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Phosphorus dynamics and solubilizing microorganisms in acid soils under different land uses of Lesser Himalayas of India

Ranjan Paul · Raj Deo Singh · A. K. Patra · D. R. Biswas · Ranjan Bhattacharyya · K. Arunkumar

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Abstract Although chemical and some soil physical properties have been studied under different land uses of the Lesser Himalayas of India, very limited information is available on soil biochemical properties. Hence we investigated phosphorus (P) fractions [total P (TP), inorganic P (P_i), organic P (P_o), available P, microbial biomass P (MBP)], enzyme activities [dehydrogenase, phosphatases, phytase], phosphate solubilizing bacteria (PSB) and fungi (PSF), and their correlations of acid soils (0–15 and 15–30 cm depths) under different land uses (viz, organic farming, maize–wheat, apple orchard, undisturbed oak forest and uncultivated land of the Indian Himalayas). All land use systems differed significantly for the P fractions, except TP. The highest values for TP, P_i, available P and MBP were found in soils under oak forest and lowest in uncultivated land. However, P_o content was highest in apple orchard. The organic farming (organic manures field under garden pea-french bean cropping system for > 10 years) maintained highest activities of dehydrogenase, acid phosphatase and alkaline phosphatase. The highest phytase activity and highest numbers of PSB (99×10^3 g^{-1} soil) and PSF (30×10^3 g^{-1} soil) were observed in the rhizosphere soils of oak forest. Significant relationships between soil P fractions and enzyme activities, except alkaline phosphatase, were recorded in surface soil layer. PSB and PSF population were also correlated significantly with P fractions and enzyme activities. This would lead us to understand the level of degradation of P pools due to cultivation over forest system and the suitable management practices needed for soil quality restoration.

Keywords Acid soils · Himalayas · Phosphorus · Soil enzymes · Phosphate solubilizers · Organic farming

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R. Paul (✉) · R. D. Singh · D. R. Biswas
Division of Soil Science and Agricultural Chemistry, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India
e-mail: ranjan.reliance@gmail.com

Present Address:
R. Paul
Division of Soil Resource Studies, ICAR-National Bureau of Soil Survey and Land use Planning, P.O. University Campus, Amravati Road, Nagpur, Maharastra 440033, India

A. K. Patra
ICAR-Indian Institute of Soil Science, Bhopal, M.P 462038, India

R. Bhattacharyya
ICAR-Centre for Environmental Science and Climate Resilient Agriculture, New Delhi 110012, India

K. Arunkumar
School of Agriculture, Lovely Professional University, Phagwara, Punjab 144411, India

Introduction

In recent years, fast changes in land-use and cropping systems in the Lesser Himalayas of India (600–2000 m above mean sea level) (Ram et al. 2013) are being witnessed due to a combination of several factors, including increased demographic pressure (Vision 2050, VPKAS 2015). This has resulted high demand for food, fodder, fuel wood and shelter along with much increased industrial activities. The region is endowed with diverse vegetation at altitudes, but acidic soil properties in the region often limit the biomass production. One of the major reasons of decreased biomass productivity is less P availability, due to its fixation in these acidic soils (Khan et al. 2007). Phosphorus fixation and precipitation in soils are generally highly dependent on pH and soil type. Low pH in hills leads to fixation of applied P in arable systems due to high activities of Al and Fe (Bucher et al. 2001).

Microorganisms are also involved in a variety of processes that affect the transformation of soil P. They enhance the P availability to plants by mineralizing organic P in soils and by solubilizing precipitated phosphates (Chen et al. 2006). Thus, management of P solubilizing microorganisms in soils plays a significant role to improve P availability. Furthermore the release of P by phosphate solubilizing microbes from insoluble and fixed/adsorbed forms is extremely important for P availability in soils. Microorganisms increase the availability of native P for plants through a variety of mechanisms, like the release of organic acids and hydrogen ions, production of siderophores and phosphatase enzymes to hydrolyze soil organic P (Surange et al. 1995; Dutton and Evans 1996; Nahas 1996).

To understand P availability, research is needed particularly on the potential phosphate solubilizing organisms, which are environmental friendly and economically feasible to farmers.

The hilly and mountainous areas in India vastly distributed all over the country with a larger area located in the Himalayas, extending up to 2500 km in length and 250–400 km in breadth and is distributed in 23 states. The majority of these areas (35% of the total geographical area of the country) has > 15% slope (Barah 2010). Himalayas having 90% forest and 10% arable land are capable of supporting production of a number of crops, because of varied agro-climatic conditions. Soils of the diverse agro-eco systems of the Indian Himalayas harbor a diverse group of adaptable and potential phosphate solubilizers that can be utilized for making agriculture sustainable in the region (Tomer et al. 2017). However, in recent times, the environmental degradation (due to faster deforestation, unrestricted grazing and destruction of vegetation) poses a threat to hill agriculture (Das et al. 2016).

Many studies in plain lands have confirmed that changes in land cover have an impact on soil biological properties and nutrient cycling, consequently affecting the organic P content (Aguiar et al. 2013; Maranguit et al. 2017; Prakash et al. 2017; Von Sperber et al. 2017). Soil enzyme activities are affected by change in soil management practices and land uses in plain lands (Li et al. 2014; Tian et al. 2016; van Leeuwen et al. 2017). However, limited information is available on the impacts of land use on soil P fractions and P cycling enzymes in the hilly regions, especially in the Indian Himalayas. Hence, the hypothesis was that soil P fractions would differ under different land use systems and management practices in arable systems. The present study aims to investigate oxidizable soil organic C, P fractions and P cycling enzyme activities in 0–15 and 15–30 cm soil layers and to quantify relationships between P cycling enzymes and selected soil chemical properties (CEC, pH, organic C and different P fractions) of different land use systems of Almora district of Uttarakhand, located in Lesser Himalayas of India.

Materials and methods

Site description and soil sampling

All soil samples were collected from different land use systems (viz, uncultivated barren land, organic farming plot, maize–wheat cropping system, undisturbed oak forest and apple orchard) at the Hawalbagh experimental farm (29°36′N, 79°40′E, and altitude: 1250 m amsl), ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS; In English: Vivekananda Institute of Hill Agriculture), located in the Indian Himalayas (http://www.vpkas.nic.in/) (Fig. 1). The map presented in Fig. 1 has been prepared with the help of Arc map 10.1 using GIS (Geographic Information System) tool.

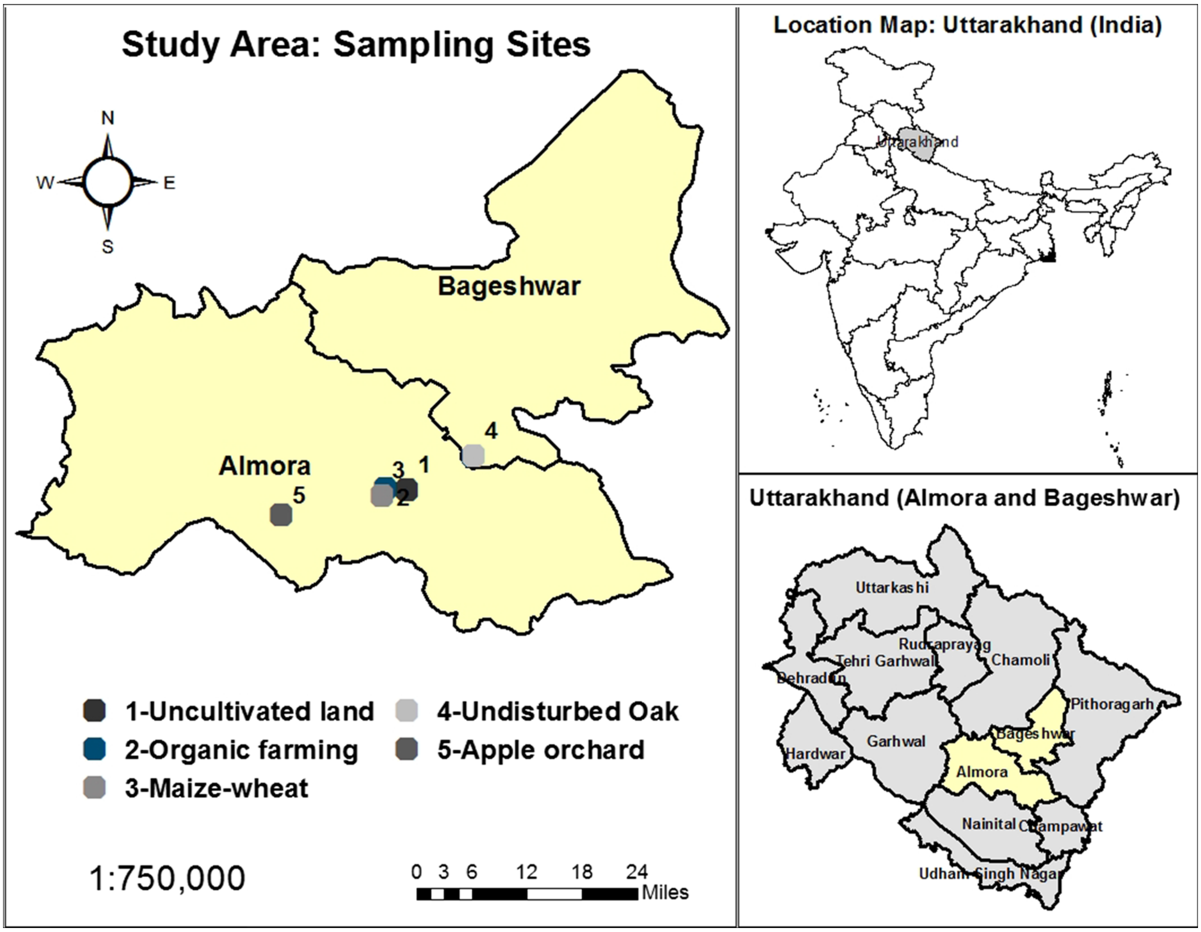

Fig. 1 Location of different land use systems

The Hawalbagh farm is about 13 km away from Almora, Uttarakhand. The climate of the region is sub-temperate, characterized by a moderate summer (May–June), extreme winter (Dec–Jan) and the southwest monsoon season (June–September) (Bhattacharyya et al. 2011). The precipitation ranges from 1000 to 1150 mm and the mean precipitation of the area is 1047 mm (mean of 30 years) (Bhattacharya et al. 2008). Precipitation increases soil moisture content that aids in soil organic matter decomposition process when temperature is optimum. During extreme winter, temperature becomes sub-optimal and gets snowfall at high altitudes. The main crops grown in these areas are wheat (*Triticum aestivum* L.), rice (*Oryza sativa*), barley (*Hordeum vulgare* L.), red kidney bean (Rajma; *Phaseolus vulgaris*) and black soyabean (*Gycine max* Merr.) (Bhattacharya et al. 2008). Taxonomically the soils of the study area is Typic Haplaquept (Ram et al. 2013). The agriculture in the area depends mainly on precipitation. The cropping and vegetation pattern of different land use systems are mentioned in Table 1.

Soil collection

The soil samples of two different depths (0–15 cm and 15–30 cm) were collected from all five different land use systems. Three composite soil samples were collected from each site for each depth. For making one composite sample, at least five soil cores were collected and pooled. Each sample was divided into two parts; first part of which was stored in refrigerator at 4 °C for determination of P cycling enzyme activities, and the second part was processed for other selected chemical analyses as detailed below. These samples were first air- dried in shade, ground with

Table 1 Description of different land use systems

Land use systems	Description
Uncultivated land	Barren land, no cultivation practices is followed
Organic farming	Garden Pea-French bean cropping system. Started in 2001. Input given during land preparation is FYM @ 10 Mg ha^{-1}
Maize–wheat	Rainfed maize–wheat grown in 10 years old continuous cropping system. Fertilizer dose: Maize @ 90–60–40 kg NPK ha^{-1} and wheat @ 120–60–40 kg NPK ha^{-1}
Undisturbed Oak (*Quercus* sp.) forest	Natural vegetation under oak forest
Apple orchard	More than 50 years old, surrounded by pine (*Pinus* sp.) and deodar (*Cedrus deodara*) forest. Fertilizer dose: FYM @ 10 kg per year age of tree. NPK @ 70–35–70 g year^{-1} per tree

wooden pestle and mortar, and passed through a 2 mm sieve. After grinding by wooden pastle, the samples were preserved in plastic containers for analyses.

Soil chemical analysis

Soil samples were analyzed for pH in a soil:solution of 1:2.5, using a glass electrode (Jackson 1973); oxidizable soil organic C (SOC) following Walkley and Black (Walkley and Black 1934); available P following Olsen et al. (Olsen et al. 1954); mechanical composition of experimental soils i.e., proportion of sand, silt and clay size particles following hydrometer method (Bouyoucos 1962) and cation exchange capacity (CEC) of the soil following ammonium acetate method as described by Jackson (1973). Total P (TP) in soils was determined by digestion method (Olsen and Sommers 1982); total organic P (P_o) by ignition method (Saunders and Williams 1955; Walker and Adams 1958); inorganic P (P_i) content by subtracting organic P from total P in the sample; microbial biomass P (MBP) following the method proposed by Brookes et al. (1982). For estimation of available P, acidic soil was extracted with Bray's P-I (Bray and Kurtz 1945) reagent and alkaline soil was extracted with 0.5 M $NaHCO_3$ (Olsen et al. 1954). Physico-chemical characteristics of surface as well as sub-surface soil are given in Table 2.

Analyses of soil biological properties

Enzymatic assay

Soil enzymes namely phosphatase (acid and alkaline) and phytase activities were assessed in the collected soil samples. Phosphatase activity was assayed by the method of Tabatabai and Bremner (1969) using substrate p-nitrophenyl phosphate and phytase activity by the method of Ames (1966).

Microbial population count

Serial dilution and plating technique was employed for enumerating the microbial population of soils as described by Rolf and Bakken (1987) and Chhonkar et al. (2007). Indigenous phosphate solubilizing bacteria was isolated from soil samples by enrichment culture techniques (Gaind and Gaur 1991) amended with 5% Mussourie rock phosphate (MRP). Phosphate solubilizing fungi was enumerated in rhizosphere soil samples by dilution plate count technique in Pikovskaya's agar medium (Ndiaye et al. 2000), modified by adding filter sterilized streptomycin (0.003%) and rose bengal (0.007%), to inhibit bacterial growth.

Detection of the phosphate solubilization ability of microorganisms

Phosphate solubilizing ability of microorganisms was detected using plate-screening method, in which, phosphate solubilizers produce clearing zones around the microbial colonies in media, and these were isolated (Pikovskaya 1948). Then, colonies were isolated and was put in petri-plates containing Pikovskaya's agar medium along with bromo-phenol blue, which produced yellow halos following a pH drop.

Table 2 General characteristics of the surface soil (0–15 cm) and sub-surface (15–30 cm) soil in different land use systems (each value is the mean of triplicate soil samples)

Properties	Land use systems				
	Uncultivated land	Apple plantation	Oak forest	Organic farming	Maize–wheat
Surface soil (0–15 cm)					
Sand (%)	53.7	49.7	29.6	25.6	17.6
Silt (%)	23.9	12.0	31.9	31.9	39.9
Clay (%)	22.4	38.3	38.5	42.5	42.6
Texture	Sandy clay loam	Sandy clay	Clay loam	Clay loam	Silty clay
pH	6.1^{a}	5.0^{c}	5.7^{ab}	6.2^{a}	6.0^{a}
EC(dS m^{-1})	0.2^{b}	0.1^{c}	0.1^{c}	0.3^{a}	0.2^{b}
Walkley and Black C (%)	0.6^{d}	0.8^{c}	3.6^{a}	1.2^{b}	0.9^{c}
CEC [cmol(p^{+}) kg^{-1}]	7^{e}	10^{cd}	13^{b}	17^{a}	11^{c}
Sub-surface (15–30 cm)					
Sand (%)	57.7	65.9	33.6	21.6	13.6
Silt (%)	23.9	11.7	35.9	31.9	43.9
Clay (%)	18.4	22.4	30.5	46.5	42.5
Texture	Sandy loam	Sandy clay loam	Clay loam	Silty clay	Silty clay
pH	6.4^{a}	5.1^{c}	5.9^{ab}	6.5^{a}	6.7^{a}
EC(dS m^{-1})	0.2^{a}	0.1^{b}	0.1^{b}	0.2^{a}	0.2^{a}
Walkley and Black C (%)	0.3^{c}	0.4^{c}	1.7^{a}	0.9^{b}	0.8^{b}
CEC [cmol(p^{+}) kg^{-1}]	6.0^{d}	8.0^{c}	10^{b}	14^{a}	9.0^{bc}

Values are means of the replications. Means with same lowercase letters within a row are not significantly different at $P < 0.05$, according to DMRT

Statistical analysis

Data were assessed by Duncan's multiple range tests ($P < 0.05$). Differences between mean values were evaluated by a two-way analysis of variance (ANOVA) (Gomez and Gomez 1984), using the software SAS 9.1.3. Pearson correlation analyses were performed using the SPSS programme (SPSS version 16.0).

Result and discussion

Soil P fractions

Total P

Mean of total P for 0–15 and 15–30 cm soil layers for all land use systems varied significantly ($P < 0.05$). Soils (0–15 and 15–30 cm layers) under oak forest (3405 kg ha^{-1}) had the highest TP followed by organic farming (2783 kg P ha^{-1}), and the lowest was in soils under uncultivated land (Fig. 2a). Similar type of results were obtained by Prakash et al. (2017), who observed significantly higher total P fractions in agroforestry system than maize–wheat and cotton-wheat cropping systems (Prakash et al. 2017). The study clearly revealed that surface soil (0–15 cm) had higher TP than sub surface soil (15–30 cm) by 12.4, 18.6, 19.9, 23.9 and 7.4% for uncultivated barren land, apple plantation, oak forest, organic farming and maize–wheat, respectively. Among major nutrients, P is the least mobile element. This is one of the main reasons for its low availability in the 15–30 cm soil layer, as organic matter inputs and fertilizer application activities are done at soil surface.

Total inorganic P

Mean of P_i under different land use systems varied from 1137 to 2764 kg ha^{-1} in the 0–15 cm soil layer (Fig. 2b). Soils (0–15 cm) under organic farming

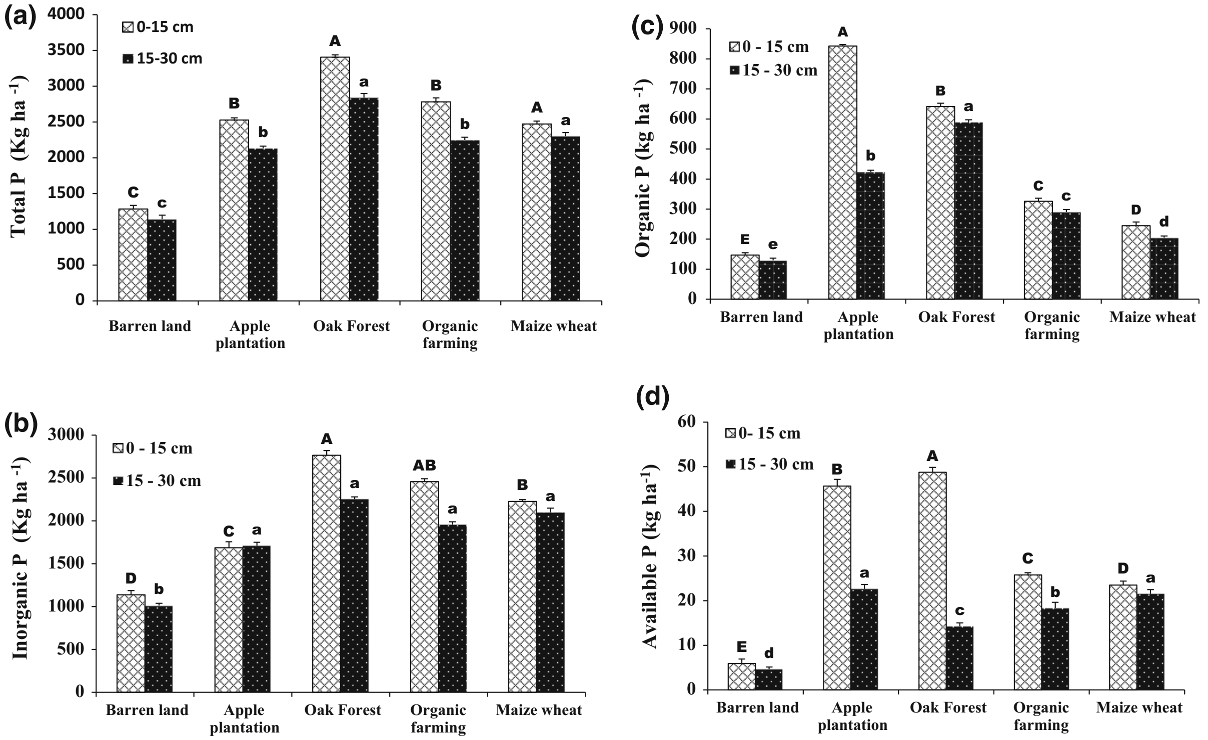

Fig. 2 **a** Total P (kg ha^{-1}), **b** total inorganic P (kg ha^{-1}), **c** total organic P (kg ha^{-1}), **d** available P (kg ha^{-1}) in soils under two depths as influenced by different land use systems in the Lesser Himalayan region of India. Bars with different letters are significant at $P < 0.05$, according to DMRT

contained more P_i (2457 kg ha^{-1}) than both apple plantation (1686 kg ha^{-1}) and maize–wheat (2227 kg ha^{-1}). Significantly lower proportion of P_i in soils under apple plantation may be due to relatively lower fertilizer P application than the other land-uses (Table 1) and higher uptake of P by apple trees (Prakash et al. 2017). Another reason might be immobilization of P in the apple rhizosphere by microorganisms for their structural build up to mineralize the organic residues added into the soil (Xavier et al. 2011). All land use systems showed significant differences in P_i in the 0–15 cm depth, whereas the differences were non-significant in the 15–30 cm depth. Surface soil contained significantly higher P_i than sub-surface soil and the differences were 12.3, 22.8, 25.7 and 6.2% for barren land, oak forest, organic farming and maize–wheat, respectively. Only soils under apple plantation and uncultivated land had similar P_i in surface and sub-surface soils.

Total organic P

Organic phosphorus (P_o) content varied widely in the 0–15 cm soil layer under different land use systems (Fig. 2c). Surface soil contained 13.2, 99.2, 8.9, 12.2 and 19.7% higher P_o than sub-surface soil (15–30 cm) for barren land, apple plantation, oak forest, organic farming and maize–wheat, respectively. The P_o in the 0–15 cm depth layer followed the order: apple plantation > oak forest > organic farming > maize–wheat > barren land. Continuous deposition of root and leaf litters on the surface and the reduced tillage makes the forest and apple plantation soil richer in terms of P_o than plots under organic farming (which is only 15 years old and cultivated) (Lobato et al. 2014). On the other hand, barren land without planting remained low in terms of P_o.

Available P

Mean data of available P content showed wide variations (from 5.9 kg ha^{-1} in barren land to 48.7 kg ha^{-1} in oak forest) under different land use systems in the soil surface (Fig. 2d). Oak forest had the highest available P, followed by apple plantation (45.6 kg ha^{-1}). Soils under oak forest, apple plantation, organic farming and maize–wheat system contained 721, 669, 333 and 295% more available P, respectively, than uncultivated land in the 0–15 cm surface soil. Available P content for surface soil in all land use systems were in the moderate to high range (Singh et al. 2005). However, for sub-surface soil, it was in the medium range. Possibly swift rates of P cycling in oak forest at surface via decomposition and the mineralization of more P rich litter helps to maintain greater concentrations of available P in the forest system (Bunemann et al. 2004), until uptake and accumulation in living biomass and leaching removes P from this cycle.

Microbial biomass P

Microbial biomass P (MBP) showed wide variations under different land use systems. Mean data of MBP varied from 2.5 (uncultivated land) to 27.1 µg P g^{-1} soil (oak forest) in soil surface (Fig. 3a). Organic farming system showed ~ 31% higher MBP than apple plantation (14.7 µg P g^{-1} soil) in soil surface. Surface soils contained 29, 84, 33, 75 and 339% higher MBP than sub-surface soils for barren land, apple plantation, oak forest, organic farming and maize–wheat, respectively.

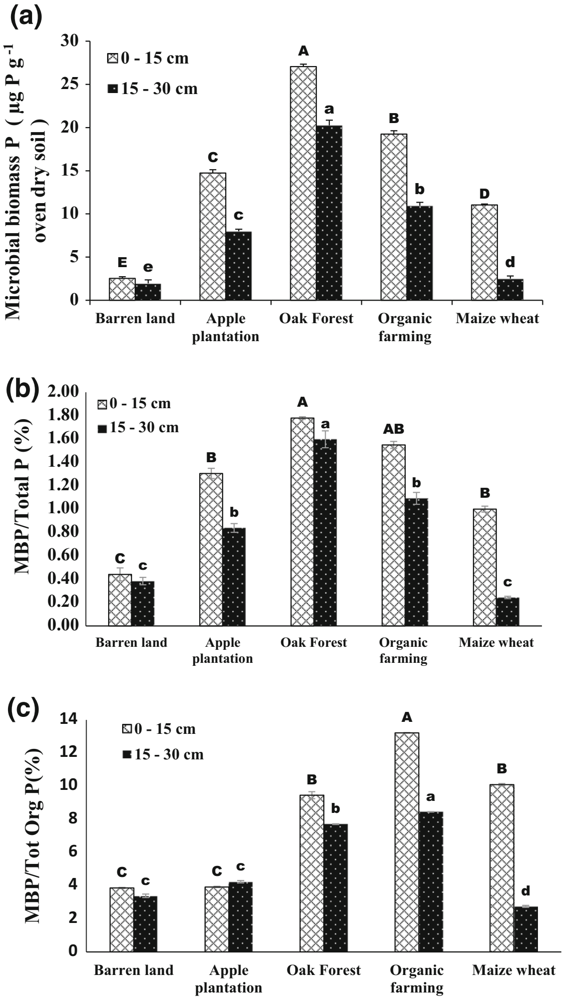

Fig. 3 **a** Microbial biomass P (kg ha^{-1}), **b** ratio of microbial P to total P (%), **c** ratio of microbial P to organic P (%) in soils under two depths as influenced by different land use systems in the Lesser Himalayan region of India. Bars with different letters are significant at $P < 0.05$, according to DMRT

Oak forest system had highest microbial P to total P ratio than the other land use systems (Fig. 3b). But, in the sub-surface layer, the ratio became more in soils under uncultivated land than the maize–wheat system. The significance of the microbial biomass as a pool for organic P is apparent from the microbial P to organic P ratios. Ratio of microbial P to organic P under different land use systems varied from 1.7 to 6.0% in the 0–15 cm soil (Fig. 3c). Soils under organic farming were having more ratio than apple plantation, oak forest and maize–wheat. All land use systems showed significant differences in the ratio in both depths. In apple plantation, P application near to apple tree along with FYM might have resulted in multiplication of microbes, which is known to swiftly immobilize substantial amounts of P when labile C is easily available (Silveira et al. 2010). Litter inputs and chemical composition of the litter may be crucial driver to alter the soil P concentrations in oak forest due to natural litter-fall and its recycling. Highest MBP in oak forest might be due to the deciduous nature of such type of forest. This enables microorganisms to decompose easily the leaf-litters, which are low in alkaloid content and easy to assimilate more and more P. It is also possible that the P mineralized was immediately fixed by the soil microbial community (Silveira et al. 2010) and also it can be attributed to nutrient pumping effect of trees

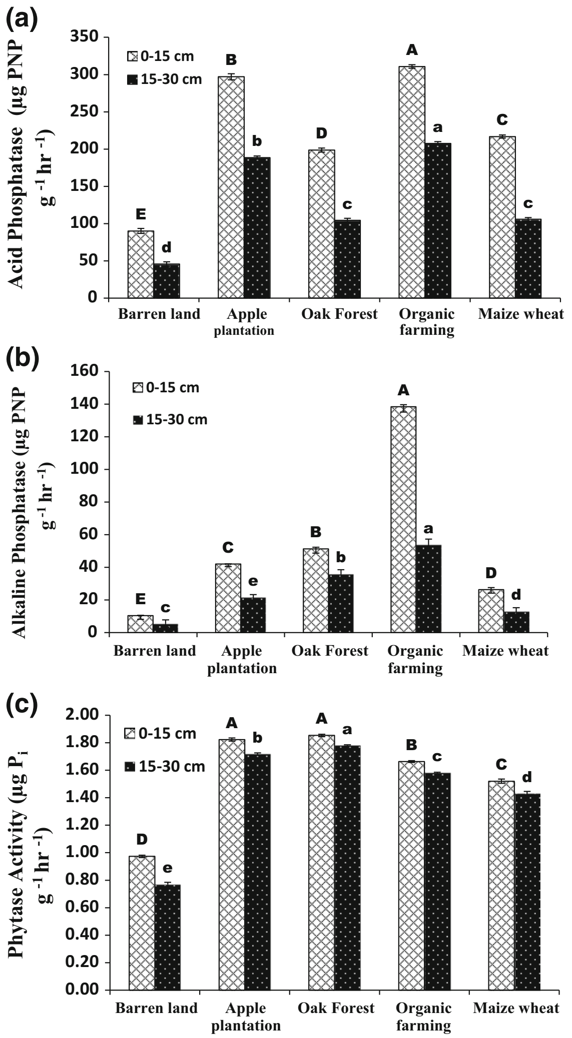

Fig. 4 Depth-wise distribution of **a** acid phosphatase activity, **b** alkaline phosphatase activity and **c** phytase activity in soils under different land use systems. Bars with different lowercase letters are significant at $P < 0.05$, according to DMRT

bringing the nutrients from lower soil horizons, which are redistributed to the surface soil through leaf fall (Farley and Kelly 2004).

P cycling enzyme activities

Mean data of acid phosphatase activity in the surface soil layer ranged from 90.1 µg PNP g^{-1} h^{-1} in the uncultivated land to 310.8 µg PNP g^{-1} h^{-1} in soils under organic farming system (Fig. 4a). All land use systems showed higher acid phosphatase activity in the 0–15 cm depth and a significant decrease in lower depth (15–30 cm) was observed. Thus, the acid phosphatase activities under different land use systems in the 0–30 cm soil depth (pooled data of two layers) followed the order: Organic farming > apple plantation > maize–wheat > oak forest > barren land. In the present investigation, acid phosphatase activity was found to be much higher than the alkaline phosphatase activity. This is due to the acidic reaction of the soils. Previous studies also verified that the phosphatase activity was strongly affected by soil pH (Mandal et al. 2007). Usually higher plants and microorganisms lack alkaline phosphatase (Kramer and Green 2000). Okur et al. (2009) reported that improved activity of soil enzymes in organic soils with FYM was due to the effects of increased organic C concentration in these soils on the size of the soil microbial population (Böhme and Böhme 2006). Masto et al. (2006) similarly reported that FYM addition to soil aids for C source, greater microbial biomass and phosphatase activity. Acid phosphatase activity was more in those systems, which received manure and it was supported by Saha et al. (2008) and Haynes and Swift (1988), who reported decreased acid phosphatase activity of soils with fertilization.

The average alkaline phosphatase activity ranged from 10.1 µg PNP g^{-1} h^{-1} in the uncultivated land to 140.2 µg PNP g^{-1} h^{-1} in soils under organic farming system (Fig. 4b). Alkaline phosphatase activities under different land use systems in the 0–15 cm soil depth followed the order: organic farming > oak forest > apple plantation > maize–wheat > barren land. The significantly greater activities of alkaline phosphatase in the organic farming systems and forests were due to enhanced microbial activity and diversity caused by organic matter inputs over the years. Manure addition to soils changes the persistence of soil enzymes (Parham et al. 2002). The decreased activities of both enzymes with increasing depth may be due to the declined microbial activity with depth. The declined activity with depth was highest in case of maize–wheat cropping system. This may be due to decreased rhizospheric effect with small increase in soil depth. Population of PSM was highest in oak forest system. This is in agreement with earlier studies conducted in Garhwal Himalayas, where the number of potential PSM varied depending on the soil characteristics, and the highest number was in oak forest (Pal 1998).

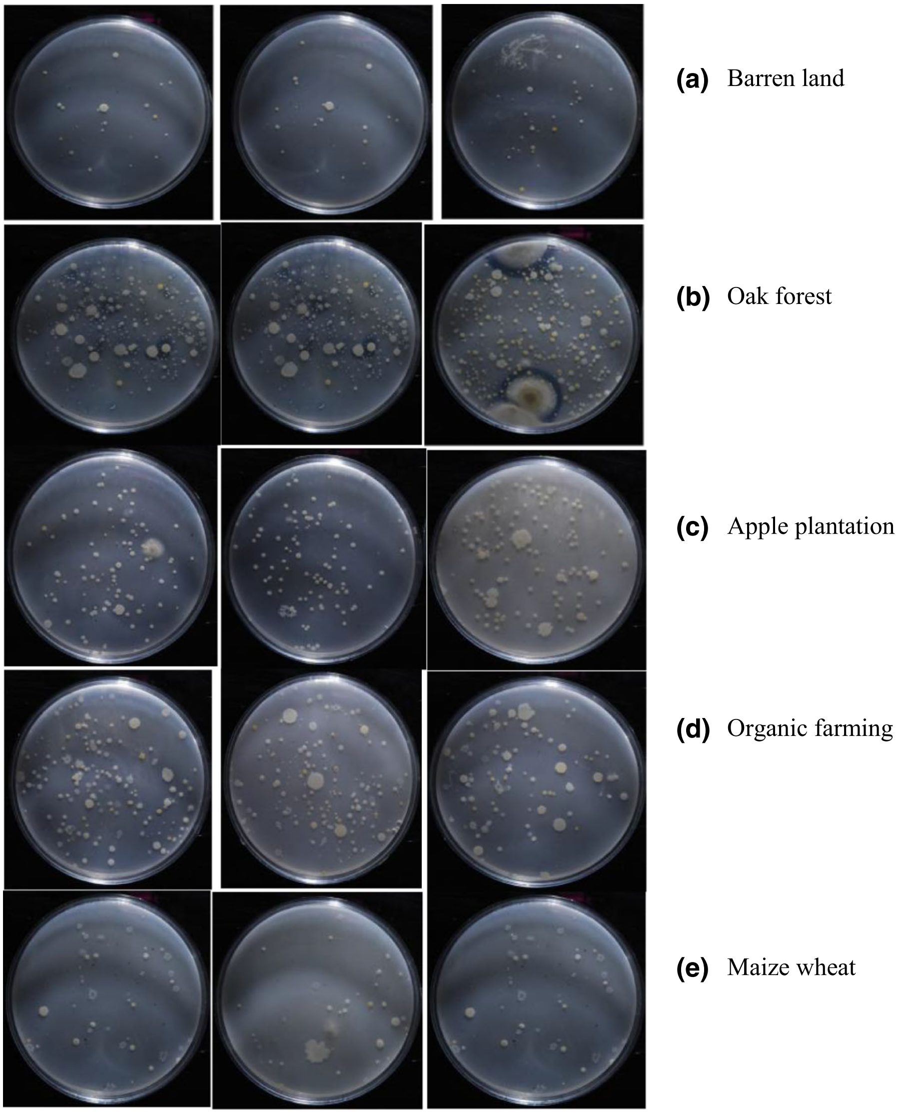

Fig. 5 Appearance of colonies of different groups of phosphate solubilizing microorganisms on Pikovskaya's agar plate (selective medium) in soils (0–15 cm depth) of different land use systems of the north-western Indian Himalayan region

Table 3 Total phosphate solubilizing bacteria (PSB) and phosphate solubilizing fungi (PSF) population in soils under different land use systems in the north-western Indian Himalayan region

	PSB ($\times 10^3$ g^{-1} soil)		PSF ($\times 10^3$ g^{-1} soil)	
	0–15 cm	15–30 cm	0–15 cm	15–30 cm
Barren land	45^{C}	32^{d}	4.1^{D}	0.9^{c}
Apple plantation	83^{B}	50^{c}	10^{C}	7.2^{b}
Oak forest	99^{A}	65^{b}	30^{A}	18^{a}
Organic farming	85^{B}	70^{a}	28^{A}	21^{a}
Maize–wheat	45^{C}	32^{d}	17^{B}	8^{b}

Values are means of the replications. Means with same upper or lowercase letters within a column are not significantly different at $P < 0.05$, according to DMRT

Oak forest system had higher phytase activity than apple plantation, organic farming and maize–wheat system (Fig. 4c) in the surface soil layer. All land use systems showed higher phytase activity in the 0–15 cm soil depth and the activity significantly decreased with increasing depth. The phytase activities under different land use systems in the 0–30 cm soil depth followed the order: oak forest > apple plantation > organic farming > maize–wheat > barren land. Phytases play an important role in the mineralization of P. Phytic acid is the prime storage form of P in many seeds and pollen (Mega 1982). Phytase is important in mobilizing organic P reserves for the growing seedlings and pollen germination (Walker 1974). The overall phytase activity is higher in oak forest followed by other land use systems. The forest system can be compared to a no-till condition, which promotes storage of water and nutrients in soils (Havlin et al. 1990), but also increased phytase activities, thereby increasing the P cycling in soils. This is supported by the results showing that microbial growth is favored in this kind of soil environment, resulting in greater enzyme activities (Dick 1994).

P solubilizing microorganisms

Population of phosphate solubilizing microorganisms (PSM) has been recorded from their growth on the culture media in petri-plates (Fig. 5). In the surface soil, PSB counts of apple plantation system (83×10^3 CFU g^{-1} soil) and organic farming system (85×10^3 CFU g^{-1} soil) were similar, but that was greater than maize–wheat and uncultivated land (Table 3). All land use systems showed higher PSB counts in the 0–15 cm layer and a significantly decreased in the lower layer.

The average PSF count ranged from 4.1×10^3 CFU g^{-1} soil in barren land to 30×10^3 CFU g^{-1} soil in oak forest in surface soil layer (Table 3). Organic farming system (28×10^3 CFU g^{-1} soil) had higher PSF count than maize–wheat system (17×10^3 CFU g^{-1} soil) and apple plantation (10×10^3 CFU g^{-1} soil). Organic matter addition in the form of green manure fertilization increased the fungal biomass as well as its diversity in the soil, when compared with chemical fertilizer addition (Kataoka et al. 2017). Therefore, the variations of PSF in different soils may be due to differences in organic matter contents in different ecosystems. The reasons for higher PSMs in the apple plantation systems is not clear from this study, thus warrants further investigation.

Relationships among soil P fractions, P cycling enzymes and P solubilizing bacteria

Correlation studies of soil enzyme activities with soil P fractions have shown a significant relationship ($P < 0.01$) between soil P fractions and enzyme activities, excepting for alkaline phosphatase in surface soils (Tables 4, 5). Soil bacteria, actinomycetes, and fungi hydrolyze organic P compounds and release phosphatases there. The enzyme activities significantly correlated with each other. Soil P fractions were significantly correlated with acid phosphatase, phytase and dehydrogenase in soil surface. Microbial biomass P was significantly correlated with all enzyme activities and other P fractions at both soil depths. Phosphate solubilizing bacteria and fungi were significantly correlated with enzyme activities, except for alkaline phosphatase in the surface soil; reasons being all studied soils were acidic. PSB is highly correlated with P fractions in both depths and significant correlation between acid phosphatase activity and organic P was observed. The results are very similar to the findings observed by Tarafdar and Jungk (1987) in wheat and clover rhizospheric soils. Acid phosphatase and total P content in different land use systems were significantly correlated. These results are in agreement with the earlier findings by Gianfreda et al. (2005). Soil P was apparently related closely to soil phosphatase activity. Generally soil microorganisms and plants produce extracellular phosphatase, which mineralizes and organically bound P and when available P is deficient in soils, micro-biota increase the production of extracellular phosphatase to enhance supply of inorganic P. However, higher P concentration inhibits the organisms to produce phosphatase enzyme. Such relationship has been explained that soil P supply and phosphatase activities were regulated by the negative feedback mechanisms (Olander and Vitousek 2000).

Conclusions

Thus, all P fractions viz., total P, inorganic P, organic P, available P and microbial biomass P in soils were

Table 4 Pearson correlation matrix among P solubilizing organisms, P pools and soil enzyme activities in the surface soil (0–15 cm layer) under different land uses

Properties	PSB	PSF	Av-P	Org-P	Inorg-P	TP	MBP	Ac-ase	Alk-ase	Phytase
PSB	1	0.68**	0.83**	0.63*	0.78**	0.89**	0.92**	0.54*	NS	0.87**
PSF		1	NS	NS	0.52*	0.57*	0.74**	0.78**	NS	0.52*
Av-P			1	0.92**	0.58*	0.83**	0.82**	0.57*	NS	0.94**
Org-P				1	NS	0.57*	0.59*	0.53*	NS	0.78**
Ignore-P					1	0.93**	0.88**	0.54*	NS	0.72**
TP						1	0.96**	0.60*	NS	0.91**
MBP							1	0.54*	0.54*	0.87**
Ac-ase								1	0.71**	0.78**
Alk-ase									1	NS
Phytase										1

PSB Phosphate solubilizing bacteria, *PSF* Phosphate solubilizing fungi, *Av-P* Available P, *Org-P* Organic P, *Inorg-P* Inorganic P, *TP* Total P, *MBP* Microbial Biomass P, *Ac-ase* Acid Phosphatase, *Alk-ase* Alkaline phosphatase

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Table 5 Pearson correlation matrix among P solubilizing organisms, P pools and enzyme activities in the 15–30 cm soil layer under different land uses

Properties	PSB	PSF	Av-P	Org-P	Inorg-P	TP	MBP	Ac-ase	Alk-ase	Phytase
PSB	1	0.93**	NS	0.58*	0.77**	0.78**	0.78**	NS	0.82**	0.68**
PSF			NS	0.56*	0.72**	0.74**	0.77**	0.57*	0.94**	0.70**
Av-P				NS	0.64**	0.61*	NS	0.74**	NS	0.77**
Org-P					0.60*	0.76**	0.91**	NS	NS	0.82**
Ignore-P						0.97**	0.59*	NS	0.54*	0.81**
TP							0.73**	NS	0.57*	0.89**
MBP								NS	0.69**	0.71**
Ac-ase									0.72**	0.69**
Alk-ase										0.64*
Phytase										1

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

significantly affected by different land uses. Changes in enzyme activities in different systems were much higher than P fractions, indicating that enzymes activities are much more sensitive to land use changes. Soils under Oak forest were best in terms of having the enzyme activities, P fractions and PSB. Regularly manured apple orchard and organic farming were next to oak forest. Correlation matrices among different parameters of enzyme activities, P pools and PSM were positive, indicating that these were strongly associated in soils irrespective of land use and crop management practices. A good population of PSM has been observed almost in all systems. Thus, this study has generated information on the extent of P pools, enzyme activities and P solubilizers of different land use systems of Almora district of Uttarakhand, located in the Lesser Himalayas of India. Further research may concentrate on impacts of other land use systems on soil P dynamics in winter and summer seasons of the Indian

Himalayas with sampling in more area. Also there is need for isolation and identification of PSM in this area to provide information of new species, which may have high potential to enhance P solubilization from the fixed or mineral- P in the acid soils of the region, and subsequent use as P-biofertilizers.

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Compliance with ethical standards

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References

Aguiar AD, Cândido CS, Carvalho CS, Monroe PH, de Moura EG (2013) Organic matter fraction and pools of phosphorus as indicators of the impact of land use in the Amazonian periphery. Ecol Ind 30:158–164

Ames BN (1966) Assay of inorganic phosphate, total phosphate and phosphatases. Methods Enzymol 8:115–118

Barah BC (2010) Hill agriculture: problems and prospects for mountain agriculture. Indian J Agric Econ 65:584–601

Bhattacharya R, Kundu S, Prakash V, Gupta HS (2008) Sustainability under combined application of mineral and organic fertilizers in a rainfed soybean-wheat system of the Indian Himalayas. Eur J Agron 28:33–46

Bhattacharyya R, Kundu S, Srivastva AK, Gupta HS, Prakash V, Bhatt JC (2011) Long term fertilization effects on soil organic carbon pools in a sandy loam soil of the Indian sub-Himalayas. Plant Soil 341:109–124

Böhme L, Böhme F (2006) Soil microbiological and biochemical properties affected by plant growth and different long-term fertilization. Eur J Soil Biol 42:1–12

Bouyoucos GJ (1962) Hydrometer method improved for making particle size analysis of soils. Agron J 54:464–465

Bray RH, Kurtz LT (1945) Determination of total, organic and available forms of phosphorus in soils. Soil Sci 59:39–45

Brookes PC, Powlson DS, Jenkinson DS (1982) Measurement of microbial biomas phosphorus in the soil. Soil Biol Biochem 14:319–329

Bucher M, Rausch C, Daram P (2001) Molecular and biochemical mechanisms of phosphorus uptake into plants. J Plant Nutr Soil Sci 164(2):209–217

Bunemann EK, Bossio D, Smithson PC, Frossard E, Oberson A (2004) Microbial community composition and substrate use in a highly weathered soil as affected by crop rotation and P fertilization. Soil Biol Biochem 36:889–901

Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tri-calcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Chhonkar PK, Bhadraray S, Patra AK, Purakayastha TJ (2007) Experiments in Soil Biology and Biochemistry. Westville Publishing House, New Delhi, p 182

Das A, Ramkrushna GI, Makdoh B, Sarkar D, Layek J, Mandal S, Lal R (2016) Managing soils of the lower Himalayas. Encyclopedia of Soil Science, 3rd edn. Springer, New York

Dick RP (1994) Soil enzyme activities as indicators of soil quality. In: Doran JW, Coleman DC, Bezdicek DF, Stewart BA (eds) Defining soil quality for a sustainable environment. Soil Science Society of America, Madison, pp 107–124

Dutton VM, Evans CS (1996) Oxalate production by fungi: its role in pathogenicity andecology in the soil environment. Can J Microbiol 42:881–895

Farley KA, Kelly EF (2004) Effects of afforestation of a pa´ ramo grassland on soil nutrient status. Forest Ecol Manag 195:271–290

Gaind S, Gaur AC (1991) Thermotolerant phosphate solubilizing microorganisms and their interactions in mung bean. Plant Soil 133:141–149

Gianfreda L, Rao MA, Piotrowska A, Palumbo G, Colombo C (2005) Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution. Sci Total Environ 341:265–279

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research. Wiley, New Jersey

Havlin JL, Kissel DE, Maddux LD, Claassen MM, Long JH (1990) Crop rotation and tillage effects on soil organic carbon and nitrogen. Soil Sci Soc Am J 54:448–452

Haynes RJ, Swift RS (1988) Effects of lime and phosphate additions on changes in enzyme activities, microbial biomass and levels of extractable nitrogen, sulphur, and phosphorus in an acid soil. Biol Fertil Soils 6:153–158

Jackson ML (1973) Soil chemical analysis. Prentice Hall of India, New Delhi, pp 10–114

Kataoka R, Nagasaka K, Tanaka Y, Yamamura H, Shinohara S, Haramoto E, Hayakawa M, Sakamoto Y (2017) Hairy vetch (*Vicia villosa*), as a green manure, increases fungal biomass, fungal community composition, and phosphatase activity in soil. Appl Soil Ecol 117:16–20

Khan MS, Almas Z, Parvaze AW (2007) Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. Agron Sustain Dev 27:29–43

Kramer S, Green DM (2000) Acid and alkaline phosphatase dynamics and their relationship to soil microclimate in semiarid woodland. Soil Biol Biochem 32:179–188

Li Q, Liang JH, He YY, Hu QJ, Yu S (2014) Effect of land use on soil enzyme activities at Karst area in Nanchuan. Plant Soil Environ 60:15–20

Lobato EMSG, Fernandes AR, Lobato AKS, Guedes RS, Netto JRC, Moura AS, Marques DJ, Avila FW, Borgo JDH (2014) The chemical properties of a clayey oxisol from Amazonia and the attributes of its phosphorus fractions. J Food Agric Environ 2:1328–1335

Mandal A, Patra AK, Singh D, Swarup A, Masto RE (2007) Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. Biores Technol 98:3585–3592

Maranguit D, Guillaume T, Kuzyakov Y (2017) Land-use change affects phosphorus fractions in highly weathered tropical soils. Catena 149:385–393

Masto RE, Chhonkar PK, Singh D, Patra AK (2006) Changes in soil biological and biochemical characteristics in a long-term field trial on a sub-tropical Inceptisol. Soil Biol Biochem 38:1577–1582

Mega JA (1982) Phytate: its chemistry, occurrence, food interactions, nutritional nutritional significance, and methods of analysis. J Agric Food Chem 30:1–9

Nahas E (1996) Factors determining rock phosphate solubilization by microorganism isolated from soil. World J Microb Biotechnol 12:18–23

Ndiaye EL, Sandeno JM, Mcgrath D, Dick RP (2000) Integrative biological indicators for detecting changes in soil quality. Am J Alternative Agric 15:26–36. https://doi.org/10.1017/S0889189300008432

Okur N, Altindisli A, Cengel M, Gocmez S, Kayikcioglu HH (2009) Microbial biomass and enzyme activity in vineyard soils under organic and conventional farming systems. Turk J Agric Forestr 33:413–423

Olander LP, Vitousek PM (2000) Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry 49:175–191

Olsen SR, Sommers LE (1982) Phosphorus. In: Page AL, Miller RH, Keeney DR (eds) Methods of Soil Analysis Part 2 Chemical and Microbiological Properties. American Society of Agronomy, Madison, Wisconsin, pp 403–442

Olsen SR, Cole CV, Watanable FS, Dean LA (1954) Estimation of availablephosphorus in soil by extraction with sodium bicarbonate. US Department of Agriculture, Washington

Pal SS (1998) Interactions of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. Plant Soil 198:169–177

Parham JA, Deng SP, Raun WR, Johnson GV (2002) Long-term cattle manure application in soil I. Effect on soil phosphorus levels, microbial biomass C, and dehydrogenase and phosphatase activities. Biol Fertil Soils 35:328–337

Pikovskaya RI (1948) Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiologiya 17:362–370

Prakash D, Benbi DK, Saroa GS (2017) Land-use effects on phosphorus fractions in Indo-Gangetic alluvial soils. Agroforestr Syst. https://doi.org/10.1007/s10457-016-0061-6

Ram J, Singh SP, Jadav RC, Mahapatra SK, Sidhu GS, Sarkar D, Sharda VN (2013) Soil Erosion in Uttarakhand. NBSS Publ. 156, NBSS&LUP, Nagpur, p 53

Rolf AO, Bakken LR (1987) Viability of soil bacteria: optimization of plate-counting technique and comparison between total counts and plate counts within different size groups. Microb Ecol 13:59–74

Saha S, Prakash V, Kundu S, Kumar N, Mina BL (2008) Soil enzymatic activity as affected by long term application of farm yard manure and mineral fertilizer under a rainfed soybean wheat system in N-W Himalaya. Eur J Soil Biol 44:309–315

Saunders WMH, Williams EG (1955) Observations on the determination of total organic phosphorous in soil. J Soil Sci 6:254–267

Silveira ML, Vendramini JMB, Sollenberger LE (2010) Phosphorus management and water quality problems in grazing land ecosystems. Int J Agron 2010:517603

Singh D, Chhonkar PK, Dwivedi BS (2005) Manual on soil. Plant and water analysis. Westville Publishing House, New Delhi, p 200

Surange S, Wollum AG, Kumar N, Nautiyal CS (1995) Characterization of Rhizobium from root nodules of leguminous trees growing in alkaline soils. Can J Microbiol 43:891–894

Tabatabai MA, Bremner JM (1969) Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 4:301–307

Tarafdar JC, Jungk A (1987) Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biol Fertil Soils 3:199–204

Tian J, Wei K, Condron LM, Chen Z, Xu Z, Chen L (2016) Impact of land use and nutrient addition on phosphatase activities and their relationships with organic phosphorus turnover in semi-arid grassland soils. Biol Fertil Soils 52 (5):675–683

Tomer S, Suyal DC, Shukla A, Rajwar J, Yadav A, Shouche Y, Goel R (2017) Isolation and characterization of phosphate solubilizing bacteria from Western Indian Himalayan soils. 3 Biotech 7(2):95

van Leeuwen JP, Djukic I, Bloem J, Lehtinen T, Hemerik L, de Ruiter PC, Lair GJ (2017) Effects of land use on soil microbial biomass, activity and community structure at different soil depths in the Danube floodplain. Eur J Soil Biol 79:14–20

Vision 2050 (2015) Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora 263601, Uttarakhand, Indian Council of Agricultural Research, India (http://vpkas.nic.in/vision2050.pdf)

Von Sperber C, Stallforth R, Du Preez C, Amelung W (2017) Changes in soil phosphorus pools during prolonged arable cropping in semiarid grasslands. Eur J Soil Sci. https://doi.org/10.1111/ejss.12433

Walker KA (1974) Change in phytic acid and phytase during early development of *Phaseolus vulgaris* L. Planta 116:91–98

Walker TW, Adams AFR (1958) Studies on soil organic matter: I. Influence of phosphorous content of parent materials on accumulation of carbon, nitrogen, sulphur and organic phosphorous in grassland soils. Soil Sci 85:307–318

Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci 37:29–38

Xavier FAS, Almeida EF, Cardoso IM, Mendonca ES (2011) Soil phosphorus distribution in sequentially extracted fractions in tropical coffee-agroecosystems in the Atlantic Forest biome, Southeastern Brazil. Nutr Cycl Agroecosyst 89:31–44