Research Article **AJAR (2020), 5:77**

American Journal of Agricultural Research (ISSN:2475-2002)

Mineralization of phosphorous by phosphate solubilising bacteria isolated from a vertisol

Vivek Singh, Bharati Kollah, Santosh Ranjan Mohanty*

Indian Institute of Soil Science, Nabibagh, Bhopal, India 462038

ABSTRACT

The current experiment unravels P solubilisation potential of soil under long term fertilizer application. Soil samples collected from a 20 years old long term experimental field. Treatments included fallow (no fertilizer, no crop), control (no fertilizer, with crop), 100% N, 100% NP, 100% NPK, and 100% NPK+FYM. P solubilisation potential of soils determined using Ca3(PO4)2 as inorganic insoluble P source. Abundance of total bacteria, phosphate solubilising bacteria (PSB) estimated along with the efficient PSB isolated to evaluate P solubilisation potential using Ca3(PO4)2, rock phosphate and sodium phytate as P sources. P solubilisation rate was highest in 100% NP and lowest in fallow. Abundance of total eubacteria and phosphate solubilizing bacteria (PSB) was high in 100% NP and low in fallow. The 16S rRNA sequences of the isolates were homologues to Paraburkholderia sp. The efficient PSB isolate solubilised Ca3(PO4)2, rock phosphate as well as sodium phytate. Acid phosphatise activity was highest in Ca3(PO4)2 and lowest in sodium phytate. Study concludes that P solubilisation in vertisol under long term fertilizer application is regulated by nutrients, particularly P fractions and abundance of PSB. The PSB solubilise different P sources by reducing pH of medium as well as through acid phosphatase attributes.

Keywords: Phosphorus solubilisation; long term fertilizer; vertisol; bacteria; 16S rRNA

***Correspondence to Author:**
Santosh Ranjan Mohanty
Indian Institute of Soil Science, Nabibagh, Bhopal, India 462038

How to cite this article:
Vivek Singh, Bharati Kollah, Santosh Ranjan Mohanty. Mineralization of phosphorous by phosphate solubilising bacteria isolated from a vertisol. American Journal of Agricultural Research, 2020,5:77.

eSciPub LLC, Houston, TX USA.
Website: https://escipub.com/

Introduction

The mobilization of orthophosphate from soil P complexes and then diffusion of P within soil solution represents a major challenge to meet plant's P requirement (Menezes-Blackburn et al. 2018). To resolve this issue, phosphate solubilizing microorganisms solubilize insoluble P compounds uptake (Alori et al. 2017). Understanding the microbial contribution to plant P nutrition and approaches for manipulating certain microorganisms to enhance P availability in soil is therefore have been of considerable interest over many decades. Recently, this area of research is accentuated, as P deficiency is recognized as a major bottle neck for crop production. Hence government is taking serious steps particularly in areas of weathered and tropical soils where the P limitation possesses serious threat for sustainable agriculture (Van Vuuren et al. 2010).

Long term experiments are crucial for understanding P dynamics in soil, because of the high phosphorus buffering capacity of soils (Beck and Sanchez 1994). Therefore, the effect of different management practices, fertilizer application on P availability can be most convincingly assessed only after their long-term application. Depending on the soil type, soil organic C content, nutrients, the effect of P on soil and plant can be observed only after about a decade or more (Hofmann et al. 2016). Thus, long-term field experiments are very valuable tools to understand P dynamics in a better way.

The current experiment is based on hypotheses that long term fertilizer application influences P solubilisation. P solubilisation potential of a soil depends on the nutritional status, minerals and abundances of phosphate solubilising bacteria. The potential of PSB to solubilise P fractions depends on the type of P sources. Experiments were carried out using soils of a long term fertilizer experimental fields, to evaluate the structure of the microbial community involved in phosphorous solubilisation in vertisol under long term fertilizer application, to quantify the abundance of phosphorous mineralizing microbial population in response to long term fertilizer application, and to estimate the mineralization efficiency of phosphorus solubilizers in relation to mineralogical and nutritional status of soil.

Materials and methods

Experimental field is situated as long term fertilizer experimental (LTFE) fields of the Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhatisgarh, India (21^{o}14 N, 81^{o}42 E, 267.91 m above mean sea level. The soil is a clayey vertisol (*typic* Chromustert). Soil sampling was done during April 2018. In this study, soil samples were collected from the following treatments : fallow, control; 100% N, 100% N and P; NPK 100%, NPK 100% + FYM. Sampling was done from surface layer (0-15 cm) with the help of Screw Auger from different locations according to the distribution of treatments and their replications. All the soil samples were kept in separate polythenes and marked properly.

Experiments were carried out in a completely randomized design using 24 vials (6 treatments x 4 replicates). Soils were amended with either none or one of the P sources including $Ca_3(PO_4)_2$, rock phosphate, and sodium phytate. Briefly, a portion of dry soil (20g) was placed in 130 ml sterile vials. The soils were moistened with distilled water to provide 60% moisture holding capacity and allowed to equilibrate with the ambient air for 1d in the dark in an incubator at 30±2^{o}C. The P sources were applied at 40 µg P g^{-1} soil. At the day of sampling vials were opened and soils (2g) were taken out for available P estimation.

Quantitative analysis of phosphate solubilization was accomplished by applying vanadomolybedate phosphoric method (Jackson 1958). Enumeration of bacteria and phosphate solubilising bacteria (PSB) was carried out using nutrient agar and Pikovskayas agar media. Incubation period for bacteria was 3 days and for PSB was 7 days at 28ºC. Number of colonies appeared in nutrient agar media were counted as abundance of bacteria. Colonies growing in Pikovskayas agar with halo zone

considered positive for PSB. The phosphate solubilising efficiency of PSB strains were calculated by following formula D-d/d*100. Colonies with different efficiency level were enumerated. Isolate with highest efficiency was identified through 16S rRNA sequencing.

Analysis of acid phosphatase activity of PSB strains was accomplished by using method suggested by Tabatabai and Bremner(1969). 1 ml of centrifuged bacterial cell free culture taken and 4 ml of Modified universal buffer (pH6.5), 1 ml of p-nitrophenyl phosphate solution and 1-2 drop of toluene were added to restrict microbial activity. Incubation of 1 hr at 37 ºC were given to solutions. After incubation 4 ml 0.5 M NaOH and 1 ml 0.5 M $CaCl_2$ added and immediate filtration through Whatman no 2 had been done. Color intensity of yellow color appeared measured in spectrophotometer at wavelength 420.

Results

Soil chemical properties of the long term fertilizer experimental fields are given in Table 1. Soil pH was in neutral range and varied from 7.2 to 7.6. No significant difference was observed among the treatments. Electrical conductivity ranged from 0.20 to 0.26 dS m^{-1}. Organic C varied from 0.45 % to 0.66%, lowest in 100% N and highest in 100% NPK+FYM. Total N, P, and K was lowest in control and highest in 100% NPK + FYM.

Phosphorous solubilisation rate of soils under different long term fertilizer application was examined by incubating soil with insoluble $Ca_3(PO_4)_2$ and the concentration of available P was estimated to determine P solubilisation rate (Fig 1). P solubilization rate (µg P solubilized g^{-1} soil d^{-1}) was lowest in fallow (0.032) and highest in 100% NP (0.080) (Fig 1). P solubilization rate of 100% NP was at par with NPK 100% and NPK100%+FYM. In general the P solubilization rate followed a trend as fallow <control< 100% N< 100% NP. P solubilisation rate of 1005 NP, 100% NPK, and 100% NPK + FYM were at par. To reveal, whether pH of broth is decreased during incubation which caused P solubilization, experiments were carried out by incubating soil samples in Pikovaskaya broth. Broth pH decreased from neural to acidic range (4.36 to 4.78) in all treatments. Among the treatments pH of broth was lowest (4.36) in 100% NP (Fig 1).

Total number of eubacteria and phosphate solubilising bacteria (PSB) in soils of long term fertilizer experiment were enumerated (Fig 2). Bacterial count varied from 25 x 10^6 to 48 x 10^6 CFU g^{-1} soil. Lowest bacterial abundance was in 100% N while their abundances were highest and at par in the treatments of 100% NP, 100% NPK, and 100% NPK + FYM. Abundance of PSB varied from 6.33 x 10^4 to 23.67 x 10^4 CFU g^{-1} soil. Lowest was in control and highest in 100% NP.

P solubilising bacteria were enumerated through enrichment on Pikovaskaya agar. Based on the cultural characteristics, diversity of PSB was estimated. Colonies having clear zone around were considered as P solubilizers, as these bacteria were capable of solubilising insoluble $Ca_3(PO_4)_2$ causing halo zone around the colonies. Colonies with larger zones are efficient P solubilizers than of narrow zones. P solubilisation efficiency zone (D/d) varied from 0.2 to 1.8. Colonies with D/d <0.25 were 18%, D/d 0.25-5 were 45%, D/d 0.75 to 1 were 5% and D/d of 1.25 to 1.5 and > 1.5 were 9%. Colonies with highest D/d was selected for identification and estimation of P solibilization potential.

P solubilisation potential

Efficiency of P solubilizers were evaluated by incubating Pikovaskaya broth with different P sources including $Ca_3(PO_4)_2$, rock phosphate, sodium phytate at 40 µg ml^{-1} concentration (Fig 3). Isolated phosphate solubilizer was inoculated at 10^6 cells ml^{-1}. Solubilization of P sources progressed linearly during incubation period of 5 days. P solubilisation potential or rate (µg P solubilised d^{-1}) was 0.219 for $Ca_3(PO_4)_2$, 0.213 for rock phosphate, and 0.195 for sodium phytate (Table 2). Acid phosphatase activity estimated from the samples (Table 2). Acid phosphatase was highest (1406 µg PNP released g^{-1} soil) in $Ca_3(PO_4)_2$ and lowest (758 µg PNP released g^{-1} soil) in sodium phytate.

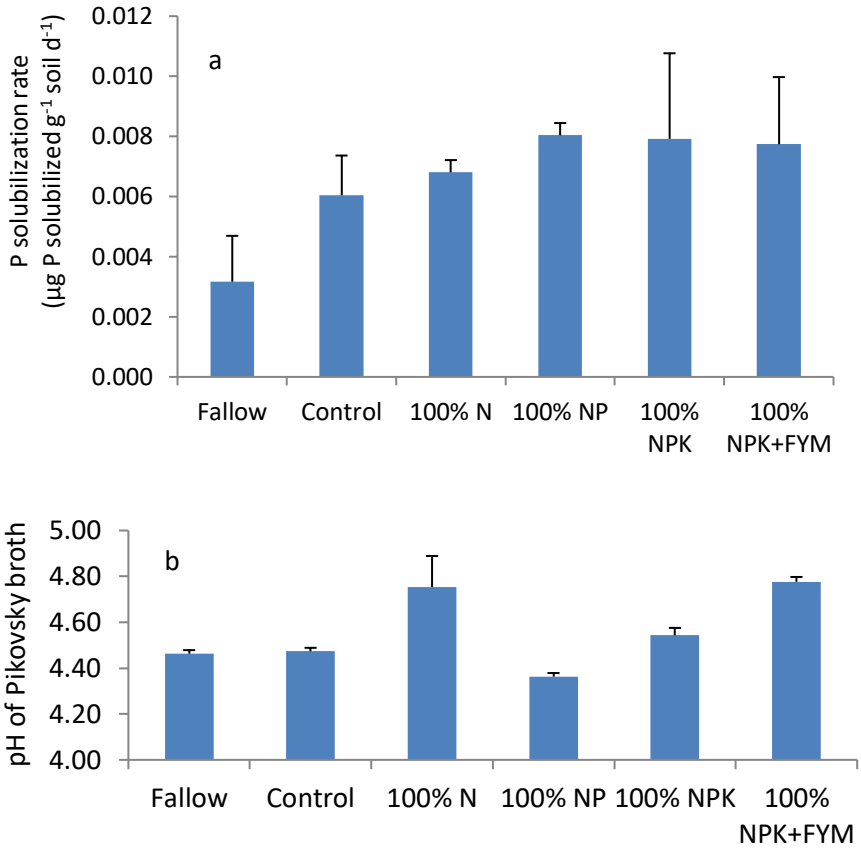

Fig 1. Phosphorous mineralization rate of soils under long term fertilizer application (a). Soil samples were amended with $Ca_3(PO_4)_2$ and available P was estimated over incubation period. Y axis represents rate of P mineralization rate. Effect of P mineralization by phosphate solubilising organisms on soil pH (b). Soils from long term fertilizer experiments were added to Pikovskayas broth to estimate change in pH. X axis represent treatments of long term fertilizer experiment. Each data point represents arithmetic mean with error bar as standard deviation of 3 replicated observations.

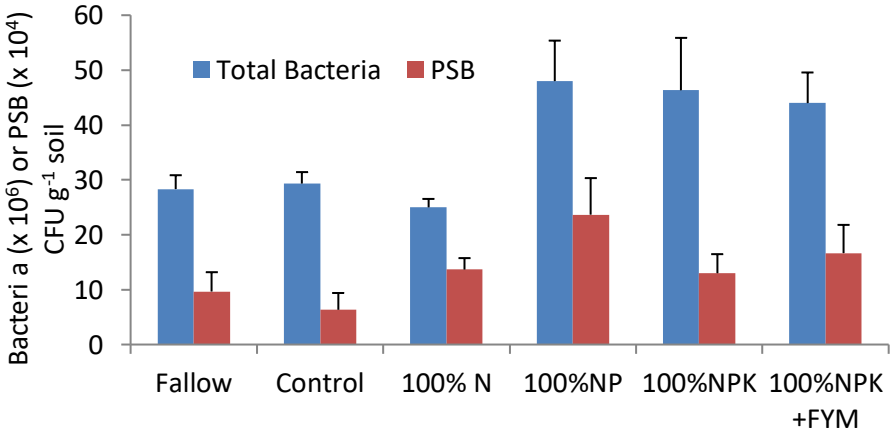

Fig 2. Abundances of total aerobic bacteria and phosphate solubilising bacteria (PSB) in the soils of long term fertilizer experiment. Soil samples from long term fertilizer experimental fields were freshly collected and bacteria abundances were estimated. Nutrient agar was used to enumerate total aerobic bacteria and Pikovskayas agar to estimate abundances of PSB. Colonies exhibited clear zone around the colonies were considered as PSB. Each data point represents arithmetic mean with error bar as standard deviation of 3 replicated observations. Y axis represents colony forming units (CFU) of bacteria or PSB and X axis represents various treatments of long term fertilizer experiment. Each data point represents arithmetic mean with error bar as standard deviation of 3 replicated observations.

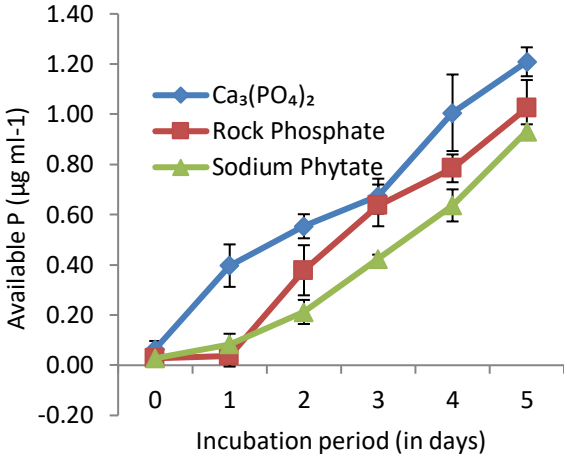

Fig 3 Mineralization or solubilisation of different P sources by phosphate solubilising bacteria (PSB) isolated from vertisol under long term fertilizer experiment. Isolate was cultured in Pikovskayas broth containing $Ca_3(PO_4)_2$, rock phosphate, and sodium phytate as P sources and applied at 40 µg P ml^{-1}. Change in concentration of available P was estimated periodically. X axis represents days of incubation and Y axis represents concentration of available P (µg ml-1. Each data points represent arithmetic mean and error bar as standard deviation of three replicated observations.

Table 1. Chemical properties of soil under long term fertilizer experiment.

Treatment	pH	EC (dS m^{-1})	Organic C (%)	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
Fallow	7.3	0.20	0.64	188	9.55	386
Control	7.4	0.22	0.60	180	8.24	377
100%N	7.2	0.22	0.45	220	8.68	381
100% NP	7.4	0.24	0.55	208	24.74	360
100% NPK	7.6	0.23	0.61	230	27.34	442
100% NPK+ FYM	7.3	0.26	0.66	256	28.65	468

Table 2. Phosphate mineralization rates and acid phosphatase activities of phosphate solubilising bacteria (PSBs). Isolate was grown with different P sources at 40 µg P ml^{-1}. Available P was estimated during the incubation period. Rate of P mineralization was derived from the slope of change in P concentration over incubation period. Acid phosphates determined after the end of incubation. Each data presented as arithmetic mean ±standard deviation of three replicated observations. Letter followed by same letters are not statistically different at p<0.05.

P sources	P mineralization rate (µg P mineralized g^{-1} soil d^{-1})	Acid phosphatise (µg PNP released g^{-1} soil)
$Ca_3(PO_4)_2$	0.219 ± 0.026^a	1406 ± 184^a
Rock phosphate	0.213 ± 0.015^b	758 ± 46^c
Sodium phytate	0.195 ± 0.036^c	965 ± 58^b
Tukeys HSD (P< 0.05)	0.051	61

Discussion

Soil chemical properties were altered by the long term fertilizer application. In general, soil pH was neutral and did not change by long term fertilizer application. Vertisol has high buffering capacity, therefore pH change was not observed. Electrical conductivity was highest (0.26 dS m^{-1}) in NPK 100% + FYM and lowest in fallow. This could be due to the availability of nutrients and probably NPK and FYM contributed to high nutrient availability. Total N, P, and K was highest in 100% NPK + FYM.

Mineralization of P was highest in 100% NP and lowest in fallow. Secondly, it was observed that the P mineralization rates in 100% NP, 100% NPK, and 100% NPK +FYM were at par, indicated that amendment of P increased mineralization of insoluble P fractions. Phosphorous was applied to the fields in available P form such as single super phosphate. Probably, addition of P stimulated microbial metabolism essential to initiate mineralization of insoluble P forms. Albeit the added P gets fixed in soil compartments rich in Ca, Mg etc. However, it is unclear how the P and in which form stimulated PSB. pH of Pikovaskaya broth decreased during P mineralization in all treatments. Soil pH was least (4.36) in the treatment of 100% NP, strongly supported that mineralization of $Ca_3(PO_4)_2$ proceeded due to decline in pH of medium. P mineralization occur either through production of organic acids and/or through phosphatase activity. Organic acids are produced by PSB when the P sources are complex inorganic forms. The principal mechanism by which P is solubilised is through the production of organic acids, siderophores, protons, hydroxyl ions and CO_2 (Rodriguez and Fraga 1999). Organic acids and the corresponding carboxyl and hydroxyl ions chelate cations or reduce the pH to release P (Tallapragada and Seshachala 2012); The organic acids are produced in the periplasmic space by the direct oxidation pathway (Zhou et al. 2015). The excretion of these organic acids is accompanied by a drop in pH of medium results in the acidification of the microbial cells and the surroundings, leading to P ions release by substitution of H^+ for Ca^{2+} (Goldstein 1995).

Abundance of bacteria and PSB was low in fallow, control and 100% N indicated that soils were low in the microbial population. Cropped fields mostly have higher microbial population than fallow and similar results has been observed in other long term field experiments (Drijber et al. 2000). Application of only N can affect the soil biological function reducing the abundance of total bacteria. In a study on the long-term effects of imbalanced fertilization on the composition and diversity of soil bacterial community, it was found that N promotes some bacterial groups, which are involved in the degradation of materials; however, only N application had an overall negative impact on the abundance of several groups (Eo and Park 2016). The abundance of both bacteria and PSB was high in 100% NP, 100% NPK, and 100% NPK +FYM, indicated that these treatments favoured bacterial growth. It is also learned that P is prerequisite for enhancing abundance of bacteria and PSB. Therefore, the treatment of 100% NP, and 100% NPK exhibited similar result on the abundance of bacteria. The effect of mineral N, P, K fertilizer and organic manure on soil microbial community and diversity was studied in a 21 year long term experiment. The experiment included organic manure, organic manure plus fertilizer NPK, fertilizer NPK, fertilizer NP, fertilizer NK, fertilizer N, fertilizer P, fertilizer K, and the control (without fertilization). The amounts of PLFAs representing total bacteria, Gram-negative and actinobacteria were highest in the OM + NPK treatment, followed by the OM treatment, whilst least in the N treatment. The amounts of Gram-positive and anaerobic PLFAs were least in the P treatment and the control, respectively. The amounts of aerobic and fungal PLFAs were highest in the NPK treatment whilst least in the N and P treatment, respectively (Zhong et al. 2010).

To estimate P solubilization potential of the isolate, the P sources were no P, $Ca_3(PO_4)_2$, rock phosphate and sodium phytate. The no P was used as blank. The treatment of $Ca_3(PO_4)_2$ and rock phosphate was to test if the PSB mineralize the inorganic insoluble P sources. $Ca_3(PO_4)_2$ as well as rock phosphate are widely used to isolate potential PSB (Sharon et al. 2016). A large proportion (57.8%) of the total P in the soils was in organic form (Horii et al. 2013). In this study, sodium phytate was used as organic P source (Li et al. 2003). The soil organic-P fraction is composed chiefly of phytic acid or phytin (Alori et al. 2017). The organic phosphate is of special interest for plants P uptake, because it is the principal storage form of phosphorus in mature seeds of both monocot and dicot plants. Phytin is a calcium magnesium salt of phytic acid (inositol phosphate). Inositol phosphate may have one to six phosphorus atoms per inositol unit (Li et al. 2016). In order to become available to plants, organic-P compounds must first be hydrolyzed by phosphatases (Yoon et al. 1996). In soil, this process is predominantly mediated by the activity of soil microorganisms, and plant roots. Approximate recoveries of P from different organic P compounds are insoluble phosphorus (2-50%). To cope up with this P limitations, most bacteria, yeast and fungi produces extracellular phosphatases. Phosphatases are group of enzymes that catalyze the hydrolysis of both esters and anhydrates of H_3PO_4. These enzymes are classified as acid and alkaline phosphatases because their maximum activities occur at low (pH 6.5) and high (pH 11) pH ranges. Acid phosphatases are produced by both, microorganisms.

Solubilization of P from $Ca_3(PO_4)_2$, rock phosphate, and sodium phytate progressed linearly as the isolate solubilised insoluble P sources effectively. P solubilisation rate was highest in rock phosphate followed by $Ca_3(PO_4)_2$ and then sodium phytate. Three P sources were tested. Both $Ca_3(PO_4)_2$ and rock phosphate are insoluble inorganic in nature, while the sodium phytate is organic form of P source (Feng et al. 2003). Isolate is potential to solubilise both inorganic and organic form of P sources. *Burkholderia sp* is known to solubilise inorganic P sources. In a study Burkholderia sp has been used to increase the crop yield by enhancing inorganic P in rice soil (Stephen et al. 2015). *Burkholderia cepacia* CC-Al74 has been reported as a high solubilizer of $Ca_3(PO_4)_2$. The strain Al74 demonstrated P-solubilization as high as 200 µg ml^{-1} during exponential growth, when the pH decreased from 8 to 3. Solubilization of $Ca_3(PO_4)_2$ was mainly caused by the release of gluconic acid (about 16.3 mM). At this concentration, gluconic acid was capable of solubilizing 376 µg ml−1 of $Ca_3(PO_4)_2$ whereas water at pH 3 only solubilized 35 µg ml^{-1} (Lin et al. 2006). Acid phosphatase was highest in $Ca_3(PO_4)_2$ and lowest in sodium phytate. High acid phosphatese denoted high P solubilisation potential of the isolate.

Conclusion

The current research outlined the P solubilisation potential of soil under long term fertilizer application. The treatment of 100% NP exhibited highest P solubilisation and fallow as lowest. Abundance of total eubacterial population as well as phosphate solubilizing bacteria (PSB) was high in 100% NP and low in fallow. The isolated PSB solubilised $Ca_3(PO_4)_2$, rock phosphate as well as sodium phytate. Based on the above findings it was clear that P mineralization potential of soil depends on the nutrient status of soil. Amendment of P is essential to stimulate PSB activity. In vertisol *Paraburkholderia sp* could be the dominant and effective P solubilizers. In addition, there were several PSB with varying P solubilisation activities, which need to be identified through metagenomic approach. Bridging these knowledge gaps will contribute to efficient exploration of PSB improving agriculture.

Acknowledgements

Authors declare no conflict of interest of any type associated with this manuscript.

References

1. Alori ET, Glick BR, Babalola OO (2017) Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front Microbiol 8:971
2. Beck MA, Sanchez PA (1994) Soil phosphorus fraction dynamics during 18 years of cultivation on a Typic Paleudult. Soil Sci Soc Am J 58:1424–1431
3. Cosgrove DJ (1963) The chemical nature of soil organic phosphorus. I. Inositol phosphates. Soil Res 1:203–214
4. Drijber RA, Doran JW, Parkhurst AM, Lyon DJ (2000) Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biol Biochem 32:1419–1430
5. Duncan EW, King KW, Williams MR, et al (2017) Linking soil phosphorus to dissolved phosphorus losses in the Midwest. Agric Environ Lett 2:
6. Eo J, Park K-C (2016) Long-term effects of imbalanced fertilization on the composition and diversity of soil bacterial community. Agric Ecosyst Environ 231:176–182
7. Feng G, Song YC, Li XL, Christie P (2003) Contribution of arbuscular mycorrhizal fungi to utilization of organic sources of phosphorus by red clover in a calcareous soil. Appl Soil Ecol 22:139–148
8. Foltran EC, Rocha JHT, Bazani JH, et al (2019) Phosphorus pool responses under different P inorganic fertilizers for a eucalyptus plantation in a loamy Oxisol. For Ecol Manag 435:170–179
9. Goldstein AH (1995) Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by gram negative bacteria. Biol Agric Hortic 12:185–193
10. Hermans C, Hammond JP, White PJ, Verbruggen N (2006) How do plants respond to nutrient shortage by biomass allocation? Trends Plant Sci 11:610–617
11. Hofmann K, Heuck C, Spohn M (2016) Phosphorus resorption by young beech trees and soil phosphatase activity as dependent on phosphorus availability. Oecologia 181:369–379
12. Horii S, Matsuno T, Tagomori J, et al (2013) Isolation and identification of phytate-degrading bacteria and their contribution to phytate mineralization in soil. J Gen Appl Microbiol 59:353–360
13. Hsu P-CL, O'Callaghan M, Condron L, Hurst MR (2018) Use of a gnotobiotic plant assay for assessing root colonization and mineral phosphate solubilization by Paraburkholderia bryophila Ha185 in association with perennial ryegrass (Lolium perenne L.). Plant Soil 425:43–55
14. Jackson ML (1958) Soil Chemical Analysis. Prentice- Hall, Inc, Englewood, Cliffs, New Jersey
15. Li L, Tang C, Rengel Z, Zhang F (2003) Chickpea facilitates phosphorus uptake by intercropped wheat from an organic phosphorus source. Plant Soil 248:297–303
16. Li W, Angel R, Kim S-W, et al (2016) Impacts of dietary calcium, phytate, and nonphytate phosphorus concentrations in the presence or absence of phytase on inositol hexakisphosphate (IP6) degradation in different segments of broilers digestive tract. Poult Sci 95:581–589
17. Lin T-F, Huang H-I, Shen F-T, Young C-C (2006) The protons of gluconic acid are the major factor responsible for the dissolution of tricalcium phosphate by Burkholderia cepacia CC-Al74. Bioresour Technol 97:957–960
18. Liu Z, Li YC, Zhang S, et al (2015) Characterization of phosphate-solubilizing bacteria isolated from calcareous soils. Appl Soil Ecol 96:217–224
19. Menezes-Blackburn D, Giles C, Darch T, et al (2018) Opportunities for mobilizing recalcitrant phosphorus from agricultural soils: a review. Plant Soil 427:5–16
20. Miao Y, Stewart BA, Zhang F (2011) Long-term experiments for sustainable nutrient management in China. A review. Agron Sustain Dev 31:397–414
21. Nannipieri P, Giagnoni L, Landi L, Renella G (2011) Role of phosphatase enzymes in soil. In: Phosphorus in action. Springer, pp 215–243
22. Roberts TL, Johnston AE (2015) Phosphorus use efficiency and management in agriculture. Resour Conserv Recycl 105:275–281
23. Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339
24. Schlesinger WH, Bruijnzeel LA, Bush MB, et al (1998) The biogeochemistry of phosphorus after the first century of soil development on Rakata Island, Krakatau, Indonesia. Biogeochemistry 40:37–55
25. Sharon JA, Hathwaik LT, Glenn GM, et al (2016) Isolation of efficient phosphate solubilizing bacteria capable of enhancing tomato plant growth. J Soil Sci Plant Nutr 16:525–536
26. Shen J, Li R, Zhang F, et al (2004) Crop yields, soil fertility and phosphorus fractions in response

to long-term fertilization under the rice monoculture system on a calcareous soil. Field Crops Res 86:225–238

27. Song O-R, Lee S-J, Lee Y-S, et al (2008) Solubilization of insoluble inorganic phosphate by Burkholderia cepacia DA23 isolated from cultivated soil. Braz J Microbiol 39:151–156
28. Stephen J, Shabanamol S, Rishad KS, Jisha MS (2015) Growth enhancement of rice (Oryza sativa) by phosphate solubilizing Gluconacetobacter sp.(MTCC 8368) and Burkholderia sp.(MTCC 8369) under greenhouse conditions. 3 Biotech 5:831–837
29. Tallapragada P, Seshachala U (2012) Phosphate-solubilizing microbes and their occurrence in the rhizospheres of Piper betel in Karnataka, India. Turk J Biol 36:25–35
30. Van Vuuren DP, Bouwman AF, Beusen AH (2010) Phosphorus demand for the 1970–2100 period: a scenario analysis of resource depletion. Glob Environ Change 20:428–439
31. Wang Q, Wang W, He X, et al (2017) Changes in soil properties, X-ray-mineral diffractions and infrared-functional groups in bulk soil and fractions following afforestation of farmland, Northeast China. Sci Rep 7:12829
32. Yoon SJ, Choi YJ, Min HK, et al (1996) Isolation and identification of phytase-producing bacterium, Enterobacter sp. 4, and enzymatic properties of phytase enzyme. Enzyme Microb Technol 18:449–454
33. Zhang ZY, Huang L, Liu F, et al (2016) Characteristics of clay minerals in soil particles of two Alfisols in China. Appl Clay Sci 120:51–60
34. Zhong W, Gu T, Wang W, et al (2010) The effects of mineral fertilizer and organic manure on soil microbial community and diversity. Plant Soil 326:511–522
35. Zhou Z, Hu D, Ren W, et al (2015) Effect of humic substances on phosphorus removal by struvite precipitation. Chemosphere 141:94–99