Variations in Soil Properties Rather than Functional Gene Abundances Dominate Soil Phosphorus Dynamics under Short-Term Nitrogen Input

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Research Article

Keywords: Nitrogen input, Microbial functional genes, Soil phosphorus dynamics, Soil properties

Posted Date: February 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-168974/v1

Version of Record: A version of this preprint was published at Plant and Soil on September 29th, 2021.
See the published version at https://doi.org/10.1007/s11104-021-05143-0.

Abstract

Background and aims Microorganisms play a vital role in regulating soil phosphorus (P) dynamics in terrestrial ecosystems. However, how nitrogen (N) inputs trigger the functional traits of P transformation-related microorganisms to affect P fates in soil needs to be explored further. Our aims were to reveal the soil microbial functional profiles for P turnover in response to N input and to explore the relationships between soil P dynamics, soil properties and functional genes.

Methods We collected soil samples from field experiments with three levels of N input over three years in an alpine meadow of the Qinghai-Tibet Plateau to determine soil P dynamics and other properties and functional genes via metagenomics.

Results The soil available P and microbial biomass P were significantly affected by N inputs and significantly associated with soil properties (including soil pH, alkaline phosphatase activity, and soil total N and NO_3^--N contents). Meanwhile, high N input decreased the relative abundance of the *pstS* gene, and low N input reduced the relative abundances of *ugpQ* and *C-P* lyase genes. The *pstS* gene was a determinant of soil microbial biomass P and significantly correlated with soil pH. Moreover, *Alphaproteobacteria* with *C-P* lyase and *Actinobacteria* related to alkaline phosphatases and phosphate-specific transport were the most abundant taxa but not affected by N input.

Conclusions We found relationships between the *pstS* gene, microbial biomass P and soil pH, and the microbial functional gene abundance was less important than soil properties in regulating soil P dynamics under short-term N inputs.

Introduction

Phosphorus (P) is a critical macronutrient for plants. However, a large proportion of P is fixed or immobilized by nonlabile compounds in terrestrial ecosystems, and only a limited amount of available P can be adsorbed and utilized (Shen et al., 2011). The growth of soil microorganisms also requires P uptake, which results in them competing with plants for soil available P (Lupwayi et al., 2007). Therefore, soil available P is generally taken as a good indicator for the bioavailability of P in soil (Bai et al., 2013; Shen et al., 2014).

Many factors influence the P availability for soil, plants and microorganisms (Cross and Schlesinger, 2001; Daly et al., 2015; Baumanna et al., 2018). Among them, the dynamics of nitrogen (N) deposition are key in influencing P dynamics through their effects on other factors. Li et al. (2019a) reported that soil NO_3^--N was the most important driver of labile organic P change. Previous studies demonstrated that N deposition stimulated the P demand of plants by increasing plant production, which caused P limitation in terrestrial ecosystems (Braun et al., 2010; Deng et al., 2017). The changes in soil properties under N deposition determined soil P dynamics (Heuck et al., 2018). Long-term N input induces soil acidification through the accumulation of nitrate (Rustad et al., 1993), which promotes the dissolution of phosphate

and increases soil P availability in calcareous soil (Robles-Aguilar et al., 2019). In contrast, decreases in the soil pH mobilizes soil metal cations, including aluminium and iron, and then reduces available P and the mineralization of organic matter in acidic soil (Carreira et al., 2000). Furthermore, changes in the soil pH resulted in N input indirectly affecting the bacterial community (Ling et al., 2017), which is the main source of alkaline phosphatase (Fraser et al., 2015).

In addition to the soil available P, microbial biomass P accounts for a large proportion of the total P in soil (Achat et al., 2010). Under N input, increases in microbial biomass require immobilization of additional available P to maintain a stable N:P ratio and reduce soil available P (Lupwayi et al., 2007). Nevertheless, other studies also revealed that exogenous N addition may limit microbial biomass and activity due to C restriction and reduce microbial P fixation ability and microbial biomass P concentration (Demoling et al., 2008).

In recent years, the methods of studying microorganisms have been continuously updated (Hill et al., 2000; Roesch et al., 2007; Ouyang et al., 2018), with metagenomic sequencing providing detailed information on multiple genes involved in soil nutrient cycling (Ranjan et al., 2016). Therefore, metagenomics is used to verify the genetic mechanisms by which soil microbial genes manipulate soil P transformations and to enhance the understanding of the functional potential of microbial communities (Neal et al., 2017). In arable, forest and grassland soils, the changes in soil pH caused by N input were verified to be a key driver of a *phoX*-harbouring community structure (Ragot et al., 2016). In response to long-term N input, the potential activity of alkaline phosphatase (Aalp) and the abundance of the bacterial *phoD* gene were significantly reduced, and the ability of bacteria to mineralize nonlabile organic P was enhanced in agricultural ecosystems (Chen et al., 2019). Here, long-term N input reduced the relative abundances of total microbial genes related to P solubilization and some microorganisms containing alkaline phosphatase genes in four agroecosystems (Dai et al., 2020). However, in other agricultural soils, the addition of fertilizers had no significant effect on the relative abundances of genes related to P turnover (Grafe et al., 2018).

The Qinghai-Tibetan Plateau is sensitive to climate change and human activities owing to its high altitude and geographical characteristics (Sun *et al.*,2020). Thus, the change in N deposition might have a greater effect on P dynamics than those in other regions do. However, whether N input altered the abundances of soil microbial P transformation genes and whether these genes were linked to changes in soil P availability remain unclear, especially in alpine regions. Exploring the changes in microbial functional genes associated with P cycling under N inputs could further explain P cycling in alpine meadows. Therefore, we hypothesized that (1) N input increased the relative abundances of genes coding for inorganic P solubilization or organic P mineralization to facilitate plant utilization and (2) N input indirectly changed the abundances of microbial genes related to P dynamics via decreases in soil pH.

Materials And Methods

Study site and experimental design

The experiment was conducted in the N deposition simulation platform located in Haibei, Qinghai Province, China (36° 55'N, 100° 57' E, 3040 m elevation). This region has a continental plateau climate with a -0.45°C mean annual temperature that is characterized by a long, cold winter with a minimum monthly mean air temperature of -29°C and a short, cool summer with an extreme maximum temperature of 27°C in July. The mean annual precipitation is 400 mm, which mainly occurs from June to August. The soil is classified as Mat-Gryic Cambisol with a clay loam texture under the Chinese soil classification system (Ma et al., 2017), and the initial plant species are dominantly *Stipa capillata*, *Potentilla chinensis Poa pratensis*, *Agropyron cristatum*, *Elymus dahuricus*, *Artemisia scoparia*, *Ajuga lupulina* Maxim, and *Potentilla anserine*. As previously reported, the soil background properties were a total N of 3.53 g kg^{-1}, a total P of 0.29 g kg^{-1}, an inorganic N of 15.68 mg kg^{-1} and an available P of 5.94 mg kg^{-1} (Li et al., 2019b).

The experiment plots were established in 2017. Three levels of nitrogen addition (CK, control without N input; LN, low N addition with 5 g N m^{-2} yr^{-1}; HN, high N addition with 10 g N m^{-2} yr^{-1}) were used with three replications. Briefly, nine plots were included in the experiment with a randomized block design, and each plot area was 3 m×3 m. Buffer strips 1-m wide were set between plots. Ammonium nitrate was used as the N source and applied by hand in May of each year.

Soil sampling and analyses

Five cylindrical soil cores (0–10 cm in depth and 5 cm in diameter) were randomly sampled from each plot in August 2019. Soil samples from the same plot were composted into one sample, and plant roots and litter within the soil core were removed. Each mixed soil sample was then divided into two parts: one part was stored at 4°C for biogeochemical analysis, and one part was frozen at -80°C for DNA extraction and metagenome sequencing.

Biogeochemical analysis included determination of pH, ammonium (NH^{4+}-N), nitrate (NO_3^--N), total C, total N, total P, available P, microbial biomass P and the Aalp. Soil pH was determined by a pH meter (Orion Star A215, ThermoFisher Scientific, USA). The concentrations of NH^{4+}-N and NO_3^--N were determined by a flow-solution analyser (Flowsys, Ecotech, Germany). Soil total C and total N were measured by a carbon-nitrogen element analyser (Elementar, Hanau, Germany). Soil total P was obtained using the $HClO_4$-H_2SO_4 digestion-molybdenum antimony colorimetric method (MURPHY and RILEY, 1962). Soil available P was determined by colorimetric measurement. Microbial biomass P was measured by the fumigation-extraction method and calculated from the difference between the P contents of fumigated and nonfumigated soils (Brookes et al., 1985). Aalp was measured after extraction by culturing 5 g of dry soil for 2 h at 37°C in an Erlenmeyer flask with methylbenzene and disodium phenyl phosphate. The suspensions were analysed by measuring the absorbance at 510 nm using a spectrophotometer with buffer solution, amino antipyrine solution and potassium ferricyanide.

DNA extraction and sequencing

Commercial kits were used to extract genomic DNA according to the manufacturer's instructions, and 1% agarose gels were used to monitor the integrity and purity of DNA. Qubit 2.0 (Thermo Fisher Scientific, USA) and Nanodrop One (Thermo Fisher Scientific, Weihao, USA) instruments were used to determine the DNA concentration and purity. Sequencing libraries were generated using the NEBNext Ultra DNA Library Prepkit for Illumina (New England BioLabs, MA, USA), and index codes were added as recommended by the manufacturer. Library quality was assessed using a Qubit 3.0 fluorometer (Life Technologies, Grand Island, NY) and an Agilent 4200 (Agilent, Santa Clara, CA) system. Finally, a library was generated on the Illumina HiSeq X Ten platform, and 150-bp paired-end reads were obtained. Scaftigs (≥ 500 bp) assembled from both single and mixed combinations of predicted ORFs by MetaGeneMark (Version 3.38, http://exon.gatech.edu/GeneMark/metagenome/ Prediction) using default parameters to remove information for sequences shorter than 90 nt from the prediction results. CD-HIT (version: 4.7, http://www.bioinformatics.org/ cd-HIT/) deletes redundant genes to obtain a unique initial catalogue (here, the gene nucleotide sequence was encoded by unique and contiguous genes) that exhibits an identity of 95%, a coverage of 90%, clusters, and the longest single selection sequence. The clean data for each sample were mapped to the initial gene directory using BBMAP software (http://jgi.doe.gov/data-and-tools/bbtools/) to obtain the gene-mapped readings for each sample. According to the number of labelled reads and the lengths of genes, the abundance of each gene in each sample was calculated. Basic statistics, core-pan gene analysis, sample correlation analysis and Venn diagram analysis of gene numbers were all based on the abundances of each gene in each sample in the gene catalogue. DIAMOND software was used to inject unigenes into the functional database. The functional databases include the KEGG database (http://www.kegg.jp/kegg/) and the eggNOG database (http://eggnogdb.embl.de/). Analysis was carried out with the optimal BLAST results. The genes involved in the P transformation of soil microbes were sought in datasets based on previous publications (Dai et al., 2020; Liang et al., 2020). A total of 41 genes related to P transformation and their corresponding KEGG orthology (KO) numbers were collected. According to their functional roles in soil P cycling, we classified these genes into four groups: the P-starvation response regulation gene group (group 1), organic P mineralization gene group (group 2), inorganic P solubilization gene group (group 3), and P transport system gene group (group 4). The names, functions, and classifications of the genes associated with P cycling are listed in Table S1.

Statistical analysis

One-way ANOVA and least significant difference (LSD) tests were employed to verify the effect of N input on soil properties, soil total P, available P, microbial biomass P, and the relative abundances of functional genes and microorganisms coding for P transformation (SPSS 19.0 for Windows). Redundancy analysis (RDA) was used to analyse the relationships among functional microorganisms and soil P dynamics. First, we selected the microorganisms with increased abundance or significant changes under N input. Then, the selected microbial variables and soil P dynamics were analysed by RDA in the “vegan” package in R (R 4.0.2 for Windows). Pearson correlation analysis was used to explore the relationships between soil P dynamics and soil properties and the relationships between soil properties and microbial genes and microorganisms harbouring functional genes using the package “corrplot” in R (R 4.0.2 for Windows).

Random forest analysis was performed to identify the main functional genes influencing soil available P and microbial biomass P.

Results

Responses of main soil properties to N input

The concentration of soil total P was not significantly affected by N input (P> 0.05, Fig. 1). Compared to CK treatment, LN and HN inputs significantly increased the soil available P by 21.74% and 27.27%, respectively (P< 0.05, Fig. 1); however, the microbial biomass P decreased significantly by 22.49% and 61.92%, respectively (P< 0.05, Fig. 1).

Soil pH decreased significantly in HN compared with CK and LN (P< 0.05, Table 1). Soil Aalp remarkably increased under the N inputs (P< 0.05, Table 1). Soil inorganic N and NO_3^--N were different (P< 0.05) between CK and HN, whereas total C, total N and NH^{4+}-N were not different among the three treatments (P> 0.05, Table 1). The soil C: N, C:P and N:P ratios did not change with N addition level (P> 0.05, Table 1). These results indicated that N input notably influenced the soil phosphorus dynamics and some soil properties.

Table 1
Soil properties under different N addition levels. Values are presented as mean± SE of three plot per N input level

Soil properties	CK	LN	HN	P value
Moisture (%)	16.92±0.72a	14.49±0.05b	15.35±0.42b	<0.05
pH	8.16±0.01a	8.16±0.01a	8.09±0.01b	<0.05
Total C (g Kg^{-1})	40.787±0.655a	41.913±0.912a	41.230±0.324a	0.297
Total N (g Kg^{-1})	3.083±0.072a	3.350±0.142a	3.240±0.092a	0.136
Inorganic N (mg Kg^{-1})	4.771±0.202b	5.709±0.207ab	6.050±0.538a	<0.05
NH^{4+}-N (mg Kg^{-1})	1.560±0.056a	1.596±0.066a	1.593±0.121a	0.785
NO_3^--N (mg Kg^{-1})	3.211±0.157b	4.113±0.203ab	4.457±0.417a	<0.05
Aalp (mg g^{-1} $24h^{-1}$)	1.202±0.178b	1.532±0.979a	1.640±0.650a	<0.05
Soil total C: N	13.23±0.10a	12.53±0.26a	12.74±0.28a	0.083
Soil total C: P	66.43±1.14a	68.80±3.14a	67.90±0.35a	0.434
Soil total N: P	5.02±0.21a	5.50±0.37a	5.34±0.15a	0.219
a, b, c Stages not sharing the same letter are significantly different from each other (ANOVA, P < 0.05)				

Responses of genes involved in soil Pcycling to N input

The genes in functional gene group 4 were the most abundant genes (Fig. 2). N input had no significant impact on the total relative abundances of the genes coding for P-starvation response regulation, inorganic P solubilization, organic P mineralization and P uptake and transport ($P > 0.05$, Fig. S2).

The relative abundances of genes involved in P-starvation response regulation (including phoB, phoR and phoU) showed a decreasing trend under N input ($P > 0.05$, Fig. 2). Within group 2, the relative abundance of the ugpQ gene significantly decreased by 10.5% in LN compared to CK ($P < 0.05$), and genes coding for the C-P lyase subunit (phn FGHIJKLMNOP) showed lower abundances in LN than in CK and HN ($P < 0.05$, Fig. 2). N input had no significant effect on the relative abundances of some specific genes coding for acid phosphatase (e.g., phoN, olpA and aphA) and alkaline phosphatase (e.g., phoD, phoA and phoX) ($P > 0.05$, Fig. 2). Within group 4, pstA, pstB, pstC and pstS showed relatively higher abundances. The relative abundances of the pstA, pstB and pstC genes were not different among treatments, but the pstS gene in HN was 8.7% less abundant than that in CK. The relative abundances of some specific genes coding for glycerol-3-phosphate transporter systems (ugpABCE) and phosphonate transporter systems (phnCED) showed no difference among treatments.

Taxonomic assignments of genes involved in P transport and mineralization

The taxonomic assignment of investigated genes was based on KEGG database results. The results are shown at the class level and reflect the overall abundance of taxa in the metagenomic data sets. Within taxa containing genes coding for alkaline phosphatase, N input reduced the relative abundance of Gammaproteobacteria in the short term ($P < 0.05$). Actinobacteria were dominant but exhibited no significant response to N input ($P > 0.05$, Fig. 3). Among the genes involved in C-P lyase, those in Alphaproteobacteria were most abundant, but they had no significant change under N input ($P > 0.05$); the relative abundance of Betaproteobacteria significantly decreased under N input ($P < 0.05$, Fig. 3). Among P-cycle genes, genes corresponding to phosphate-specific transport systems were the most abundant. For genes involved in phosphate-specific transport systems, those in Actinobacteria and Deltaproteobacteria were most abundant, but they had no significant change under N input ($P > 0.05$), and those of Thermomicrobia significantly decreased under N input ($P < 0.05$, Fig. 3).

Relationships among soil P, soil properties and functional genes

Soil available P had a significant positive correlation with inorganic N, NO_3^--N and Aalp ($P < 0.05$). Soil microbial biomass P showed a positive correlation with soil pH and a negative relationship with Aalp ($P <$

0.05, Fig. 4).

The random forest analysis showed that 9 of 41 P-related genes were the determinants of available soil P concentration (Fig. 5a). Among these genes, only ugpQ showed a significant decrease under LN conditions (P< 0.05; Fig. 2). In addition, 12 of 41 P-related genes were the determinants of microbial biomass P concentration (Fig. 5b). Among them, the pstS gene was the most important, and it had a significant decrease under HN input (P< 0.05; Fig. 2).

The concentration of microbial biomass P showed a positive relationship with Gammaproteobacteria with alkaline phosphatase genes and Deltaproteobacteria involved in phosphate-specific transport systems (Fig. 6). Actinobacteria involved in alkaline phosphatase and phosphate-specific transport systems showed a positive correlation with available P and total P. Alphaproteobacteria with C-P lyase genes had no relationship with available P, total P and microbial biomass P (Fig. 6).

The relative abundances of 11 genes were significantly correlated with soil parameters (P< 0.05). Most of the investigated P transformation genes were positively correlated with the soil total N concentration and C:N ratio (Table S3). The relative abundance of Gammaproteobacteria with alkaline phosphatase genes showed significant positive correlations with soil moisture and the soil C:N ratio and negative correlations with total N and Aalp (P< 0.05, Table S4). The relative abundances of Betaproteobacteria responsible for C-P lyase were positively correlated (P < 0.05) with soil moisture (Table S5). The relative abundance of Deltaproteobacteria with phosphate-specific transport systems was negatively correlated with Aalp (P< 0.05). Actinobacteria with alkaline phosphatase genes and phosphate-specific transport systems, Alphaproteobacteria with C-P lyase genes and Thermomicrobia with phosphate-specific transport systems were not significantly correlated with soil properties (P >0.05, Table S6).

Discussion

Shifts in individual microbial genes associated with phosphorus dynamics under N input

Notably, metagenomic analysis showed that HN input decreased the relative abundance of the *pstS* gene, which encodes a high-affinity phosphate-specific transporter (Fig. 2) that allows inorganic P assimilation under P-low conditions (Hsieh and Wanner, 2010). The *pstS* gene is required for the high-affinity acquisition of phosphate and is expressed only when environmental phosphate is limiting (Vuppada et al., 2018). In our results, the soil available P increased under N input, which did not meet the environmental conditions for gene expression. This finding was also supported by a lower relative abundance of *pstS* in P-rich soil than in P-depleted soils (Bergkemper et al. 2016). In the restoration of degraded land, the *pstS* gene was more relatively abundant in an unamended layer of reclaimed tailings with lower concentrations of total P and available P (Liang et al., 2020).

Our study suggested that the microbial function of hydrolysing glycerol phosphate generally decreased under LN addition since the relative abundances of the *ugpQ* genes were lower in LN than in CK and HN (Fig. 2). The protein encoded by the *ugpQ* gene is a glycerophosphoryl diester phosphodiesterase that hydrolyses only diesters (Luo et al., 2009), and glycerol phosphate is taken up with *ugp* transporter systems (*ugp ABCE*) in P-limiting environments (Brzoska,1994). The *ugpQ* gene was not adopted the environment of increased available P content.

In contrast to our hypothesis, the total relative abundances of functional gene groups involved in soil P transformation were not significantly influenced by N input in the short term. In agricultural soils, the relative abundances of genes related to P turnover were also not significantly affected by fertilizer inputs at different sites and seasons (Grafe et al., 2018). These results indicate that microbial potentials for mineralization, solubilization and uptake of phosphorus in soils are stable under short-term N inputs. However, long-term N input (over 35 years) significantly reduced the relative abundances of total genes coding for microbial P solubilization in agroecosystems (Dai et al., 2020). This contrasting result indicated that different durations of treatment might cause varied changes in microbial functional genes (Dai et al., 2020).

In addition, we found that N input did not significantly increase the relative abundances of genes coding for alkaline phosphatase, while the Aalp had a positive response to N addition. One possible reason for this difference is that there are significant differences in the presence and expression of genes involved in P turnover among some taxa (Ragot et al. 2016).

Microbial community performing P transformation

Due to the significant change in available P, we performed taxonomic assignments of genes involved in organic P mineralization and microorganisms containing genes related to alkaline phosphatase and *C-P* lyase. Alkaline phosphatase contributes greatly to the mineralization of soil organic P (Kageyama et al., 2011). The *phoD* gene, encoding alkaline phosphatase, is mainly found in the bacterial phylum *Actinobacteria* (Ragot et al., 2015), which is relatively abundant in soil and water (Luo et al., 2017; Tan et al., 2013). In our study, we discovered that the relative abundance of *phoD*-harbouring *Actinobacteria* was high. *C-P* lyase performs *C-P* cleavage in organic phosphonates (Rodríguez et al., 2006). In our study, *C-P* lyase significantly decreased under LN conditions. Most *C-P* lyases are harboured by *Alphaproteobacteria*, which emphasizes the significance of *Proteobacteria* among P-solubilizing bacteria (Widdig et al., 2019). Among *Proteobacteria*, the *Gammaproteobacteria* were the most frequently reported to harbour *phoX* (Ragot et al., 2016), and this class's abundance significantly decreased under N input, which was supported by findings of Dai et al. (2020).

Because the most-abundant genes were related to the P uptake and transport systems, we determined the taxonomic assignments of genes involved in phosphate-specific transport systems in our study. These genes enable microorganisms to effectively compete with plants for available phosphorus from soil solution (Richardson and Simpson, 2011). Most of the genes coding for phosphate-specific transport

systems were harboured by *Actinobacteria*, which indicated that the highly abundant phylum *Actinobacteria* influenced soil microbial P turnover (Bergkemper et al., 2016).

Increases in P availability caused by N input

Soil P dynamics in this study were strongly related to soil properties (i.e., pH, inorganic N, NO_3^--N) and the Aalp. This result indicated that the changes in edaphic environments regulated the changing patterns of soil available P and microbial biomass P.

Numerous studies have reported that N fertilization stimulates plant growth, increases P uptake by plants, and finally decreases soil inorganic P availability (Vitousek et al., 2010; Yang et al., 2015). However, in our study, the soil available P significantly increased in the LN and HN treatments compared to the CK treatment. This might be because a large amount of N stimulated the secretion of phosphatase and further promoted the accumulation of available P in soil (Tian et al., 2016). The Aalp in our study further proved this finding. A meta-analysis of 34 fields with N addition found that plants and microbes under nitrogen addition can allocate additional N to produce phosphatase enzymes to accelerate the mineralization of soil organic P to mitigate P limitation (R. *et al.*, 2012). In general, P availability is considered regulated by soil pH (Adhikari et al., 2017). N input can decrease soil pH by inducing significant accumulation of nitrate (Guo et al., 2010; Wang et al., 2017; Yang et al., 2015), which increases dissolution of phosphate and enhances soil P availability. In our study, we found that the concentration of soil NO_3^--N significantly increased and the P availability increased under N addition and the soil pH significantly decreased under HN addition.

P availability is also regulated by microorganisms (Alori et al., 2017). On the one hand, microorganisms directly mineralize and solubilize soil phosphorus through the release of hydrolase enzymes, thereby increasing plant available phosphorus (Richardson and Simpson, 2011); on the other hand, microorganisms can efficiently utilize P and then immobilize P into their biomass (Achat et al., 2010). Our study showed that the soil microbial biomass P significantly decreased under HN addition. N deposition increased soil total N and available N contents, limiting microorganism growth as a result of C restriction and then inhibiting the ability of microbes to fix P (Demoling et al., 2008; Deng et al., 2017). P fixation by microorganisms mainly occurs in soil (Wei et al., 2018), and decreases in fixed P by microorganisms could theoretically increase the soil available P.

The significant positive correlations between the relative abundance of the *pstS* gene and soil pH under N input (Table S3) indicate that soil acidification had a greater influence than other factors on the potential capacity of soil microbial P uptake and transport (Hsieh and Wanner, 2010). The random forest analysis revealed that the concentration of microbial biomass P was determined by the relative abundance of the *pstS* gene (Fig. 5).

It is likely that the reduction in pH caused by N input directly decreased the microbial capacity for P uptake and transport (Dai et al., 2020) and thus decreased microbial biomass P. However, a direct positive correlation between soil available P and the abundances of functional genes related to P cycling was not

observed. Similar findings regarding the rates of soil C and N mineralization also revealed that it was difficult to find a clear relationship between functional genes and correlated nutrient cycling processes via only investigation of functional genes (Zhang et al., 2019). One possible reason was probably because the metagenomics approach could obtain only the functional potentials of the microbial community and not their real activities (Grafe et al., 2018). Future studies should combine transcriptomic and proteomic characterization methods to demonstrate the mechanisms affecting functional genes.

Conclusions

The highlight of our study was considering the role of functional genes in exploring the effects of N inputs on P availability in alpine grassland soil. Compared to the abundance of microbial functional genes, soil properties played important roles in regulating soil P dynamics. Shifts in soil pH, Aalp, and soil total N and NO_3^--N contents were determinants of soil available P and microbial biomass P. The relative abundances of the *ugpQ* and *pstS* genes were reduced in environments with increased P availability. In addition, positive relationships existed between the soil pH, *pstS* gene and microbial biomass P. Future studies are required to further explore the relationship between microbial functional genes and different soil P fractions.

Declarations

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 31971746), The Innovation Platform Construction Project of Qinghai Province of China (Grant No. 2017-ZJ-Y20), and Platform of Adaptive Management on Alpine Grassland-livestock System (Grant No. 2020-ZJ-T07).

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 31971746), The Innovation Platform Construction Project of Qinghai Province of China (Grant No. 2017-ZJ-Y20), and Platform of Adaptive Management on Alpine Grassland-livestock System (Grant No. 2020-ZJ-T07).

Conflicts of interest/Competing interests

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability

The authors make sure that all software application support their published claims and comply with field standards

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Bing Han and Jingjing Li. The first draft of the manuscript was written by Bing Han and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

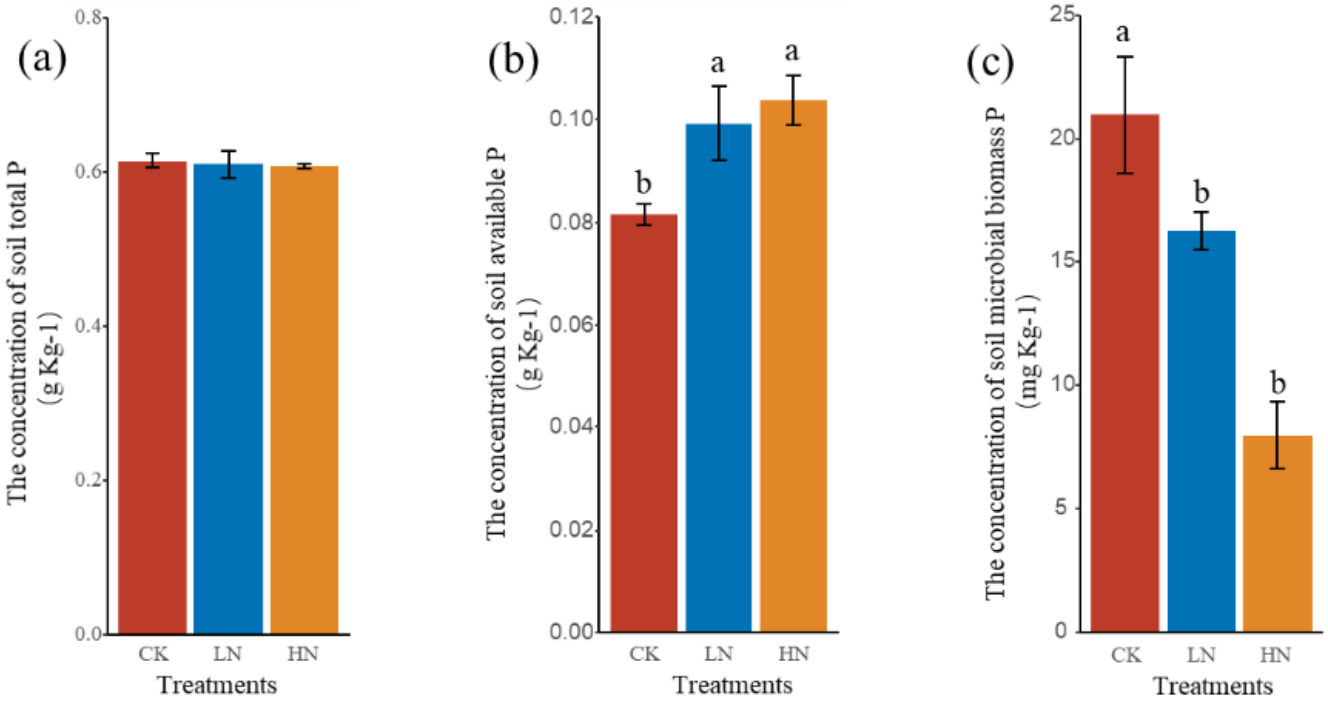

Figure 1

The concentration of soil total P, available P and microbial biomass P under different N input levels Different lowercase letters mean significant difference from each other (ANOVA, $P < 0.05$)

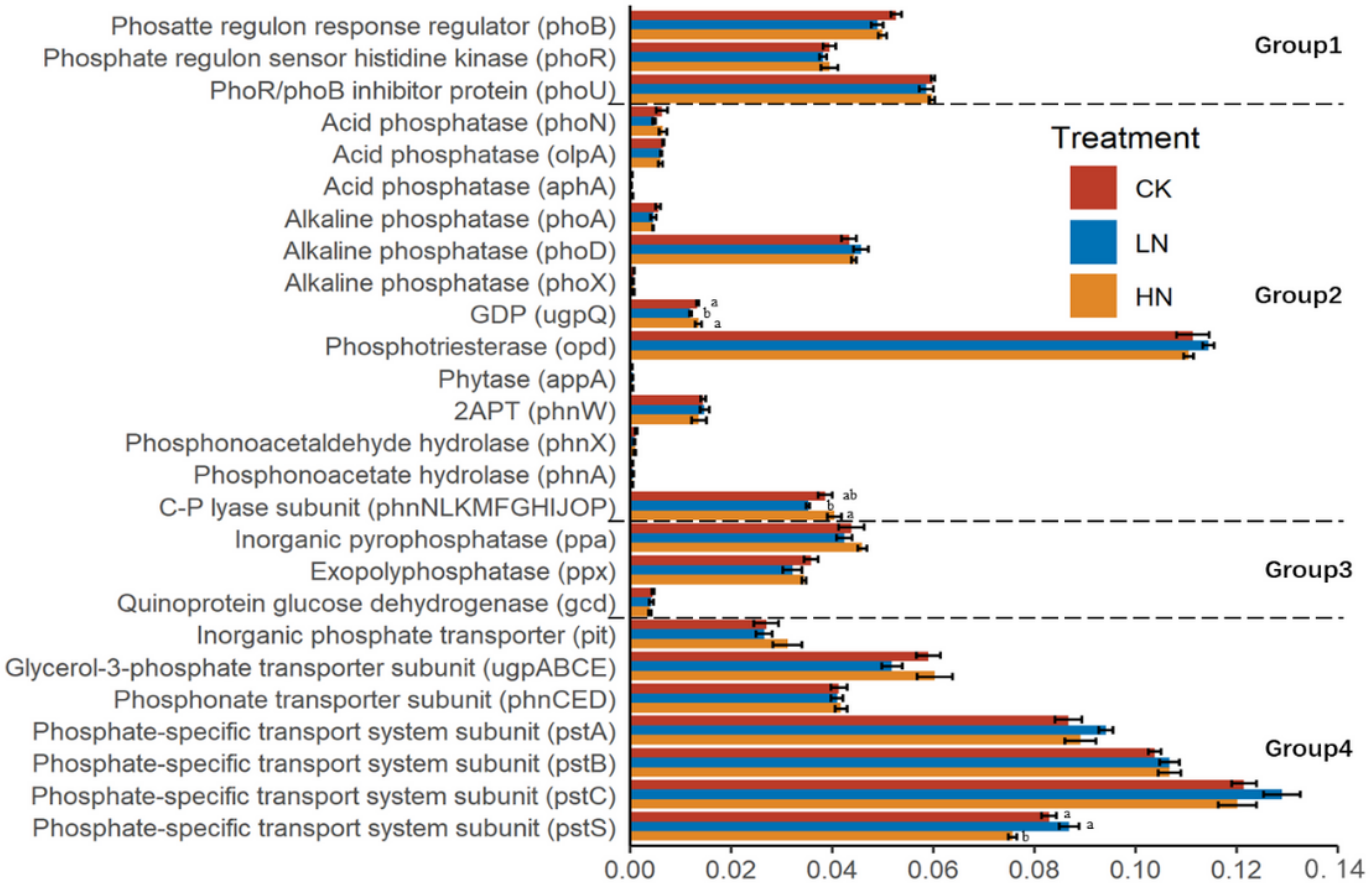

Figure 2

The variation of the relative abundance of representative genes within different groups under different N input levels Group 1 includes P-starvation response regulation genes, group 2 includes organic P-mineralization genes, group 3 includes inorganic P solubilization genes, and group 4 includes P-uptake and transport genes. Different lowercase letters mean significant difference from each other (ANOVA, $P < 0.05$). The relative abundance of ugp transporter systems was calculated as the average abundances of gene ugpA, ugpB, ugpC, and ugpE; the phn transporter systems was calculated as the average abundances of gene phnC, phnE, and phnD; the C-P lyase subunit was calculated as the average abundances of gene phnF, phnG, phnH, phnI, phnJ, phnK, phnL, phnM, phnN, phnO, and phnP

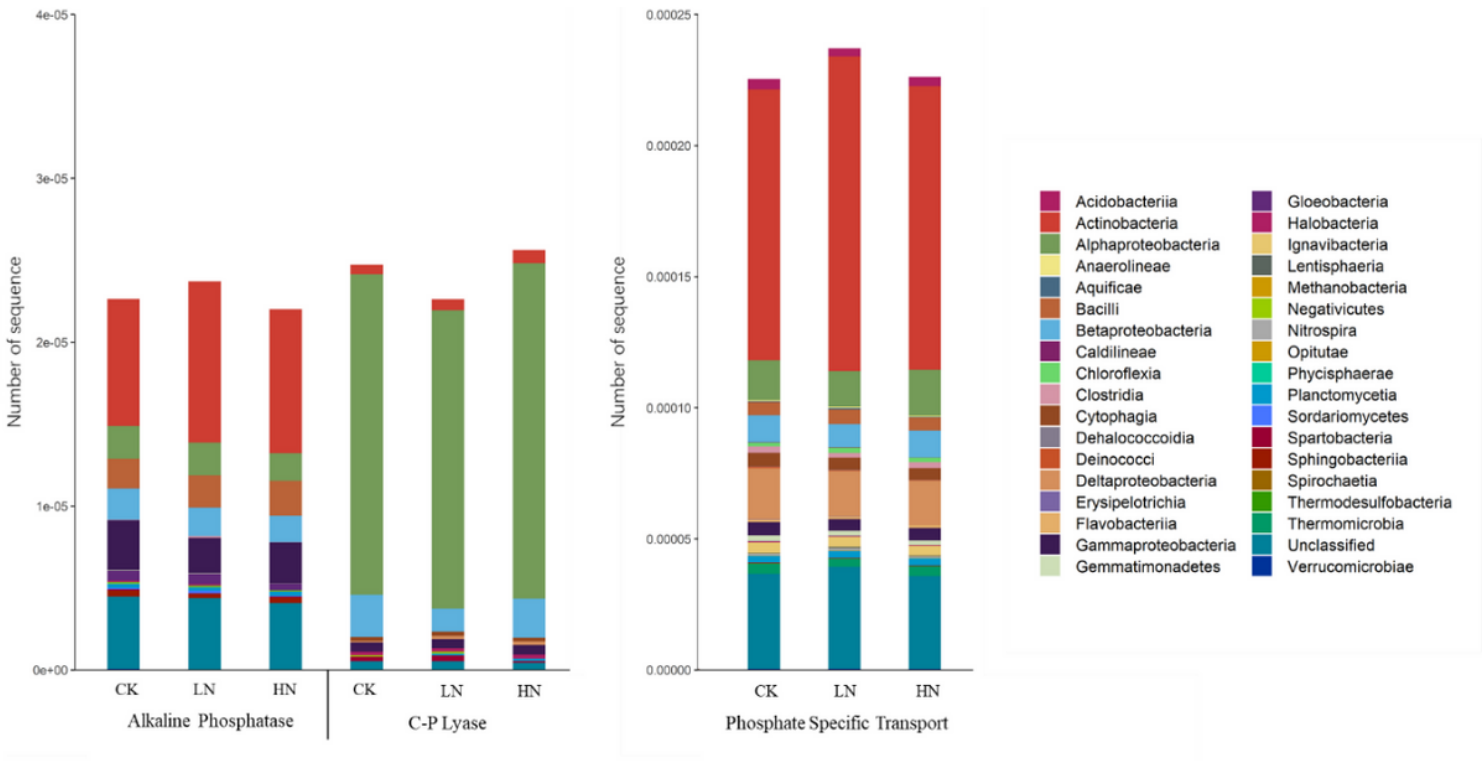

Figure 3

Taxonomic assignment of microbial genes coding for P-mineralization and phosphate specific transport Metagenomic data sets of three treatments soils were assigned on functional level using DIAMOND against the KEGG database. Sequences coding for microbial P-mineralization and phosphate specific transport systems (pooled subunits) was taxonomically assigned (DIAMOND against NCBI Non-redundant protein sequences (nr) database)

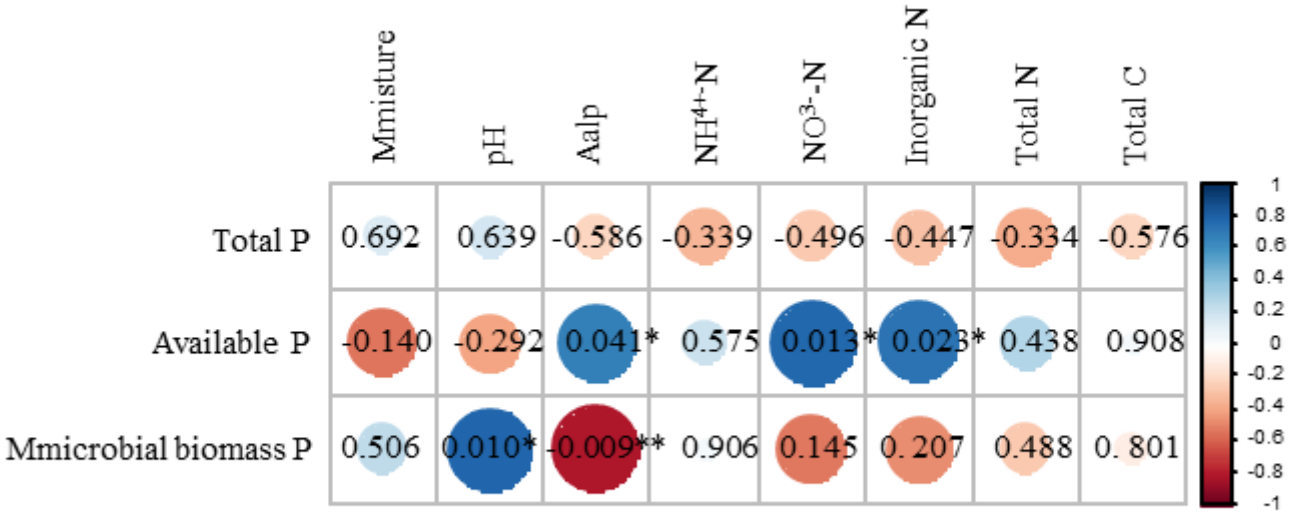

Figure 4

Pearson correlation coefficients between soil P dynamics and soil properties Significance levels: *P < 0.05, **P < 0.01

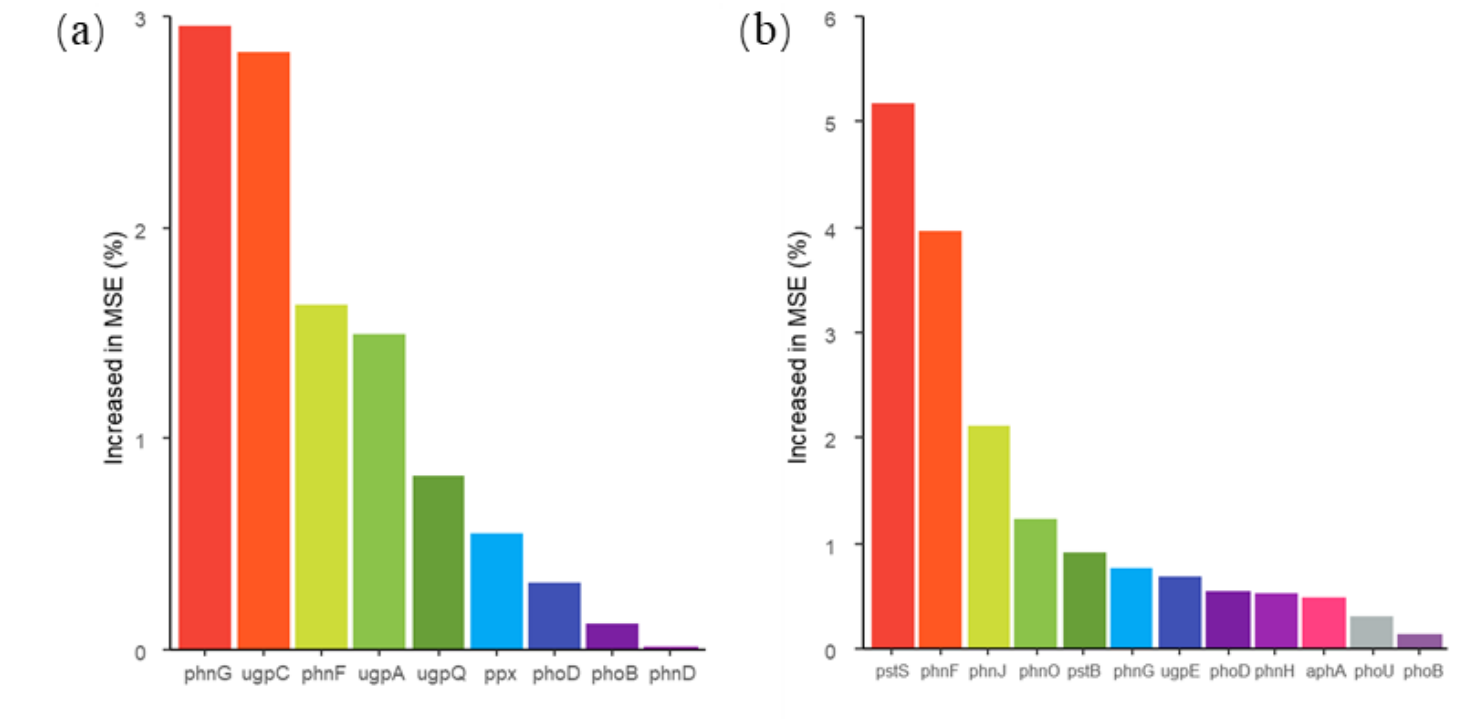

Figure 5

The linkages between genes responsible for soil microbial P-cycling potential and soil P status Panel shows the gene predictors of soil available P (a) and microbial biomass P (b), identified by random forest analysis

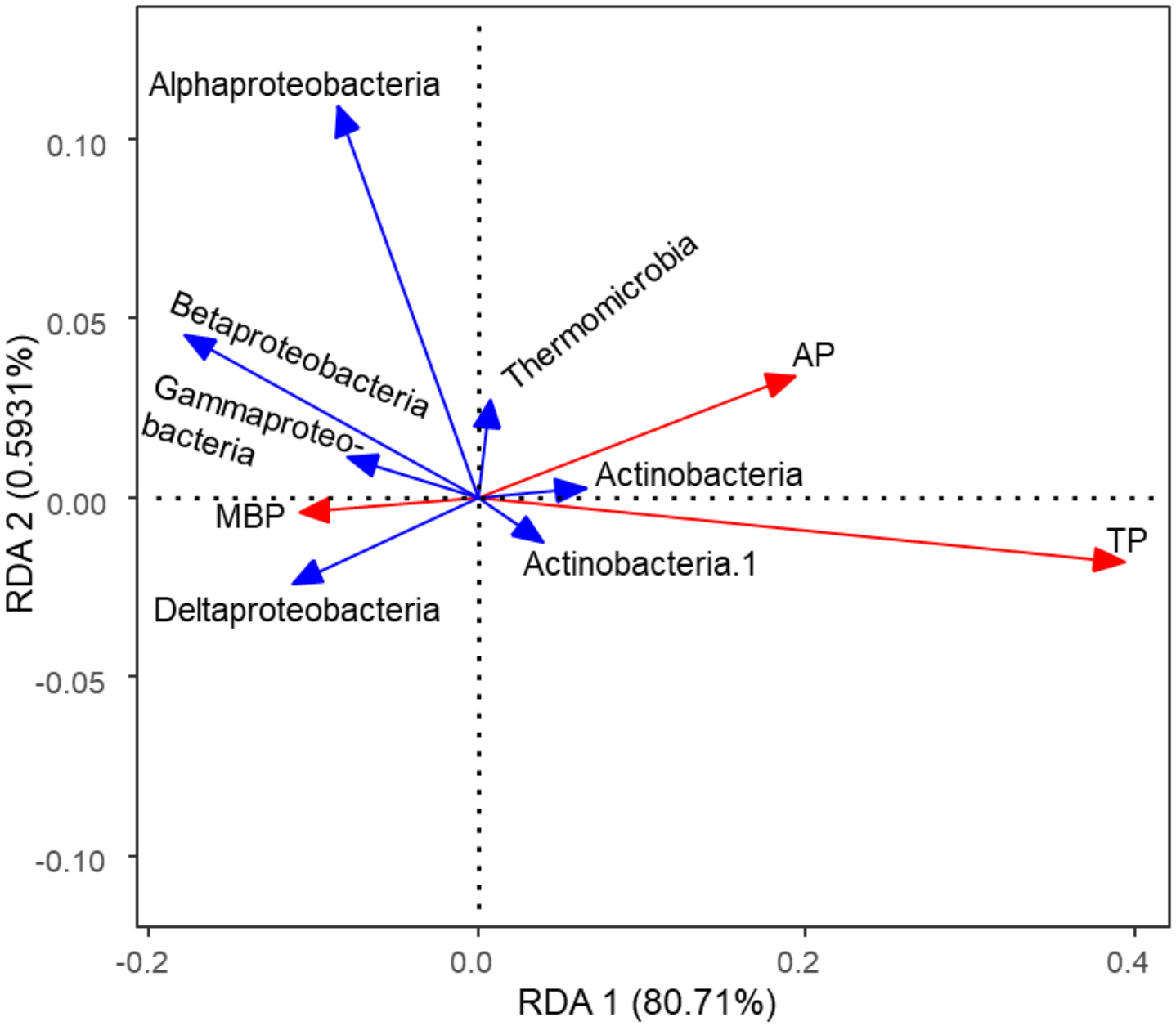

Figure 6

RDA (Redundancy Analysis) ordination maps based on the relative abundance of functional microorganisms Gamaproteobacter and Actinobacteria harbored the genes coding for Alkaline phosphatase. Betaproteobacteria and Alphaproteobacterial harbored the genes coding for C-P lyase. Thermomicrobia, Deltalproteobacteria and Actinobacterial.1 harbored the genes coding for phosphate-specific transport systems

Supplementary Files

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