

Fate of low-molecular-weight organic phosphorus compounds in the Prich and P-poor paddy soils

LI Bao-zhen; Gunina, Anna; Zhran, Mostafa; Jones, Davey L.; Hill, Paul W.; HU Ya-jun; GE Ti-da; WU Jin-shui

JOURNAL OF INTEGRATIVE AGRICULTURE

DOI:

10.1016/S2095-3119(20)63310-X

Published: 01/09/2021

Publisher's PDF, also known as Version of record

Cyswllt i'r cyhoeddiad / Link to publication

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA): LÍ Bao-zhen, Gunina, Ă., Zhran, M., Jones, D. L., Hill, P. W., HU Yà-jun, GE Ti-da, & WU Jinshui (2021). Fate of low-molecular-weight organic phosphorus compounds in the P-rich and P-poor paddy soils. *JOURNAL OF INTEGRATIVE AGRICULTURE*, 20(9), 2526-2534. https://doi.org/10.1016/S2095-3119(20)63310-X

Hawliau Cyffredinol / General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
 - You may not further distribute the material or use it for any profit-making activity or commercial gain
 You may freely distribute the URL identifying the publication in the public portal?

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



Available online at www.sciencedirect.com

ScienceDirect



RESEARCH ARTICLE

Fate of low-molecular-weight organic phosphorus compounds in the P-rich and P-poor paddy soils

LI Bao-zhen¹, Anna GUNINA^{2, 3}, Mostafa ZHRAN^{1, 4}, Davey L. JONES⁵, Paul W. HILL⁵, HU Ya-jun¹, GE Tida¹, WU Jin-shui^{1, 6}

Abstract

Continuous application of organic fertilizers can cause accumulation of organic phosphorus (P) in soil, especially in the low-molecular-weight organic phosphorus (LMWOP) forms. This organic P pool represents a potentially important source of P for both plants and microorganisms. To understand the effect of long-term fertilization (30 years) (P-rich soil) vs. fallowing (P-poor soil) on the bioavailability and fate of LMWOP in subtropical paddy soils, we determined the sorption and mineralization of ¹⁴C-labeled adenosine, adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP) in each soil. The contents of carbon, nitrogen, and P in the P-rich soil were more than two times greater than those in the P-poor soil. The mineralization rates of the LMWOP compounds were faster in the P-rich soil compared to the P-poor soil, and followed the order AMP>ADP>ATP. Using sterilized soil, all forms of adenosine-P were strongly sorbed to the solid phase and reached saturation in a short time, with the adsorbance increasing with the number of phosphate groups. We concluded that the mineralization of LMWOP compounds was repressed slightly by sorption to the solid phase, but only in the short term. Thus, LMWOP compounds serve as readily available sources of C for microorganisms, making P available for themselves as well as for the plants. However, P accumulation and the progressive saturation of the P sorption sites in highly fertile soils may increase the potential risk of P runoff.

Keywords: rice paddy, phosphatase, phosphorus cycling, microbial community

Received 20 March, 2020 Accepted 23 June, 2020 LI Bao-zhen, Tel: +86-731-84619736; E-mail: bzli@isa.ac.cn; Correspondence WU Jin-shui, Tel: +86-731-84615224; Fax: +86-731-84612685, E-mail: jswu@isa.ac.cn

© 2021 CAAS. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). doi: 10.1016/S2095-3119(20)63310-X

1. Introduction

Phosphorus (P) is one of the most important nutrients for crops. The fate of the inorganic P pool, including its movement in the soil and its adsorption/desorption reaction, has been intensively studied (Kögel-Knabner *et al.* 2010; Yan *et al.* 2013, 2017). Organic forms of P comprise 20–80%

¹ Key Laboratory of Agro-ecological Processes in Subtropical Region/Changsha Research Station for Agricultural & Environmental Monitoring, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, P.R.China

² Department of Environmental Chemistry, University of Kassel, Nordbahnhofstrae 1a 37213, Witzenhausen, Germany

³ Department of Soil Biology and Biochemistry, Dokuchaev Soil Science Institute, Moscow 119017, Russian Federation

⁴ Soil and Water Research Department, Nuclear Research Center, Atomic Energy Authority, Abou-Zaabl 13759, Egypt

⁵ School of Natural Sciences, Bangor University, Bangor, Gwynedd LL57 2UW, UK

⁶ University of Chinese Academy of Sciences, Beijing 100049, P.R.China

of the total P in the soils, and they represent a potential source of P for both plants and microorganisms (Chen *et al.* 2003; Fransson and Jones 2007; Zhang *et al.* 2014; Arruda *et al.* 2018; Hu *et al.* 2018). Fertile soils are generally rich in organic-P fractions, which are labile or moderately labile, including compounds such as nucleic acids, phospholipids, inositol phosphates, phosphoproteins, and P-containing metabolic compounds, such as adenosine phosphates (Lee *et al.* 2004; Fransson and Jones 2007). These compounds may represent up to 50% of the organic P pool in soils (Turner *et al.* 2003; Vats *et al.* 2005; Li *et al.* 2016). Thus, in order to develop sustainable agricultural systems by improving P utilization, it is essential to understand the factors that regulate the dynamics of the major forms of labile organic-P in soil.

Cycling of organic-P in soil is largely regulated by the activity of plant roots and microorganisms (Chen et al. 2003; Parham 2014; Yokoyama et al. 2017; Wei et al. 2019a), which produce enzymes to mineralize P-containing organic compounds (e.g., phosphatases), as well as being sources of these compounds (e.g., in root and microbial turnover). For instance, ATP is one of the dominant forms of P in plant tissues (i.e., ATP contents of pea shoots and roots are 113 and 98 mmol g⁻¹; Smyth and Black 1984) and microbial cells (i.e., ATP content in bacterial cells is 0.6-4.2 mmol L-1; Yaginuma et al. 2014). ATP can be released from dead cells but is also excreted into the environment by Gram-positive and Gram-negative bacteria (Mempin et al. 2013) and by plant roots (from actively growing root regions where cell expansion is occurring; Kim et al. 2006; Ge et al. 2019). The role of the ATP released by living bacterial cells is still under discussion, but ATP is a possible source of nutrients or a signaling molecule for bacterial communities (Mempin et al. 2013). ATP contains not only C and P, but also N, therefore the products of its degradation can serve as sources of multiple nutrients for both microorganisms and plants.

The availability of P in the soil depends on a combination of environmental and biological factors, including: i) the amount and type of clay minerals, i.e., presence of gibbsite and goethite increases the sorption of inorganic P, making it unavailable for plants (Li et al. 2016); ii) oxygen content, i.e., frequent changes in soil moisture (pulse redox conditions) mobilizes labile forms of organic P (Yevdokimov et al. 2016; Gu et al. 2018; Wei et al. 2019b); and iii) pH conditions, i.e., the acidification of the rhizosphere by root exudates (i.e., by carboxylic acids) increases P availability (Bending 2017). The type of fertilizer applied also directly and indirectly affects soil microbial community composition and activity (Yao et al. 2016; Wei et al. 2017, 2019a; Yu et al. 2019), which in turn is responsible for P mineralization via the production of hydrolytic enzymes. However, it is still not clear what regulates the turnover of dissolved organic-P

(DOP) in rice paddy soils, because: i) The Eh and pH are frequently fluctuating (Yan et al. 2017), ii) the contents of Al and Fe are high, and iii) these soils are always fertilized with either organics, mineral fertilizers, or a combination of the two (Lan et al. 2012; Dong et al. 2014). For low-molecular-weight compounds (sugars, carboxylic and amino acids), it is known that microbial utilization overcomes sorption on the mineral matrix (Fischer et al. 2010; Gunina et al. 2014). However, it is not clear whether the same patterns could be observed for organic-P compounds present in soil solutions in paddy environments.

Thus, the present research hypothesized that the sorption and mineralization of organic P substrates in soils will be affected by: i) the number of phosphate groups within the organic-P compound, because this part of molecule can be sorbed to the mineral phase, and ii) soil fertility level (contents of total carbon (C), nitrogen (N) and P), because it directly affects microbial activity. To test these hypotheses, the base compound (adenosine) with either 1, 2, or 3 phosphate groups — namely adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP) (all labeled with ¹⁴C) — were obtained, and their fates in P-rich (fertilized) and P-poor (fallow) paddy soils were studied in a short-term laboratory experiment.

2. Materials and methods

2.1. Soil samples

Soil samples were collected from the Changsha Research Station for Agricultural and Environmental Monitoring (113°19′52′′E, 28°33′04′′N) in Jingjin County, Hunan Province, China. Two soil samples were collected from: i) fertilized soil, as the P-rich soil from a site that was cultivated for rice (Oryza sativa L.) and fertilized with a combination of pig manure and inorganic fertilizer that contained 120 kg N ha⁻¹, 40 kg P_2O_5 ha⁻¹, and 100 kg K_2O ha⁻¹ in every growing season, and ii) a P-poor (control) soil, which was under fallow for the same period of time. Soil samples were collected from a depth of 0-20 cm in four replicates, sieved (<2 mm), and stored dry prior to analysis. The soil was a typical Stagnic Anthrosol developed from granitic red parent material, and had 6.1% clay, 61.1% silt, and 32.8% sand for the control, and 5.5% clay, 50.6% silt, and 43.9% sand for the P-rich soil. The main characteristics of the soils are presented in Table 1. Importantly, the Olsen-P content was significantly higher in the P-rich than in the P-poor soil (42.45 vs. 3.87 mg kg⁻¹).

2.2. Chemical analyses

The pH and electrical conductivity (EC) were determined

Table 1 Chemical characteristics for the P-rich and P-poor paddy soils at 0-20 cm depth¹⁾

| Paddy soil | рН | EC | TN | SOC | Olsen-P | TP | DOC | MBC |
|------------|-----------|------------------------|-----------------------|-----------------------|------------------------|-----------------------|------------------------|------------------------|
| | | (µs cm ⁻¹) | (g kg ⁻¹) | (g kg ⁻¹) | (mg kg ⁻¹) | (g kg ⁻¹) | (mg kg ⁻¹) | (mg kg ⁻¹) |
| P-poor | 5.27±0.01 | 69.7±0.21 b | 1.48±0.01 b | 14.8±0.17 b | 3.87±0.02 b | 0.42±0.04 b | 79.9±1.74 b | 150.4±14.0 b |
| P-rich | 5.04±0.01 | 138.7±0.21 a | 2.44±0.08 a | 29.8±0.83 a | 42.5±1.34 a | 1.03±0.02 a | 118.3±6.16 a | 175.9±8.56 a |

¹⁾ EC, electrical conductivity; TN, total nitrogen; SOC, soil organic carbon; Olsen-P, available phosphorus; TP, total phosphorus; DOC, dissolved organic carbon; MBC, microbial biomass carbon.

with a standard electrode with a soil/water ratio of 1:2.5 (w/v). Soil moisture content was determined by drying at 105° C for 24 h, and total C and N were obtained by the dry combustion method (Vario MAX, Elementar Analysen System GmbH, Germany). Total P content in soil was measured by a molybdate blue method after the soil samples were digested in a mixture of nitric and perchloric acids. Olsen-P was extracted into 0.5 mol L⁻¹ NaHCO₃ (pH 8.5) solution by shaking at 205 r min⁻¹ for 30 min (Ding *et al.* 2012). Soil dissolved organic C (DOC) was extracted with K₂SO₄ (0.5 mol L⁻¹) and measured with a liquid-TOC analyzer (Phoenix-8000). Soil microbial biomass C (MBC) was determined by chloroform fumigation-extraction (Wu *et al.* 1990).

2.3. Organic-P mineralization

Five grams of dry soil was placed into a 50 mL centrifuge tube, hydrated to 50% of WHC, and later saturated to over 100% by placing a water table 1 cm above the soil surface, and pre-incubated for 2 weeks at 20°C. There were 20 tubes for each soil. After pre-incubation, 0.5 mL of a ¹⁴C-labeled solution (0.2 kBq mL⁻¹) of either [8-¹⁴C]adenosine (Sigma-Aldrich Corp, USA), [U-14C]-adenosine monophosphate (AMP; NEN-Dupont, USA), [8-14C]adenosine diphosphate (ADP; NEN-Dupont), or [8-14C]adenosine triphosphate (ATP; NEN-Dupont) were added to each soil type at five concentrations: 10, 50, 100, 500 and 1 000 µmol L⁻¹. To collect the ¹⁴CO₂ producted from mineralization of the added compounds, a 1 mol L-1 NaOH trap (1 mL) was placed inside the tube which was then tightly sealed and incubated in the dark at 20°C for 168 h. The NaOH traps were replaced after 1, 3, 6, 24, 48, 72 and 168 h. The amount of ¹⁴CO₂ captured was determined by liquid scintillation counting (Wallac EG & G, Milton Keynes, UK). After 168 h, 25 mL of 0.5 mol L⁻¹ KH₂PO₄ was added to all samples and the tubes were shaken for 30 min in order to extract any free 14C organic compounds still remaining in the soil. Subsequently, a 1 mL aliquot of the 0.5 mol L⁻¹ KH₂PO₄ extract was removed and centrifuged (18 000×g for 5 min) to remove microorganisms/particles from the solution. The radioactivity of the solution was determined as described above.

2.4. Organic-P sorption

Sorption experiments were conducted using the soils sterilized by autoclave at 121°C for 30 min (Serrasolsas and Khanna 1993). The autoclaving eliminated microorganisms that could degrade the ¹⁴C-labelled substrates and denatured any phosphatases that existed in the soil (Fransson and Jones 2007; Roberts et al. 2007). Solid phase sorption of each substrate was determined by shaking 2.5 g of soil with 5 mL of each ¹⁴C-labeled compound for periods of up to 3 h. The same ¹⁴C-labeled compounds listed above were used, namely: [8-14C]-adenosine, [U-14C]-AMP, [8-14C]-ADP, or [8-14C]-ATP. The 14C-labeled solutions (0.2 kBq mL-1) were added at five concentrations: 10, 50, 100, 500 and 1000 µmol L⁻¹ into the soil. After shaking for either 0.25, 1, 3, or 6 h, an aliquot of solution (350 µL) was removed from the soil suspensions. Aliquots were centrifuged at 18000×g for 5 min and 14C activity in the supernatant was determined as described above.

2.5. Statistical and data analysis

The mineralization of substrates is bi-phasic, with a rapid first phase followed by a second slow phase (Hill *et al.* 2011). The ¹⁴CO₂ efflux from the soil after the addition of ¹⁴C-labelled substrates was best fitted to a first order double exponential decay equation (Hill *et al.* 2011):

$$y=\alpha_1 \times (1-\exp^{-k_1 \times t}) + \alpha_2 \times (1-\exp^{-k_2 \times t})$$
 (1) where k_1 and k_2 are the coefficients describing the fast and slow mineralization phases, respectively, and a_1 and a_2 describe the sizes of the pools. The $t_{1/2}$ of the soil solution substrate pool (a_1) was calculated as:

$$t_{1/2} = \ln(2)/k_1$$
 (2)

Freundlich isotherm was fitted to the data from the sorption experiment and the amount of sorbed compounds was calculated as follows:

$$S=\alpha \times ESC^b$$
 (3)

where S is the amount of sorbed (μ mol g⁻¹) compounds, a and b are empirically derived parameters (Roberts et al. 2007), ESC is the equilibrium solution concentration at the end of the experiment (μ mol L⁻¹), The partition coefficient (K_d) was calculated as follows:

$$K_d = S/ESC$$
 (4)

Values represent mean±SE (n=4). Significant differences at P<0.05 level are shown by different letters within the same column.

All data represented the means of four replicates with their standard errors. The analysis of significant differences was performed by one-way ANOVA at the 95% confidence level (*P*<0.05). Residuals were checked for normality and homogeneity. All analyses were conducted with SigmaPlot 10.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Soil characteristics

The two paddy soils used in the experiments differed significantly in their chemical characteristics (Table 1). The EC, and total N, C, and P in the P-rich soil were *ca*. two times higher compared to those in the P-poor soil, whereas

Oslen-P was 11 times higher.

3.2. Mineralization of substrates

Mineralization curves of adenosine and the three organic P substrates showed a biphasic $^{14}\mathrm{CO}_2$ evolution (Fig. 1). The total amount of ADP mineralized in the P-rich soil was 1.5 times greater than in the P-poor soil (Fig. 1) with similar values being observed for adenosine, AMP, and ATP. Generally, less than 1% of the initially applied $^{14}\mathrm{C}$ was recovered in a 0.5 mol L $^{-1}$ K $_2\mathrm{SO}_4$ extract at the end of a 7-day incubation period, which suggested a nearly complete utilization of all compounds by the microbial community.

There was a significant divergence in mineralization rates among the substrates during the initial utilization phase

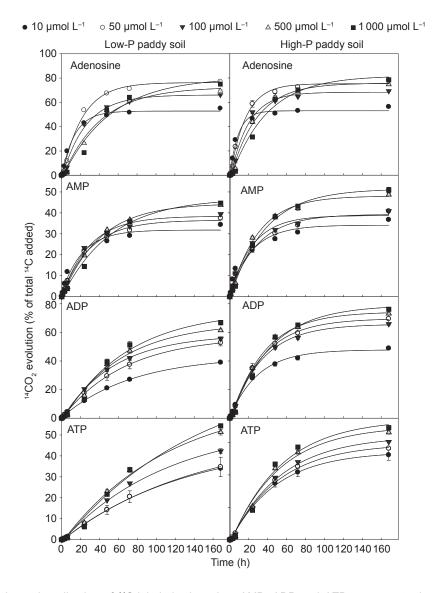


Fig. 1 Time-dependent mineralization of 14 C-labeled adenosine, AMP, ADP and ATP at concentrations ranging from 10 to 1 000 μ mol L⁻¹ in P-rich and P-poor paddy soils in comparison to their non-phosphorylated counterparts (10 to 1 000 μ mol L⁻¹ of adenosine). Values represent mean±SE (n=3).

(first 48 h): for the first hour, mineralization rates followed the order AMP>ADP>adenosine>ATP in both paddy soils (Fig. 2). After 48 h, the mineralization rates followed a different trend: adenosine>ADP>ATP>AMP in both soils (Fig. 2). Moreover, the mineralization rates increased for adenosine and ATP, but decreased for AMP in both soils. These results indicated that the soil microorganisms utilized the AMP and ADP as C or P sources first, and then adenosine and ATP. Moreover, the mineralization rate of P-containing substrates was always higher in the P-rich soil than in the P-poor soil as a function of incubation time.

3.3. Half-life of adenosine substrate-derived C in soil

The half-life $(t_{1/2})$ of C derived from each adenosine substrate increased with increasing the applied concentration in both soils (Table 2). The $t_{1/2}$ of the adenosine substrates increased to 3–10 times with increasing the number of phosphate groups for both soils depending on the concentration (Table 2). The $t_{1/2}$ of ADP- and ATP-derived C was 1.5 and 3 times slower in the P-rich soil than in the P-poor soil, whereas $t_{1/2}$ of AMP-C and adenosine-C was the same in both soils. Thus, the increasing $t_{1/2}$ of adenosine compounds with increasing the number of phosphate groups can reflect the effect of sorption on the mineral matrix, which can partially affect microbial mineralization. The faster $t_{1/2}$ of the studied compounds in the P-rich soil indicated higher microbial activity than in the P-poor soil.

3.4. Sorption of organic-P compounds

The sorption of the ¹⁴C-labeled adenosine compounds to the solid phase was time-dependent and followed the same pattern in both paddy soils: ATP=ADP>AMP>adenosine (Fig. 3). The sorption was very fast for all compounds and reached the maximum after 15 min. The Freundlich sorption isotherm showed that saturation was not reached over the applied

concentration range of the substances (10 to 1 000 µmol L⁻¹) (Fig. 4). The Freundlich parameter a increased with the number of phosphate groups, whereas parameter b decreased in both paddy soils (Table 3). The solid-to-solution partition coefficients $K_{\rm d}$ for AMP, ADP, and ATP were concentration-dependent and decreased with substrate concentration in both soils, but there was no dependency for adenosine (Table 3). Moreover, the $K_{\rm d}$ increased with an increasing number of phosphate groups from adenosine to ATP. Additionally, $K_{\rm d}$ was always greater for ATP in the

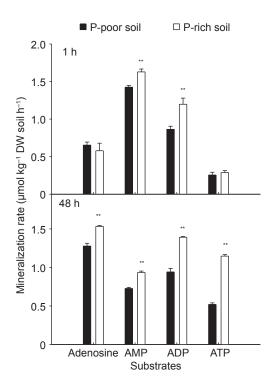


Fig. 2 Mineralization rates of 14 C-labeled adenosine, AMP, ADP, and ATP at a concentration of 1000 µmol L⁻¹ after 1 h and 48 h of incubation in the P-rich and P-poor paddy soils. Values represent mean±SE (n =4). "represent significant differences of same substrate between two paddy soils at the P<0.01 level.

Table 2 Half-life $(t_{1/2})$ of ¹⁴C-labeled adenosine, AMP, ADP and ATP in P-poor and P-rich soils¹⁾

| | 1/2/ | | - | • | | | | | |
|--------|---------------------------------------|----------------------|-------|----------------------|-------|----------------------|-------|----------------------|-------|
| Paddy | Concentration (µmol L ⁻¹) | Adenosine | | AMP | | ADP | | ATP | |
| soil | | t _{1/2} (h) | R^2 |
| P-poor | 10 | 9.7±0.6 | 0.99 | 15.1±1.0 | 0.97 | 51.7±1.8 | 0.99 | 91.6±1.5 | 0.99 |
| | 50 | 15.0±0.3 | 0.99 | 21.1±2.5 | 0.99 | 49.5±6.4 | 0.99 | 78.6±4.5 | 0.99 |
| | 100 | 17.6±0.6 | 0.99 | 20.3±0.3 | 0.99 | 38.4±1.1 | 0.99 | 78.6±4.5 | 0.99 |
| | 500 | 28.1±0.3 | 0.98 | 27.8±0.6 | 0.99 | 43.4±1.6 | 0.99 | 79.3±3.2 | 0.99 |
| | 1 000 | 35.4±1.2 | 0.98 | 35.7±0.9 | 0.99 | 48.2±1.8 | 0.98 | 105.1±7.5 | 0.99 |
| P-rich | 10 | 6.4±0.3 | 0.99 | 16.7±2.9 | 0.96 | 20.6±0.5 | 0.99 | 33.7±0.6 | 0.99 |
| | 50 | 12.3±0.6 | 0.99 | 20.5±2.1 | 0.98 | 24.6±1.4 | 0.99 | 35.9±1.0 | 0.99 |
| | 100 | 13.4±0.6 | 0.99 | 16.9±0.5 | 0.99 | 23.7±0.8 | 0.99 | 37.3±0.5 | 0.99 |
| | 500 | 19.3±0.7 | 0.99 | 20.6±0.5 | 0.99 | 24.7±0.7 | 0.99 | 37.3±0.5 | 0.99 |
| | 1 000 | 26.6±0.3 | 0.99 | 25.8±1.6 | 0.99 | 29.2±0.5 | 0.99 | 37.1±0.7 | 0.98 |

¹⁾The R^2 coefficients represent the goodness of fit of a double first-order kinetic decay model to the experimental data. Values represent mean±SE (n=4).

P-poor soil than in the P-rich soil, except when ATP was applied at concentrations above 500 μ mol L⁻¹. The results showed that organic P sorption increased with the number of phosphate groups and decreased with increased nutrient availability.

4. Discussion

Long-term fertilization can improve soil fertility of paddy surface soils (Abdi et al. 2014; Yan et al. 2017; Liu et al.

2019; Wang et al. 2019; Zhang et al. 2019) by increasing the total C, N, and P contents, as well as increasing the size of the microbial biomass (Table 1). Mineralization of organic-P compounds in both paddy soils was similar for the fast stage (0–24 h) followed by a slower mineralization stage (24–168 h), which was consistent with the report of a previous study (Fransson and Jones 2007). The results indicated that high rates of phosphatase activity and dephosphorylation of the added compounds occur almost immediately, which makes adenosine available for uptake by soil microbes (Fransson

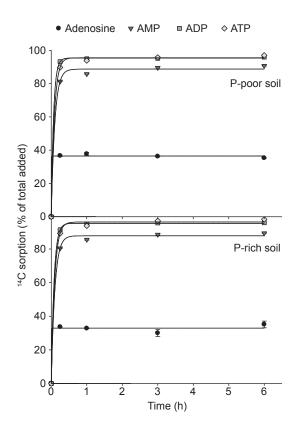


Fig. 3 Sorption of 14 C-labeled adenosine, AMP, ADP, and ATP in P-poor and P-rich paddy soils. The initial concentration was 500 µmol L $^{-1}$. Values represent mean \pm SE (n=3).

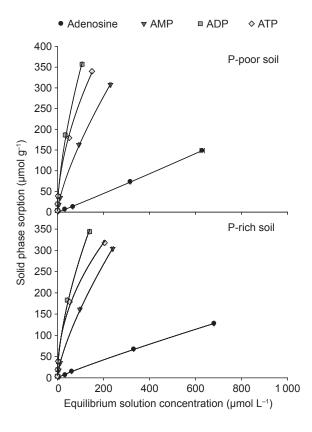


Fig. 4 Sorption isotherms for ¹⁴C-labeled adenosine, AMP, ADP, and ATP in P-poor and P-rich paddy soils. Values represent mean±SE (*n*=3).

Table 3 Parameters of Freundlich sorption isotherm fits to the sorption of different ¹⁴C-labeled substrates to the solid phase at different concentrations in P-poor and P-rich soils¹⁾

| Paddy soil | Substrate | а | b | K_{d} for various substrate concentrations | | | | | |
|------------|-----------|------------|-----------|--|-------------------------|--------------------------|--------------------------|---------------------------|--|
| | | | | 10 µmol L ⁻¹ | 50 µmol L ⁻¹ | 100 µmol L ⁻¹ | 500 µmol L ⁻¹ | 1000 µmol L ⁻¹ | |
| P-poor | Adenosine | 0.18±0.02 | 1.05±0.02 | 0.32 | 0.22 | 0.19 | 0.23 | 0.24 | |
| | AMP | 5.82±0.45 | 0.73±0.02 | 4.57 | 3.15 | 2.83 | 1.74 | 1.34 | |
| | ADP | 18.81±3.28 | 0.63±0.04 | 14.43 | 10.18 | 9.40 | 5.47 | 3.32 | |
| | ATP | 21.36±3.66 | 0.55±0.04 | 160.70 | 85.82 | 17.23 | 3.49 | 2.27 | |
| P-rich | Adenosine | 0.35±0.041 | 0.91±0.02 | 0.22 | 0.20 | 0.24 | 0.20 | 0.19 | |
| | AMP | 5.74±0.45 | 0.73±0.02 | 3.62 | 2.98 | 2.85 | 1.65 | 1.26 | |
| | ADP | 18.11±3.27 | 0.60±0.04 | 12.38 | 8.98 | 8.53 | 4.28 | 2.46 | |
| | ATP | 25.43±3.56 | 0.48±0.03 | 36.49 | 15.61 | 13.62 | 3.34 | 1.55 | |

¹⁾ The *a* and *b* values are empirically derived parameters for calculation of the amounts of sorbed compounds, and *K*_d is the solid-to-solution partition coefficient for adenosine compounds for the various substrate concentrations.

and Jones 2007). The adsorption capacity of organic P is closely related to the number of phosphate groups (Condron 2005). Consistent with our first hypothesis, the $K_{\rm d}$ was the lowest for adenosine, followed by AMP and ADP, and the highest for ATP. Higher adsorption leads to lower microbial accessibility (Cabrita *et al.* 2002; Giannecchini *et al.* 2005; Tozzi *et al.* 2006). As a result, the mineralization rates of organic P substrates decreased with the increasing number of phosphate groups, regardless of soil fertility. The mineralization rates of ADP and ATP were accelerated over time, potentially indicating that phosphatase activity was increased with prolonged incubation times.

As expected by our second hypothesis, the mineralization rates of added LMWOP compounds were profoundly affected by soil fertility, which was significantly higher in the P-rich than P-poor soil. Many studies have found that the addition of exogenous nutrients or increasing of the soluble orthophosphate concentration in soil could potentially increase phosphatase activity and accelerate the mineralization process of organic P (Fox and Comerford 1992; Marklein and Houlton 2012; Wei et al. 2018). The results most likely attributed to the stimulatory effect of increased P availability on phosphatase activity. In additional, the LMWOP was much more accessible to microorganisms in P-rich soil, supported by the greater K_{d} of LMWOPs in P-poor soil than in P-rich soil (Table 3). Also, organic matter accumulation could compete with P for absorption sites, therefore decreasing P adsorption capacity and increasing P availability in soil (Mikutta et al. 2006; Lindegren and Persson 2009; Pavinato et al. 2009; Fink et al. 2016). In our study, the SOC in P-rich soil was 2 times higher than that in P-poor soil (Table 1). Consequently, P sorption in the P-rich soil was smaller than in the P-poor soil (the strongest for ATP) (Table 3). However, long-term fertilization in soil enhances P accumulation and decreases the soil P saturation capacity (Wang et al. 2012; Yan et al. 2013, 2017). This can also have a negative consequence for highly fertile paddy soils, leading to an increased risk of P runoff during rice cultivation (Zhang et al. 2003; Shan et al. 2005; Wang et al. 2012; Yan et al. 2017).

The sorption of organic-P on the solid phase was largely completed within 15 min (Fig. 3). The sorption did not greatly interfere with the mineralization of AMP, especially at the low substrate concentrations where sorption to the solid phase was the strongest (Table 3). These results were consistent with previous findings (Fransson and Jones 2007), which showed that microorganisms can promote desorption of adenosine compounds from the mineral matrix and utilize them. P-solubilizing microorganisms are common in soil, which produce organic acids and H $^+$ during the metabolism of organic C (Alori *et al.* 2017). The $t_{1/2}$ of ADP- and ATP-derived C were two times faster in the P-rich soil than in

the P-poor soil. However, a small variation for $t_{\scriptscriptstyle 1/2}$ of both compounds between the applied concentrations was found (Table 2). These observations indicated that phosphatase activity was high in both soils, with an additional increase in the production of phosphatases in the P-rich soil. The $t_{\scriptscriptstyle 1/2}$ of AMP-C was even higher at some of the applied concentrations, which suggested that phosphatase activity did not limit the utilization of LMWOP compounds if only one phosphate group was present (Fransson and Jones 2007).

5. Conclusion

Long-term fertilization of paddy soils not only increased the contents of essential nutrients (C, N, and P) but also improved LMWOP mineralization. Mineralization rates of adenosine phosphates were the highest for AMP and the lowest for ATP in both soils, indicating that the number of phosphate groups can partially affect this process. However, the microbial activity was higher in the P-rich than P-poor soil, as indicated by the faster mineralization rates of ADP and ATP. The sorption of AMP was lower than ADP and ATP, and each respective adenosine phosphate showed the same sorption trend in both soils. Thus, these results suggested that on the one hand soil fertilization can improve the capacity of the microbial community to mineralize LMWOP, and thus promote plant nutrition; but on the other hand, it can increase the risk of P runoff.

Acknowledgements

This work was funded by the Natural Science Foundation of Hunan Province, China (2020JJ4563), the National Natural Science Foundation of China (4181101348), the Innovation Groups of Natural Science Foundation of Hunan Province (2019JJ10003), the Chinese Academy of Sciences President's International Fellowship Initiative to Anna Gunina (2019VCC0003), and the Talented Young Scientist Program (TYSP) to Mostafa Zhran supported by the China Science and Technology Exchange Center (Egypt-19-004). We thank Dr. Zhu Zhenke and Dr. Wei Xiaomeng of the Institute of Subtropical Agriculture, Chinese Academy of Sciences for their advice on writing and data analyses.

Declaration of competing interest

The authors declare that they have no conflict of interest.

References

Abdi D, Cademenun B J, Ziadi N, Parent L E. 2014. Long-term impact of tillage practices and phosphorus fertilization on soil phosphorus forms as determined by P nuclear magnetic

- resonance spectroscopy. *Journal of Environmental Quality*, **43**, 1431–1440.
- Alori E T, Glick B R, Babalola O O. 2017. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology*, **8**, 971.
- Arruda B, Dall'orsoletta D J, Heidemann J C, Gatiboni L C. 2018. Phosphorus dynamics in the rhizosphere of two wheat cultivars in a soil with high organic matter content. *Archives of Agronomy & Soil Science*, **64**, 1011–1020.
- Bending G D. 2017. The rhizosphere and its microorganisms. In: Thomas B, Murray B G, Murphy D J, eds., *Encyclopedia of Applied Plant Sciences*. 2nd ed. Elsevier, UK. p. 347–351.
- Cabrita M A, Baldwin S A, Young J D, Cass C E. 2002. Molecular biology and regulation of nucleoside and nucleobase transporter proteins in eukaryotes and prokaryotes. *Biochemistry and Cell Biology*, **80**, 623–638.
- Chen C R, Condron L M, Davis M R, Sherlock R R. 2003. Seasonal changes in phosphorus and associated microbial properties under adjacent grassland and forest in New Zealand. Forest Ecology and Management, 177, 539–557.
- Condron L M, Turner B L, Cade-Menun B J. 2005. Chemistry and dynamics of soil phosphorus. In: Sims J T, Sharpley A N, eds., *Phosphorus: Agriculture and the Environment. American Society of Agronomy*. Crop Science Society of America, and Soil Science Society of America. pp. 87-121.
- Ding L J, Wu J S, Xiao H A, Zhou P, Syers K J. 2012. Mobilisation of inorganic phosphorus induced by rice straw in aggregates of a highly weathered upland soil. *Journal of* the Science of Food and Agriculture, 92, 1073–1079.
- Dong W Y, Zhang X Y, Dai X Q, Fu X L, Yang F T, Liu X Y, Sun X M, Wen X F, Schaeffer S. 2014. Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China. *Applied Soil Ecology*, 84, 140–147.
- Fink J R, Inda A V, Bavaresco J, Barrón V, Torrent J, Bayer C. 2016. Adsorption and desorption of phosphorus in subtropical soils as affected by management system and mineralogy. *Soil & Tillage Research*, **155**, 62–68.
- Fischer H, Ingwersen J, Kuzyakov Y. 2010. Microbial uptake of low-molecular-weight organic substances out-competes sorption in soil. *European Journal of Soil Science*, **61**, 504–513
- Fox T R, Comerford N B. 1992. Rhizosphere phosphatase activity and phosphatase hydrolyzable organic phosphorus in two forested spodosols. *Soil Biology and Biochemistry*, **24**, 579–583.
- Fransson A M, Jones D L. 2007. Phosphatase activity does not limit the microbial use of low molecular weight organic-P substrates in soil. *Soil Biology and Biochemistry*, **39**, 1213–1217.
- Ge T, Luo Y, He X H. 2019. Quantitative and mechanistic insights into the key process in the rhizodeposited carbon stabilization, transformation and utilization of carbon, nitrogen and phosphorus in paddy soil. *Plant and Soil*, **445**, 1–5.
- Giannecchini M, Matteucci M, Pesi R, Sgarrella F, Tozzi M G, Camici M. 2005. Uptake and utilization of nucleosides for energy repletion. *International Journal of Biochemistry &*

- Cell Biology, 37, 797-808.
- Gunina A, Dippold M A, Glaser B, Kuzyakov Y. 2014. Fate of low molecular weight organic substances in an arable soil: From microbial uptake to utilisation and stabilisation. *Soil Biology and Biochemistry*, **77**, 304–313.
- Gu S, Gruau G, Malique F, Dupas R, Petitjean P, Gascuel-Odoux C. 2018. Drying/rewetting cycles stimulate release of colloidal-bound phosphorus in riparian soils. *Geoderma*, **321**. 32–41.
- Hill P W, Farrell M, Roberts P, Farrar J, Grant H, Newsham K K, David W H, Richard D, Davey L J. 2011. Soil- and enantiomer-specific metabolism of amino acids and their peptides by Antarctic soil microorganisms. *Soil Biology and Biochemistry*, **43**, 2410–2416.
- Hu Y, Xia Y, Sun Q, Liu K, Chen X, Ge T, Zhu B, Zhu Z, Zhang Z, Su Y. 2018. Effects of long-term fertilization on phoDharboring bacterial community in Karst soils. *Science of the Total Environment*, **628–629**, 53–63.
- Kim S Y, Sivaguru M, Stacey G. 2006. Extracellular ATP in plants, visualization, localization, and analysis of physiological significance in growth and signaling. *Plant Physiology*, **142**, 984–992.
- Kögel-Knabner I, Amelung W, Cao Z, Fiedler S, Frenzel P, Jahn R, Kalbitz K, Kölbl A, Schloter M. 2010. Biogeochemistry of paddy soils. *Geoderma*, **157**, 1–14.
- Lan Z M, Lin X J, Wang F, Zhang H, Chen C R. 2012. Phosphorus availability and rice grain yield in a paddy soil in response to long-term fertilization. *Biology and Fertility* of Soils, 48, 579–588.
- Lee C H, Lee I B, Kim P J. 2004. Effects of long-term fertilization on organic phosphorus fraction in paddy soil. *Soil Science and Plant Nutrition*, **50**, 485–491.
- Li B, Ge T, Xiao H, Zhu Z, Li Y, Shibistova O, Liu S, Wu J, Inubushi K, Guggenberger G. 2016. Phosphorus content as a function of soil aggregate size and paddy cultivation in highly weathered soils. *Environmental Science and Pollution Research*, **23**, 7494–7503.
- Lin S, Wang S, Si Y, Yang W, Zhu S, Ni W. 2017. Variations in eco-enzymatic stoichiometric and microbial characteristics in paddy soil as affected by long-term integrated organicinorganic fertilization. *PLoS ONE*, **12**, e0189908.
- Lindegren M, Persson P. 2009. Competitive adsorption between phosphate and carboxylic acids: Quantitative effects and molecular mechanisms. *European Journal of Soil Science*, **60**, 982–993.
- Liu K L, Huang J, Li D M, Yu X C, Ye H C, Hu H W, Hu Z H, Huang Q H, Zhang H M. 2019. Comparison of carbon sequestration efficiency in soil aggregates between upland and paddy soils in a red soil region of China. *Journal of Integrative Agriculture*, **18**, 1348–1359.
- Marklein A R, Houlton B Z. 2012. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytologist*, **193**, 696–704.
- Mempin R, Tran H, Chen C, Gong H, Ho K K, Lu S. 2013. Release of extracellular ATP by bacteria during growth. *BMC Microbiology*, **13**, 301–301.
- Mikutta R, Kleber M, Torn M S, Jahn R. 2006. Stabilization of soil organic matter: Association with minerals or chemical

- recalcitrance? Biogeochemistry, 77, 25-56.
- Parham R. 2014. Phosphorus cycling in organic systems. MSc thesis, University of Saskatchewan, Canada.
- Pavinato P S, Merlin A, Rosolem C A. 2009. Phosphorus fractions in Brazilian Cerrado soils as affected by tillage. *Soil & Tillage Research*, **105**, 149–155.
- Roberts P, Bol R, Jones D L. 2007. Free amino sugar reactions in soil in relation to soil carbon and nitrogen cycling. *Soil Biology and Biochemistry*, **39**, 3081–3092.
- Serrasolsas I, Khanna P K. 1993. Changes in heated and autoclaved forest soils of SE Australia. 2. Phosphorus and phosphatase-activity. *Biogeochemistry*, **29**, 25–41.
- Shan Y H, Yang L Z, Yan T M, Wang J G. 2005. Downward movement of phosphorus in paddy soil installed in large-scale monolith lysimeters. *Agriculture Ecosystems & Environment*, **111**, 270–278.
- Smyth D A, Black C C. 1984. Measurement of the pyrophosphate content of plant tissues. *Plant Physiology*, **75**, 862–864.
- Tozzi M G, Camici M, Mascia L, Sgarrella F, Ipata P L. 2006. Pentose phosphates in nucleoside interconversion and catabolism. *FEBS Journal*, **273**, 1089–1101.
- Turner B L, Mahieu N C, Leo M. 2003. Quantification of myo-inositol hexakisphosphate in alkaline soil extracts by solution ³¹P NMR spectroscopy and spectral deconvolution. *Soil Science*, **168**, 469–478.
- Vats P, Bhattacharyya M S, Banerjee U C. 2005. Use of phytases (myo-inositolhexakisphosphate phosphohydrolases) for combatting environmental pollution: A biological approach. *Environmental Science & Technology*, **35**, 469–486.
- Wang D, Zhu Z, Shahbaz M, Chen L, Liu S, Inubushi K, Wu J, Ge T. 2019. Split N and P addition decreases straw mineralization and its priming effect in the paddy soil ecosystem: A 100-day incubation experiment. *Biology and Fertility of Soils*, **55**, 701–712.
- Wang S X, Liang X Q, Chen Y X, Luo Q X, Liang W S, Li S, Huang C L, Li Z Z, Wan L L, Li W, Shao X X. 2012. Phosphorus loss potential and phosphatase activity under phosphorus fertilization in long-term paddy wetland agroecosystems. *Soil Science Society of America Journal*, **76**, 161–167.
- Wei K, Sun T, Tian J H, Chen Z H, Chen L J. 2018. Soil microbial biomass, phosphatase and their relationships with phosphorus turnover under mixed inorganic and organic nitrogen addition in a *Larix gmelinii* plantation. *Forest Ecology and Management*, **422**, 313–322.
- Wei X, Hu Y, Peng P, Zhu Z, Atere C T, O'Donnell, Anthony G, Wu J, Ge T. 2017. Effect of p stoichiometry on the abundance of nitrogen-cycle genes in phosphorus-limited paddy soil. *Biology and Fertility of Soils*, **53**, 767–776.
- Wei X, Hu Y, Razavi B S, Zhou J, Shen J, Nannipieri P, Wu J S, Ge T D. 2019a. Rare taxa of alkaline phosphomonoesterase encoding microorganisms mediate soil phosphorus mineralization. *Soil Biology and Biochemistry*, **131**, 62–70.

- Wei X, Razavi B S, Hu Y, Xu X, Zhu Z, Liu Y, Kuzyakov Y, Li Y, Wu J, Ge T. 2019b. C/P stoichiometry of dying rice root defines the spatial distribution and dynamics of enzyme activities in root-detritusphere. *Biology and Fertility of Soils*, **55**, 251–263.
- Wu J S, Joergensen R G, Pommerening B, Chaussod R, Brookes P C. 1990. Measurement of soil microbial biomass C by fumigation-extraction An automated procedure. *Soil Biology and Biochemistry*, **22**, 1167–1169.
- Yaginuma H, Kawai S, Tabata K V, Tomiyama K, Kakizuka A, Komatsuzaki T, Noji H, Imamura H. 2014. Diversity in ATP concentrations in a single bacterial cell population revealed by quantitative single-cell imaging. *Scientific Reports*, **4**, 6522.
- Yan X, Wang D, Zhang H, Zhang G, Wei Z. 2013. Organic amendments affect phosphorus sorption characteristics in a paddy soil. *Agriculture Ecosystems & Environment*, **175**, 47–53.
- Yan X, Wei Z, Hong Q, Lu Z, Wu J. 2017. Phosphorus fractions and sorption characteristics in a subtropical paddy soil as influenced by fertilizer sources. *Geoderma*, **295**, 80–85.
- Yao H, Huang S, Qiu Q, Li Y, Wu L, Mi W, Dai F. 2016. Effects of different fertilizers on the abundance and community structure of ammonia oxidizers in a yellow clay soil. *Applied Microbiology and Biotechnology*, **100**, 6815–6826.
- Yevdokimov I, Larionova A, Blagodatskaya E. 2016. Microbial immobilisation of phosphorus in soils exposed to dryingrewetting and freeze-thawing cycles. *Biology and Fertility* of Soils, 52, 685–696.
- Yokoyama D, Imai N, Kitayama K. 2017. Effects of nitrogen and phosphorus fertilization on the activities of four different classes of fine-root and soil phosphatases in Bornean tropical rain forests. *Plant and Soil*, **416**, 463–476.
- Yu Z H, Hu X J, Wei D, Liu J J, Zhou B K, Jin J, Liu X B, Wang G H. 2019. Long-term inorganic fertilizer use influences bacterial communities in Mollisols of Northeast China based on high-throughput sequencing and network analyses. *Archives of Agronomy and Soil Science*, **65**, 1331–1340.
- Zhang H C, Cao Z H, Shen Q R, Wong M H. 2003. Effect of phosphate fertilizer application on phosphorus (P) losses from paddy soils in Taihu Lake region: I. Effect of phosphate fertilizer rate on P losses from paddy soil. *Chemosphere*, **50**, 695–701.
- Zhang L, Fan J, Ding X, He X, Zhang F, Feng G. 2014. Hyphosphere interactions between an arbuscular mycorrhizal fungus and a phosphate solubilizing bacterium promote phytate mineralization in soil. *Soil Biology and Biochemistry*, **74**, 177–183.
- Zhang W W, Zhan X Y, Zhang S X, Lbrahima K H M, Xu M G. 2019. Response of soil Olsen-P to P budget under different long-term fertilization treatments in a fluvo-aquic soil. *Journal of Integrative Agriculture*, 18, 667–676.