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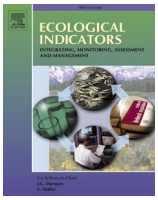

Original Articles

Land use change alters phosphatase enzyme activity and phosphatase-harboring microbial abundance in the subalpine ecosystem of southeastern Qinghai-Tibet Plateau, China

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ABSTRACT

In the presence of limited phosphorus (P) in terrestrial ecosystems, exploring how land use change (LUC) affects phosphatase enzyme activity and microbial communities is important for managing soil P, because microorganisms carry out the majority of P cycling in the soil through producing phosphatase enzymes. In this study, we explored the impact of LUC on P availability, phosphatase enzyme, the abundance of phosphatase-encoding genes, and microorganisms. We collected 168 soil samples at soil depths of 0–20 cm and 20–40 cm from seven sampling sites, each of which represented by four different land uses: artificial forests (AF), farmlands (FL), natural forests (NF), and shrubland (SL). We analyzed phosphatase-encoding genes and microbes from metagenome datasets. Results indicated that P availability substantially increased following NF to FL conversion. In contrast, phosphatase enzyme activity significantly decreases when NF is converted to different land uses, due to the decline of soil organic carbon (SOC), moisture content (MC) and total nitrogen (TN). We have also detected 13 P solubilizing and mineralizing genes. The *phoD* and *gcd* were the dominant mineralizing and solubilizing genes, respectively. Farmland had higher *gcd* gene abundance, while NF had significantly higher *phoD* gene abundance. The *gcd* gene abundance were mainly governed by pH and total P, whereas pH and available P were the primary factors controlling *phoD* gene. MC, SOC and TN regulated other genes detected in this study. With regard to the dominant *gcd*-harboring phyla, Acidobacteria, Proteobacteria, Bacteroidetes and Gemmatimonadetes were the dominant, while Proteobacteria, Acidobacteria, Actinobacteria, and Candidatus_Rokubacteria were the dominant *phoD*-harboring phyla. The majority of *gcd* and *phoD*-harboring microorganisms were primarily controlled by pH, available P and total P. However, some phyla also regulated by MC, SOC, and TN. In general, our findings suggested that LUC significantly alters phosphatase enzymes and the abundance of phosphate-encoding genes and microbes. These changes have significant implications for soil P cycling.

1. Introduction

The size and coverage of natural forests has diminished tremendously for the last couple of decades (Bonan, 2008; Heino et al., 2015). The loss in natural forests primarily attributed to the increased demand for alternative land-use patterns such as urban and agricultural land expansion and plantation forests (Busch and Ferretti-Gallon, 2017). Conversion of natural forests to different land uses may alter land cover, biota, and biogeochemical cycles. Previous studies on the effect of the loss of natural forests have demonstrated that land-use change has an

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impact on soil physical, chemical and biological properties (McGrath et al., 2001). Phosphorus (P) has been identified as an important macronutrient for living organisms, however most of P is found in insoluble forms, which limits plant and microbial growth (Vitousek et al., 2010). Changes in land use pattern influence the chemical makeup of distinct P species in soil and, thus their availability (Condron et al., 2005). Losses of available P due to land use change (LUC) will reduce the ecosystem's productivity. Given the scarcity of P in terrestrial ecosystems (Vitousek et al., 2010), understanding the effect of LUC on available P has paramount important to establish soil P management strategies and increase land productivity. In addition, it is crucial to examine the role of microorganisms in the facilitation of soil P cycle.

Previous studies by (Alori et al., 2017; Richardson and Simpson, 2011) identified that microorganisms can facilitate the bioavailable soil P and have a substantial role on the soil P cycle. Phosphorus in soils can occur in a variety of inorganic and organic forms that are difficult for plants to absorb due to its high reactivity. As a result, the presence of microbes appears to have a significant impact on the availability of a more diversified metabolic capacities that increase the bioavailability of various insoluble P forms in soils (Alori et al., 2017; Richardson and Simpson, 2011). For example, phosphate solubilizing microorganism (PSM) is a diverse group of microorganisms distributed among bacteria, fungi, and archaea, and have the ability to convert recalcitrant P forms into available P (Chen et al., 2006; Ragot et al., 2017). These organisms are predominantly solubilizing inorganic P and mineralize organic P. Organic P mineralization is essential to increasing P availability that is easily absorbed by plants and microorganisms, because organic P concentration in soil accounts large proportion of total P (between 30 and 65%) (Condron et al., 2005). In this regard, regulating the function of PSM is an effective method to manage soil P deficiency and improve soil P availability (Sharma et al., 2013). Despite this, LUC may significantly impact the abundance and composition of soil microorganisms. As previous studies report, LUC has an impact on soil physical and chemical properties via changing vegetation composition and other related attributes (Tellen and Yerima, 2018). There are also substantial correlations between soil conditions and various microbes (Hermans et al., 2017). As a result, land-use change may put significant selection pressure on the soil microbial community, leading to short-term adaptation of present community members and, over time, a shift in community composition toward species that are better adapted to the new environment. There are several studies on the ecology of soil microorganisms including role of microbial organisms to the soil biogeochemical cycle (Merloti et al., 2019; Powlson et al., 2001) and effects of changing land use and climate on soil microbial communities (Liu et al., 2022; van Leeuwen et al., 2017). However, there is very scant information on the influence of LUC on abundance of PSM. Therefore, investigating the effects of LUC on PSM in soil populations is believed to have a crucial role for establishing measures to ensure the sustainable productivity of each land use.

Furthermore, PSMs influence the composition of P forms and are important drivers of P transformation by producing extracellular phosphatase enzymes (Fraser et al., 2015b; Hu et al., 2020). Phosphatase enzymes are classified into acid phosphatase (ACP) and alkaline phosphatase (ALP). Acid phosphatase is mostly produced by microbes and plant root exudates, while ALP is mainly produced by microbes (Nannipieri et al., 2011). Alkaline phosphatase is an essential enzyme that hydrolyzes organic P in the soil to release available orthophosphate P for plant and microbe utilization (Ragot et al., 2015). It is also crucial to note that there are three genes that encodes ALP homologues (*phoD, phoA* and *phoX*) that have been identified in terrestrial ecosystems (Fraser et al., 2015b). In terms of abundance, *phoD* is identified as the most abundant gene in the soil metagenome and is thought to be an important ALP-encoding gene (Ragot et al., 2015; Tan et al., 2013). *phoD* gene is also used as an important molecular marker to estimate the abundance and community composition of the microorganisms involved in organic P mineralization, and the abundance of the gene can be used to assess the activity of ALP (Hu et al., 2020, 2018). Similarly, inorganic P solubilization is another way of increasing available P. Phosphate-solubilizing microorganisms secrete organic acids (formic acid, gluconic acid, malic acid, citric acid, and oxalic acid) to dissolve insoluble inorganic P (Alori et al., 2017; Richardson and Simpson, 2011). Among them, gluconic acid is an important phosphoric acid that helps solubilize insoluble inorganic P (Li et al., 2019). It is controlled by glucose dehydrogenase, a major enzyme involved in gluconic acid production and encoded by *gcd* microbes (An et al., 2016). The *gcd* gene was proposed as a molecular marker for studying the abundance and community composition of the P-solubilizing microorganisms.

Previous findings revealed that environmental factors (land use type, climate and pH) and agricultural managements have strong influences on phosphatase enzyme activities and diversity of phosphatase harboring microorganisms (Fraser et al., 2015a; Neal et al., 2017; Ragot et al., 2017). For example, when forest lands are converted to other land use the soil microclimate and soil properties are altered (Anamulai et al., 2019). Soil microclimates have such a strong influence on microbial populations and root growth, which are the most important enzyme producers in the soil systems, this leads to a change in the soil enzyme activities. Soil enzymes produced by microbes strongly correlates with soil properties including pH, moisture content and soil organic matter, etc. (Błońska et al., 2017). A change in soil organic matter will have a substantial effect on soil enzymes produced by microbes since it provides substrates and energy for enzyme-producing microbes (Zhang et al., 2020). pH in soil is also a determinant factor especially for phosphatase since it regulates the extent and type of their activities (Dick et al., 2000). In addition, several studies demonstrated that changes in pH, SOC, available P and total N as a result of climate change, soil management and land use have a substantial impact on the diversity and composition of phosphatase-harboring microorganisms as well as the genes abundance that express phosphatase in the soil (Fraser et al., 2015a; Neal et al., 2017; Ragot et al., 2017; Tan et al., 2013). For instance, Neal et al. (2017) found that land use type (grassland, arable and bare fallow) has shown a clear difference on the compositions and diversity of microorganisms which involved in soil P cycling and some functional genes including phosphatase and phytase genes. However, it is unclear whether converting natural forest to other land use has significant effect on phosphatase-encoding genes and phosphatase harboring microbes involved in P cycling, especially in highly experienced LUC areas such as Qinghai Tibetan plateau, China. Therefore, examining the effect of LUC on phosphatase enzyme activity, the abundance of genes encoding phosphatase enzymes and phosphatase-harboring microbes is important to generate meaningful information on the influence of LUC on P cycling.

The Qinghai-Tibetan Plateau (QTP), often referred to as the world third pole, is the highest plateau on Earth, with an average elevation around 4000 m above sea level (Hao et al., 2021). The plateau is estimated to cover up to 2.5 million km^2, or 25% of all China's land area (Hao et al., 2021). The largest elevation gradient in the region increases habitat heterogeneity while serving as a crucial support for a diverse range of flora and fauna. The region's ecosystem is fragile, and it is especially sensitive to environmental change brought on by climate changes and human activities (Pan et al., 2017; Wischnewski et al., 2011). According to Wang et al. (2008) a massive increase in human activity in the region have had a significant impact on LUC. Deforestation has taken place in this area over the past 60 years due to logging (Studley, 1999), which has reduced the function and services of the natural forest ecosystems (Hu et al., 2008). Moreover, most of the degraded forest areas have been converted into artificial forest (He et al., 2017), farmland and shrublands (Wang et al., 2019), which may have a substantial effect on soil ecosystems. Because LUC have an effect on the vegetation type and inputs, which in turn modifies the nutrients in the soil (Wu et al., 2020). Soil microbes also provide a vital link between soils and plants, which is crucial for maintaining the functioning of the ecosystems (Chen et al., 2019). On the other hand, it is widely

recognized that soil properties including moisture content, pH, soil organic carbon and nitrogen have substantial effect on the diversity and composition of soil microorganisms. Numerous studies so far have studied the effects of LUC on soil C and N stock (Justine et al., 2020), P fractions (Azene et al., 2022), and diversity and community composition of microorganisms (Luo et al., 2020) in the Tibetan plateau. Nonetheless, to the best understanding of researcher of this study, there is no study that has been conducted on the influence of LUC on the abundance of phosphatase-encoding genes and phosphatase-harboring microorganisms. A better understanding how changes in land use affect phosphatase-encoding genes, phosphatase-harboring microbes, and phosphatase activity will improve future soil P management. With its distinctive nature of vegetation, altitude and soil, the QTP is a prime location to study the effects of LUC on P availability, phosphatase enzyme activity, abundance of phosphatase encoding genes and microorganisms.

Therefore, the present study aims; to assess the response of soil P availability and phosphatase enzyme activity to LUC; to quantify the influence of LUC on the abundance of genes which involved in P mineralization and solubilization process; to examine the effect of LUC on the abundance of phosphatase harboring microorganisms; and to determines the main environmental factors explaining the shifts in PSM abundance. We hypothesized that 1) Soil P availability and enzyme activity will decrease as NF is converted to other land uses due to decline of organic material inputs. 2) Land use change, which is a significant factor in changing microorganisms, will have an effect on the abundance of phosphatase encoding genes and phosphatase harboring microorganisms. 3) Soil pH and P content will be a key factor affecting the abundance of phosphatase encoding genes and phosphatase harboring microorganisms.

2. Material and methods

2.1. Study site and sampling

The study sites are situated in Songpan county (32° 06′–33° 09′ N and 102° 38′-104° 15′ E), Sichuan province, Southwest China (Fig. 1). The area experiences alpine climate with cold winter and hot summer. The area's annual average temperature is 2.8 °C. Annual rainfall of the area is approximately 718 mm, with more than 72% of it falling between June to August (Shi et al., 2010).

For this study, soil samples were taken at the end of June 2021 from seven sites; Each site was represented by four adjacent land uses types including artificial forest (AF), farmland (FL), natural forest (NF) and shrublands (SL). In each land use, three replicated 20 m × 20 m main plots were established with five subplots (1 m × 1 m) in the four corners and the center of the main plot. Soil samples were taken from the center of the subplots at a depth of 0–20 cm and 20–40 cm using a soil auger. Soil samples taken from the similar layers at each plot were thoroughly mixed to make one composite sample for each soil layer. In total, 168 samples were collected from seven sampling sites, four land use types, three replications and two soil depths. The composited soils samples were further split into two parts; one of which was used to measure the soil moisture content, enzyme activities, phosphatase encoding genes and microorganism. The remaining soil part was air dried, removed the plant debris, and stones, sieved through 2 mm and then used to determine the soil physicochemical properties. For this study, data such as moisture content (MC), pH, total nitrogen (total N), soil organic carbon (SOC), and total P were used from our published paper (Azene et al., 2022).

2.2. Determination of available P

Available P was measured from the soil in a 1:20 ratio with a 0.5 M $NaHCO_3$ (the solution pH was 8.5) solution and after shaking for 30 min (Olsen et al., 1954). The concentration of available P was determined after filtering the solution through filter paper using a blue molybdate method and a spectrophotometer set to 700 nm (Murphy and Riley, 1962).

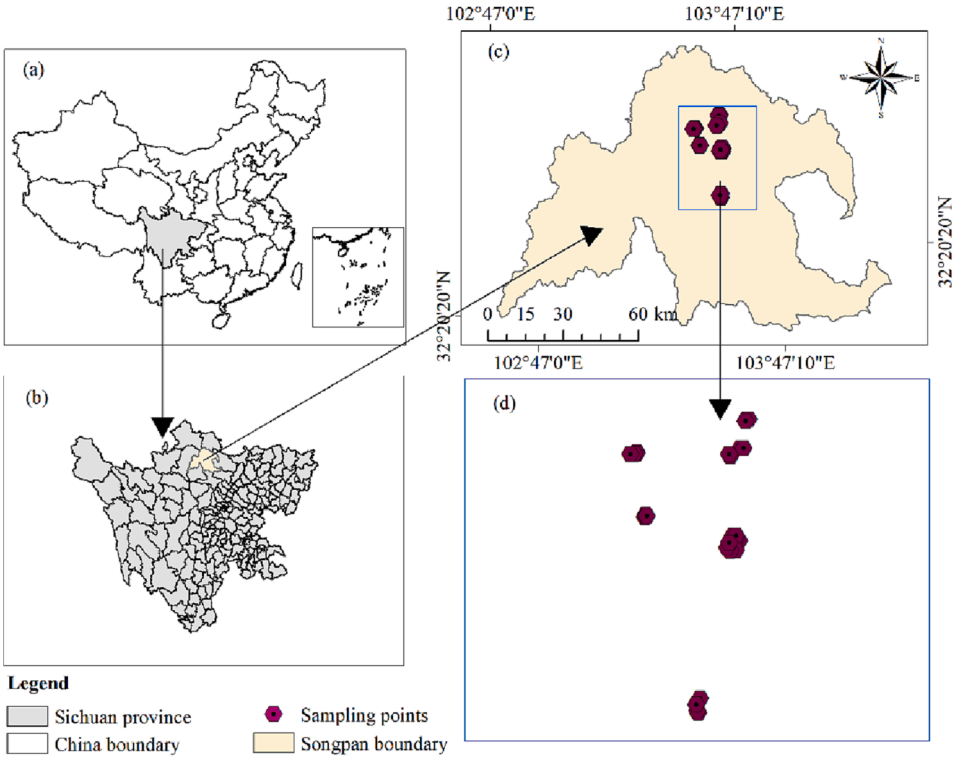

Fig. 1. Map of the study area. a) Map of China, b) Sichuan province c) Songpan county, d) sampling points.

2.3. Analysis of acid and alkaline phosphatase

Acid (ACP) and alkaline phosphatase (ALP) enzymes were measured using *para*-nitrophenyl phosphate (*p*NPP) as a substrate according to Tabatabai and Bremner (1969) method, which was later improved by Eivazi and Tabatabai (1977). Briefly, a 0.2 g of moist soil was taken for each sample and incubated using a modified universal buffer solution (for acid phosphatase assay at a pH of 6.5 and for alkaline phosphatase assay at pH 11) with a 0.115 M *para*-nitrophenyl phosphate (*p*NPP) substrate at 37℃ for 1 h. Then we stopped the process by adding solution (0.5 M $CaCl_2$ and 0.5 M NaOH) to the flask and spinning it for a few seconds. We also added deionized water to dilute the sample solutions and the soil suspension, and it was filtered to eliminate any possible precipitates. A spectrophotometer was used to measure phosphatase enzyme activity (at wavelengths of 400 nm). Finally, the activity of acid and alkaline phosphatase was reported as μg pNP per gram of dry soil and incubation time (h).

2.4. DNA extraction and metagenomic sequencing

Three replicates of each soil sample were used to extract DNA using FastDNA® Spin Kit for Soil (MP Biomedicals, USA) following the manufacturer's instructions. A 1% agarose gel electrophoresis was used to evaluate isolated DNA quality. For further analysis, all DNA samples were kept at −20 °C.

About 1 μg of qualified DNA from each soil sample was used for library building and sequencing on an Illumina NovaSeq 6000 platform (Majorbio, Shanghai, China) using the PE150 by metagenomic shotgun techniques (Mantri et al., 2021).

Low-quality raw reads were clipped, to ensure the quality of data. The 3′ and 5′ adapter sequences were trimmed using the Fastp software (v0.20.0) and reads less than 50 bp or containing N were removed (ambiguous bases). single-end reads, and pair-end reads of excellent quality were kept. Clean reads were then combined using MEGAHIT software (v1.1.2) (Li et al., 2016). The ORF of contigs was predicted using MetaGene software. For each sample, genes with nucleic acids greater than or equal to 100 bp were chosen and translated into amino acid sequences to construct a statistical table of the results of gene prediction. A non-redundant ORF set was constructed using the CD-HIT software (v4.6.1) based on the parameters (90% coverage and 90% identity were the default parameters) (Li and Godzik, 2006). The longest gene in each class used as the model sequence for generating a non-redundant gene. Finally, SOAPaligner (soap2.21 release, identity 95%) was used to align each sample's high-quality reads to its non-redundant ORFs and then calculated the abundance of genes in each sample.

2.5. Analysis of the abundance of P mineralizing and solubilizing genes and microbes

The soil microbial organic P-mineralization and inorganic P-solubilization genes were collected from datasets based on earlier studies (Bergkemper et al., 2016; Fraser et al., 2015b; Puehringer et al., 2008). Gene names, classifications and functions related to P cycling are listed in Table S1. DIAMOND was used to allocate predicted gene sequences from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database against the NCBI non-redundant protein sequences database in order to determine the taxonomic assignments of specific genes (Buchfink et al., 2014). The total relative abundance of dominant species, key pathways, and functional genes between samples was calculated using the proportion of matched reads per kilobase million in the total effective reads per kilobase million.

2.6. Data analysis

Statistical analysis was performing using IBM SPSS version 26 (Yang and Lu, 2022). All data were checked for normality. However, the test for normality assumption has failed even after data transformations. As a result, we used Kruskal-Wallis analyses to assess the effects of LUC on soil P availability, phosphatase enzyme activities, phosphatase encoding genes and microorganisms. We have also applied Mann-Whitney U to test differences in available P and phosphatase enzyme activity between soil depths. Spearman correlation was performed to see the relation between phosphatase enzyme activity and soil properties.

To evaluate the relations between soil properties, the abundance of phosphatase-encoding genes, and phosphatase-harboring microorganisms, we performed redundancy analysis (RDA) using Canoco version 5.0 (the length of the first DCA axis of the data was <1 standard deviation). Finally, GraphPad Prism 9 software and excel were used to create graphs and significance difference was set at $p \leq 0.05$.

3. Result

3.1. Soil phosphorus availability and phosphatase enzyme activities

Soil available P differed significantly among land uses and soil depth ($p \leq 0.05$) (Fig. 2). Available P was significantly increased after conversion of NF to FL in both soil depths (41.10 mg kg^{-1}, 0–20 cm, and 23.58 mg kg^{-1}, 20–40 cm) (Fig. 2). Similarly, the top layer (0–20 cm) of the FL soil had much higher available P than the bottom layer (20–40 cm). However, conversion of NF to AF and SL land had no significant effect on available P (Fig. 2).

The activity of ACP significantly decreased following land use changes from NF to FL and from NF to SL soils in both soil depths, while ALP activity significantly decreased after converting NF to FL soil at 0–20 cm soil depth (Fig. 3, Table S2). The maximum ACP and ALP activities were found in the NF soil (1668.80 and 1152.40 μg p-NP g^{-1} dry soil h^{-1} in 0–20 cm, respectively), whereas FL and AF soils had the lowest ACP and ALP, respectively (Fig. 2, Table S2). Similarly, ACP and ALP activities decreased significantly with increasing soil depths in AF, NF and SL soils ($p \leq 0.05$), whereas there was no difference in FL soils ($p \leq 0.05$) (Table S2). Generally, FL soils have the lowest amount of ACP and ALP, while NF soils had the highest amount in the 0–40 cm soil depths (Table S2). The activity of ACP and ALP enzymes decreased in these orders due to land use change; NF < AF < SL < FL and FL < AF < SL < NF, in 0–40 cm, respectively (Table S2).

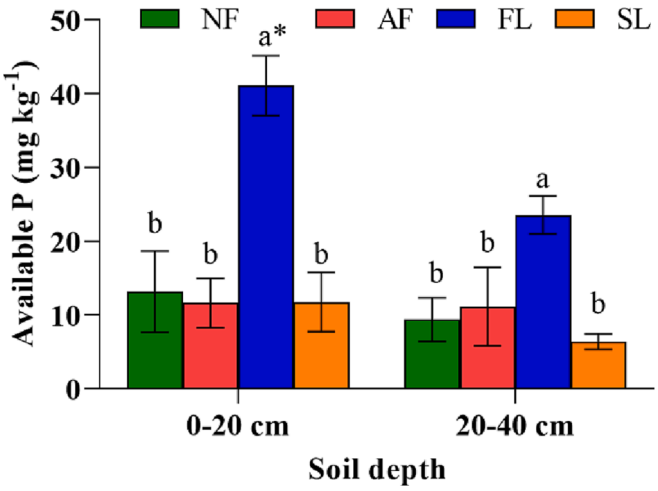

Fig. 2. The concentration of available phosphorus in four land use types at 0–20 and 20–40 cm soil depths. Data represents the sample mean values (n = 21). Significant difference among land uses is represented by different lowercase letters ($p \leq 0.05$). Asterisk (*) denote statistically significant difference between soil depths within the same land use type. Note: AF: artificial forest, FL: farmland, NF: natural forest, SL: shrubland.

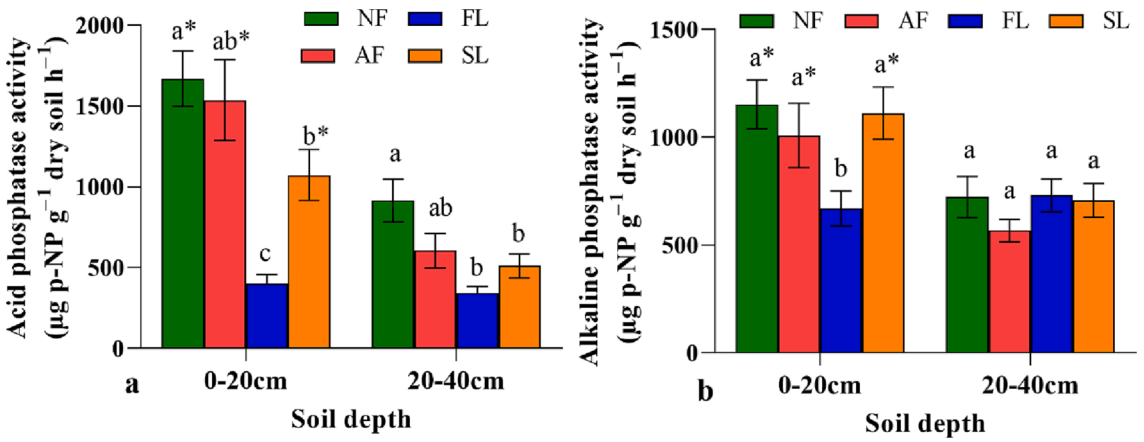

Fig. 3. The amount of acid (a) and alkaline (b) phosphate at 0–20 and 20–40 cm soil depth in four land uses. Data represent the sample mean values ($n = 21$). Significant difference among land uses is represented by different lowercase letters ($p \leq 0.05$). Asterisks (*) denote statistically significant difference between soil depths within the same land use type. Note: AF: artificial forest, FL: farmland, NF: natural forest, SL: shrubland.

3.2. Abundance of genes and microbes involved in P mineralization and solubilization

The total relative abundance of genes in the sampled soils to P mineralizing and solubilizing were displayed in Fig. 4. Totally, 13 P solubilizing and mineralizing genes (*gcd, ppa, pqqA, pqqB, pqqC, pqqD, pqqE, phoA, phoD, phoX, phoN, aphA, and olpA*) were detected in the study area. The results also showed that *gcd and ppa* genes were significantly higher relative abundance in FL soil as compared to NF and AF soil, while *phoA, phoD and phoN* genes were significantly higher relative abundance in NF followed by AF soils compared to other land uses *($p \leq 0.05$)*. In addition, *pqqB, pqqC, pqqD* and *pqqE* encoding genes were substantially higher in NF in comparison with FL soil *($p \leq 0.05$)* (Fig. 4). The most abundant P solubilizing and mineralizing genes were *gcd* and *phoD*, respectively, while the lowest abundant genes in this study were *pqqA and olpA* encoding genes (Fig. 4).

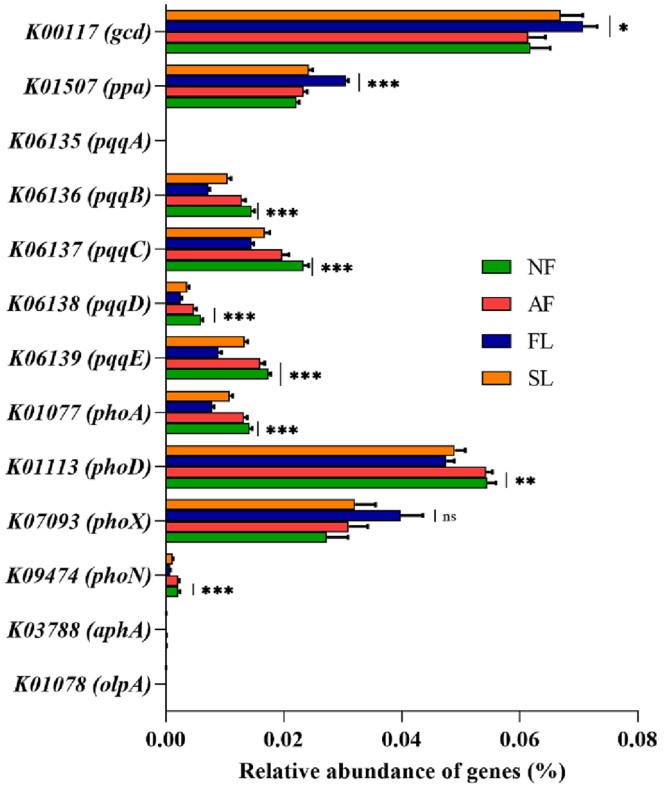

Fig. 4. The total relative abundance of P mineralizing and solubilizing encoding genes in four land uses at 0–20 cm soil depths. Statistically significant differences in the total relative abundance of genes among land uses are determined at $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***) and ns indicates no significance.

Moreover, the taxonomic distribution of P solubilizing and mineralizing harboring microbes at the phylum level are presented in Fig. 5. In all land uses, we found 20 *gcd*-harboring phyla, 36 *ppa*-harboring phyla, one *pqqA*-harboring phylum, 13 *pqqB*-harboring phyla, 14 *pqqC*-harboring phyla, eight *pqqD*-harboring phyla and nine *pqqE* -harboring phyla, 20 *phoD*-harboring phyla, 14 *phoA*- harboring phyla, 19 *phoX*-phyla, five *phoN*-harboring phyla, two *aphA*-harboring phyla and two *olpA*-harboring phyla (Table S3). The dominant phyla that harbored the *gcd* gene were Acidobacteria and Proteobacteria, accounting for 59.13% and 27.34%, respectively, while the dominant *phoA*-harboring phyla were Proteobacteria 50.55%, Verrucomicrobia (21.42%) and Actinobacteria (14.73%). The dominant *phoD* harboring phyla were Proteobacteria (52.42%), Actinobacteria (22.33%) and Acidobacteria (10.49%). Similarly, the dominant *phoX*-harboring phyla were Proteobacteria and Actinobacteria, while for aphA-harboring phyla were Proteobacteria and Actinobacteria. The dominant *pqqB, pqqC, pqqD* and *pqqE* harboring phylum were Proteobacteria, Actinobacteria, Candidatus_Rokubacteria, Verrucomicrobia and Acidobacteria (Fig. 5).

3.3. Effect of land use change on dominant genes (gcd and phoD) harboring microorganisms

The result indicated that the relative abundance of Acidobacteria phylum was significantly higher in SL and FL soils, while the relative abundances of Proteobacteria and Candidatus_Rokubacteria phyla were significantly higher in the NF and AF soils *($p \leq 0.001$)* (Fig. 6). Moreover, the relative abundances of microorganisms that harbor *gcd* genes, such as Bacteroidetes, Gemmatimonadetes, Verrucomicrobia, Thaumarchaeota and Planctomycetes phyla were significantly higher in FL soils *($p \leq 0.01$)* (Fig. 6a). Similarly, among *phoD* harboring microbes, Actinobacteria, Cyanobacteria, Firmicutes and Nitrospirae were significantly higher relative abundance in FL soils *($p \leq 0.01$)* (Fig. 6b).

Results also demonstrated that the relative abundance of dominant *gcd*-harboring Acidobacteria increased after converting NF (51.75%) to AF (52.94%), FL (63.91%), and SL (67.91%) soils, while the relative abundance of Proteobacteria decreased after converting NF (37.53%) to AF (34.35%), FL (17.91%), and SL (19.55%) soils (Fig. 7) Similarly, the relative abundance of the dominant *phoD* harboring Proteobacteria decreased following conversion of NF to FL, AF and SL, while the relative abundance of Actinobacteria increased after conversion of NF

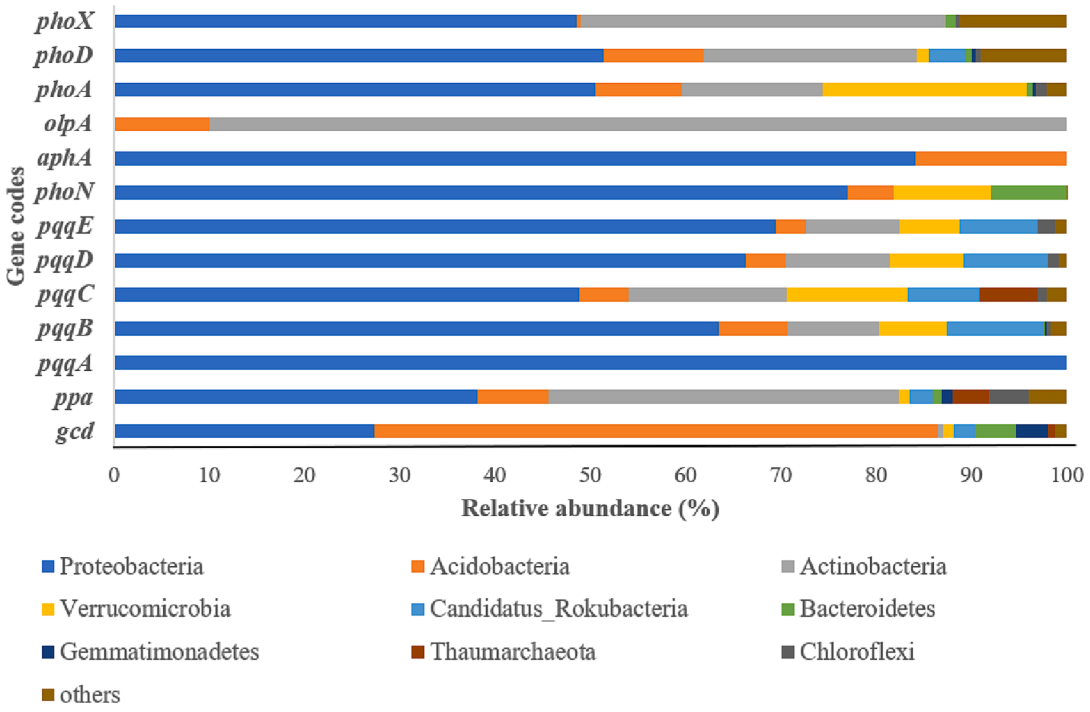

Fig. 5. Taxonomic composition of phosphorus solubilizing and mineralizing functional genes based on the metagenomic datasets in all land uses.

(19.20%) to AF (21.39%), FL (25.00%) and SL (23.70%) (Fig. 7).

3.4. Relation between phosphatase enzymes and microbial genes with soil properties

The correlation analysis indicated that a substantial positive relationship was found between soil phosphatase enzymes and MC ($r = 0.89$ with ACP, $r = 0.70$ with ALP), SOC ($r = 0.70$ with ACP $r = 0.71$ with ALP), Total N ($r = 0.65$ with ACP, $r = 0.69$ with ALP) *($p \leq 0.01$)* (Table 1). ACP activity had a negative correlation with pH, available P and total P *($p \leq 0.01$),* while ALP had a negative relation with available P *($p \leq 0.05$)* (Table 1).

The RDA analysis between P mineralization and solubilization genes, as well as *gcd* and *phoD*-harboring microorganisms with soil properties are displayed in Fig. 8. The analysis between P mineralization and solubilization genes with soil properties indicated that 67.25% of total variations were explained in the two RDA axes (Fig. 8a). The first and second axes accounted 57.49% and 9.76% of the total variation, respectively. The *gcd* and *ppa* encoding genes had positive relation with soil pH, total P and available P, but negative relation with MC, SOC, total N, ACP and ALP. Similarly, *phoD* gene shows a positive relationship with soil pH and ALP and a negative correlation with available P. The *phoX* gene also had positive relation with pH and total P. Other genes found in this study have positive correlations with MC, SOC, total N, ACP and ALP but have negative relation with pH, total P and available P (Fig. 8a). Moreover, the RDA result between *gcd* and *phoD*-harboring microbes and soil properties showed that the first axis explained 51.88% and 40.59% of variations in the *gcd* and *phoD* microbial communities, while 7.95% and 12.47% were explained in the second axis, respectively (Fig. 8b,c). The effect of selected soil properties on *gcd* and *phoD*-harboring microorganisms were similar. The majority of the PSM communities were strongly positively correlated with pH, available P and total P. Similarly, other soil properties such as MC, SOC, total N, ACP and ALP strongly correlated with some of the PSM communities, including Proteobacteria, Candidatus Rokubacteria, Firmicutes and Chloroflexi.

4. Discussion

4.1. Response of soil available phosphorus and phosphatase enzyme activity to land use change

Understanding the effect of land use change on available P could facilitate sustainable land management and productivity. Land use change has been demonstrated to have a considerable impact on the amount of total P and the proportion of its bioavailability (Liu et al., 2018). For instance, several studies demonstrated that when natural forests were converted to other land uses, changes in the composition of the vegetation and organic material inputs resulted in a reduction in the availability of P (Solomon et al., 2002). Conversely, in this study, conversion of NF to FL soil significantly increases the availability of P (Fig. 2), this could be due to the use of chemical fertilizers on farmland soil (Wang and Zhang, 2019). According to Liu et al. (2018), chemical fertilizer application in croplands results in additional P accumulating in these ecosystems, leading to increased available and total P in croplands compared to other land uses. Similar results have also been reported on the regional scale, particularly in China and Europe, where the use of chemical fertilizer in agricultural areas has been higher (Viscarra Rossel and Bui, 2016; Zhang et al., 2020). The other reason could be the higher abundance of the *gcd* and *ppa* genes in FL soils (Fig. 4), which solubilizes inorganic P to available P. Therefore, our findings suggested that applying chemical fertilizers or a higher abundance of *gcd or ppa*-encoding genes in specific land use would considerably improve the amount of available P.

Furthermore, soil enzymes are well known to be a good sign of soil quality (Karaca et al., 2010); thus, understanding the effect of LUC on soil enzyme activities is critical to maintaining soil fertility. Earlier studies have found that changes in land use have a significant impact on the activity of soil enzymes by altering soil physicochemical and biological properties (Wang et al., 2012). Similarly, changes in land use affect the habitat characteristics, which has an effect on the activity of the soil enzymes and related microorganisms. Among others, acid and alkaline phosphatases are commonly used enzymes to evaluate the change of soil qualities (Trasar-Cepeda et al., 2008). Consistence with

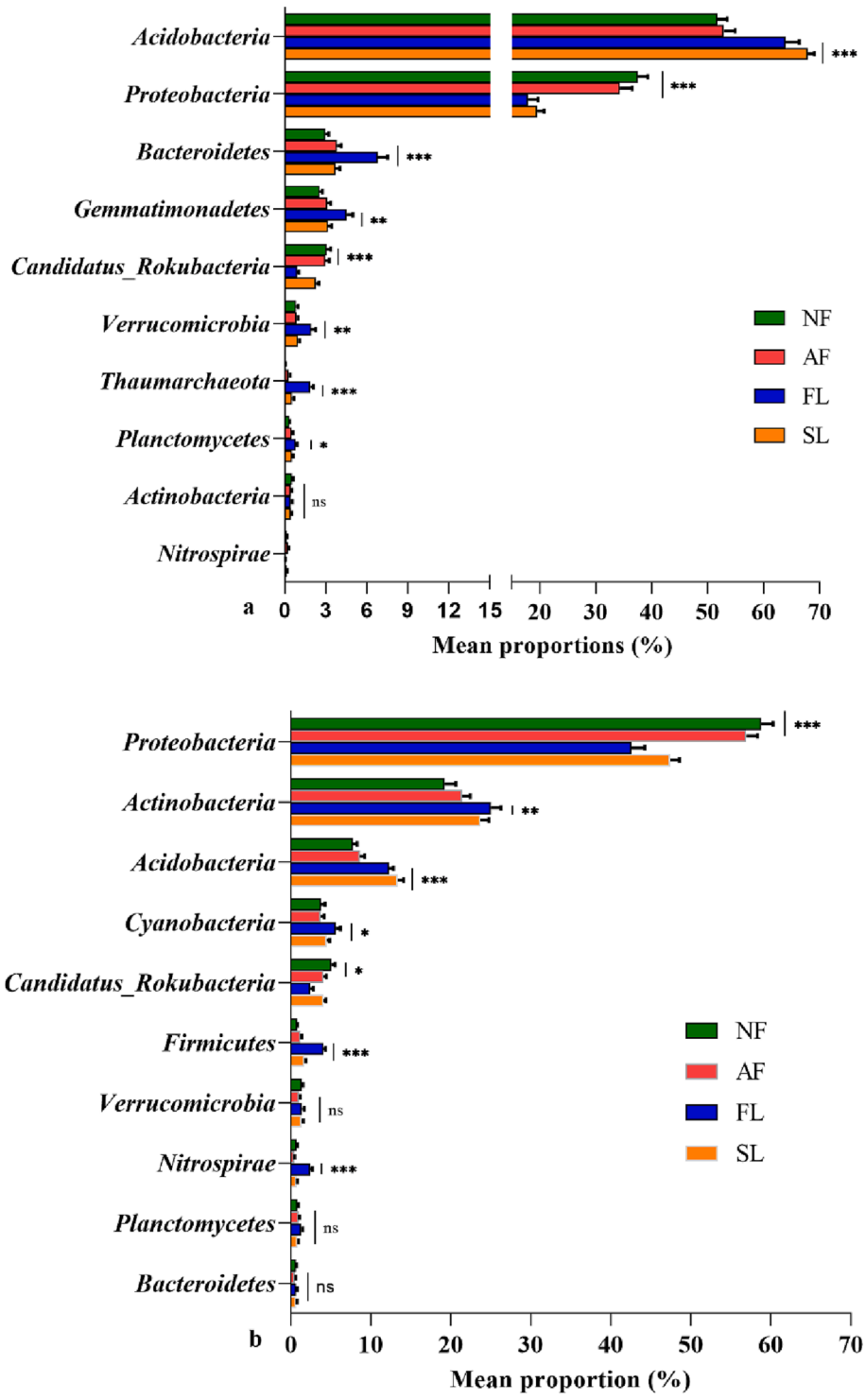

Fig. 6. Response of ten dominant *gcd*-harboring microbial phyla (a) and *phoD* harboring microbial phyla (b) to land use changes at 0–20 cm soil depths. Data represents the sample mean values ($n = 21$) and error bars represents the standard error of mean. Asterisks (*) represents the significant differences in the relative abundance of each phylum among land uses (* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ and ns indicates no significance.

our hypothesis, the activity of phosphatase enzymes decreased following conversion of NF to AF, FL and SL (Fig. 3). The most likely cause of the decrease in soil enzyme activities was a reduction in litter inputs, soil organic matter, and a change in soil microclimates (Raiesi and Beheshti, 2014). The amount of soil organic matter in soil regulate phosphatase enzyme activity (Margalef et al., 2017). This argument is supported by the strong positive association between phosphatase enzyme activity and MC, SOC, and TN (Table 1). This implies that the reduction of MC, SOC, and TN content following NF conversion to other land uses may reduce microbial activity, resulting in a decrease in phosphatase enzyme secretion. Moreover, converting natural forest to other land use increase soil disturbance, which reduces the activity of soil enzyme (De Barros et al., 2020). According to Meena and Rao (2021), the availability of microbial substrates is limited in disturbed soils, which lowers the activity of soil enzymes. This suggested that the limited supply of nutrients after converting NF to other land uses may stress microbes' ability to

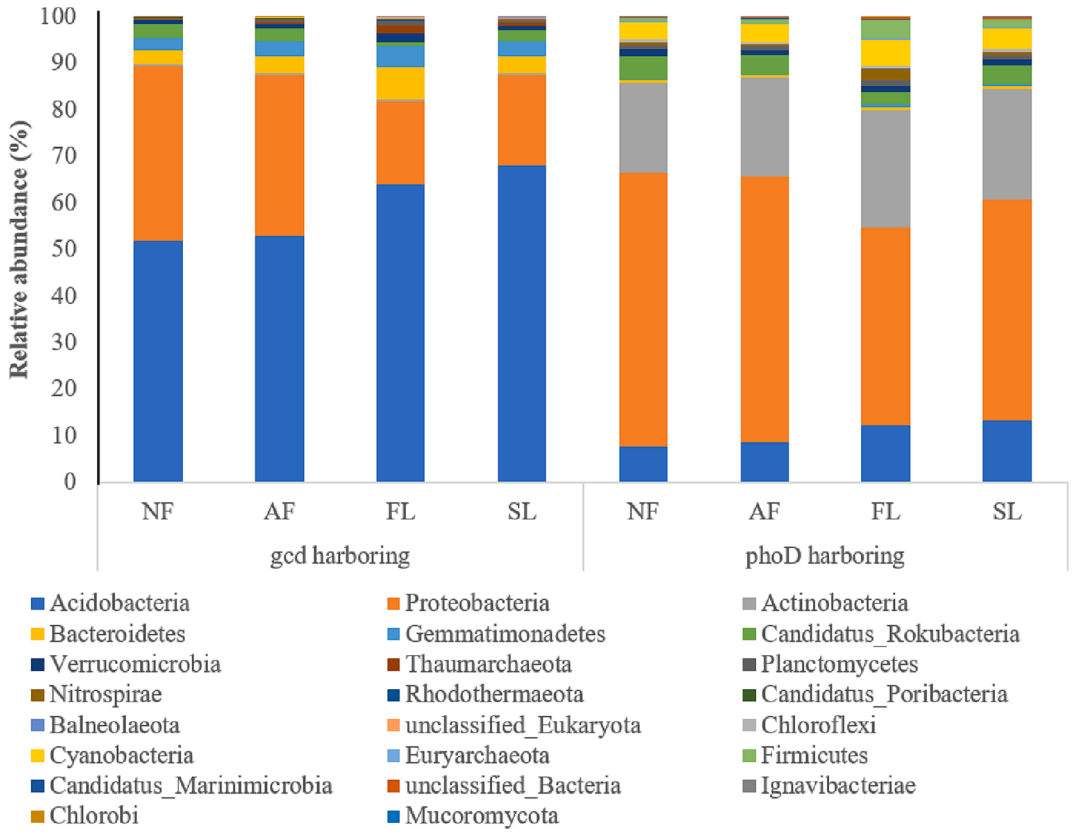

Fig. 7. Relative abundance of the *gcd*-harboring microbes and *phoD*-harboring microbes in a metagenomic dataset of four land use soils. AF: artificial forest: FL: farmland: NF: natural forest, SL: shrubland.

Table 1
Correlation between phosphatase enzyme activity and selected soil properties at 0–20 cm soil depth.

Soil property	pH	MC	SOC	Total N	Available P	Total P
ACP	−0.56**	0.89**	0.70**	0.65**	−0.38**	−0.37**
ALP	0.05	0.70**	0.71**	0.69**	−0.26*	−0.01

Note: SOC: soil organic carbon; MC: moisture content; Total N: Total nitrogen; Total P: Total Phosphorus; ACP: acid phosphatase; ALP: Alkaline phosphatase. Asterix's represents the degree of significance level (* $p \leq 0.05$and ** $p \leq 0.01$).

produce enzymes compared to NF soils. Another explanation may be P deficient sites are stimulated by phosphate-harboring microbes to generate and secrete phosphate enzymes that mineralize organic P into available P (Bergkemper et al., 2016). Fraser et al. (2015b) also noted that the production of ALP could be promoted by bacteria in low available P sites because phosphate deficiency may stimulate the phosphate starvation regulon, which is made up of numerous genes involved in AP uptake and delivery. This result is supported by the positive association between ALP and alkaline phosphate genes (*phoA and phoD*) (Fig. 8a) and the negative correlation between phosphate enzymes and available P (Table 1).

Soil pH is another important factor affecting phosphatase enzyme activities (Dick et al., 2000). A significant negative correlation between ACP and pH could reduce the acid phosphatase level in AF, FL and SL soils because they have slightly higher pH values than NF soil (Azene et al., 2022). This finding is in agreement with (Dick et al., 2000), who found that ACP decreases with increasing pH. In this study, the level of ACP activity was higher than ALP activities in NF and AF soil due to the acidic nature of the soil studied (Fig. 3a), implying that ACP has more prevalent than ALP in acidic soil. Our findings also revealed that phosphatase enzyme activities decreased with increasing soil depth (Fig. 3). The decrease in enzyme activity is due the loss of organic matter as soil depth increases (Venkatesan and Senthurpandian, 2006). For example, the higher level of phosphatase activity in 0–20 cm could be attributed to a higher amount of soil nutrients (SOC and TN) and MC, which increases microbe activity to secret enzymes. However, the reduction of soil nutrients (SOC and TN) in the 20–40 cm soil depth could reduce microbe activity, resulting in a decrease in phosphatase activity. Overall, our result suggested that land use conversion from natural forest to others decreases the level of phosphatase enzyme activity by altering MC, SOC and total N in the Qinghai–Tibetan Plateau. In addition, the level of phosphatase enzyme activity decreases with increasing soil depth due to changes in MC, SOC and TN.

4.2. *Land use change affect soil P solubilizing and mineralizing genes*

Exploring the effect of land use change on some specific microbial functional groups may provide insights into potential influences on the processes carried out by those groups. Microbial groups involved in P cycling are particularly interesting due to their importance in ecosystem maintenance. In this study, we assessed how the abundance of P mineralizing and solubilizing genes responded to land use change. Our result indicated that 13 P solubilizing and mineralizing genes were found in all land uses, and that land uses had a significant effect on all genes, with the exception of *pqqA, phoX, aphA and olpA* genes (Fig. 4). In terms of P-solubilization, the *gcd* gene was stood out among them the most dominant gene. This gene is the most prevalent phosphatase solubilizing gene in terrestrial ecosystems (Liang et al., 2020), and used as a molecular marker to investigate the abundance and diversity of microorganisms involved in inorganic P solubilization process (Bergkemper et al., 2016; Liang et al., 2020). However, different factors such as soil properties, land use, and soil management have a substantial impact on its abundance (Siles et al., 2022; Yang and Lu, 2022). Our finding

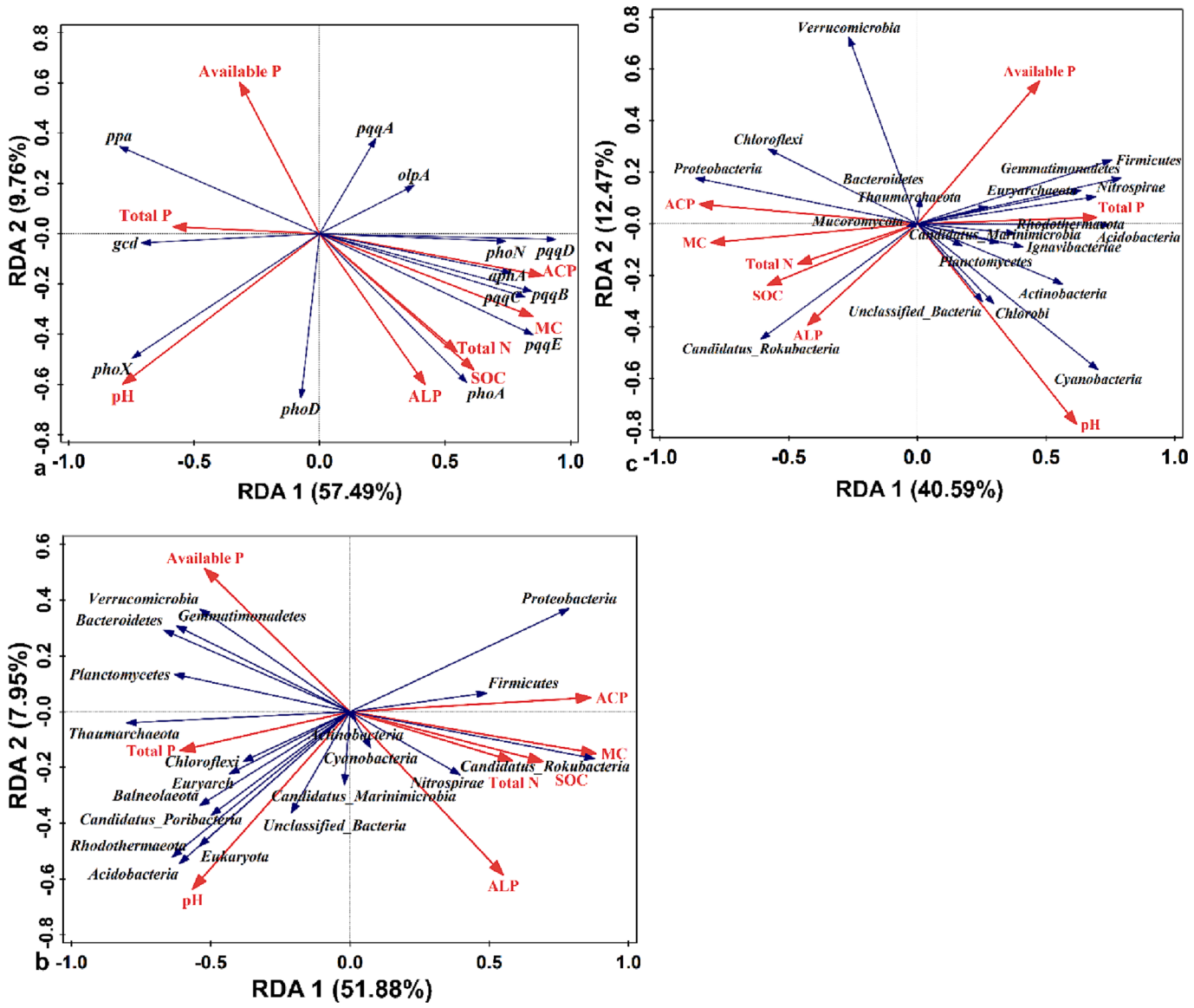

Fig. 8. RDA plot analysis between the relative abundance of phosphorus solubilizing and mineralizing genes (a), *gcd* (b) and *phoD* (C) harboring microbes with selected soil properties. Note: Available P; available phosphorus; MC, moisture content; SOC, soil organic carbon; Total N, total nitrogen; ACP, acid phosphatase; ALP, alkaline phosphatase.

revealed that the *gcd* gene's relative abundance increased significantly after the conversion of NF to FL soils (Fig. 4). The higher *gcd* gene abundance in the FL soil could be due to the greater amount of soil pH and total P, which was supported by the strong correlation between *gcd* gene abundance and concentration of soil pH and total P (Fig. 8a). Liang et al. (2020) reported that the *gcd* gene is more prevalent in P-rich soils. As a result, the higher P content in FL soil may help in the growth of the *gcd*-harboring microbes, which results in an increase in the *gcd* gene abundance.

Moreover, previous studies have suggested that inorganic P solubilization is an important process in soil P cycling in inorganic P-containing soils (Bergkemper et al., 2016). In this regard, we can conclude that the *gcd* gene contributes more to P availability by solubilizing inorganic P in P-dominated lands. On the other hand, *phoD* was the dominant P mineralizing gene in the study area (Fig. 4), most likely due to its strong correlation with pH. Soil pH was the key factor controlling the abundance and composition of *phoD*-harboring microbes (Hu et al., 2018; Ragot et al., 2015), which in turn affects the *phoD* gene abundance. In addition, our result indicated that the NF had significantly higher relative abundance of *phoD* gene compared to FL soil, probably due to the lower concentration of available P, which is confirmed by the negative correlation between them (Fig. 8a). Several studies have found that the *phoD* gene is influenced by the level of P in the soil (Fraser et al., 2015a; Hu et al., 2018). For example, when P is scarce, microbes can enhance the expression of particular functional phosphatase genes such as *phoD* gene (Vershinina and Znamenskaya, 2002). Similarly, the abundance of genes encoding *phoA, phoN, pqqB, pqqC, pqqD,* and *pqqE* in NF soils was significantly higher than in FL soils (Fig. 4). The most likely explanation for this finding is a higher concentration of MC, SOC, and TN in NF, which is supported by the positive correlation between the aforementioned genes and soil properties (Fig. 8a). Similar studies also found that the abundance of P mineralizing and solubilizing genes are mostly influenced by SOC, MC and total N in terrestrial ecosystems (Chen et al., 2021; Nahas, 2007; Ragot et al., 2015). In general, our findings suggested that P solubilizing and mineralizing genes significantly influenced by land use change, but the effect is mostly determined by soil pH and level of P condition.

4.3. Abundance of gcd and phoD-harboring microorganisms

The *gcd* and *phoD* gene are mostly found in soil microorganisms belonging to the bacterial phyla Acidobacteria, Proteobacteria, Actinobacteria, Candidatus_Rokubacteria, Gemmatimonadetes and Verrucomicrobia (Liang et al., 2020; Luo et al., 2017; Tan et al., 2013; Zhao et al., 2022), which is in agreement with our findings (Fig. 5). As a result, our findings suggested that changing environmental conditions had no significant impact on the dominance of the *gcd* and *phoD*-harboring phyla.

Although *gcd* and *phoD*-harboring microbes have been studied in different environmental contexts (Fraser et al., 2015a; Liang et al., 2020; Ragot et al., 2017; Zhao et al., 2022), it is unclear how changing NF to other land use may affect these vital microorganisms. In this study, we hypothesized that land use change will modify the abundance of *gcd* and *phoD*-harbor microbes by altering different soil physicochemical properties. The results confirmed our hypothesis that the relative abundance of microorganisms that harboring *gcd* and *phoD* was significantly affected by land use changes (Fig. 6). For example, the total relative abundance of Acidobacteria phylum considerably increased after conversion of NF to SL and FL soil (Fig. 6). The main reason for the greater abundance of Acidobacteria phylum in SL and FL was due to the higher amount of soil pH, which was confirmed by the strong relationship between the abundance of Acidobacteria and pH (Fig. 8bc). According to earlier researches, pH is a key determining factor for the abundance and composition of *gcd* and *phoD*-harboring microorganisms in different ecosystems (Hu et al., 2018; Ragot et al., 2015). Moreover, the total relative abundance of Gemmatimonadetes, Bacteroidetes, Verrucomicrobia, Planctomycetes and Thaumarchaeota phyla which harbor the *gcd* gene was significantly increased following converting NF to FL.

Similarly, Actinobacteria, Cyanobacteria, Firmicutes and Nitrospirae phyla which harbor *phoD* gene were significantly increased after the conversion of NF to FL soil. These could be due to the higher content of pH, available P, and total P in FL soils. This finding is supported by the significant correlation between the abundance of the above-mentioned *gcd* and *phoD*-harboring microorganism and pH, available P, and total P (Fig. 8bc). Several studies conducted in various ecosystems proved that pH, total P and available P are the key factors to shift the composition and abundance of *gcd* and *phoD*-harboring microbes (Hu et al., 2018; Ragot et al., 2015). On the other hand, the abundance of Proteobacteria and Candidatus_Rokubacteria phyla considerably decreases after a LUC from NF to FL soil. The possible reason for the decline of the abundance of these phyla was due to the decline of SOC, MC and total N. The result is confirmed by the strong correlation between Proteobacteria and Candidatus_Rokubacteria phyla and SOC, MC and total N, inferring that the lowest amount of MC, SOC and TN after conversion of NF to FL soil could explain this change. In this study, pH, total P and available P were the key environmental drivers of the abundance of the majority of *gcd*- and *phoD*-harboring microorganisms, whereas some of the dominant phyla such as Proteobacteria and Candidatus_Rokubacteria were substantially controlled by MC, SOC and TN. Generally, our findings suggest that pH, MC, available P, SOC, total P and total N are significant factors for shifting the abundance of phosphatase microbial organisms in the soil ecosystems.

4.4. The relation between phosphatase activity, gene abundance and soil properties

The correlation and RDA analysis demonstrated that selected soil properties had a substantial correlation with phosphatase enzyme activities, the abundance of genes and microorganisms (Table 1, Fig. 8). For example, phosphatase enzyme activities had a positive correlation with MC, SOC and total N, indicating that high content of MC, SOC and total N can stimulate the growth of microorganisms, which in turn increases the release of phosphatase enzymes. Moisture content, SOC and total N have strong association with the phosphatase enzyme activities (Dick et al., 1988; Saha et al., 2008; Sardans et al., 2006). On the other hand, phosphatase enzymes had a negative correlation with available P, indicating that the level of available P inhibited phosphatase enzyme production. Many studies concur with our findings, which show that sites with more available P suppressed the production of phosphate enzymes (Fraser et al., 2015b, 2015a; Nannipieri et al., 2011; Zhang et al., 2012).

Similarly, soil pH had a negative correlation with ACP activity, implying that ACP activity is more prevalent in low pH soil or acidic soil (Dick et al., 2000; Nannipieri et al., 2011). Moreover, the *gcd* encoding gene had a positive association with pH and total P, while it had a negative correlation with MC, SOC, TN, ALP and ACP (Fig. 8a). This could be because the *gcd* gene is a known P solubilizer that thrives under P-rich soils (Liang et al., 2020). In our study, FL soils had higher *gcd* gene abundance due to higher soil pH and P content. As a result, our findings suggested that soil pH and P level are the primary regulators of *gcd* gene abundance. Similarly, abundance of *phoD* gene had a positive relation with soil pH, which is the primary factor controlling the *phoD* gene abundance in the terrestrial ecosystems (Hu et al., 2018; Ragot et al., 2015). In addition, inconsistent results were reported in the relation between abundance of *phoD* gene and ALP activities. Fraser et al. (2015b) and Hu et al. (2018) found a positive relation between abundance of *phoD* gene and ALP activities, while Ragot et al. (2017) demonstrated no association between abundance of *phoD* gene and ALP in various land use soils. In this study, we found that the abundance of *phoD* gene had a positive relation with ALP. Similarly, ALP had positive relation with other alkaline phosphate-encoding genes (*phoA*). Our result suggested that both *phoD* and *phoA* genes have a substantial role in ALP activity in this study area.

5. Conclusion

This study showed that LUC has a major impact on P cycling by altering phosphatase enzyme activity, the abundance of phosphatase encoding genes and phosphatase-harboring microbes. The study revealed that available P increased after converting NF to FL soils, due to the fertilizer application in FL or the higher abundance of the *gcd* gene, which solubilizes inorganic P to available P. Moreover, phosphatase enzyme activity significantly decreases following the conversion of NF to FL soils, likely due to the decline of MC, SOC and TN. Another explanation is that the higher amount of available P in FL soils inhibited the synthesis and secretion of phosphatase enzyme. This result was supported by the negative correlation between phosphatase enzyme and available P. Thirteen phosphatase solubilizing and mineralizing genes were detected in the study area. Among them, *gcd* and *phoD* were the dominant solubilizing and mineralizing genes, respectively. Farmland soil has a higher *gcd* gene abundance than NF, while NF soil has a higher abundance of *phoD* gene. pH and total P were the key factors for controlling the abundance of *gcd,* whereas pH and available P were the primary factors governing *phoD* genes. Moreover, MC, SOC and TN were the main factors governing other genes found in this study area. The majority of *gcd*-harboring microbes belonged to the phyla of Acidobacteria, Proteobacteria, Bacteroidetes and Gemmatimonadetes, while Proteobacteria, Actinobacteria, Acidobacteria and Candidatus_Rokubacteria were the dominant *phoD*-harboring phyla. pH, available P and total P were the key factors influencing the abundance of the majority of *gcd* and *phoD*-harboring microbes. Similarly, MC, SOC and TN have also significant roles in the structure of phosphatase solubilizing and mineralizing genes. In conclusion, this study has improved our understanding of how changes in land use affects the availability of P, phosphatase enzyme activity, phosphatase-encoding genes, and phosphatase-harboring microbes.

CRediT authorship contribution statement

Belayneh Azene: Conceptualization, Methodology, Data curation,

Formal analysis, Writing – original draft. **Renhuan Zhu:** Conceptualization, Methodology, Data curation, Formal analysis, Writing – review & editing. **Kaiwen Pan:** Conceptualization, Methodology, Supervision, Funding acquisition, Writing – review & editing. **Xiaoming Sun:** Writing – review & editing. **Yalemzewd Nigussie:** Writing – review & editing. **Piotr Grub:** Writing – review & editing. **Ali Raza:** Writing – review & editing. **Awoke Guadie:** Writing – review & editing. **Xiaogang Wu:** Writing – review & editing. **Lin Zhang:** Writing – review & editing.

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110416.

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