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ORIGINAL RESEARCH

Farming practices and crop species influence the population of total and alkaline phosphatase gene harboring bacteria in tropical agro-ecosystem

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Abstract: The alkaline phosphatase (ALP) enzyme is encoded by the *phoD* gene reported majorly in bacteria. In the present study, we investigated variation in the *phoD* gene abundance, and the relationship between *phoD* gene abundance, ALP enzyme activity and available P under different farming practice (organic vs conventional), crop species (chickpea, mustard, soybean and maize) and their growth stages (pre-vegetation, vegetative, flowering, maturation and post-harvest). The qPCR analysis revealed variation in total bacterial and *phoD* gene copy number (copies g^{-1}dws), ranging from 1.40×10^9 to 9.16×10^{10} and 1.72×10^5 to 1.43×10^7, respectively. The farming practices suggested significant effect with increased activity of ALP, and abundance of *phoD* and 16S rRNA genes in organic farming than the conventional one. The 16S rRNA and *phoD* gene abundance varied significantly along different growth stages of crops in the order: flowering > maturation > vegetative > post-harvest > pre-vegetation stages with maximum in maize and lowest in soybean in both the farming practice. In conclusion, farming practices, crop types and crop growth stages influenced soil available P and significantly affected ALP activity by regulating *phoD* bacterial population in agroecosystem.

Keywords: phoD gene copy number; alkaline phosphatase activity; available P; farming practices; crops

1. Introduction

Plant metabolic activities require phosphorus (P) as an indispensable element that plays a vital role in maintaining the soil fertility as well as crop productivity. In soil, abundant P is present, mainly in two forms as organic and inorganic P (Brady & Weil, 2007). However, in soil solution microbes and plants utilize P as inorganic orthophosphate (HPO_4^{2-} or $H_2PO_4^-$). To enhance crop productivity, both mineral and organic P fertilizers are used in agroecosystems. Soon after application some amount of inorganic P is readily taken up by the microbes and plants whereas the unused phosphorous is immobilized which leads to the accumulation of P in soil (Richardson, 2001). Since phosphorous is the main limiting macronutrient in agricultural soil, many bacteria play an indispensable role in P-cycle to solubilize insoluble inorganic P (Pi) by producing organic acids, and mineralizing the organic form of P (Po) by releasing extracellular enzymes like acid and alkaline phosphatases (ALP) (Ragot et al., 2015). Researches have well acknowledged that acid phosphatase is produced mainly by plants, fungi and bacteria whereas, alkaline phosphatase is excreted by soil microbes mainly by bacteria (Nannipieri, Giagnoni, Landi, & Renella, 2011). The activity of phosphatase is generally higher in rhizosphere soil with increased microbial activity as compared to bulk soil (Fraser et al., 2017). It is also reported that the organic matter application in the soil increases the ALP activity, as a result, concentration of available P is increased (Mandal et al., 2007). There are some reports that the production and activity of alkaline phosphatase

enzyme correlated negatively with available P in soil (Chen et al., 2019a; Fraser et al., 2017; Long et al., 2018). According to Apel et al. (2007) the Pho regulon has been found mostly in bacteria, and consist of set of genes that are useful to synthesize and exudate phosphatase enzymes comprising alkaline phosphatase. As a segment of Pho regulon, alkaline phosphatase enzyme is encoded by 3 different genes (homologous): *phoD* (Sakurai et al., 2008), *phoX* (Wu et al., 2007), and *phoA* (Zappa et al., 2001) . The bacteria harbouring *phoD* genes are abundant among the different environments predominantly in the soil while *phoX* and *phoA* gene harboring bacteria are present in aquatic ecosystems (Hu et al., 2018). Previous studies showed that *phoD* gene is the most frequently present ALP gene in the terrestrial environment and has been used as an effective functional marker to study the relationship between ALP activity and available phosphorous (Ragot et al., 2016), and the abundance of ALP gene inhabiting the soil (Fraser et al., 2015a;b).

Several studies reported that the abundance of *phoD* gene containing bacterial community is affected by various environmental factors and agricultural management practices (Neal et al., 2017), soil pH (Ragot et al., 2016), organic matter (Chen et al., 2017), mineral fertilizer (Chen et al., 2017; Hu et al., 2018), cover crops (Hallama et al., 2022). A positive correlation was found between *phoD* gene abundance and ALP activity in response to chemical P fertilizer and manure application in soil but this correlation was not significant (Fraser et al., 2015a;b). Moreover, increased activity of ALP enzyme and abundance of *phoD* gene was reported in relation to long-term addition of organically amended soil (composted bean cake) as compared to chemically treated soil (Luo et al., 2017). Besides, it was reported that the effect of the addition of P (phosphate and phytate) on the total and ALP gene (*phoD*) harboring bacterial populations inhabiting the rhizosphere microsites (root tip and mature zone) of ryegrass. The qPCR of *phoD, phoX* and *16S rRNA* gene showed increased abundance in root tip microsites amended with phytate as compared to phosphate (Lagos et al., 2016). Chen et al. (2019a) studied the effect of long-term mineral-P inputs in continuous maize cropping. The study showed the negative correlation of alkaline phosphatase enzyme with soil P availability and positive correlation with *phoD* gene abundance.

In the tropical agro-ecosystems of countries like India, there are very few studies available on the activity of soil ALP enzyme. Mandal et al. (2007) conducted a field experiment to study the long-term effect of NPK fertilizer and farmyard manure on microbial biomass C, N, phosphatase (alkaline/acid) and dehydrogenase in soil. In another study, the effect of combined and individual input of various organic supplements and chemical P fertilizer was evaluated on the alkaline phosphatase activity in maize cropping (Garg et al., 2008). Saha et al. (2008) studied the effects of continuous treatment of manure and chemical fertilizer on the enzymatic activities involved in mineralization of carbon, nitrogen and phosphorus. Bhat et al. (2017) compared the biochemical activity in soil under long-term organic and conventional farming systems related to P-availability in vertisols of Central India grown with soybean and wheat. The study concludes that organic farming soil support increased biological activity. These studies limit the information on changes in microbial population lined to P-mineralization. Literature survey suggests that there are very few studies carried out on alkaline phosphatase encoding *phoD* gene abundance and most of the studies have been performed in temperate agroecosystems. It is therefore inevitable to understand the changes in *phoD* gene containing bacterial population and their relation with available P in tropical agroecosystem for sustainable agriculture. Most of the researches have restricted primarily on the activity of alkaline phosphatase enzyme in the diverse environment. Our understanding of bacterial *phoD* gene abundance, ALP activity, available P and their relationship under the influence of different crop species and fertilizer treatment in tropical agroecosystem is poorly understood and require further study.

In the present study, we hypothesized that different farming practices, crop species and crop growth stages can regulate the P availability in soil and influence *phoD* gene abundance and the soil ALP activity. The present study aimed to (1) determine the abundance of the *phoD* and 16S *rRNA* genes (2) study the variation in ALP activity and available P content, and (3) investigate the relationship between P-availability, ALP activity and *phoD* gene abundance in a tropical agro-

ecosystem soils managed with different farming practices and crop species at different growth stages of the crops.

2. Materials and methods

2.1. Study site

The study was conducted for consecutive two years (2017-2019) at the Dagmagpur agricultural farm in the district of Mirzapur, Eastern Uttar Pradesh, India (83°34′E, 25°09′N), 80 m above mean sea level. The soil at the site is Alfisol with a silty sandy texture (32% sand, 64% silt and 4% clay). The climate of this area is seasonally tropical monsoonal and average annual precipitation of 849.9 mm. The average temperature (minimum-maximum) varies from 8°C (January) to 42°C (June). The agricultural farm was managed by the farmer and the site has been used for intensive agriculture practices for a long time (≈30 years).

2.2. Experimental design

For the current study, two agricultural farms/fields having different farming practices were chosen: one field was treated with compost (organic farming) and the other with mineral fertilizers (conventional farming). The experimental design for sample collection consists of a randomized complete block design with three blocks at each site (5×4 m each with 1 m gap) and a treatment combination of 4 crops × 2 farming practices (total 24 blocks). The conventional and organic farming plots were separated by 100 m distance to avoid edge effects. In the conventional field, as per standard farming practice, mineral fertilizers, NPK (120,40 and 60 kg ha^{-1} for *Rabi* crops and 20, 40 and 60 kg ha^{-1} for *Kharif* crops) was applied once both during *Rabi* and *Kharif* cropping as a basal dose. The organic farming field was treated with compost which was used as organic supplement and comprised of cattle dung and crop residues prepared by NADEP (Narayan Deorao Pandharipande) technique (Chandra et al., 2007). The ripe compost was distributed manually (at the rate of 15 tonnes ha^{-1}) and ploughed to incorporate up to 15 cm depth before *Rab*i and *Kharif* cropping. Any other supplement was not added except for diluted cow urine (1:50; cow urine: water ratio) as the source of nitrogen (Singh et al., 2012). To ensure comparability under both the farming system the cultivation practices (soil preparation, sowing of seed, and irrigation) were managed uniformly. The *Rabi* cropping extends from November to March and *Kharif* from July to October. The seed sowing rate is depicted in Table S1. Irrigation was done as per the standard norms i.e. before flowering and at the time of grain formation for chickpea, mustard and maize, and before the emergence of plant and during pod formation for the soybean crop. The *Rabi* crops comprised Chickpea (*Cicer arietinum L*. var Pusa-256) and mustard (*Brassica campestris* var. T-151) and *Kharif* crop includes Soybean (*Glycine max* var.PS-1225) and maize (*Zea mays* var. Ganga II). Pesticides/ fungicides were not applied in both experimental fields, and weeds were removed manually. Conventional tillage was performed and ploughed up to depth of 10-15 cm under both the farming practices.

2.3. Soil sampling

The experimental plots for each crop in different farming practices (organic vs conventional) comprised of three blocks and from each block, soil samples in triplicate were sampled at different time intervals of cultivation period i.e. vegetative, flowering, maturation including pre-vegetation and post-harvest stage. Rhizosphere soil samples were collected from the vegetative, flowering and maturation stage of the all test crops. Three plants from each plot of four crops (chickpea, mustard, soybean and maize) were uprooted and soil adhering roots were shaken gently to dislodge the soil clumps in a plastic bag and bulk soil samples were collected at pre-vegetation and post-harvest stage from 0-10 cm depth using a soil corer (5 cm diameter) and carried to the laboratory within 12 hours of sampling. The soil samples were sieved with a 2 mm sieve to remove plant debris and stone. The samples (pooled) were separated into two parts: one kept (4°C) for soil alkaline phosphatase (ALP) enzyme activity and the other (at -20°C) for

molecular study (qPCR). All the measurements were carried out in triplicate for two consecutive years.

2.4. Soil variables and microbial biomass

Soil pH was determined by using a pH meter. Soil characteristic parameters such as texture, water holding capacity (WHC) and bulk density were determined by the standard method as described by Bhardwaj et al. (2020). Soil organic carbon (SOC) was estimated as per Walkley et al. (1947) and total nitrogen (TN) is measured by the micro Kjeldahl method (Jackson et al., 1958). Total P was analyzed by the method described by Allen et al. (1986). Triacid mixture ($HCl0_4$, HNO_3, and H_2SO_4 at 1:5:1) was used to digest the soil, and total P was analyzed spectrophotometrically using protocol based on ammonium molybdate-stannous chloride. Soil available P content was estimated as per Olsen et al. (1954). MBC (microbial biomass carbon), MBN (microbial biomass nitrogen) and MBP (microbial biomass phosphorus) were measured as per standard protocol based on chloroform fumigation extraction method.

2.5. Crop biomass

To measure crop biomass, plants were collected at the vegetative, flowering and maturation stages of each test crop plant in three replicates and cleaned with water to remove the adhered soil. The root and shoot of these crop plants were segregated and dried constantly in the oven (at 80°C) and measured to determine crop (root and shoot) biomass in g plant^{-1} on an oven dry-weight basis (Wang et al., 2017).

2.6. Alkaline phosphatase (ALP) enzyme activity assay

Potential ALP activity of soil samples was determined as per the method described by Tabatabai and Bremner (1969). The soil samples (1g) in triplicate were incubated (1 hour at 37 °C) with 1 ml modified universal buffer and *para*-nitrophenol phosphate as substrate at pH 11. Samples were filtered (Whatman 42 filter paper) after 1 hour of incubation and the synthesis of *p*-nitrophenol (*p*-NP) was measured using spectrophotometer (at 420 nm). The values were presented as μmol of *p*-NP g^{-1}soil h^{-1}.

2.7. Soil DNA extraction, quantification of phoD gene and 16S rRNA gene copy number (abundance)

Genomic DNA was extracted from soil (0.5 g) in triplicate by Fast DNA Spin Kit (MP Biomedicals, Ohio, USA) using a bead beater (FastDNAprep, MP Bio, USA) following manufacturer's protocol. The concentration and purity of DNA was determined using Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., USA).

The abundance of bacterial *phoD* gene and 16S *rRNA* was performed by qPCR using iCycler iQ5 thermocycler (Bio-Rad). Gene copy number (*phoD* gene and 16S *rRNA* gene) was quantified using a specific set of primer (*phoD* gene: ALPS-F730; ALPS-R1101; *16S rRNA*: Eub 338F, Eub 518R) as described in Sakurai et al. (2008) and Muyzer et al. (1993), respectively. The PCR reaction mixture contained 10 μl of PowerUp™ SYBR Green Master Mix (Applied Biosystem), 0.5 μl of each primer (10 μM), and 2 μl of DNA template and DNAase/RNAase free water to maintain final volume upto 20 μl. The PCR reaction was performed in duplicate for each sample and for plasmid standards, the samples were analyzed in triplicates.

The PCR cycling conditions for bacterial *phoD* gene: three min at 94°C subsequently by 40 cycles of denaturation for 1 min (at 94°C), annealing (at 61°C) for 45 secs and eventually extended at 72°C for 1 min. For bacterial 16S *rRNA* gene, the PCR conditions were 3 min at 95°C followed by 40 cycles of 45 sec of melt at 95°C and annealing at 60°C and extension at 72°C for 45 sec. To check the reaction specificity, analysis of melting curve was performed with heat denaturing by increasing the temperature from 50°C to 95°C for 40 cycles and fluorescence was recorded for every 0.5°C increase. The plasmid DNA harboring the marked gene from the soil samples containing ALP and 16S rRNA gene was taken as the standard DNA in qPCR analysis by cloning using the specific primer described above. The plasmid was isolated using HiPurA

(TM) Plasmid DNA Miniprep Purification Kit (Himedia) using the manufacturer's protocol. The standard curve was plotted in triplicate with 10-fold dilutions of cloned plasmid DNA. As per the standard curves, the gene copy number of *phoD* and 16S *rRNA* were expressed in g^{-1}dws (dry weight soil).

2.8. Statistical analysis

Multivariate analysis of variance (MANOVA) with Tukey post hoc test ($p < 0.05$) was used to analyze the significant effect of farming practices and crop species on properties of soil and gene copy number. The effect of growth stages on the properties of soil and abundance of genes was evaluated by repeated-measures ANOVA. Regression analysis was used to determine the correlation between *phoD* gene abundance, PO_4^{-}-P and activity of alkaline phosphatase in rhizosphere soil. Statistical data analyses were conducted through SPSS 20.0 software (IBM, SPSS, Inc., NY, USA).

3. Results

3.1. Soil variables, microbial biomass (C, N, and P)

The total organic C (TOC), total P (TP) and total N (TN) contents were higher in soils subjected to organic farming in comparison to conventional ones (Table 1). The available P content was highest in conventional farming at the pre-vegetation stage and lowest at the flowering stage of all the four crops and the values were highest in soybean grown fields (Table 2). The effect of crops, farming practices and growth stages showed a significant ($p < 0.05$) effect. Mineral-N was highest in conventional farming at the flowering stage and lowest at the pre-vegetation stage for all the crops. There was a significant ($p < 0.05$) effect of farming practice and growth stages on variation in mineral-N and available P (Table 2). Soil MBC, MBN and MBP were significantly higher ($p < 0.05$) in organic farming than the conventional farming. MANOVA revealed a significant ($p < 0.05$) effect of farming practice, crop type and crop growth stages on microbial biomass C, N and P (Table 2).

3.2. Alkaline phosphatase (ALP) activity of soil

Alkaline phosphatase activity ranged from 1.75 to 3.59 μmol *p*-nitrophenol g^{-1}soil h^{-1} in organic farming and 1.20 to 2.97 μmol *p*-nitrophenol g^{-1}soil h^{-1} in conventional farming soil. The ALP activities were highest in organic farming than the conventional farming practice. Among different crops, maize crop showed the highest ALP enzyme activity followed by chickpea, mustard and the lowest in soybean (Fig. 1). Irrespective of crops similar trend of variation in the ALP activity in soil was seen between the crop growth stages and the values were highest at the flowering stage followed by maturation > vegetative > post-harvest > pre-vegetation stages. MANOVA showed a significant ($p < 0.001$) effect of farming practice and crop species and repeated measures ANOVA showed significant ($p < 0.001$) effect of crop growth stages on ALP enzyme activity during both year of study.

3.3. Abundance of phoD gene and 16S rRNA gene

The qPCR result revealed the abundance of 16S rRNA gene and *phoD* gene copy number for both years are shown in Fig. 2 and 3 respectively. The abundance of the 16S rRNA gene was higher in organic farming which ranged between 5.03×10^{9} and 9.16×10^{10} copies g^{-1} dws and from 1.40×10^{9} and 1.57×10^{10} copies g^{-1} dws in conventional farming fields. The abundance of *phoD* gene ranged from 7.72×10^{5} to 1.43×10^{7} copies g^{-1} dws in organic farming and 1.86×10^{5} to 1.10×10^{7} copies g^{-1} dws in conventional farming. The *phoD* gene abundance was higher in organic farming practice than the conventional one. The population size of 16S rRNA and *phoD* gene was recorded highest in soils samples of maize crop followed by chickpea, mustard, and least in soybean grown soils. Among the crop growth stages, *phoD* and 16S rRNA gene abundance showed the same trend as ALP activity i.e. the flowering stage showed the maximum abundance followed by maturation > vegetative > post-harvest > pre-vegetation stages. The

MANOVA showed significant (p < 0.001) effect of farming practices, and crops and repeated measures ANOVA showed significant (p < 0.001) effect attributable to crop growth stages on the abundance of total bacterial (16S rRNA) and *phoD* gene abundance.

3.4. Relationship between available P, ALP activity and phoD gene abundance

Soil samples with higher *phoD* gene abundance exhibited correspondingly increased rates of soil ALP activity and decreased available P. The results of regression analysis revealed that there was significant positive correlation (R^2 = 0.66, P < 0.001) between ALP enzyme activity and *phoD* gene abundance (Fig.4a). On the other hand, a negative correlation was observed between ALP activity and available P content of soil (R^2 = 0.40, P < 0.001) and also *phoD* gene copy number and available P (R^2 = 0.60, P < 0.001) (Fig. 4b and c).

3.5. Crop biomass

The crop root biomass ranged from 0.65 to 4.11 and 3.68 to 37.20 g plant^{-1}in organic farming and 0.54 to 2.49 and 3.02 to 32.35 g plant^{-1} in conventional farming practice, respectively (Table 3). The result showed higher root and shoot biomass in organic farming practice irrespective of crops. There were significant (p < 0.05) variations (crop-wise) observed in root and shoot biomass with maize having the highest, and lowest in soybean crop. Stage-wise differences in all the crops were significant (p < 0.05). Among the different crop growth stages, the flowering stage showed the highest biomass and least in the vegetative stage.

4. Discussion

4.1. Soil variables

The long-term organic farming practice promotes SOC, TN and TP in soil (Table 1). MBC, MBN and MBP increase with respect to the organic farming practice and enhance the soil biological activity compared to a conventional counterpart at different growth stages of test crops i.e. chickpea, mustard, soybean and maize crops (Table 2). The soil amended with organic manure was well-established to enhance organic matter (Edmeades et al., 2003). Organic farming soil showed increased level of organic matter (Kaufman et al., 2020) and the soil rich in organic matter have long-term potential to sustain nutrient release (Rochette et al., 1999) and crop productivity in agro-ecosystems (Guo et al., 2012). The compost-treated soil showed a slightly higher pH in comparison to conventional farming soil. This decline in pH in chemical fertilizer treated soil may be due to nitrification of NH_4^+ thus H^+ ion is produced and enhances the soil acidity. This result seems consistent as per the outcomes of McAndrew et al. (1992). Chakraborty et al. (2011) also showed similar findings in which they reported that the increase in the application of chemical fertilizer considerably decreased the soil pH. In the present study, the available P significantly (p < 0.05) increased in conventionally treated soil (Table 2). This trend is in accord with Liu et al. (2010). However, Fraser et al. (2015a;b) observed an opposite trend. This may be because of confounding factors like the proportion of P in mineral fertilizers, several management practices and crop requirements for P (Fraser et al., 2015a;b; Welsh et al., 2009). Apart from farming practices, crops and crop growth stages also influence nutrient availability by altering the degree of root exudates and root biomass. In this study, a significant (p < 0.05) effect of different developmental stages of crops on available P was observed. This may be due to disparity among the amount of available nutrients between the crop growth stages might be credited to plant-microbe competition for nutrient assimilation (Devare et al., 2004).

4.2. Effect of farming practice on soil alkaline phosphatase activity and abundance of phoD gene

The present study provides insight into the assessment of P availability, ALP activity and alkaline phosphatase gene (*phoD*) quantification in organic and conventionally treated rhizosphere soil. The present study showed increased ALP activity in the plots with organic farming which show

increased activity of ALP gene harboring bacteria in response to compost application than in conventional farming plots. This result was consistent with the earlier studies which reported enhanced ALP activity (Mandal et al., 2007; Yang et al., 2006) and bacterial *phoD* gene abundance (Chen et al., 2019b) in the fields managed under organic farming practices. This may be because of the high organic matter content in the soil treated with compost. Crecchio et al. (2001) studied that organic matter amendment in the soil enhances the activity of several soil enzymes like nitrate reductase, dehydrogenase, etc. Saha et al. (2008) communicated that cattle manure amendment in soil results in a significant increase in alkaline phosphatase activity. Moreover, from providing substrate for hydrolysis of the enzyme, organic matter content in the manure possibly increase in binding sites for enzyme accumulation in the soil (Burns et al., 1982).The total bacterial (16S rRNA gene) gene copy number ranged from 1.40×10^{9} to 9.16×10^{10} copies g^{-1} dws with the highest values observed in organic farming and lowest in conventional one. Singh et al. (2012) observed similar range of bacterial 16S rRNA gene abundances (9.60×10^{9} to 1.44×10^{10} copies g^{-1} dws) in soil samples in compost treated rice cultivated field of tropical soil. The abundance of 16S rRNA gene in Chilean extreme environments ranged between 8.6×10^{7} and 19×10^{10} copies g^{-1} dws (Acuña et al., 2016) .The increased number of total bacteria in organic farming practice may be because organic fertilizers not only carry various forms of organic compounds, as well as indigenous bacteria present in manure that may reside in soil (Pershina, 2015).The studies on the population distribution of *phoD* gene harboring bacteria in tropical agroecosystem are almost negligible. Therefore, our study can be compared with available data of other agroecosystems with similar techniques used for the study of population abundance. Fraser et al. (2015a;b) reported *phoD* abundance values ranging between 3×10^{6} and 1×10^{7} copies g^{-1} soil in long-term organic fertilizer management. The qPCR analysis of alkaline phosphatase gene (*phoD*) harboring bacteria, we observed abundance in the range between 1.86×10^{5} and 1.43×10^{7} gene copies g^{-1} dws. In organic farming treated field soils, the abundance of *phoD* genes was highest. Similar results were obtained in the long-term manure fertilized soil (Fraser et al., 2015a;b).It is observed that significant ($p < 0.05$) highest abundance of *phoD* gene was observed in organic treatment soil with highest ($p < 0.05$) alkaline phosphatase activity and highest SOC which shows increased organic matter content and consequently increases bacterial abundance (Sun et al., 2015). In organic farming, the organic fertilizers low in available P content and high in C rich substance may be responsible for the stimulation of various *phoD* gene encoding microbes and as a result increases the *phoD* gene abundance and ALP activity (Luo et al., 2017). Similarly, Chen et al. (2019b) reported that ALP activity and bacterial *phoD* gene abundance were reduced significantly in mineral fertilizer treated soil. This may be due to several factors including increased soil acidity. The ALP activity and *phoD* gene abundance have been related to soil available P in manure and inorganic fertilizer treated soil (Chhabra et al., 2013; Sakurai et al., 2008). It is well established that there is a negative correlation between soil available P and alkaline phosphatase activity and *phoD* gene abundance. These observations are in accordance with the widely consented fact that activity of alkaline phosphatase is governed by the soil available P and increased in response to P starvation (Chen et al., 2019b; Zhang et al., 2014). Consistent with the above fact, in the present study, soil available P is negatively correlated with ALP activity and *phoD* gene abundance (Fig. 4b and c) and a positive correlation was found between ALP activity and bacterial *phoD* gene copy number (Fig. 4a). This result corroborates the previous study where a correlation (negative) was found between PO_4^{-}- P and *phoD* gene abundance in an upland soil ecosystem (Luo et al., 2017; Tan et al., 2013). In another study, a similar negative relationship was reported between soil available P and bacterial alkaline phosphatase activity, while a positive relationship between ALP activity and gene abundance, in the rhizosphere soil from plants of extreme environment of Chile (Acuña et al., 2016) . This result suggests that alkaline phosphatase enzyme production is induced in the environment with low available P (Fraser et al., 2015a;b).

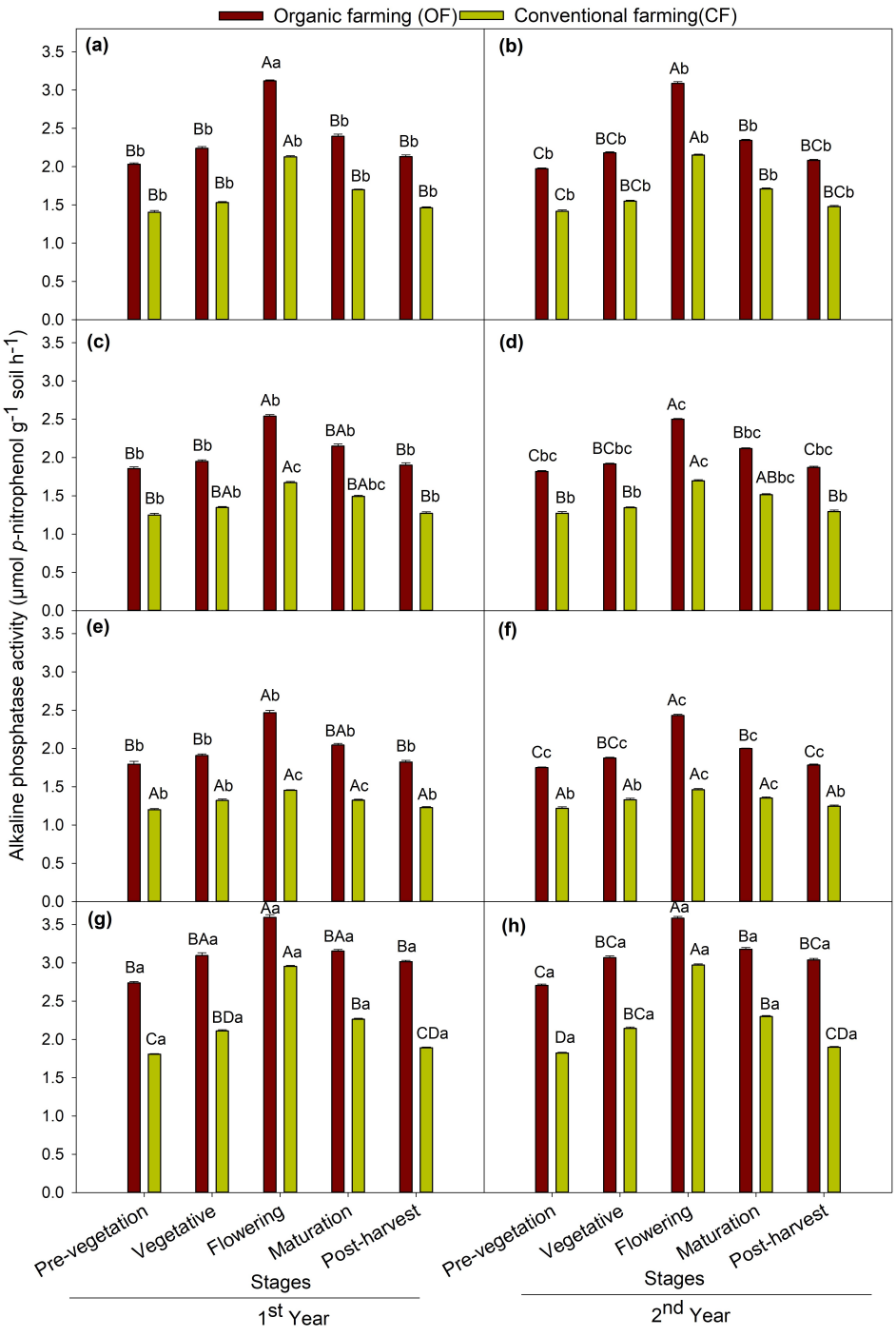

Figure 1. The alkaline phosphatase (ALP) activity (a-b chickpea; c-d mustard; e-f soybean; g-h maize) in organic farming (OF) and conventional farming (CF) for two consecutive years. Different upper cases denote significant differences ($p < 0.05$) between crop growth stages within same crop in both farming practice and different lower cases denote significant difference between different crop species in same growth stage estimated by Tukey's HSD test.

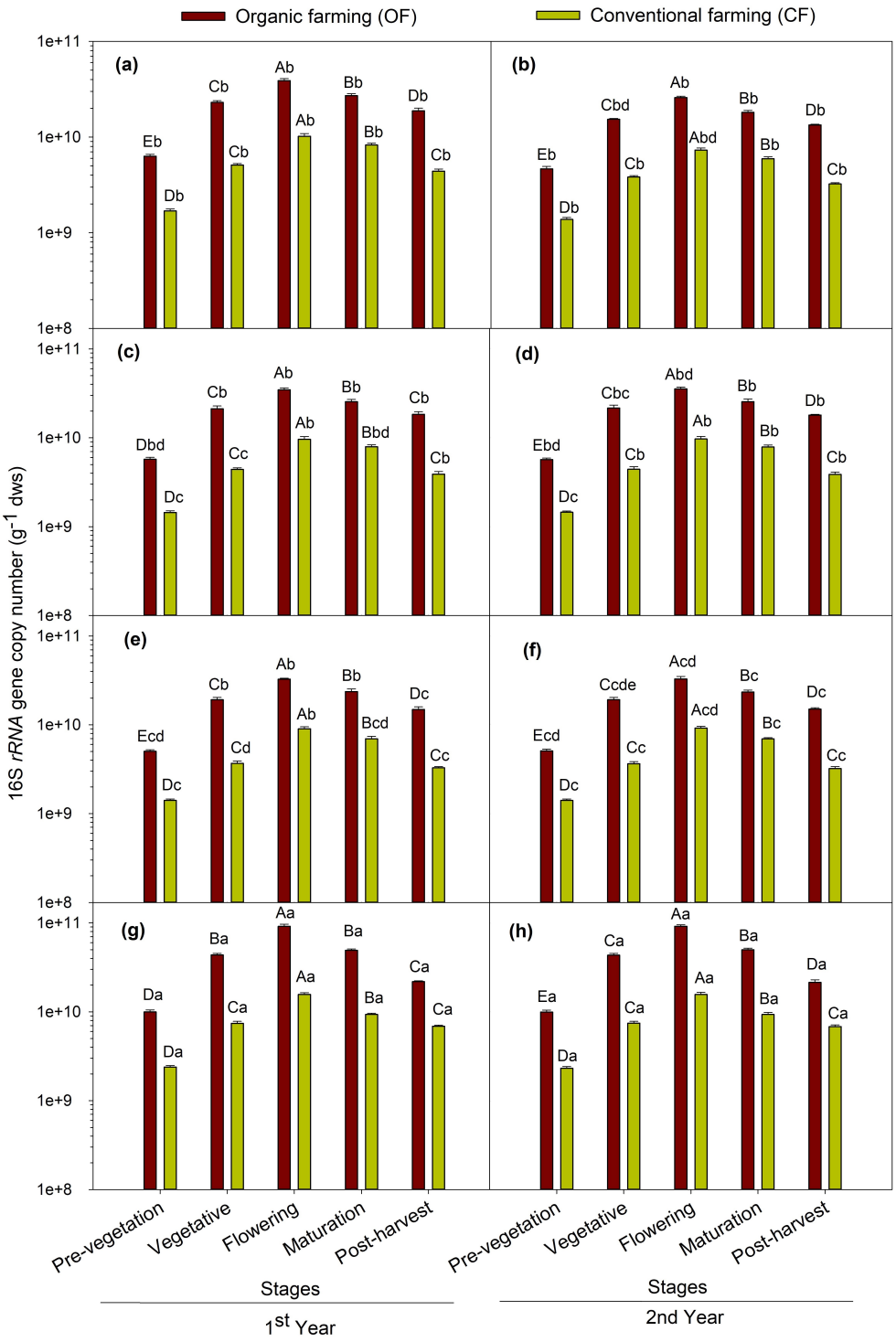

Figure 2. 16S rRNA gene abundance (copies g^{-1} dws) (a-b chickpea; c-d mustard; e-f soybean; g-h maize) in organic farming (OF) and conventional farming (CF) for two consecutive years. Different uppercase denote significant differences ($p< 0.05$) between crop growth stages within same crop in both farming practice and different lower cases denote significant difference between different crop species in same growth stage estimated by Tukey's HSD test.

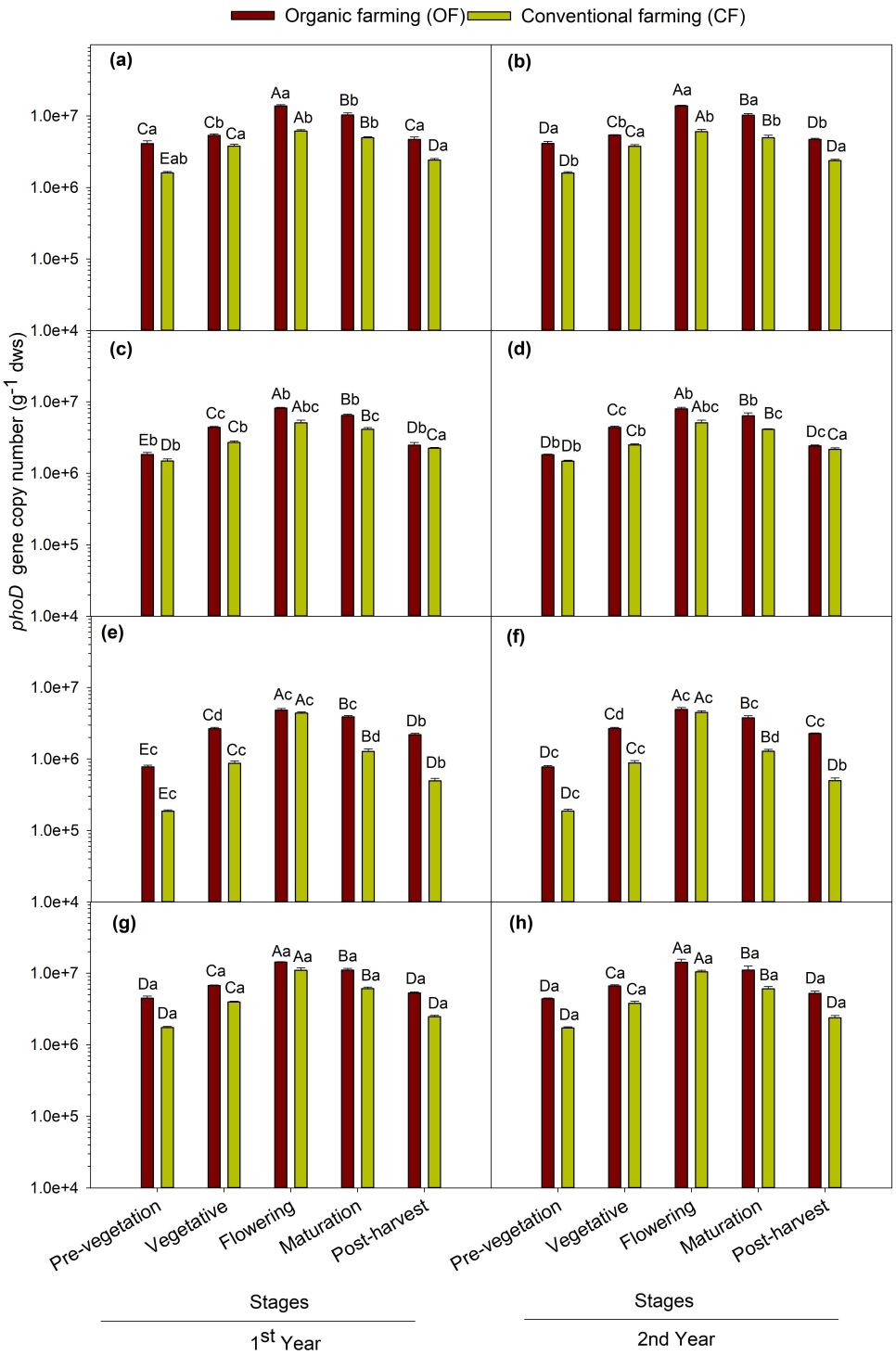

Figure 3. *phoD* gene abundance (copies g^{-1}dws) (a-b chickpea; c-d mustard; e-f soybean; g-h maize) in organic farming (OF) and conventional farming (CF) for two consecutive years. Different upper cases denote significant differences ($p< 0.05$) between crop growth stages within same crop in both farming practice and different lower cases denote significant difference between different crop species in same growth stage estimated by Tukey's HSD test.

Table 1. Soil physico-chemical properties (mean ± SD)

	Farming practice	
Soil parameters	Organic	Conventional
Soil texture (%) (sand:silt:clay)	32:64:4	32:64:4
Water holding capacity (%)	35.93 ± 1.31	35.17 ± 0.83
Bulk density (g cm-3)	1.25 ± 0.02	1.34 ± 0.03
Total organic carbon (%)	0.83 ± 0.02	0.59 ± 0.02
Total nitrogen (%)	0.18 ± 0.002	0.10 ± 0.003
Total P (μg g−1)	152.17 ± 12.37	127.08 ± 6.60
pH	7.38 ± 0.15	7.30 ± 0.15

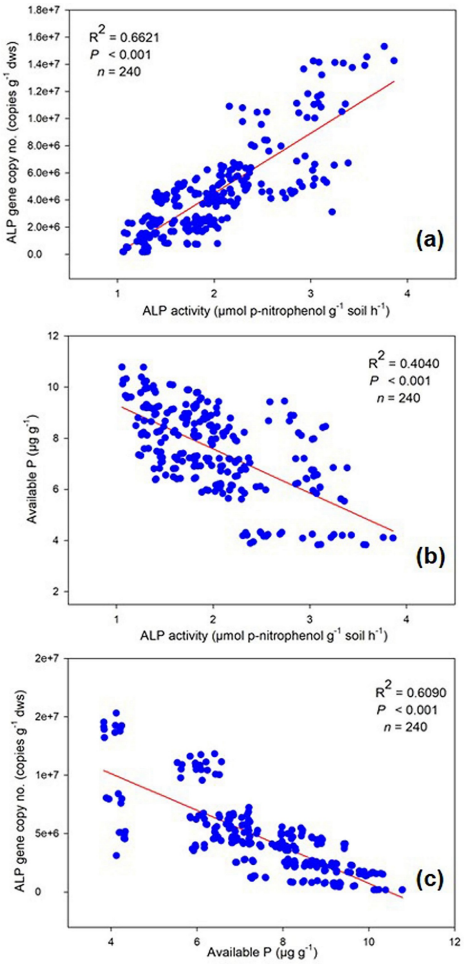

Figure 4. Relationship between ALP activity and ALP gene (*phoD* gene) copy number in organic and conventional farming and four crops at five different growth stages. Fig. 4b. Relationship between ALP activity and available P in organic and conventional farming and four crops at five different growth stages. Fig. 4c. Relationship between available P and ALP gene (phoD gene) copy number in organic and conventional farming and four crops at five different growth stages.

Table 2. Variation in mineral-N, available P, MBP, MBC, MBN with different crops at different growth stages in two different farming practices (organic farming (OF) and conventional farming (CF)) for two consecutive years. Different upper cases across column denote significant differences ($p < 0.05$) between crop growth stages within same crop and different lower cases denote significant difference ($p < 0.05$) between crops in same growth stage estimated by Tukey's HSD test.

Farming practice (FP)		Organic Farming (OF)					Conventional Farming (CF)				
Stages	Crop species	Mineral-N (μg g-1 dws)	Available-P (μg g-1 dws)	MBP (μg g-1 dws)	MBC (μg g-1 dws)	MBN (μg g-1 dws)	Mineral-N (μg g-1 dws)	Available-P (μg g-1 dws)	MBP (μg g-1 dws)	MBC (μg g-1 dws)	MBN (μg g-1 dws)
1st year											
1	Chick-pea	9.51 aD	9.04 aA	7.26 bD	202.05 bC	20.89 bD	10.01 aC	9.97 abA	5.24 bD	165.09 bC	18.86 bC
	Mus-tard	9.61 aD	9.06 aA	6.27 bD	191.45 bcD	17.76 cC	10.11 aC	10.03 abA	5.04 bD	124.36 cD	17.85 bD
	Soy-bean	9.58 aD	9.17 aA	5.88 bD	172.30 cC	14.47 dD	10.05 aC	10.45 aA	4.72 bD	122.83 cC	15.41 cD
	Maize	9.72 aD	8.99 aA	8.80 aD	238.43 aD	25.65 aD	10.33 aC	9.73 bA	6.32 aC	193.03 aC	21.01 aD
2	Chick-pea	11.40 aC	6.96 aC	12.10 bC	268.00 abB	25.24 cC	12.74 aB	8.14 aC	11.42 abBC	220.56 bB	21.97 bB
	Mus-tard	11.44 aC	7.01 aC	11.35 bcC	247.93 bC	22.59 cB	12.82 aB	8.36 aC	10.70 bcB	190.18 cB	20.34 bcC
	Soy-bean	11.97 aC	7.11 aC	10.02 cC	243.67 bB	18.51 bC	12.91 aB	8.45 aC	9.05 cBC	182.69 cB	19.11 cC
	Maize	11.81 aC	6.93 aC	14.71 aBC	291.73 aBC	35.40 aC	13.02 aB	7.94 aB	13.29 aB	246.90 aB	25.04 aC
3	Chick-pea	14.92 aA	4.07 aE	18.37 abA	401.06 aA	40.70 bA	15.31 aA	6.49 aE	14.63 abA	312.91abA	30.43 abA
	Mus-tard	14.59 aA	4.10 aE	16.51 bcC	389.52 aA	33.34 cA	15.18 aA	6.55 aE	13.94 bA	300.24 bA	28.50 bcA
	Soy-bean	14.70 aA	4.28 aE	15.43 cA	379.24 aA	24.88 dA	15.14 aA	6.60 aE	12.24 cA	261.89 cA	26.84 cA
	Maize	14.95 aA	4.04 aE	19.51 aA	416.64 aA	52.68 aA	15.29 aA	6.38 aD	15.74 aA	338.22 aA	32.63 aA
4	Chick-pea	12.71 aB	5.92 aD	15.30 abB	294.71 aB	29.39 bB	13.32 aB	7.24 aD	12.46 abAB	234.7 bB	26.67 bB
	Mus-tard	12.46 aB	5.99 aD	14.50 abB	264.52 abB	23.79 cB	13.41 aB	7.27 aD	10.91 bcB	202.73 cB	22.83 cB
	Soy-bean	12.81 aB	6.01 aD	13.87 bB	258.74 bB	21.83 cB	13.09 aB	7.41 aD	10.19 cB	196.39 cB	21.04 cB
	Maize	12.95 aB	5.89 aD	16.11 aB	322.69 aB	40.12 aB	13.53 aB	7.12 aC	13.59 aB	264.49 aB	29.38 aB
5	Chick-pea	9.89 aD	8.22 aB	11.88 abC	223.45 bC	24.16 bC	10.63 aC	9.09 abB	9.24 bC	173.85 bC	19.62 bC
	Mus-tard	9.85 aD	8.30 aB	10.81 bcC	196.64 cC	19.04 cC	10.96 aC	9.31 aB	8.98 bC	159.34 bC	17.89 cD
	Soy-bean	10.16 aD	8.57 aB	9.71 cC	182.08 cC	15.89 dD	10.75 aC	9.40 aB	8.14 bC	130.26 cC	16.62 cD
	Maize	10.09 aD	8.20 aB	13.11 aC	256.91 aD	28.36 aD	10.64 aC	8.47 bB	11.95 aB	210.34 aC	22.76 aD

Table 2. Continue

Farming practice (FP)		Organic Farming (OF)					Conventional Farming (CF)				
Stages	Crop species	Mineral-N (μg g-1 dws)	Available P (μg g-1 dws)	MBP (μg g-1 dws)	MBC (μg g-1 dws)	MBN (μg g-1 dws)	Mineral-N (μg g-1 dws)	Available-P (μg g-1 dws)	MBP (μg g-1 dws)	MBC (μg g-1 dws)	MBN (μg g-1 dws)
2nd year											
1	Chick-pea	9.52 aD	9.05 aA	7.28 bD	202.75 bC	21.19 bD	10.05 aC	10.02 aA	5.26 bD	166.73 bC	18.75 bC
	Mus-tard	9.63 aD	9.07 aA	6.29 bD	190.53 bcC	18.06 cC	10.13 aC	10.05 aA	5.03 bD	123.56 cD	18.01 bD
	Soy-bean	9.60 aC	9.15 aB	5.89 bD	173.68 cC	14.77 dD	10.07 aC	10.38 aA	4.73 bD	121.44 cC	15.35 cD
	Maize	9.76 aD	9.01 aB	8.81 aD	240.37 aD	25.66 aD	10.29 aC	9.75 aA	6.31 aC	192.05 aC	21.35 aD
2	Chick-pea	11.38 aC	6.94 aC	12.12 bC	267.88 aB	25.11 bC	12.79 aB	8.12 aC	11.41 abBC	218.87 bB	21.92 bB
	Mus-tard	11.44 aB	7.02 aC	11.37 bcC	248.23 abB	22.38 cB	12.85 aB	8.34 aC	10.71 bcB	189.63 cB	20.88 bcC
	Soy-bean	11.99 aC	7.10 aC	10.03 cC	242.28 abB	18.66 dC	12.94 aB	8.50 aC	9.07 cBC	181.31 cB	18.98 cC
	Maize	11.89 aA	6.94 aC	14.73 aC	290.88 aBC	35.50 aC	13.01 aB	7.92 aB	13.30 aB	247.49 aB	24.96 aC
3	Chick-pea	14.96 aA	4.05 aE	18.37 abA	400.75 abA	40.11 bA	15.38 aA	6.47 aE	14.64 abA	313.24 abA	30.70 abA
	Mus-tard	14.47 aA	4.12 aE	16.53 bcA	391.16 abA	33.46 cA	15.09 aA	6.57 aE	13.95 bA	298.79 bA	28.60 bcA
	Soy-bean	14.69 aA	4.27 aE	15.45 cA	376.47 bA	24.38 dA	15.07 aA	6.56 aE	12.24 cA	262.22 cA	26.40 cA
	Maize	14.83 aA	4.06 aE	19.53 aA	415.74 aA	52.60 aA	15.35 aA	6.41 aD	15.75 aA	337.85 aA	32.30 aA
4	Chick-pea	12.75 aB	5.90 aD	15.32 abB	293.37 abB	30.04 bB	13.34 aB	7.26 aD	12.47 abAB	235.34 bB	26.71 bB
	Mus-tard	12.44 aB	6.02 aD	14.52 abB	265.40 bB	24.13 cB	13.46 aB	7.25 aD	10.92 bcB	199.88 cB	23.09 cB
	Soy-bean	12.89 aB	6.02 aD	13.88 bB	260.10 bB	21.55 cB	13.07 aB	7.39 aD	10.20 cB	197.67 cB	21.29 cB
	Maize	12.97 aB	5.82 aD	16.11 aB	323.94 aB	40.88 aB	13.55 aB	7.15 aC	13.60 aB	263.04 aB	29.31 aB
5	Chick-pea	9.91 aD	8.20 aB	11.90 abC	221.99 bC	24.18 bC	10.66 aC	9.12 aB	9.26 bC	172.06 bC	19.84 bC
	Mus-tard	9.87 aD	8.32 aB	10.82 bcC	195.76 cC	19.07 cC	10.98 aC	9.33 aB	9.00 bC	160.06 bC	17.58 cD
	Soy-bean	10.10 aC	9.73 aA	8.58 cC	183.77 cD	15.92 dD	10.79 aC	9.38 aB	8.16 bC	129.49 cC	16.70 cD
	Maize	10.14 aD	13.09 aA	8.18 aC	257.22 aD	28.47 aD	10.67 aC	8.43 bB	11.96 aB	211.21 aC	22.30 aD

Stages;1- pre-vegetation; 2- vegetative; 3-flowering; 4- maturation; 5-post-harvest; MBC: microbial biomass carbon; MBN:microbial biomass nitrogen; MBP: microbial biomass phosphorous

Table 3. Variation in root and shoot biomass with different crops at different growth stages in two different farming practices (organic farming (OF) and conventional farming (CF)) for two consecutive years. Different uppercases across column denote significant differences ($p < 0.05$) between crop growth stages within same crop and different lowercases denote significant difference ($p < 0.05$) between crops in same growth stage estimated by Tukey's HSD test.

		1^{st} year				2^{nd} year			
Farming practice (FP)		Organic Farming (OF)		Conventional Farming (CF)		Organic Farming (OF)		Conventional Farming (CF)	
Stages	Crop species	Root biomass (g plant^{-1})	Shoot biomass (g plant^{-1})	Root biomass (g plant^{-1})	Shoot biomass (g plant^{-1})	Root biomass (g plant^{-1})	Shoot biomass (g plant^{-1})	Root biomass (g plant^{-1})	Shoot biomass (g plant^{-1})
1	Chick-pea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mus-tard	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Soy-bean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Maize	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	Chick-pea	0.92 bC	6.59 bC	0.69 bC	3.86 bC	0.91 bC	6.61 bC	0.71 bC	3.87 bC
	Mus-tard	0.75 cC	4.49 cC	0.61 bcC	3.51 bcC	0.77 bC	4.50 bC	0.62 bcC	3.53 bcC
	Soy-bean	0.65 dA	3.68 dA	0.54 cB	3.02 cB	0.66 bA	3.65 bA	0.56 cB	3.04 cB
	Maize	1.79 aC	10.39 aC	1.47 aC	8.79 aC	1.81 aC	10.40 aC	1.48 aC	8.81 aC
3	Chick-pea	2.73 bA	15.79 bA	2.15 bA	11.01 bA	2.78 bA	15.80 bA	2.16 bA	10.99 bA
	Mus-tard	2.56 bA	12.82 bA	2.11 bA	11.37 bA	2.54 bA	12.83 bA	2.13 bA	11.36 bA
	Soy-bean	1.34 cA	10.54 cA	1.08 cA	9.66 cA	1.35 cA	10.56 cA	1.09 cA	9.68 cA
	Maize	4.11 aA	37.20 aA	2.49 aA	32.35 aA	4.09 aA	37.23 aA	2.51aA	32.36 aA
4	Chick-pea	1.84 bB	9.59 bB	1.37 cB	7.89 cB	1.85 bB	9.65 bB	1.38 cB	7.90 cB
	Mus-tard	1.69 cB	8.55 cB	1.52 bB	8.02 bB	1.70 cB	8.56 cB	1.53 bB	8.04 bB
	Soy-bean	1.23 dA	8.11 dA	1.11 dA	7.83 dA	1.23 dA	8.10 dA	1.11 dA	7.85 dA
	Maize	2.45 aB	31.24 aB	2.08 aB	26.38 cB	2.46 aB	31.25 aB	2.10 aB	26.41 aB
5	Chick-pea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mus-tard	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Soy-bean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Maize	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Note: Stages;1- pre-vegetation; 2- vegetative; 3- flowering; 4-maturation; 5-post-harvest

4.3. Effect of crop types and growth stages

A significant variation ($p < 0.05$) was observed between crop species and their growth stages for both ALP activity and *phoD* gene abundance. Total 16S rRNA gene abundance was also significantly ($p < 0.05$) varied between different crops and growth stages. The soil samples under the maize crop exhibited the highest ALP activity and *phoD* gene abundance followed by chickpea, mustard and least in soybean grown soils (Fig. 1 and 3). The previous studies have shown that the effect of different plant species on soil microorganisms display variability in plant physiological traits involving root exudates (Eisenhauer et al., 2017) which differ with varying crop and physiological stages (Bardgett et al., 1999; Grayston et al., 1998). This may be due to differences in plant root biomass between different crops. The plant root biomass was maximum in maize crop followed by chickpea, mustard and soybean (Table 3). This study shows maximum ALP enzyme activity and population of bacterial ALP genes in maize crop. The reason behind this may be because maize crops result in greater root biomass which may secrete enhanced root exudates and therefore have pronounced effects on microbial populations. This outcome is in accordance with those of Wang et al. (2017) in which they reported that the plant root biomass showed a positive effect on soil microbial functions and structure. Neal et al. (2021) also reported that crop types impose greater impact on the abundance of rhizosphere phosphohydrolase gene in Brazilian soils.

Furthermore, the crop growth stages also significantly ($p < 0.05$) affected the ALP activity, *phoD* gene and 16S rRNA abundance. The maximum abundance of alkaline phosphatase gene harboring bacteria was detected at the flowering stage (Fig. 3) followed by maturation > vegetative > post-harvest and least during the pre-vegetation stage in all the four test crops. The highest *phoD* gene copy number as observed at the flowering stage in all the crops distinctly indicates the enhancement of total and *phoD* bacterial gene abundance at the flowering stage of crop due to optimal availability of nutrients. The results corroborate the findings of others (Smalla et al., 2001) and also supports the findings of Singh et al. (2013) which showed the influence of plant growth stage on 16S rRNA gene copy number and reported the similar trend to the present study. In contrary to the present study, Tamilselvi et al. (2015) reported maximum total culturable bacteria in maize crops active during the vegetative growth stage and decreased thereafter at harvest. This discrepancy in the result could be accredited to the disparity in growth conditions, soil type, climatic condition, methods used.

5. Conclusion

The present study documents the impact of different farming practices, crops and their growth stages on variations in alkaline phosphatase (ALP) activity, abundance of 16S rRNA and *phoD* gene. The population of bacterial *phoD* gene and potential ALP activity decreased significantly under conventional farming practice. The *phoD* gene abundance was correlated positively to activity of ALP enzyme but negatively with soil available P. Also, different crop species and growth stages exhibited significant effects on changes in bacterial population size and enzymatic activity indicating variations in the rhizosphere chemistry of different crops and their growth stages. This study provides in deep insight into changes in *phoD* copy number to unravel the understanding of processes involved in phosphorous turnover in a tropical agroecosystem. The comparative study of phosphorous mobilizing bacterial abundance in organically and conventionally managed crops will provide opportunity for development of more P-efficient sustainable agriculture system. Outcome derived through this investigation will be useful to enhance the nutrient use efficiency of available P in agroecosystem.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Acuña, et al. (2016). Bacterial alkaline phosphomonoesterase in the rhizospheres of plants grown in Chilean extreme environments. *Biology and Fertility of Soils*, *52*, 763-773.

Allen, et al. (1986). Chemical Analysis. *Methods of Plant Ecology*, 285-344.

Apel, et al. (2007). Phosphate control of phoA, phoC and phoD gene expression in Streptomyces coelicolor reveals significant differences in binding of PhoP to their promoter regions. *Microbiology*, *153*, 3527-3537.

Bardgett, et al. (1999). Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. *Functional Ecology*, *13*, 650-660.

Bhardwaj, et al. (2020). Variations in microbial community in a tropical dry deciduous forest across the season and topographical gradient assessed through signature fatty acid biomarkers. *Ecological Research*, *35*, 139-153.

Bhat, et al. (2017). Soil biological activity contributing to phosphorus availability in vertisols under long-term organic and conventional agricultural management. *Frontiers in Plant Science*, *8*, 1523-1523.

Brady, & Weil. (2007). *The nature and properties of soils*. New Jersey, USA: Prentice Hall.

Burns, et al. (1982). Enzyme activity in soil: location and a possible role in microbial ecology. *Soil Biology and Biochemistry*, *14*, 423-427.

Chakraborty, et al. (2011). Effect of long-term fertilizers and manure application on microbial biomass and microbial activity of a tropical agricultural soil. *Biology and Fertility of Soils*, *47*, 227-233.

Chandra, et al. (2007). Nutrient dynamics and decomposition rates during composting of sulphitation pressmud by different methods. *Journal of Environmental Engineering and Science*, *49*, 183-188.

Chen, et al. (2017). Response of soil phoD phosphatase gene to long-term combined applications of chemical fertilizers and organic materials. *Applied Soil Ecology*, *119*, 197-204.

Chen, et al. (2019a). Impact of long-term phosphorus fertilizer inputs on bacterial phoD gene community in a maize field. *Science of Total Environment*, *669*, 1011-1018.

Chen, et al. (2019b). Soil alkaline phosphatase activity and bacterial phoD gene abundance and diversity under long-term nitrogen and manure inputs. *Geoderma*, *349*, 36-44.

Chhabra, S., Brazil, D., Morrissey, J., Burke, J. I., O'Gara, F., & N Dowling, D. (2013). Characterization of mineral phosphate solubilization traits from a barley rhizosphere soil functional metagenome. *MicrobiologyOpen*, *2*(5), 717-724.

Crecchio, et al. (2001). Short-term effects of municipal solid waste compost amendments on soil carbon and nitrogen content, some enzyme activities and genetic diversity. *Biology and Fertility of Soils*, *34*, 311-318.

Devare, et al. (2004). Effect of Cry3Bb transgenic corn and tefluthrin on the soil microbial community: biomass, activity, and diversity. *Journal of Environmental Quality*, *33*, 837-843.

Edmeades, et al. (2003). *The long-term effects of manures and fertilisers on soil productivity and quality: a review. Nutrient Cycling in Agroecosystems*, *66*, 165-180.

Eisenhauer, et al. (2017). Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. *Scientific Reports*, *7*, 1-8.

Fraser, et al. (2015a;b). Linking alkaline phosphatase activity with bacterial phoD gene abundance in soil from a long-term management trial. *Geoderma*, *257*, 115-122.

Fraser, et al. (2017). Quantification of bacterial non-specific acid (phoC) and alkaline (phoD) phosphatase genes in bulk and rhizosphere soil from organically managed soybean fields. *Applied Soil Ecology*, *111*, 48-56.

Garg, et al. (2008). Phosphorus availability to maize as influenced by organic manures and fertilizer P associated phosphatase activity in soils. *Bioresource Technology*, *99*, 5773-5777.

Grayston, et al. (1998). Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biology and Biochemistry*, *30*, 369-378.

Guo, et al. (2012). Soil organic carbon dynamics in a dryland cereal cropping system of the Loess Plateau under long-term nitrogen fertilizer applications. *Plant and Soil*, *353*, 321-332.

Hallama, et al. (2022). The role of microbes in the increase of organic phosphorus availability in the rhizosheath of cover crops. *Plant and Soil*, 1-21.

Hu, et al. (2018). Effects of long-term fertilization on phoD-harboring bacterial community in Karst soils. *Science of Total Environment*, *628*, 53-63.

Jackson, et al. (1958). Soil chemical analysis. *NJ*, *498*, 183-204.

Kaufman, et al. (2020). Sustainability of soil organic matter at organic mixed vegetable farms in Michigan. *USA. Organic Agriculture*, *10*, 487-496.

Lagos, et al. (2016). Effect of phosphorus addition on total and alkaline phosphomonoesterase-harboring bacterial populations in ryegrass rhizosphere microsites. *Biology and Fertility of Soils*, *52*, 1007-1019.

Liu, et al. (2010). Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China. *Geoderma*, *158*, 173-180.

Long, et al. (2018). Phosphate levels influence the utilisation of rice rhizodeposition carbon and the phosphate-solubilising microbial community in a paddy soil. *Soil Biology and Biochemistry*, *118*, 103-114.

Luo, et al. (2017). Long-term fertilisation regimes affect the composition of the alkaline phosphomonoesterase encoding microbial community of a vertisol and its derivative soil fractions. *Biology and Fertility of Soils*, *53*, 375-388.

Mandal, et al. (2007). Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. *Bioresource technology*, *98*, 3585-3592.

McAndrew, et al. (1992). Long-term N fertilization of a Solonetzic soil-effects on chemical and biological properties. *Soil Biology and Biochemistry*, *24*, 619-623.

Muyzer, et al. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, *59*, 695-700.

Nannipieri, P., Giagnoni, L., Landi, L., & Renella, G. (2011).

Role of phosphatase enzymes in soil. Phosphorus in action. Springer.
Neal, et al. (2017). Land-use influences phosphatase gene microdiversity in soils. *Environmental Microbiology*, *19*, 2740-2753.
Neal, et al. (2021). Crop type exerts greater influence upon rhizosphere phosphohydrolase gene abundance and phylogenetic diversity than phosphorus fertilization. *FEMS Microbiology Ecology*, *97*, 33-33.
Olsen, et al. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate.* US Department of Agriculture.
Pershina. (2015). Comparative analysis of prokaryotic communities associated with organic and conventional farming systems. *PloS one*, *10*, 12-12.
Ragot, et al. (2015). phoD alkaline phosphatase gene diversity in soil. *Applied and Environmental Microbiology*, *81*, 7281-7289.
Ragot, et al. (2016). Total and active microbial communities and phoD as affected by phosphate depletion and pH in soil. *Plant and soil*, *408*, 15-30.
Richardson, A. E. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Functional Plant Biology*, *28*, 897-906.
Rochette, et al. (1999). Maize residue decomposition measurement using soil surface carbon dioxide fluxes and natural abundance of carbon-13. *Soil Science Society of America Journal*, *63*, 1385-1396.
Saha, et al. (2008). Soil enzymatic activity as affected by long term application of farm yard manure and mineral fertilizer under a rainfed soybean-wheat system in NW Himalaya. *European Journal of Soil Biology*, *44*, 309-315.
Sakurai, et al. (2008). Analysis of bacterial communities on alkaline phosphatase genes in soil supplied with organic matter. *Soil Science and Plant Nutrition*, *54*, 62-71.
Singh, et al. (2012). Temporal variation in methanogenic community structure and methane production potential of tropical rice ecosystem. *Soil Biology and Biochemistry*, *48*, 162-166.
Singh, et al. (2013). Bacterial community structure in the rhizosphere of a Cry1Ac Bt-Brinjal crop and comparison to its non-transgenic counterpart in the tropical soil. *Microbial Ecology*, *66*, 927-939.
Smalla, et al. (2001). Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. *Applied and Environmental Microbiology*, *67*, 4742-4751.
Sun, et al. (2015). Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. *Soil Biology and Biochemistry*, *88*, 9-18.
Tabatabai, & Bremner. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry*, *1*, 301-307.
Tamilselvi, et al. (2015). Effect of long-term nutrient managements on biological and biochemical properties of semi-arid tropical Alfisol during maize crop development stages. *Ecological Indicators*, *48*, 76-87.
Tan, et al. (2013). Long-term phosphorus fertilisation increased the diversity of the total bacterial community and the phoD phosphorus mineraliser group in pasture soils. *Biology and Fertility of Soils*, *49*, 661-672.
Walkley, et al. (1947). A critical examination of a rapid method for determining organic carbon in soils-effect of variations in digestion conditions and of inorganic soil constituents. *Soil science*, *63*, 251-264.
Wang, et al. (2017). Positive effects of plant diversity on soil microbial biomass and activity are associated with more root biomass production. *Journal of Plant Interactions*, *12*, 533-541.
Welsh, et al. (2009). High yielding organic crop management decreases plant-available but not recalcitrant soil phosphorus. *Journal of Agronomy*, *101*, 1027-1035.
Wu, et al. (2007). Cloning of the gene and characterization of the enzymatic properties of the monomeric alkaline phosphatase (PhoX) from Pasteurella multocida strain X-73. *FEMS Microbiology Letters*, *267*, 113-120.
Yang, et al. (2006). Organic phosphorus fractions in organically amended paddy soils in continuously and intermittently flooded conditions. *Journal of Environmental Quality*, *35*, 1142-1150.
Zappa, et al. (2001). Characterization of a highly thermostable alkaline phosphatase from the euryarchaeon Pyrococcus abyssi. *Applied and Environmental Microbiology*, *67*, 4504-4511.
Zhang, et al. (2014). Phosphorus composition and phosphatase activities in soils affected by long-term application of pig manure and inorganic fertilizers. *Communications in Soil Science and Plant Analysis*, *45*, 1866-1876.