoesters (mg kg$^{-1}$) |
|------------|-------------------------------|---------------------------------------|----------------------------------------|
|            | 2002                          | 2012                                  | 2002                                   | 2012   | 2002                         | 2012                         |
| IS         | 0.063±0.004D                  | 0.063±0.004C                          | 48.34±3.03E                            | 48.34±3.03D | 14.50±0.91D                  | 14.50±0.91C                  |
| CK         | 0.089±0.003C                  | 0.077±0.010C                          | 66.10±2.22D                            | 62.50±8.19D | 23.14±0.78C                  | 14.38±1.88C                  |
| NPK        | 0.164±0.008A                  | 0.241±0.022A                          | 130.96±6.34A                           | 207.70±19.28A | 32.74±1.58A                  | 33.23±3.08B                  |
| NPKM       | 0.138±0.003B                  | 0.238±0.009A                          | 109.22±2.75B                           | 172.54±6.65B | 28.40±0.72B                  | 65.57±2.53A                  |
| NPKS       | 0.089±0.002C                  | 0.180±0.015B                          | 77.94±1.66C                            | 147.14±12.70C | 10.91±0.23E                  | 32.37±2.79B                  |

Note: “IS” is the initial sample in 1989. Different capital letters after the number indicate that they are significantly different at $P < 0.01$. 

Table 2. Soil phosphorus contents for different years and treatments.
3.2.1 *Total P (TP) content variations in grey desert soil.* Although the soil TP contents cannot directly reflect the soil P supply capacity, a low content indicates that the soil is incapable of supplying sufficient P for plant growth [17]. Therefore, the TP content can be used as a potential indicator of soil P fertility. In general, the TP content in farmland does not change greatly over the short term. However, the results of a 23-year old long-term located experiment (Figure 2) show that the TP contents in grey desert soil changed significantly under the different fertilizer regimes during the monitoring period ($P < 0.01$). The TP content increased significantly along the years after fertilization ($P < 0.01$) (Figure 2). The TP contents in NPK, NPKM and NPKS fertilization treatments after 13 (2002 year) and 23 (2012 year) years increased by 2.60 and 3.82 times, 2.18 and 3.78 times, and 1.41 and 2.85 times, respectively. And it compared with CK, the values increased by 1.84 and 3.13 times, and 1.55 and 3.09 times, and 1.00 and 2.33 times. The TP content in CK increased at first, but then decreased. After 13 years, the TP content in CK significantly increased by 1.42 times ($P < 0.05$). Over 23 years (2012 year), the TP content in CK decreased at first, but then increased by 1.22 times (Table 2) compared to the relative basal values in 1989.

3.2.2 *IOP content variations in grey desert soil.* Generally speaking, the soil IOP compounds are all orthophosphates [18] and the IOP concentration accounts for 50%–80% of the soil TP [17]. The P concentration level is directly related to the soil P supplying capacity, and the IOP concentration was significantly affected by fertilization. The results showed that the concentrations of IOP in grey desert soil after the different fertilization treatments changed significantly during the 23 years ($P < 0.01$). Figure 2 shows that the concentrations of IOP in grey desert soil treated with fertilizers increased significantly year by year ($P < 0.01$). However, the concentration of IOP in CK, which received no additional fertilizer, significantly increased at first ($P < 0.05$), but then slightly decreased ($P > 0.05$). After 13 (2002 year) and 23 (2012 year) years, the relative basal values for IOP in NPK, NPKM and NPKS treatments had increased by 2.71 and 4.30 times, 2.26 and 3.57 times, and 1.61 and 3.04 times, respectively. The three treatments increased P after 13 and 23 years by 1.98 and 3.32 times, 1.65 and 2.76 times, and 1.18 and 2.35 times, respectively, compared to CK. The IOP concentration in CK significantly increased at first by 1.37 times (2002 year) ($P < 0.05$), but then the concentration decreased by 1.29 times compared to the relative basal values (2012 year) (Table 2).

3.2.3 *OP content variations in grey desert soil.* The soil OP content generally accounts for 20%–50% of the soil TP [18]. The OP content and variation have an important effect on the P supplying capacity of a soil and P absorption efficiency of plants. The long-term (23 year) fertilization treatment had a considerable impact on OP in grey desert soil (Figure 2). The OP in NPKM treatment significantly increased each year ($P < 0.01$), and the change trend for OP in grey desert soil after NPKS treatment showed that it remained constant at first (2002 year) ($P > 0.05$), but then increased significantly (2012 year) ($P < 0.01$). The results for the NPK treatment were the opposite. However, the results for CK without fertilization showed that there was at first a significant increase (2002 year) ($P < 0.01$) followed by a significant decrease in OP (2012 year) ($P < 0.01$). The OP content in NPKM after 13 (2002 year) and 23 (2012 year) years increased by 1.96 and 4.52 times, respectively; the OP content in NPKS after
23 years increased by 2.23 times and in NPK after 13 years increased by 2.26 times, but remained almost constant over the following 10 years. The OP content in CK significantly increased by 1.6 times after 13 years, but then decreased significantly over the following 10 years. However, the OP content changes in CK were not significantly different to the base values (Figure 2, Table 2).

3.3 Variation in relative OP contents in grey desert soil

With the increase number of years, the relative soil OP content in CK significantly increased, but then significantly decreased (Figure 3). It decreased significantly year by year in NPK treatment. In NPKM and NPKS treatments, the relative contents of the soil OP decreased significantly, but then significantly increased. The relative OP content change trend in each treatment was consistent with the absolute OP content (Figure 1 and Figure 3). In NPK and CK treatments (Figure 3 left), the relative OP content decreased extremely significantly, but it increased significantly in NPKM and NPKS as the years increased. In 2002, the relative soil OP content was significantly highest in CK (Figure 3 right), but was lowest in NPKS treatment ($P < 0.01$). The relative content values for OP in NPK and NPKM treatments were between CK and NPKS, but the difference between them was not significant. The relative content in CK significantly increased because the OP content was significantly higher. In contrast, the significant increase in TP content meant that the relative OP content significantly decreased in NPKS, NPKM and NPK treatments.

In 2012, the relative OP content significantly increased in NPKM, but significantly decreased in CK, NPK and NPKS treatments. The main reason is that the OP content in NPKM significantly increased, and the ratio of OP to TP was greater than in CK, NPK and NPKS. Soil OP content was basically constant, but the TP content significantly increased in NPK, which meant that the relative OP contents in NPK and CK treatments significantly decreased. In 2012, compared with 2002, the relative OP content was significantly higher in NPKM and lower in NPK compared with other treatments. The relative contents of CK and NPKS treatments were between NPKM and NPKS, but there was no significant difference between CK and NPKS.

4. Discussion
Under the different long-term fertilization conditions in grey desert soil, Xinjiang, China, the phosphate monoester was the main form of OP, and no other forms of OP were detected, which may be due to the low soil organic P content. In this study, the content of total P and its composition in non-fertilization treatment increased from 1989 to 2002, and gradually decreased from 2002 to 2012. Maybe, the mainly reason was that P supplementation of crop stubble and other organics in early stage, but it gradually declined over time as crop production goes down.

In the same way, the IOP and OP contents in NPKS were lower than in NPKM and NPK treatments, which was the main reason why the TP content in NPKS was significantly lower than in NPK and NPKM. The IOP and OP contents in NPKM were between NPK and NPKS, which also meant that the TP content of NPKM was between the NPK and NPKS treatments.

In 2012, the OP content in NPKM was significantly higher than in NPKS and NPK, but there was not significantly difference between NPKS and NPK. This was mainly because the TP in NPK and NPKM were not significantly different, but were very significantly greater than in NPKS. The TP content in CK decreased, mainly due to the decreases in IOP and OP. The OP content significantly decreased, which had a great effect on TP content. However, the TP contents in NPKS, NPKM and NPK significantly increased. The increases in TP contents in NPK and NPKS were mainly due to the significant increases in IOP and OP contents. In particular, IOP significantly increased in NPK. This showed that over the long term monitoring experiment, applying fertilizer and non-fertilizer application regime had a considerable impact on the OP contents in the soil.

5. Conclusion
The $^{31}$P NMR spectra showed that the phosphate monoester was the only form of soil organic phosphorus composition under the different fertilization treatments. The phosphate monoester organic phosphorus form in grey desert soil was not affected by long-term fertilization and the fertilizer regimes. Under long-term organic fertilization, the organic phosphorus content in grey desert soil treated with fertilizers significantly increased, and NPKS (nitrogen, phosphorus, potassium and crop straw returned to the field), NPKM (nitrogen, phosphorus, potassium and manure) by 2.23 and 4.52 times, respectively, compared to non-fertilization.

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