Effects of the continuous use of organic manure and chemical fertilizer on soil inorganic phosphorus fractions in calcareous soil

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A 4-year field trial with three treatments and three types of annually rotated vegetables was conducted in calcareous soil in a greenhouse using a phosphorus (P) fractionation method based on the inorganic P fraction classification system described by Jiang-Gu. With the same nutrient input, vegetable yields and P uptake were more stable under the chemical fertilizer (CF) treatment than under the organic manure (OM) treatment, and the average utilization rate of P fertilizer (URP) values were 5.27% and 11.40% under the OM and CF treatments, respectively, over the 4 years. Compared with the values in 2009, the values of the inorganic P (Pi) fractionation, including Ca-P, Al-P and Fe-P, significantly increased over time by 310.89 mg·kg\(^{-1}\), 36.21 mg·kg\(^{-1}\), and 18.77 mg·kg\(^{-1}\), respectively, with OM treatment and by 86.92 mg·kg\(^{-1}\), 175.87 mg·kg\(^{-1}\), and 24.27 mg·kg\(^{-1}\) with CF treatment. These results suggest that 1) large amounts of P were released from Ca\(_2\)-P, Ca\(_8\)-P and Al-P and were taken up by vegetables in the calcareous soil, and 2) the excessive application of P fertilizer, especially OM, resulted in a substantial accumulation of Pi (Ca\(_2\)-P, Ca\(_8\)-P and Al-P), which increased the risk of pollution from organic farming diffusing into the surface water.

In China, the growing prices of chemical fertilizers (CFs) and the concerns about soil quality have triggered an interest in applying organic manure (OM) to restore fertility. Moreover, the use of CFs is prohibited in organic farming; thus, OM serves as an important resource not only to supply nutrients for plants but also to replenish the organic matter content of most cultivated soils. Evidence from many studies in similar fields worldwide\(^1,2\) clearly indicates the advantages of OM, which include reducing the use of CF, preventing non-point source pollution, improving soil quality and reducing the environment load. However, the disadvantages of OM compared with CF are also obvious, including lower fertilizer efficiency, lower crop yields and an imbalance of nitrogen (N), phosphate and potassium fertilizer (NPK). On average, the proportion of NPK content in OM is not suitable for plant growth, which results in N deficiency but phosphorus (P) accumulation.

Due to a slower nutrient release and the out-of-proportion NPK content, crop yields are lower under OM farming than under CF farming. To achieve high yields and a high economic output, the heavy dressing of OM has been adopted in China. As a result, excess fertilization under OM farming is frequently observed, especially in vegetable greenhouses. In many areas, the excessive application of fertilizer and manure has increased soil P levels\(^3,4\). It has also been established that only one-quarter to one-third of the P fertilizer is utilized, while large amounts of P accumulate in the soil\(^5\). Excessive P can lead to plant toxicity and the immobilization of trace metals in the soil, as well as ground and surface water pollution through runoff.

Compared with other nutrients, P is the least mobile and least available for plants in soil, especially in calcareous soil. Therefore, P was greatly affected by fertilization. P fractionation schemes have been used to evaluate...
the effects and relationship between the P fraction and the P that is available for plants. Therefore, it is important to understand the forms and characteristics of P in soil for developing long-term management strategies. The method developed by Chang and Jackson\(^6\) and Buehler et al.\(^7\) has been widely used to estimate the soil P fractionation in non-calcareous soil. Soil P in non-calcareous soil was divided into different fractions, including Ca-P, Al-P, Fe-P and occluded P (O-P); in calcareous soil, Ca-P was further divided into Ca\(_2\)-P, Ca\(_8\)-P and Ca\(_{10}\)-P according to the solubility and availability of P\(^{4,9}\) because the Ca-P content was relatively higher in calcareous than in non-calcareous soil.

The effects of CF or OM on P fractionation, inorganic P (Pi) transformation in soil and Pi availability and recovery by crops have been investigated in several studies. These studies have reported the accumulation of a large amount of Pi or organic P in the soil due to the continuous application of P fertilizer or OM for many years\(^{10-12}\), but most studies on calcareous soils have been related to wheat, rice or maize\(^{13,14}\). There have been few reports on soil P fractionation and Pi transformation in response to the fertilization of vegetables, especially greenhouse vegetables; therefore, the purpose of the current study was as follows: 1) to investigate the response of vegetable yields to different fertilization inputs, such as OM and CF; 2) to evaluate Pi availability with different fertilization inputs; and 3) to study the transformation of Pi fractions in calcareous soil under excess fertilizer application and vegetable uptake in the greenhouse.

Materials and Methods

Study site. The study site was located at the Dahong vegetable experimental station of Shanghai Academy of Agriculture Sciences, Pudong New District (31°11′27″N, 121°42′20″E), in eastern Shanghai, China. This area has a subtropical monsoon climate and an ocean coastline with an annual average precipitation of 1100 mm and an annual average temperature of 15.8 °C. The experimental station covers an area of 4.5 hm\(^2\). Vegetables have been cultivated for more than 10 years, and the majority are planted in greenhouses. The topsoil (0–30 cm) at the research site is vegetable garden soil and evolved from silt loam.

Experimental design. A comparative greenhouse experiment was conducted from 2009–2013 to examine vegetable yields, P fractionation and Pi availability in response to different fertilizer inputs to calcareous soil. Two fertilization modes with one CK treatment were included in the experiment: (1) OM, (2) CF, and (3) no fertilizer (CK). According to the organic cultivation mode in China, only OM was applied in the OM treatment, while only CF was used in the CF treatment. No fertilizer was applied on the CK plots during the period of the experiment. The three treatments were applied in one greenhouse, which was newly built and based on an open vegetable field in the experiment station. The topsoil contained organic matter, 22.67 g kg\(^{-1}\); bulk density, 1.32 g kg\(^{-1}\); total N, 1.85 g kg\(^{-1}\); alkali-hydrolysable N, 76.08 mg kg\(^{-1}\); available P, 55.33 mg kg\(^{-1}\); and available K, 148.34 mg kg\(^{-1}\); and the pH was 7.46 (water:soil ratio = 5:1). Each experimental treatment had three plots with an area of 30 × 2 = 60 m\(^2\) each, and the same farming practices were applied in each treatment.

Experimental procedure. Three types of vegetables were rotated successively each year of the experiment: carrot (Zheluo No. 1), cucumber (Hu No. 58) and purple cabbage (Xiaguang); all vegetables were provided by the Shanghai Horticulture Research Institute, China. Plantings were conducted four times for each type of vegetable from 2009–2013. Carrot was planted from March to June, cucumber was planted from July to November, and purple cabbage was planted from December to February. Vegetable cultivation and management were synchronous in all treatment plots. There was no fertilization in the CK treatment. OM was applied at 30,000 kg hm\(^{-2}\) for cucumber and 15,000 kg hm\(^{-2}\) for purple cabbage and carrot each year as a basal fertilizer in the OM treatment; only one type of OM was used during the experimental period, and it contained N at 11.5 mg kg\(^{-1}\); P\(_2\)O\(_5\) at 20.4 mg kg\(^{-1}\); and K\(_2\)O at 15.1 mg kg\(^{-1}\). However, NPK was added in the form of urea, calcium superphosphate and potassium chloride at the rate of 46% N, 16% P\(_2\)O\(_5\) and 50% K\(_2\)O in the CF treatment. P and K fertilizers were all applied as basal fertilizers for each type of vegetable along with 50% N as the basal fertilizer and 50% as the top-dressing. The rate of each type of CF was determined according to the level of pure nutrients from the OM that was applied in the OM treatment (Table 1). Except for fertilization, all other aspects were equal in the different treatments.

Soil sampling and analysis. The initial soil sample was a composite consisting of 5 soil cores, which were collected in an “S” pattern from the 0–20 cm topsoil layer in each greenhouse with a soil corer (5 cm in diameter) after the rice harvest, and after vegetable cultivation, soil samples were collected annually in the same way in November after the carrot harvest. Each composite sample was mixed and air dried for 10 days after the crop roots and pebbles were discarded. All the samples were passed through 2-mm and 0.149-mm sieves to analyse the various indicators.

Table 1. Varieties and amounts of fertilizer each year under the different treatments.
The soil organic matter was measured by wet oxidation. The soil pH was measured with a glass electrode after mixing the soil with water (1:5 wt/vol soil:water), and the soil bulk density was determined by pushing a 100 cm³ stainless steel cylinder into the soil vertically and oven drying the soil samples to a constant weight at 105 ± 2 °C (normally within 24 h). The total N was determined by a micro-Kjeldahl digestion method and the total P was digested with H₂SO₄ and HClO₄ and measured using molybdenum blue spectrophotometry. Alkali-hydrolysable N was measured using an alkali solution diffusion method; available P was measured via the Olsen-P method; and the available K was determined using atomic absorption spectrophotometry.

The P fractionation method was based on the Pi fraction classification systems described by Jiang and Gu. Six Pi fractions (Ca₂-P, Ca₈-P, Al-P, Fe-P, O-P and Ca₁₀-P) were extracted from the soil samples using a sequential extraction procedure. Ca₂-P was removed using a sodium bicarbonate solution (c (NaHCO₃) = 0.25 mol/L, pH 7.5); (2) Ca₈-P was removed using an ammonium acetate solution (c (CH₃COONH₄) = 0.5 mol/L, pH 4.2); (3) Al-P was removed using an ammonium fluoride solution (c (NH₄F) = 0.5 mol/L, pH 8.2); (4) Fe-P was removed using 0.1 M NaOH and 0.1 M Na₂CO₃; (5) O-P was removed using a sodium citrate solution (c (Na₃C₆H₅O₇·2H₂O) = 0.3 mol/L); and (6) Ca₁₀-P was removed using a sulphuric acid solution (c (1/2H₂SO₄) = 0.5 mol/L).

Plant sampling and analysis. The vegetables were harvested after physiological maturity, and the yields were recorded. The plant samples were collected, dried and digested with H₂SO₄ and H₂O₂, and the P concentration in the digest was determined colourimetrically using the vanado-molybdate-yellow colour method. The dry-matter yields and P concentration were used to calculate the total P uptake by crops, the utilization rate of P fertilizer (URP) and the agronomic efficiency of P (AEP).

The URP under the OM and CF treatments was calculated as follows:

\[ \text{URP} = 100 \times \frac{P_{1} - P_{0}}{P_{f}} \]

where URP is the utilization rate of P fertilizer; P₁ is the P uptake under the OM or CF treatment; P₀ is the P uptake under the CK treatment; and Pᵢ is the amount of P fertilizer applied.

The AEP under the OM and CF treatments was calculated as follows:

\[ \text{AEP} = \frac{Y_{f} - Y_{0}}{P_{1}} \]

where AEP is the agronomic efficiency of P; Yᵢ is the crop yield under the OM or CF treatment; Y₀ is the crop yield under the CK treatment; and Pᵢ is the amount of P fertilizer applied.

Statistical analysis. All the experiments were repeated in triplicate, and the mean value was accepted as the final value. The data were processed using Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, USA) and then subjected to analysis of variance (ANOVA) using the SPSS 17.0 Statistical Package (SPSS Inc., Chicago, IL, USA). Multiple comparisons of the various treatments were conducted using Tukey’s method. The graphics were produced using Origin 8.0 (OriginLab Corp., Northampton, MA, USA).

Results

Vegetable yields in response to fertilization. Different fertilization modes led to regular changes in vegetable yields under the different treatments (Fig. 1). For the CF treatment, the vegetable yields remained at a relatively stable level for the first three years but began to decrease for purple cabbage in 2011 and for cucumber and carrot in 2012. Under the OM treatment, the yields of any one type of vegetable in 2013 were dramatically lower than that in 2010. However, among the 4 years, the trend of the yields for purple cabbage and cucumber decreased, while carrot yields were the highest in 2011 and gradually decreased later. Under CK treatment, the yields of all three types of vegetables rapidly decreased with increasing time during the experiment, but the decreasing trend slowed. There was a significant difference in the yields of vegetables among the three treatments.
and the yields of vegetables under the CF and OM treatments were significantly higher than under the CK treatment. After four years of cultivation, the average yields of carrot, cucumber and purple cabbage under the CF treatment improved by 89%, 50% and 193%, respectively, compared with those under the OM treatment.

Change in the total P and Olsen-P. Table 2 shows the change in Olsen-P and total P affected by different fertilization modes in the surface soil layer (0–20 cm) over time. Olsen-P had a similar concentration value, and there was no significant divergence among the treatments in 2009 before the experiment; however, the different fertilization modes strongly affected Olsen-P accumulation in the 0–20 cm soil layer. Compared with the value in 2009, the concentration of Olsen-P increased by 56.56% and 30.85% under OM and CF treatments, respectively, in 2011. The data indicated a marked accumulation of Olsen-P in the soil with the current P fertilizer amount, without an application of P fertilizer. Therefore, in the comparison of the different treatments, there was a significant difference (P < 0.05) among the OM, CF and CK treatments from 2011 and (P < 0.01) 2012. In addition, the soil total P concentrations at the 0–20 cm depth increased under the OM and CF treatments by 12.11% and 5.68%, respectively, over the 4 years of fertilization. The trend was due to the plant absorption of P in soil and the yields of vegetables under the CF and OM treatments were significantly higher than under the CK treatment.

Partial balance of P. As shown in Table 3, P input mainly occurred from fertilization, and other sources of P, including rainfall and irrigation, were neglected. Additionally, P output was only evaluated as plant uptake without considering the small leaching losses in the greenhouse, so the P input was far greater than its output under both treatments. The partial P balance budget indicated a net gain of approximately 491.26 kg·hm⁻² and 458.52 kg·hm⁻² per year under the OM and CF treatments, respectively, over the 4 years of fertilization. The amount of plant P uptake was greater under CF than OM because the vegetable yields were much higher under CF (Fig. 1). However, there was an obvious depletion of P under the CK treatment, and the amount of plant P uptake was much lower than in the fertilized control. Under the OM and CF treatments, the average URP values were 5.27% and 11.40% for 4 years, respectively. The URP is often low and rarely exceeds 20% for P in agrosystems. This condition was also observed in the present study, but the value of OM was much less than that of

Table 2. Change in total P and Olsen-P affected by different fertilization modes in the surface soil layer (0–20 cm) over time. Note: The different lowercase and uppercase letters in the same column for each parameter indicate a significant difference at the 0.05 and 0.01 levels, respectively.

| Year | Treatments | P input (kg·hm⁻²) | Plant P uptake (kg·hm⁻²) | P balance (kg·hm⁻²) | URP (%) | AEP (kg·hm⁻²) |
|------|------------|-------------------|-------------------------|---------------------|---------|-------------|
| 2010 | OM         | 534.42            | 51.00 ± 6.57B           | 483.42 ± 38.42A     | 4.64 ± 1.45B | 50.06 ± 6.48B |
|      | CF         | 534.42            | 75.83 ± 5.42A           | 458.59 ± 42.56B     | 9.29 ± 3.26A | 112.15 ± 13.24A |
|      | CK         | 0                 | 26.18 ± 3.18C           | 26.18 ± 5.44C       | —       | —           |
| 2011 | OM         | 534.42            | 45.12 ± 3.67B           | 489.30 ± 41.68A     | 5.06 ± 1.87B | 59.42 ± 5.73B |
|      | CF         | 534.42            | 76.64 ± 12.15A          | 457.78 ± 46.75B     | 10.95 ± 4.21A | 131.04 ± 15.25A |
|      | CK         | 0                 | 18.10 ± 1.14C           | 18.10 ± 3.54C       | —       | —           |
| 2012 | OM         | 534.42            | 40.10 ± 4.55B           | 494.32 ± 58.47A     | 5.75 ± 2.14B | 65.95 ± 6.14B |
|      | CF         | 534.42            | 77.80 ± 6.89A           | 456.62 ± 32.48B     | 12.81 ± 4.35A | 150.47 ± 16.28A |
|      | CK         | 0                 | 9.36 ± 1.42C            | 9.36 ± 2.56C        | —       | —           |
| 2013 | OM         | 534.42            | 36.42 ± 5.47B           | 498.00 ± 62.14A     | 5.64 ± 3.37B | 63.07 ± 4.79B |
|      | CF         | 534.42            | 73.34 ± 6.64A           | 461.08 ± 35.41B     | 12.55 ± 4.56A | 147.95 ± 15.33A |
|      | CK         | 0                 | 6.26 ± 0.89C            | 6.26 ± 3.42C        | —       | —           |
Table 4. Changes in the concentration of inorganic Pi in the surface soil layer (0–20 cm) as affected by different fertilization modes (mg·kg$^{-1}$). Note: The different lowercase and uppercase letters in the same column for each year indicate a significant difference at the 0.05 and 0.01 levels, respectively.

| Time | Fertilization mode | Ca$_2$-P | Ca$_8$-P | Al-P | Fe-P | O-P | Ca$_{10}$-P | Sum of the fractions |
|------|-------------------|----------|----------|------|------|-----|------------|---------------------|
| 2009 | OM                | 55.84±8.34aA | 68.42±12.46aA | 162.24±25.57aA | 183.43±18.33aA | 472.46±38.42aA | 51.65±6.28aA | 994.04aA |
|      | CF                | 56.42±10.57aA | 67.75±12.58aA | 161.53±32.14aA | 178.16±23.49aA | 467.56±41.64aA | 51.86±7.46aA | 983.28aA |
|      | CK                | 58.06±5.10aA | 65.01±7.33aA | 157.9±16.82aA | 172.2±15.24aA | 468.65±32.16aA | 51.43±3.15aA | 978.29aA |
| 2013 | OM                | 140.99±21.38aA | 293.36±35.24aA | 196.77±36.22aA | 198.38±21.48aA | 475.01±42.10aAB | 52.02±15.34aA | 1356.53aA |
|      | CF                | 102.4±20.84aB | 107.25±18.47bB | 333.77±75.41aA | 201.51±28.36aA | 521.87±51.27aA | 51.77±17.28aA | 1318.57aA |
|      | CK                | 16.37±2.41bC | 31.93±9.37cC | 127.15±20.54cC | 178.96±15.47bA | 455.88±23.10bB | 51.49±8.47aA | 861.78bB |

Soil inorganic phosphorus fractions. Soil Pi fractionation was conducted on the calcareous soils$^8, 15$, and the results are listed in Table 4. The Pi concentration was exactly the same among the treatments in 2009, and the dominant Pi fraction was O-P (46.08–48.99%), followed by Fe-P (17.54–19.37%), Ca-P (17.04–18.58%) and Al-P (15.17–17.11%). For Ca-P, the fractions of Ca$_2$-P accounted for 31.25–34.21%, Ca$_8$-P for 36.73–39.90%, and the dominant Pi fraction was O-P (46.08–48.99%), followed by Fe-P (17.54–19.37%), Ca-P (17.04–18.58%) and Al-P (15.17–17.11%). In addition, the order of the 6 fractions by concentration was O-P > Al-P > Fe-P > Ca$_2$-P > Ca$_8$-P > Ca$_{10}$-P.

A significant change occurred for inorganic P fractions under the treatments with different fertilization modes after the course of the four-year experiment. The concentrations of Ca$_2$-P, Al-P and Fe-P significantly increased under both OM and CF treatments by 310.89 mg·kg$^{-1}$, 36.21 mg·kg$^{-1}$, and 18.77 mg·kg$^{-1}$ in 2013 compared with 2009 under the OM treatment and 86.92 mg·kg$^{-1}$, 175.87 mg·kg$^{-1}$, and 24.27 mg·kg$^{-1}$ under the CF treatment, respectively. Significant differences were observed in the concentrations of Ca$_2$-P, Ca$_8$-P, and Al-P (P < 0.01) between the OM and CF treatments. The concentrations of Ca$_2$-P and Ca$_8$-P under the OM treatment were significantly higher than those under the CF; whereas Al-P and Fe-P were lower in the OM. In contrast, the concentrations of Ca$_2$-P, Ca$_8$-P, and Al-P decreased under the CK treatment with values significantly lower (P < 0.01) than those under both the OM and CF treatments in 2013. However, Fe-P, O-P, and Ca$_{10}$-P remained at a relatively stable level.

With the increase in P, the total P increased significantly under both the OM and CF treatments due to gains of 491.26 kg·hm$^{-2}$ (average value) and 458.52 kg·hm$^{-2}$ per year, respectively. In contrast, the reduced partial P balance explained the depletion of total P under the CK treatment.

Discussion

The difference in the vegetable yields responding to fertilization under the different treatments emphasized the importance of studying the relationship between vegetable yields and soil nutrient supply with different fertilization modes in the vegetable greenhouse$^{16}$. Fertilizer application clearly increased the yield compared with that of the control; and OM was able to activate soil P$^{20, 21}$. The removal of P by crops caused the soil P depleted more available P under the CF treatment (N, not P, was the critical factor for increasing vegetable yields, than that under the CF treatment. Therefore, more P accumulated in the OM-treated soil. The high accumulation of P in the vegetable field not only could result in Fe, Ca, Mg and...
Zn becoming unavailable for vegetables but also might be one factor negatively affecting the water in the environment. The P balance under the CK treatment was negative in every growing season because there was no P fertilizer input. Excessive fertilization resulted in a low URP under both the OM and CF treatments, ranging from 4.64 to 5.75% and 9.29 to 12.81%, respectively. The URP under the CF treatment was roughly twice that under the OM treatment because the vegetable plants absorbed more P when fertilized with CF. The same trend appeared for the AEP as well, and the absorbed P that contributed to the vegetable yield can be determined effectively based on AEP.

P exists in soil in various forms and is available to plants at different times. Figure 2 presents changes in different P fractions over time under different treatments during the experimental period.

| Treatments | Coefficient of correlation |
|------------|----------------------------|
|            | Ca$_2$-P | Ca$_8$-P | Al-P  | Fe-P  | O-P  | Ca$_{10}$-P |
| OM         | 0.991**  | 0.999**  | 0.938** | 0.831* | 0.522 | 0.642       |
| CF         | 0.988**  | 0.995**  | 0.953** | 0.846* | 0.462 | 0.476       |
| CK         | 0.967**  | 0.959**  | 0.842*  | 0.511  | 0.357 | 0.122       |

Table 5. The correlation coefficient (r) between different phosphorus fractions and Olsen-P under different treatments. *P ≤ 0.05 and **P ≤ 0.01.
Table 6. Soil fertility parameters under the different treatments after a 4-year experiment. Note: OM = organic matter; BD = bulk density; TN = total nitrogen; TP = total phosphorus; AN = alkali-hydrolysable nitrogen; AP = available phosphorus; and AK = available potassium. The different lowercase and uppercase letters in the same column indicate a significant difference at the 0.05 and 0.01 levels, respectively.

| Treatment | OM g·kg⁻¹ | pH | BD g·cm⁻³ | TN mg·kg⁻¹ | TP mg·kg⁻¹ | AN mg·kg⁻¹ | AP mg·kg⁻¹ | AK mg·kg⁻¹ |
|-----------|------------|----|-----------|-------------|-------------|-------------|-------------|-------------|
| OM        | 28.420 ± 4.62aA | 7.12 ± 1.71aA | 1.03 ± 0.07bB | 1836.77 ± 112.43aA | 1468.04 ± 87.25aA | 78.48 ± 15.26bB | 86.04 ± 24.91aA | 247.70 ± 28.48aA |
| CF        | 20.358 ± 6.34bB | 6.75 ± 2.48aA | 1.41 ± 0.12aA | 1825.26 ± 89.34aA | 1392.56 ± 104.38bB | 110.60 ± 24.18aA | 72.40 ± 25.30bB | 213.27 ± 32.17bB |
| CK        | 21.375 ± 2.17bB | 7.03 ± 1.53aA | 1.32 ± 0.10aA | 1812.63 ± 65.27aA | 1317.70 ± 54.27cC | 23.42 ± 3.46cC | 19.53 ± 5.43cC | 66.58 ± 17.55cC |

There was a significant positive correlation (P ≤ 0.01) between Olsen-P and Ca₂-P, Ca₈-P or Al-P (P ≤ 0.05) and between Olsen-P and Fe-P under both the OM and CF treatments but not between O-P and Ca₁₀⁻P (Table 5). These results reflect the relation between the different Pi fractions and Olsen-P. As available P, Olsen-P had a higher correlation coefficient with Ca₂⁻P and Ca₈⁻P under the OM treatment than under the CF treatment but a lower correlation with Al-P and Fe-P. This observation suggests the different potentials of P release and availability from these fractions with different fertilization modes. However, based on previous studies, there was no significant positive relationship between Olsen-P and Ca₁₀⁻P. The difference may be due to a large input and surplus of P fertilizer in the current study. Under the CK treatment, Olsen-P also had a significant (P ≤ 0.01) positive correlation with Ca₈⁻P or Ca₆⁻P (P ≤ 0.05) and with Al-P which could indicate that these fractions provided available P for the vegetables in the experimental soil. Both Ca₂⁻P and Ca₈⁻P were readily used by plants because these Ca-P fractions released water-soluble and citrate-soluble P under the Pi fraction scheme. There was no significant correlation between Olsen-P and O-P or Ca₁₀⁻P under any of the treatments, suggesting that both P fractions had very low availability to the plants.

As shown in Table 6 and compared with data from 2009, the soil organic carbon content increased under the OM treatment and decreased under the CF and CK treatments in 2013, which suggests that the application of OM is necessary to improve the soil organic carbon supply in the vegetable greenhouse. Other studies reported no clear change in organic carbon in the soil of rice systems even without fertilizer application, which is mainly due to crop residues remaining in the fields. However, this trend was not observed for vegetable cultivation. OM application also contributed to the maintenance of the pH value so that the pH increased under the OM treatment after 4 years of cultivation. However, a decreasing pH was observed under the CF treatments due to the heavy application of inorganic N fertilizer. There was a significant change in nutrient availability under all treatments over time. The available N content decreased significantly in the CK treatment, in which no fertilizer was applied, whereas fertilizer increased the available N content under the OM and CF treatments. However, the concentration of available N under the CF treatment was much higher than that under the OM treatment, which was consistent with the trend of vegetable yields. The result suggested that N was an important limiting factor for vegetable growth, particularly under the OM treatments. The lack of available N is a common problem in organic cultivation in China and results in lower crop yields and more P surplus in the soil because there is a much lower N content than P content in OM.
and plant N uptake and N losses are also much greater than P losses in the soil. The available soil K significantly increased under the OM and CF treatments with fertilization and decreased under the CK treatment compared with the values from 2009, which suggested that excessive fertilization could give rise to the accumulation of available K regardless of the fertilization mode.

Conclusions

The excessive application of P fertilizer resulted in Olsen-P accumulation in the soil, regardless of the use of OM or CF, during the experimental period; however, vegetable yields were significantly lower with OM than with CF, which resulted in more P surplus in the soil under the OM mode. Ca₃P and Ca₅P were the predominant inorganic forms under OM application, whereas Al-P and Fe-P were greater under CF application. CF application is prohibited in organic farming. To increase vegetable yields, continuous annual application of OM has been regarded as the only effective strategy by farmers; therefore, excessive application of fertilizer is common in vegetable greenhouses in China. In fact, the practice not only has almost no effect on vegetable yields but also results in an increase in soil P levels, which increases the risk for the diffuse pollution of surface waters. Therefore, nutrient management in the greenhouse must be carefully considered when designing high-efficiency cropping systems with P utilization and a low environmental impact in organic farming.

References

1. Rasul, G. & Thapa, G. B. Sustainability of ecological and conventional agricultural systems in Bangladesh: An assessment based on environmental, economic and social perspectives. Agric. Syst. 79, 327–351, doi:10.1016/S0308-521X(03)00090-8 (2004).
2. Bengtsson, I., Ahnström, J. & Weibull, A. C. The effects of organic agriculture on biodiversity and abundance: A meta-analysis. J. Appl. Ecol. 42, 261–269, doi:10.1111/j.1365-2664.2005.01005.x (2005).
3. Kleinmann, P. J. A. et al. Managing agricultural phosphorus for water quality protection: Principles for progress. Plant Soil 349, 169–182, doi:10.1007/s11104-011-0832-9 (2011).
4. Leinweber, P., Turner, B. L. & Meissner, R. Phosphorus. (Wallingford, UK, 2002).
5. Whalen, J. K. & Chang, C. Phosphorus accumulation in cultivated soils from long-term annual applications of cattle feedlot manure. J. Environ. Qual. 30, 229–237, doi:10.2134/jeq2001.301229x (2001).
6. Chang, S. C. & Jackson, M. L. Fractionation of soil phosphorus. Soil Sci. 84, 133–144, doi:10.1007/10001694-195708000-00005 (1957).
7. Bechler, S., Oberson, A., Rao, I. M., Friesen, D. K. & Frossard, E. Sequential phosphorus extraction of a 32P-labeled oxisol under contrasting agricultural systems. Soil Sci. Soc. Am. J. 66, 688–877, doi:10.2136/sssaj2002.0860 (2002).
8. Gu, Y. C. & Jiang, B. F. The fraction method for determining soil inorganic P in calcareous soils. Chin. Soil Sci. 22, 101–102 (1990).
9. Gu, Y. C. & Qin, S. W. Phosphorus accumulation, transformation and availability under long-term application of phosphorus fertilizer. Chin. Soil Sci. 29, 13–17 (1997).
10. Lai, L., Hao, M. D. & Peng, L. F. The variation of soil phosphorus of long-term continuous cropping and management on Loess Plateau. Res. Soil Water Conserv. 10, 68–70 (2003).
11. Samadi, A. & Gilkes, R. J. Forms of phosphorus in virgin and fertilised calcareous soils of Western Australia. Soil Res. 36, 585–602, doi:10.1071/SR99066 (1998).
12. Hirata, H. et al. Effect of continuous application of farmyard manure and inorganic fertilizer for 9 years on changes in phosphorus compounds in plow layer of an upland Andosol. Soil Sci. Plant Nutr. 45, 577–590, doi:10.1080/030387769.199415821 (1999).
13. Liu, J. Transformation of fertilizer phosphorus in calcareous soil and its influencing factors. Dissertation, China Agriculture University (1999).
14. Yang, J. E. & Jacobsen, J. S. Soil inorganic phosphorus fractions and their uptake relationships in calcareous soils. Plant Nutr. Fert. Sci. 54, 1666–1669 (2000).
15. Jiang, B. F. & Gu, Y. C. A suggested fraction scheme of inorganic phosphorus in calcareous soils. Sci. Agric. Sinica 22, 58–66 (1989).
16. Damodor, R. D., Subba Rao, A. & Takkar, P. N. Effects of repeated manure and fertilizer phosphorus additions on soil phosphorus dynamics under a soyabean/white rotation. Biol. Fertil. Soils 28, 150–155, doi:10.1007/s00374-000-0477-9 (1999).
17. Lu, R. K. Analytical methods for soil and agro-chemistry (in Chinese). (Beijing, 2000).
18. Dobermann, A., Cassman, K. G., Cruz, P. C. S., Adviento, M. A. & Pampolino, M. F. Fertilizer inputs, nutrient balance, and soil nutrient-supplying power in intensive, irrigated rice systems. II: Effective soil K-supplying capacity. Nutr. Cycl. Agroecosyst. 46, 11–21, doi:10.1007/BF00210220 (1996).
19. Wang, J., Liu, W. Z., Mu, H. F. & Dang, T. H. Inorganic phosphorus fractions and phosphorus availability in a calcareous soil receiving 21-year superphosphate application. Pedosphere 20, 304–310, doi:10.1016/S0970-7060 (1998).
20. Samadi, A. & Gilkes, R. J. Phosphorus transformations and their relationships with calcareous soil properties of southern Western Australia. Soil Sci. Soc. Aust. 63, 809–815, doi:10.2136/sssaj1999.634809x (1999).
21. Wang, S. J., Chen, Y. & Li, S. Y. Balance of soil organic matter in a long-term triple cropping system in paddy fields. Acta Pedol Sinica 39, 9–15 (2002).
22. Fixen, P. E. & Grove, J. H. Testing Soils for Phosphorus. (Madison, Soil Science Society of America, Inc., 1990).
23. Selles, F., McConkey, B. G. & Campbell, C. A. Distribution and forms of P under cultivator- and zero-tillage for continuous- and fallow- wheat cropping systems in the semi-arid Canadian prairies. Soil Till. Res. 51, 47–59, doi:10.1016/S0167-1987(99)00027-6 (1999).
24. Bhattacharjee, A., Comerford, N. B. & Johnston, C. T. Influence of oxalate and soil organic matter on sorption and desorption of phosphate onto a spodic horizon. Soil Sci. Soc. Am. J. 62, 1089–1095, doi:10.2136/sssaj1998.03615995006200040033x (1998).
25. Sarada, J. L. et al. Changes in soil fertility parameters and the environmental effects in a rapidly developing region of China. Agric. Ecosyst. Environ. 129, 286–292, doi:10.1016/j.agee.2008.10.002 (2009).
26. Smeck, N. E. Phosphorus: An indicator of pedogenic weathering processes. Soil Sci. 115, 199–206, doi:10.1007/10001694-197503000-00005 (1989).
27. Seelig, B. & Jungk, A. Utilization of organic phosphorus in calcium chloride extracts of soil by barley plants and hydrolysis by acid and alkaline phosphatases. Plant Soil 178, 179–184, doi:10.1007/BF00011581 (1996).
28. Zhang, S., Li, P., Yang, X., Wang, Z. & Chen, X. Effects of tillage and plastic mulch on soil water, growth and yield of spring-sown maize. Soil Till. Res. 112, 92–97, doi:10.1016/j.still.2010.11.006 (2011).
29. Sharma, R. P. & Verma, T. S. Effect of long-term lantana addition on soil phosphorus fractions, crop yields and phosphorus uptake in rice–wheat cropping in north-west Himalayan acid Alfisols. Ind. Soil Sci. Soc. 48, 107–112 (2000).
30. Xiang, W. S., Huang, M. & Li, X. Y. Progress on fractioning of soil phosphorus and availability of various phosphorus fractions to crops in soil. Plant Nutr. Fert. Sci. 10, 663–670 (2004).
31. Song, C., Han, X. Z. & Tang, C. Changes in phosphorus fractions, sorption and release in Udic Mollisols under different ecosystems. Biol. Fert. Soils 44, 37–47, doi:10.1007/s00374-007-0176-z (2007).
32. Bronson, K. F., Cassman, K. G., Wassmann, R., Olk, D. C. & Garrity, D. P. Soil carbon dynamics in different cropping systems in principal ecoregions of Asia. (Boca Raton, New York, CRC Press, 1997).
33. Darilek, J. L. et al. Dairy effluent phosphorus sequestration of soils in Erath County, Texas. Soil Surv. Horizons 48, 51–56 (2007).

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Author Contributions
K. Song and J. Yang designed the study; K. Song, X. Zheng and H. Qiao performed the experiments; K. Song, Q. Qin, Y. Xue and W. Lv analysed the data and wrote the manuscript.

Additional Information
Competing Interests: The authors declare that they have no competing interests.

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