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ORIGINAL PAPER

Distribution Characteristics of *phoD*-Harbouring Bacterial Community Structure and Its Roles in Phosphorus Transformation in Steppe Soils in Northern China

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Abstract
The *phoD*-harbouring bacterial community is responsible for much of the hydrolysis of organic phosphorus (P_o) in soils and is therefore significant for the improvement of soil phosphorus (P) availability. However, the distribution of *phoD*-harbouring bacterial community structure and how it regulates the soil P fractions in steppe soils remain largely unknown. It is necessary to assess these relationships to maintain sustainable development on the steppe. We sampled soils from three steppe types across Inner Mongolia, China. Illumina high-throughput sequencing was used to quantify the bacterial *phoD* gene. The dominant *phoD*-harbouring genera were *Amycolatopsis* (5–11%), *Bacillus* (6–13%), *Bradyrhizobium* (3–8%) and *Pseudomonas* (3–5%) across all steppe soils. The relative abundances of *phoD*-harbouring *Amycolatopsis* and *Bacillus* increased significantly as available P (AP) decreased, while the relative abundances of *Bradyrhizobium*, *Pseudomonas* and *Methylobacterium* were significantly positively correlated with AP content. Redundancy analysis showed that the soil stoichiometric ratio of carbon (C), nitrogen (N) and P had a strong effect on the *phoD*-harbouring bacterial community structure. Correlation analyses further indicated that only *phoD*-harbouring *Dietzia* and *Sphingomonas* had a significant correlation with alkaline phosphatase activity and that they increased P availability by mineralizing P_o. *phoD*-harbouring *Frankia* and *Methylobacterium* were positively correlated with labile-P_o and negatively correlated with non-labile inorganic P. Moreover, *phoD*-harbouring *Bacillus* and *Bradyrhizobium* promoted the conversion of the non-labile P_o pool into the labile P_o pool, which can be attributed to microbial immobilization. Not all bacteria carrying the *phoD* gene promote soil P availability through mineralization or are induced not only in a P-repressible manner. Members of the *phoD*-harbouring bacterial community employ flexible P use strategies and can be strongly activated by their nutrient preferences and environmental conditions.

Keywords Steppe types · *phoD* gene · Bacterial community · Phosphorus transformation

1 Introduction

Phosphorus (P) limitation in natural ecosystems is a global issue (Vitousek et al. 2010; Atere et al. 2018), and 30–80% of the total P (TP) in grassland soils is organic P (P_o) (Sharma et al. 2013). Because microorganisms and plants absorb P mainly as inorganic orthophosphate (HPO_4^{2-} or $H_2PO_4^-$) from the soil solution, P_o can only be absorbed directly by plants after mineralization by phosphatases (Nannipieri et al. 2011; Sun et al. 2019). Therefore, the extracellular enzymes facilitating the mineralization of P_o compounds may play an important role in plant nutrition in grassland soils (Rui et al. 2009). Previous studies have confirmed that many organisms normally produce phosphatases (i.e. acid (ACP, EC 3.1.3.2) and alkaline phosphatases (ALP, EC 3.1.3.1)), phytases and nucleotidases when facing P scarcity (Ragot et al. 2015). These are crucial enzymes that are capable of hydrolysing P_o to bioavailable P in soil (Nannipieri et al. 2011; Ye et al. 2017). ALP can hydrolyse phosphomonoesters, which are generally the dominant fraction of P_o and can represent up to 90% of the P_o in soil (Condron et al. 2005; Nannipieri et al. 2011), and therefore ALP is important driver of P_o turnover. In most bacteria, the *Pho* regulon includes functional genes encoding ALP such as *phoD*, *phoA* and *phoX* (Santos-Beneit 2015; Ragot et al. 2015). Considering that the *phoD* gene has

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been identified widely in the soil bacterial community, it is a useful molecular marker to investigate soil P_o transformation processes that are largely controlled by bacteria (Tan et al. 2013; Luo et al. 2017).

phoD-Harbouring bacteria have been detected in various ecosystems such as forests (Shange et al. 2012; Bergkemper et al. 2016), grassland (Neal et al. 2017), bare fallow (Jangid et al. 2008), bogs (Keith et al. 2012) and arable land (Neal et al. 2017). The key genera identified in grassland soils using primers designed by Ragot et al. (2015) were *Bradyrhizobium*, *Pseudomonas*, *Bacillus*, *Massilia*, *Stenotrophomonas* and *Streptomyces* (Ragot et al. 2016), while Tan et al. (2013) used primers designed by Sakurai et al. (2008) and found that the dominant *phoD*-harbouring phyla in pasture soils were included mainly Proteobacteria, Actinobacteria and Cyanobacteria. Due to the differences in primers and observed levels, the current research results are not comparable, and it is difficult to identify the core *phoD*-harbouring bacterial community in grassland soils. Therefore, further study is needed.

Studies have shown that the soil type (Ragot et al. 2017), land use (Ragot et al. 2016; Neal et al. 2017), vegetation (Wang et al. 2012) and fertilizer management (Berg and Smalla 2009) indirectly affect the *phoD*-harbouring bacterial community by affecting soil properties. Soil organic matter (SOM) is an important driver of the *phoD*-harbouring bacterial community structure in subtropical orchard soils (Cui et al. 2015; Espinosa et al. 2017), and pH has a significant effect on the *phoD*-harbouring bacterial community structure in grasslands and in cropping systems (Wang et al. 2012; Ragot et al. 2015). However, the soil pH also affects the availability of nutrients such as P by modifying adsorption and desorption reactions (Ragot et al. 2016), further influencing the microbial community structure. This makes it difficult to distinguish the effect of nutrient availability on the microbial community from that of pH. Many studies have reported that variations in soil microbial community structure can be attributed to differences in nutrient stoichiometry (Sinsabaugh et al. 2013; Dai et al. 2018; Cui et al. 2019) because different microorganisms have different nutritional preferences (Shade et al. 2014; Jousset et al. 2017). Moreover, *r*-strategists grow faster in response to available carbon (C) and require larger amounts of P than oligotrophs (Fierer et al. 2007; Luo et al. 2019). Therefore, the present studies are still very limited with regard to understanding the effects of environmental factors on the *phoD*-harbouring bacterial community structure in soils, especially in grassland soils with poor environmental homogeneity. In addition, some studies have shown that not all bacteria carrying the *phoD* gene play an important role in the secretion of ALP (Fraser et al. 2015), so how *phoD*-harbouring bacteria participate in soil P transformation remains largely unknown. To obtain a comprehensive picture of the processes through which bacteria are involved in P turnover, more information is needed about the distribution of the *phoD*-harbouring bacterial community in the soil and its roles in the transformation of soil P fractions.

The Inner Mongolia steppe represents approximately 22% of the grassland in China (Zhu et al. 2020) and has an indispensable position with regard to ecosystem protection, sandstorm prevention, soil and water conservation and climate regulation (Miu and Liu 2013). However, previous studies have shown that soil P levels have seriously restricted the development of Inner Mongolia steppe productivity (Compton et al. 2000; Elser et al. 2007) and soil P exists mainly in the form of P_o (Zhu et al. 2020). Therefore, it is urgent to improve soil P availability in this region through biotic processes such as P_o mineralization. However, the distribution and function of the P_o mineralizing bacterial community in this region remain to be elucidated. In this study, we surveyed three types of steppe soil (i.e. desert steppe, typical steppe and meadow steppe) in Inner Mongolia and investigated the *phoD* gene using primers described by Ragot et al. (2015). We aimed to (1) investigate the key bacteria harbouring the *phoD* gene in steppe soil; (2) understand the factors driving the *phoD*-harbouring bacterial community and its response to changes in soil properties; and (3) illustrate the process by which *phoD*-harbouring bacteria regulate P transformation. We hypothesized that (1) the *phoD*-harbouring bacterial community structure in the different steppe types would be different; (2) the soil available P (AP) and stoichiometric ratios of C, nitrogen (N) and P would be strong drivers of the *phoD*-harbouring bacterial community; and (3) microorganisms would employ flexible P use strategies that might vary even within the same taxon.

2 Materials and Methods

2.1 Soil Sampling and Sample Preparation

Soil samples were collected from 15 sites in the Inner Mongolia Autonomous Region (37° 24′–53° 23′ N, 97° 12′–126° 04′ E) in July 2017.The sites covered three types of steppe habitats: desert steppe, typical steppe and meadow steppe. For each steppe type, 5 sampling sites were selected. Each sample was mixed with at least 20 individual surface-soil cores (0–15 cm). These soils were mainly derived from granite. The soil type at each site was identified based on FAO-UNESCO 1974. More sampling details are showed in Table 1 and provided in Zhu et al. (2020).

After removing plant residues, roots and stones, soil subsamples were immediately stored at − 80 °C to be used for DNA extraction and at 4 °C to be used to determine soil phosphatase activity. The remaining composite soil samples were air dried and sieved to 2 mm for the analyses basic soil properties.

Table 1 The information of sampling sites

Site no.	Coordinates	MAT (°C)	MAP (mm)	NDVI	Steppe types	Soil types	Vegetation types
1	N41° 49.925′ E111° 53.842′	4.24	280.13	0.40	Desert steppe	Calcic Xerosols	*Stipa breviflora Griseb*; *Stipa klemenzii Roshev*
2	N42° 47.267′ E112° 40.77′	5.58	193.15	0.30			
3	N43° 10.317′ E112° 56.739′	5.09	180.2	0.23			
4	N43° 24.680′ E113° 07.275′	4.93	178.52	0.17			
5	N43° 57.072′ E114° 38.685′	2.49	241.35	0.36			
6	N43° 53.996′ E115° 20.438	2.16	271.3	0.47	Typical steppe	Calcic Kastanozems	*Leymus chinensis*; *Stipa grandis*
7	N44° 43.960′ E117° 27.602′	2.04	313.62	0.59			
8	N44° 50.291′ E117° 27.721′	1.99	315.57	0.56			
9	N45° 58.978′ E119° 10.382′	2.05	335.48	0.61			
10	N46° 07.554′ E119° 12.818′	−0.75	449.15	0.66			
11	N49° 30.269′ E119° 47.364′	−0.63	377.24	0.69	Meadow steppe	Calcic Chernozems	*Leymus chinensis*; *Stipa baicalensis*
12	N49° 19.213′ E119° 43.256′	−0.77	368.02	0.66			
13	N49° 18.130′ E119° 06.102′	−0.16	336.73	0.69			
14	N49° 27.104′ E118° 15.772′	−0.15	308.8	0.56			
15	N49° 26.842′ E118° 38.516′	−0.01	326.4	0.58			

[a] *MAT* mean annual temperature, *MAP* mean annual precipitation, *NDVI* the normalized difference vegetation index

2.2 Soil Analysis

2.2.1 Chemical Analyses

The soil pH was determined at a soil:water ratio of 1:2.5 ratio (w/v) (Skjemstad and Baldock 2007). SOM was measured by potassium dichromate digestion (Nelson and Sommers 1982). Soil total N (TN) was determined using an elemental analyser. Soil TP was determined by $HClO_4$-H_2SO_4 digestion followed by colorimetric analysis (Kuo 1996). Total P_o was measured by the ignition method (Saunders and Williams 1955). Soil AP was extracted by 0.5 M $NaHCO_3$ with a soil/solution ratio of 1:20 (Olsen et al. 1954), and the P in solution was determined using molybdenum blue colorimetry at 880 nm (Murphy and Riley 1962). The soil P fractions were obtained according to a previously published article (Zhu et al. 2020). Potential ALP activity at pH 11.0 was analysed using the method described by Wu et al. (2006). Briefly, 0.5 g of fresh soil was incubated with *p*-nitrophenyl phosphate (*p*NPP) as a substrate at 37 °C. ALP activity was expressed as μg pNP produced per g soil dry weight per hour. The values of all soil properties are expressed in dry weight equivalents. A detailed description of the soil properties is shown in Table 2.

2.2.2 DNA Extraction

Genomic DNA was extracted in duplicate from 0.50 g frozen soil using a PowerSoil® DNA Isolation Kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions. The DNA concentration and quality were assessed by the A260/280 and A260/230 ratios using a NanoDrop ND-2000 spectrophotometer (Thermo Scientific Wilmington, DE, USA). The isolated DNA was stored at − 80 °C for further analysis.

2.2.3 High-Throughput Sequencing of the *phoD* Gene Amplicon and Data Processing

The primer pair for the *phoD* gene and the PCR conditions was performed as previously described by Ragot et al. (2015).

Table 2 Soil chemical properties in Inner Mongolia steppe

Steppe types	pH	SOM (g kg^{-1})	TN (g kg^{-1})	TP (mg kg^{-1})	AP (mg kg^{-1})	P_o (mg kg^{-1})	ALP (μg pNP g^{-1} h^{-1})
Desert steppe	7.19±0.25 a	7.98±5.28 c	0.72±0.30 b	206.05±65.83 a	5.16±1.63 c	96.98±44.31 a	643.19±275.94 b
Typical steppe	7.27±0.09 a	24.41±3.07 b	1.69±0.24 a	236.52±84.64 a	9.12±1.53 b	139.49±21.89 a	1473.47±371.97 a
Meadow steppe	6.88±0.07 b	8.96±16.12 a	2.21±0.68 a	204.8±41.11 a	16.32±3.94 a	149.1±39.21 a	1046.38±328.66 ab
Mean	7.11	23.78	1.54	215.79	10.20	128.52	1054.35
CV (%)	3.08	65.06	47.73	28.31	50.74	30.87	42.52

[a] *SOM* soil organic matter, *TN* total nitrogen *TP* total phosphorus, *AP* available phosphorus, P_o organic phosphorus, *ALP* alkaline phosphatase, *CV* coefficient of variation

[b] The values represent the mean ± standard deviation ($n = 5$) for each steppe types. Different lowercase letters indicate significant difference ($p < 0.05$) among steppe types

Briefly, primers (*phoD*-F733: 5′-TGGGAYGATCAYGARGT-3′ and *phoD*-R1083: 5′-CTGSGCSAKSACRTTCCA-3′) were used to amplify the *phoD* gene. The PCRs were run on a Bio-Rad S1000 (Bio-Rad Laboratory, CA, USA). The amplification conditions were as follows: 95 °C for 3 min; followed by 30 cycles at 95 °C for 5 s, 58 °C for 30 s and 72 °C for 30 s; and a final extension at 72 °C for 10 min. The PCR products were purified using the EZNA Gel Extraction Kit (Omega, USA). Sequencing libraries were generated using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA) following the manufacturer's recommendations, and index codes were added. The library quality was assessed on a Qubit@2.0 fluorometer (Thermo Fisher Scientific, MA, USA) and an Agilent Bioanalyzer 2100 system (Agilent Technologies, Waldbronn, Germany). Finally, the library was sequenced on an Illumina HiSeq 2500 platform, and 250-bp paired-end reads were generated (Guangdong Magigene Biotechnology Co., Ltd., Guangzhou, China). Sequence analysis was performed using USEARCH software (V10). Sequences with ≥97% similarity were assigned to the same operational taxonomic unit (OTU).

2.3 Statistical Analysis

One-way ANOVA with steppe types as a factor was performed for the community composition of *phoD*-harbouring bacteria using SPSS 24.0. α-Diversity, measured with the Chao1 index, was used to analyse the complexity of species diversity for each sample and was calculated with QIIME (V1.9.1) and displayed with R software (V2.15.3). Principal coordinate analysis (PCoA) based on a weighted UniFrac distance matrix was performed to compare the β-diversity across all samples and was displayed by the QIIME and ggplot2 packages in R software. Additionally, to explore the linkage between environmental factors and the structure of the *phoD*-harbouring bacterial community, detrended correspondence analysis (DCA) was performed firstly with species-sample data to determine whether to use redundancy analysis (RDA) or canonical correspondence analysis (CCA). According to the result of DCA, the size of the first axis of length of gradient is 0.5, thus choosing RDA for association statistical analysis in R software (V2.15.3). The ggplot2 and RColorBrewer packages in R software were used to generate heat maps to analyse the relationship of *phoD*-harbouring genera to environmental factors. Pearson's correlation coefficient was used to investigate the potential correlation between the *phoD*-harbouring bacterial diversity and composition, ALP activity and content of soil P factions using SPSS 24.0.

3 Results

3.1 *phoD*-Harbouring Bacterial Community Structure

The dominant *phoD*-harbouring phyla in the three steppes were Proteobacteria, Planctomycetes, Firmicutes, Cyanobacteria and Actinobacteria (Fig. S1a). The average relative abundances of Proteobacteria and Cyanobacteria were more than 50%. The identified *phoD* sequences were further assigned to different taxonomic levels (> 1%) covering 6 classes, 10 orders, 16 families and 17 genera. The dominant genera were *Amycolatopsis* (5–11%), *Bacillus* (6–13%), *Bradyrhizobium* (3–8%) and *Pseudomonas* (3–5%) (Fig. 1). ANOVA showed that the *phoD*-harbouring bacterial community in the different steppes were significantly different from each other at the genus level ($p < 0.05$). The relative abundances of *Amycolatopsis* and *Bacillus* decreased significantly with the change of desert steppe to typical steppe to meadow steppe, while *Bradyrhizobium* and *Frankia* were significantly higher in the typical and meadow steppes than in the desert steppe. The relative abundance of *Dietzia* was the highest in the typical steppe.

The α-diversity of the *phoD*-harbouring bacterial community estimated by the Chao1 index revealed significant differences among the three steppes ($p < 0.05$) (Fig. 2a). The Chao1 index was the lowest in the meadow steppe and the highest in

the typical steppe. There was a positive correlation between the Chao1 index and ALP activity ($r = 0.65$, $p < 0.01$) (Fig. 3). The β-diversity comparison assessed by PCoA showed that the *phoD*-harbouring bacterial community structure in all the samples clustered into three groups according to steppe type and that these clusters explained 58.48% of the total variation (Fig. 2b). The β-diversity of the *phoD*-harbouring bacterial community showed great variation among the desert steppe sites.

3.2 Relationships of Environmental Factors to the *phoD*-Harbouring Bacterial Community

Based on the RDA, the *phoD*-harbouring bacterial community structures of all samples were clustered into three groups according to the steppe type (Fig. 4a). The examined environmental factors explained 63.8% of the total variation in the *phoD*-bacterial community structure (Table 3). RDA1 and RDA2 explained 34.16% and 14.21% of the variation in the *phoD*-harbouring bacterial community, respectively. The *phoD*-harbouring bacterial community structure was strongly correlated with ALP activity ($p < 0.01$) and the C/P ratio ($p < 0.05$); these two factors explained 36.2% of the total variation. The *phoD*-harbouring bacterial community in the desert steppe diverged from that in the other two steppes along RDA2, which was related to the C/N ratio.

The heat map indicated that 9 out of 17 *phoD*-harbouring bacteria were susceptible to environmental factors (Fig. 4b). The C/P and N/P ratios were the most influential environmental drivers, and both influenced the relative abundance of 7 out of 9 genera. There were strong, significant correlations between *Bacillus* and *Methylobacterium* and soil properties. However, we found that only *Dietzia* and *Sphingomonas* were closely correlated with ALP activity.

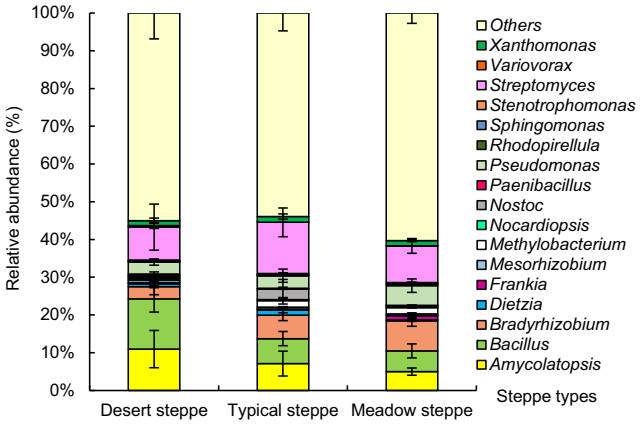

Fig. 1 Composition of *phoD*-harbouring bacterial community under different steppes at the genus level

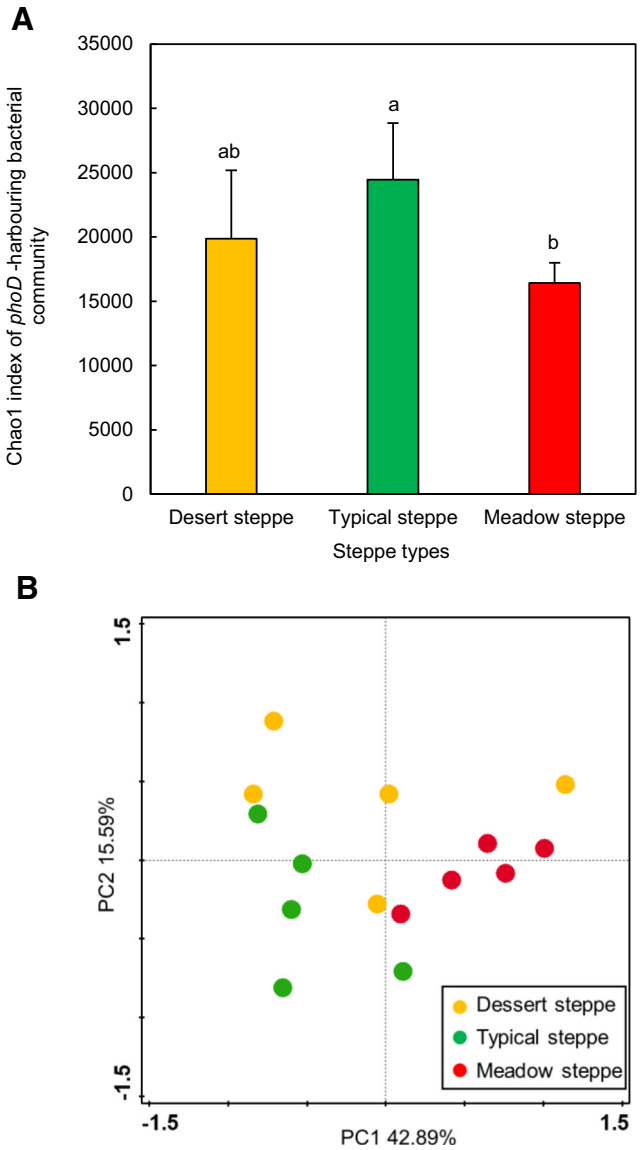

Fig. 2 α-diversity (**a**) and β-diversity (**b**) of *phoD*-harbouring bacteria under different steppes at the genus level. The α-diversity of the *phoD*-harbouring bacterial community is estimated by Chao1 index. Values are means ($n = 5$) with bars representing standard deviation. Different lowercase letters indicate significant differences among steppe types ($p < 0.05$). Colour dots represent soil samples from different steppes ($n = 5$)

3.3 The Relationships of the *phoD*-Harbouring Bacterial Community with Soil P Fractions

The soil P fractions were dominated by HCl-P_i and NaOH-P_o (Table S1). There were significant differences in the soil P fractions among the different steppe types. The content of labile-P_o increased significantly in the order desert steppe < meadow steppe < typical steppe, while HCl-P_o showed the opposite trend. The contents of NaOH-P and Phyt-P in the typical steppe and HCl-P_i in the desert steppe were the highest. There was a positive correlation between ALP activity and

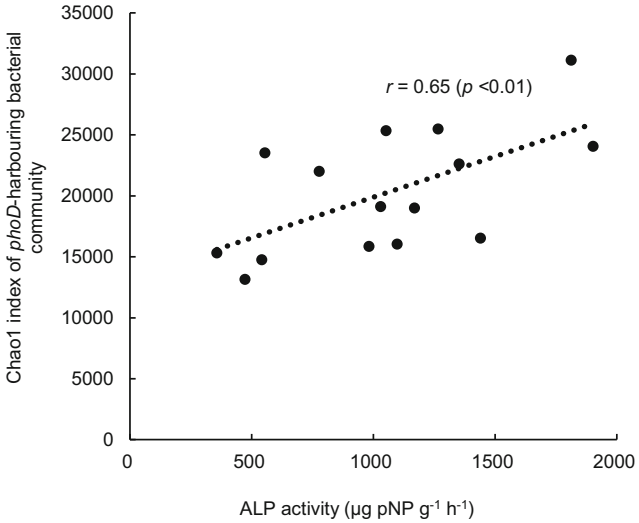

Fig. 3 The relationship between alkaline phosphatase (ALP) activity and α-diversity estimated by Chao1 index of *phoD*-harbouring bacterial community ($n = 15$). Data points represent values from soils collected from 15 sites across different steppe types

NaOH-P_o ($r = 0.89$, $p < 0.01$) (Table 4) and AP content ($r = 0.52$, $p < 0.05$) (Fig. S2). The relative abundance of *Dietzia* had a significantly positive correlation with NaOH-P_i. The relative abundances of *Bacillus* and *Bradyrhizobium* were positively correlated with labile-P_o and negatively correlated with NaOH-P_o and HCl-P_o, respectively ($p < 0.05$). The relative abundance of *Frankia* had a negative correlation with soil NaOH-P_i ($p < 0.01$) and a positive correlation with labile-P_o ($p < 0.01$). The relative abundance of *Methylobacterium* was positively correlated with labile-P_o and negatively correlated with HCl-P_i ($p < 0.05$).

Table 3 Variation portioning into *phoD*-harbouring bacterial community at the genus level explained by soil properties

Environmental factors[a]	% of all variation[b]	p[c]
ALP	19.7	0.008**
C/P	16.5	0.044*
N/P	6.6	0.272
pH	6.3	0.306
C/N	5.3	0.412
SOM	4.8	0.468
AP	4.6	0.500
Total	63.8	

[a] *SOM* soil organic matter, *AP* available phosphorus, *ALP* alkaline phosphatase, *C/P* the ratio of soil organic carbon to total phosphorus, *N/P* the ratio of total nitrogen to total phosphorus, *C/N* the ratio of soil organic carbon to total nitrogen

[b] Environmental factors' effect was assessed through variance partitioning based on the conditional effect (λ-A) of each environmental variable. Forward selection on 499 permutations was used to test the significant contributions of each factor

[c] * indicates significant difference at $p < 0.05$; ** indicates significant difference at $p < 0.01$

4 Discussion

4.1 *phoD*-Harbouring Bacterial Community Composition and Diversity

It is essential to understand the distribution of *phoD*-harbouring bacterial community in ecosystems as they influence a variety of important ecosystem processes related to soil P turnover and biogeochemical cycling (Chhabra et al. 2012; Jorquera et al. 2014). In this study, the *phoD* gene was found mainly in 5 bacterial phyla (Fig. S1a). In contrast, Ragot et al. (2015) amplified 13 phyla from grassland soils using the same primers. They observed *phoD*-harbouring Acidobacteria, Bacteroidetes, Chloroflexi, Deinococcus-Thermus, Nitrospirae, Spirochaetes and Verrucomicrobia. This difference may be explained by the smaller variation in soil properties and the lower microbial diversity in this study than in previous studies. More specifically, the dominant *phoD*-harbouring bacterial genera were *Amycolatopsis*, *Bacillus*, *Bradyrhizobium*, *Pseudomonas*, *Streptomyces* and *Xanthomonas* (Fig. 1), while *Scytonema*, *Rhodoplanes*, *Kaistia*, *Rhizobacter* and *Methylibium* were observed by Sun et al. (2015). Ragot et al. (2015) captured a particularly large diversity of *phoD*-harbouring genera, including *Actinomyces*, *Arthrobacter*, *Micrococcus*, *Streptosporangium*, *Azorhizobium*, *Rhodospirillum*, *Caulobacter* and *Variovorax*. These findings revealed differences in the *phoD*-harbouring bacterial genera in different grassland ecosystems due to the specific geographical location, climate and soil type (Ragot et al. 2017; Luo et al. 2017). However, the dominant bacterial community at both phylum and genus levels were similar in all sample plots in our study, suggesting that there was a remarkable degree of genetic similarity and a core *phoD*-harbouring bacterial microbiome across the Inner Mongolia steppe soils. This study showed that high relative abundances of *phoD*-harbouring *Amycolatopsis*, *Bacillus*, *Bradyrhizobium*, *Streptomyces* and *Pseudomonas* may have made great contributions to P_o mineralization. Previous studies have found that the genus *Pseudomonas* also solubilizes P_i (Kundu et al. 2009; Yu et al. 2011). Therefore, *Pseudomonas* may be involved in both the mineralization of P_o and the solubilization of P_i.

Although the composition of the *phoD*-harbouring bacterial community was similar among the three steppe soils, there was great variability in the relative abundances of *phoD*-harbouring bacterial genera (Fig. 1). This result was consistent with our hypothesis. For example, the relative abundance of *Bacillus* was the highest in the desert steppe, while that of *Bradyrhizobium* was the lowest. *Bacillus* is a gram-positive

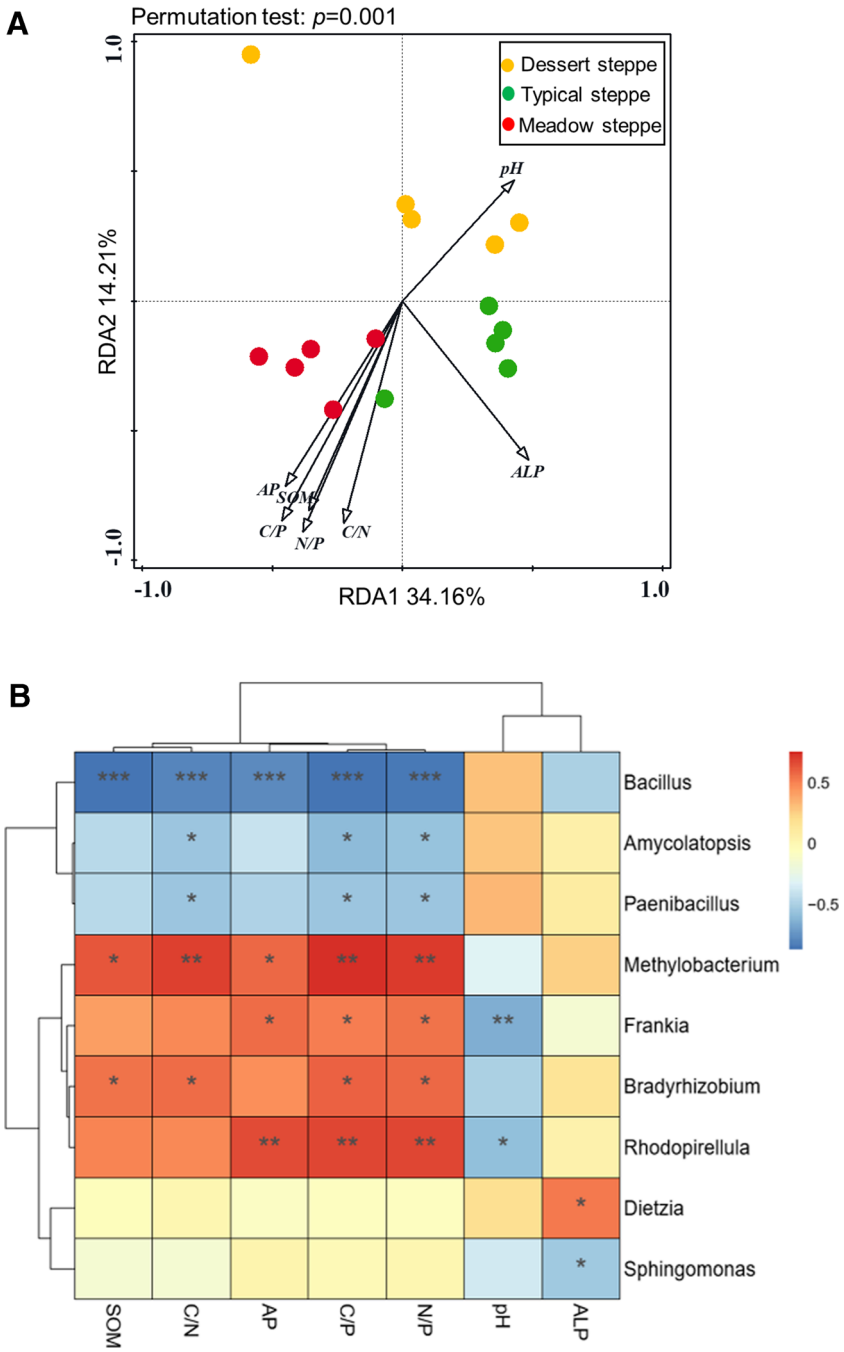

Fig. 4 The effect of environmental factors on the distribution of *phoD*-harbouring bacterial community structure (**a**) and the relationships between environmental factors and *phoD*-harbouring bacteria at the genus level (**b**). Black lines in RDA plot represent the environmental variables, and colour dots represent soil samples from different steppes ($n = 5$). SOM, soil organic matter; AP, available phosphorus; ALP, alkaline phosphatase

bacterium that exhibits resistance to adverse environments, and it was more abundant in the desert steppe soils where harsh geographic and climatic conditions are expected. *Bradyrhizobium* also encodes a N_2-fixing gene (Kaneko et al. 2002), which is closely related to soil N nutrient levels; thus, it exhibited high relative abundance in the typical and meadow steppe soils.

There was a significant difference in *phoD*-harbouring bacterial α-diversity among the three steppes (Fig. 2a). The Chao1 index was the highest in the typical steppe, suggesting that the typical steppe soils had a higher quantity of species carrying the *phoD* gene; this result is closely related to the high vegetative diversity and good hydrothermal conditions in the typical steppe (Steenwerth et al. 2002). The β-diversity of the *phoD*-harbouring bacterial community also diverged among the three steppe types (Fig. 2b), and the analysis revealed that the richness of the *phoD*-harbouring bacterial community was preserved with the change in steppe type. The zonal distribution of the climate in the Inner Mongolia steppe results in the variations in steppe type, including desert steppe, typical steppe and meadow steppe, and is mainly reflected in variations in soil properties (Wang et al. 2016). Changes in

Table 4 The relationships among alkaline phosphatase, *phoD*-harbouring bacteria at the genus level and soil P fractions

		Labile-P		HCl-P		NaOH-P	
		P_o	P_i	P_o	P_i	P_o	P_i
	ALP	0.19	0.33	−0.28	−0.03	0.89**	0.38
phoD-Harbouring bacteria	*Amycolatopsis*	−0.50	0.02	−0.04	0.70**	0.18	0.41
	Bacillus	0.65**	−0.31	0.26	0.33	−0.57*	0.03
	Bradyrhizobium	0.57*	0.31	−0.56*	−0.32	0.15	−0.34
	Dietzia	−0 .36	0.26	−0.14	0.31	0.56*	0.60*
	Frankia	0.93**	−0.16	−0.25	0.05	0.13	−0.72**
	Methylobacterium	0.55*	0.19	−0.34	−0.61*	0.24	−0.07
	Sphingomonas	−0.10	−0.08	0.02	0.18	0.64*	−0.33

[a] *ALP* alkaline phosphatase; Labile-P = H_2O-P + $NaHCO_3$-P; H_2O-P, $NaHCO_3$-P, HCl-P and NaOH-P represent phosphorus extracted by deionized water, 0.5 M $NaHCO_3$, 1 M HCl and 0.5 M NaOH, respectively; P_o organic phosphorus, P_i inorganic phosphorus

[b] The values in the table were Spearman correlation coefficients ($n = 15$). * $p < 0.05$; ** $p < 0.01$

soil properties directly affect the structure of the soil bacterial community (Cui et al. 2019) and lead to significant changes in the P_o-mineralizing bacterial community.

4.2 Environmental Drivers of the *phoD*-Harbouring Bacterial Community

The RDA results showed that the *phoD*-harbouring bacterial community structure was strongly correlated with the soil C/N ratio, N/P ratio, AP and SOM (Table S2). The results, which suggest shifts in the soil C:N:P stoichiometry, shed light on the potential for nutrient availability to influence the diversity and composition of the *phoD*-harbouring bacterial community (Zhou et al. 2017; Aanderud et al. 2018). This results also support those of other studies showing that the nutrient requirements of different microbial biomass were differ (Cleveland and Liptzin 2007; Hartman and Richardson 2013). With the change from desert steppe to typical steppe to meadow steppe, the soil AP content increased significantly (Table 2), but only a few rare *phoD*-harbouring bacteria were sensitive to the change in AP. As AP increased, the relative abundances of *Amycolatopsis* and *Bacillus* decreased significantly, while the relative abundances of *Bradyrhizobium*, *Pseudomonas* and *Methylobacterium* increased significantly, indicating that not all *phoD*-harbouring bacteria were induced in a P-repressible manner. Other *phoD*-harbouring bacteria may be correlated with C and N contents or characterized by different nutrient preferences (Shade et al. 2014; Jousset et al. 2017; Samad et al. 2017). In general, *r*-strategists (e.g. *Xanthomonas*) grow faster in response to available C and require larger amounts of P compared to oligotrophs (Elser et al. 2003; Fierer et al. 2007; Luo et al. 2019). Thus, P mineralization is inherently coupled, to a degree, with C mineralization. The microbial community tends towards optimal states, which benefits nutrient cycling. More importantly, as an indicator of the composition of the *phoD*-harbouring bacterial community, functional diversity may be more related than taxonomic diversity to ecosystem functions (Lagos et al. 2016).

In other studies, pH was also the main soil property influencing the *phoD*-harbouring bacterial community (Lauber et al. 2009; Wang et al. 2012; Ragot et al. 2015). However, similar results were not obtained in this study, which was mainly due to the narrow pH range of the three steppe types (6.88–7.19). Additionally, temporal and spatial variations often lead to differences in bacterial communities and soil properties, so the abundances of different *phoD*-harbouring bacteria may be driven by different soil properties. Nevertheless, pH was an important factor influencing the relative abundances of *phoD*-harbouring *Rhodopirellula* and *Frankia* (Fig. 4b).

4.3 The Relationships of *phoD*-Harbouring Bacterial Community Composition to Soil P Fractions

Our results showed that ALP activity was positively correlated with AP content (Fig. S2) and NaOH-P_o (Table 4). This indicates that the NaOH-P_o pool can be an important source of AP through mineralization (Yang et al. 2015), which is consistent with the findings that microorganisms promote the availability of P by mineralizing P_o (Kanchikerimath and Singh 2001; Fraser et al. 2015; Chen et al. 2019b). Although there was a significant positive correlation between the α-diversity (Chao1 index) of the *phoD*-harbouring bacterial community and ALP activity (Fig. 3), only a portion of bacteria had a significant correlation with ALP (Fig. 4b). This result suggests that not all bacteria carrying the *phoD* gene play an important role in secretion of ALP and that this portion of bacteria may be highly activated during the secretion of ALP (Chen et al. 2019a). This phenomenon may also indirectly reflect that the production of ALP in soil is not controlled by the diversity of

the *phoD* gene under low-P conditions. Therefore, although the bacteria carrying the *phoD* gene were diverse with respect to classification, ALP synthesis-related expression of the *phoD* gene was highly induced in only a few instances. The bacteria that efficiently produce ALP may be preferentially selected for, especially when the total bacterial diversity in soil is limited (Fraser et al. 2015).

The relative abundance of *Dietzia* was closely correlated with ALP and had a significantly positive correlation with NaOH-P_i (Fig. 4b), indicating that the P_i produced by *Dietzia* mineralization exists in the form of NaOH-P_i. However, the relative abundances of *Bacillus* and *Bradyrhizobium* had a negative correlation with NaOH-P_o and HCl-P_o, and both were positively correlated with labile-P_o. These results suggest that these two bacteria promoted the conversion of the non-labile P_o pool into the labile P_o pool. In addition, the relative abundances of *Frankia* and *Methylobacterium* were both positively correlated with labile-P_o and negatively correlated with non-labile P_i ($p < 0.05$), which may be attributed to microbial immobilization. Our results suggest that *phoD*-harbouring bacteria may employ flexible P use strategies (Godwin and Contner 2015).

It is also important to note that other phosphatases (i.e. phytases, phosphodiesterases) not measured in this study may be responsible for hydrolysing P compounds in the $NaHCO_3$-P fraction. In addition, although the *phoD* gene is considered to be the most abundant phosphatase gene in soil (Tan et al. 2013; Luo et al. 2017), it can provide only partial information about the microbial mineralization of P_o. Other P_o mineralization genes, such as *phoA*, *phoX*, *BPP* and *appA*, may provide more information about this process; these genes were not analysed here due to the lack of genetic tools. Moreover, the actual expression levels of soil enzymes can be explained only by integrating genomics and proteomics. Further studies are necessary to quantify the gene transcriptome to fully understand the response of ALP activity caused by *phoD*-harbouring bacteria to environmental factors.

5 Conclusions

The bacterial *phoD* gene was found to be distributed mainly across 5 phyla, 6 classes, 10 orders, 16 families and 17 genera in Inner Mongolia steppe soils. Although the *phoD*-harbouring bacterial community structure varied by steppe type, there was a core *phoD*-harbouring bacterial microbiome. The soil stoichiometric ratios of carbon, nitrogen and phosphorus were the most influential factors shaping the *phoD*-harbouring bacterial community structure. However, not all bacteria carrying the *phoD* gene play an important role in the secretion of alkaline phosphatase or are induced in a P-repressible manner. In summary, the response of *phoD*-harbouring bacteria to soil P status is asynchronous and can be highly activated by their nutrient preferences and environmental conditions. Future research should address the gene transcriptome to fully understand the microbe-related processes of organic P turnover.

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Declarations

Conflict of Interest The authors declare no competing interests.

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