Successions of Soil *phoD* And *gcd* Microbial Communities Along a Chronosequence of Sand-Fixation Forest

Fei Wang

Northeastern University

Ying Zhang

Northeastern University

Yong Xia

Northeastern University

Zhenbo Cui

Northeastern University

Chengyou Cao (✉ caochengyou@mail.neu.edu.cn)

Northeastern University College of Life and Health Sciences

Research Article

Keywords: P turnover, *phoD* gene, *gcd* gene, *Caragana microphylla*, sandy land

Posted Date: September 22nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-888240/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Revegetation by planting shrubs on moving sand dunes is widely used to control desertification in arid/semi-arid areas. The soil microbial community can gradually recover along with plantation development. The purposes of this study were (1) to investigate the responses of microbial communities involved in the mineralization of soil organic phosphorus (OP) and dissolution of inorganic P (IOP) to the development of sand-fixation plantation and (2) to discuss the interactions between P turnover microbial communities and soil properties. We detected the diversities of soil *phoD* gene (one of the Pho regulons encoding alkaline phosphomonoesterases) and *gcd* gene (encoding glucose dehydrogenase) and the compositions of *phoD* and *gcd* communities by using the high-throughput Illumina MiSeq sequencing technique in a chronosequence of *Caragana microphylla* plantations (0-, 10-, 20-, and 37-year plantations and a native *C. microphylla* shrub forest) in Horqin Sandy Land, northeast China. Soil properties including soil nutrients, enzymatic activity, and P fractions were also determined. The abundance of *phoD* and *gcd* genes linearly increased with the plantation age. However, the diversity of soil *phoD* microbes was more abundant than that of *gcd*. The *phoD* gene abundance and the fractions of total OP and IOP were positively correlated with the activity of phosphomonoesterase. Actinobacteria and Streptomycetaceae were absolutely dominant *phoD* taxa, while Proteobacteria and Rhizobiaceae were absolutely dominant *gcd* taxa. Plantation development facilitated the progressive successions of soil *phoD* and *gcd* communities resulting from the asymmetric increase in the abundance of dominant taxa. Soil total N, NH₄-N, and available K were the main factors affecting the structures of *phoD* and *gcd* communities, while pH was not the main influencing factor in such arid and nutrient-poor sandy soil. Many *phoD* or *gcd* operational taxonomic units were classified into *Rhizobium* and *Bradyrhizobium*, suggesting the coupling relationship between soil P turnover and N fixation.

Introduction

P is an essential macronutrient for all living cells and is vital to the survival of plant and soil microbes [1]. Although various forms of P are relatively abundant in soils, it is usually considered as one of the limiting nutrients for plants and soil microorganisms because most P cannot be directly utilized [2]. The forms of P in soil can be classified into organic P (OP) and inorganic P (IOP), but only the inorganic orthophosphate in soil solutions is readily available for plants [3–4]. The availability of soil P mainly depends on the mineralization of OP and the solubilization of IOP. Both processes are driven by specific soil microorganisms involved in P transformation. Phosphomonoesters, which can be hydrolyzed by phosphatases, are commonly the dominant form of OP[3]. Various phosphatases, extracellular hydrolytic enzymes produced by soil microbes or plant roots, contribute to the mineralization of OP compounds. Among phosphatases, alkaline phosphomonoesterases (ALPs) can non-specifically catalyze the hydrolysis of many orthophosphate monoesters, thereby playing an important role in soil P bioavailability [5–6]. Phosphate solubilization is mainly performed by soil phosphate-solubilizing bacteria (PSB), which secrete various organic acids to increase the solubility of IOP [5, 7].

In natural ecosystems, soil OP mainly originates from residuals of plants and animals and metabolites of microorganisms, and it is considered as an important P source for plants and soil biota. Although OP occupies a large proportion of soil total P (TP), it must be transformed into available P (AP) form before it can be assimilated [8]. Under P scarce condition, the expressions of some microbial functional genes can be upregulated by activating the *pho* regulons to meet the requirement of P, which can increase ALP production and further promote OP mineralization. The *pho* regulon is mainly composed of genes encoding high-affinity phosphate transporters, various phosphatases, and enzymes for phosphonate utilization, which are involved in the hydrolyzation of OP and assimilation and transportation of AP [5, 9]. At least three homologous genes (*phoA*, *phoX*, and *phoD*) encoding ALP were identified within the bacterial *Pho* regulon [10–12]. Zimmerman et al. (2013) reported that the production of ALP requires the expression of at least one of the three genes [13]. Among them, *phoD* was most frequently detected in soil metagenome [8, 14]. It can be activated by Ca^{2+} and encodes monomeric enzyme (ALP) that can catalyze the hydrolysis reaction of phosphomonoesters and phosphodiester [10, 15]. The *phoD* gene could be used as a key molecular marker to analyze the structure of microbial community involved in soil OP mineralization, and its abundance could be used to estimate ALP activity and ALP bacterial diversity [3, 16–18]. Some studies suggested that *phoD* microbial community is sensitive to environmental variation and that the structure of the community is affected by many factors, including soil type and properties, fertilizer application, and crop management [18–19]. However, few studies have investigated the response of soil P-transformation microbial community to long-term development of natural ecosystems. The limited understanding of the dynamics of soil microbial diversity and the transformation of OP not only restricts management practices for sustainable development of the ecosystem, but also limits the prediction of the development tendency of soil nutrient cycle and the response of ecosystem processes to nutrient changes.

Apart from the mineralization of OP, the solubilization of insoluble soil phosphate is another pathway to increase AP. In natural environments, soil P-solubilizing microbes (PSM) can release P from soil phosphate through solubilization, thereby increasing the bioavailability of soil insoluble P [20]. The principal mechanism of inorganic phosphate solubilization depends on the functions of various organic acids secreted by PSM. Organic acids can chelate Ca, Fe, and Al cations or reduce the pH to release P [20–21]. Among the various organic acids, gluconic acid is the most important component. Gluconic acid originates from glucose oxidation, which is regulated by glucose dehydrogenase (a membrane-bound quinoprotein encoded by the *gcd* gene) [22–24]. The *gcd* gene could be used as a key molecular marker to detect the composition and the structure of soil PSM community. The ability of soil phosphate solubilization may be determined by the quantity of *gcd*-harboring microbes and their physiological activity, which is related to soil properties. At present, our knowledge of *gcd*-harboring microbes mainly comes from culture-dependent methods, and some *gcd*-PSM strains of *Pseudomonas*, *Bacillus*, or *Rhizobium* have been isolated and used as soil ameliorant to promote crop growth. However, few studies have investigated the diversity of *gcd*-PSM on the basis of culture-free method in natural environments.

Horqin Sandy Land is the semi-arid steppe of northern China, with a total area of $5.18 \times 10^4 \text{ km}^2$. In recent decades, the steppe has suffered severe desertification. Revegetation on sand dunes was considered an effective approach to control the desertification. Since the 1980s, a large area of indigenous plant plantations (e.g., *Caragana microphylla*, *Artemisia halodendron*, and *Hedysarum fruticosum*) has been established to fix moving or semi-moving sand dunes. Many studies have confirmed that establishing plantations on moving sand dunes facilitated in altering the microclimate, reducing wind velocity and soil erosion, and ameliorating soil properties [25–27], and these effects increased with plantation development [28–29]. Recent studies indicated that revegetation on moving sand dunes facilitated the restoration of soil microbial community including N-cycling microbes [28, 30–32]. However, whether soil P-transformation microbial community can be restored and how the structure of the microbial community gradually evolves along with plantation development remain unknown. This information is needed to further understand the mechanism of soil P restoration in degraded sandland ecosystems.

In this study, we investigated the compositions and structures of *phoD*- and *gcd*-harboring microbial communities under an age sequence of *C. microphylla* plantation in Horqin Sandy Land. The objectives of the study were to (1) quantify the responses of *phoD* and *gcd* community compositions to plantation development and (2) discuss the associated interactions between *phoD* and *gcd* communities with soil factors. We hypothesized that the activity of soil ALP and the content of AP increase with increasing plantation age, possibly resulting from the structural changes of *phoD* and *gcd* communities through the amelioration of soil properties.

Materials And Methods

Study location and site description

This study was conducted at the Wulanaodu Station of Desertification Control (43° 02' N, 119° 39' E) of the Chinese Academy of Sciences, western Horqin Sandy Land in northeastern China. This region lies in the semi-arid temperate zone and has a typical continental monsoon climate. The annual average temperature, precipitation, and pan evaporation are 6.3 °C, 340.5 mm, and 2500 mm, respectively. The original landscape is elm steppe-woodland; however, land desertification has developed for several decades. At present, the landscape is characterized as combination of sand dune, interdune lowland, and desertified grassland. To control desertification, *C. microphylla* (an indigenous shrub) is commonly used for revegetation with the help of high-density sand-protecting barriers (1 m × 1 m squares, made of straw) on moving sandy land. At present, a large area of *C. microphylla* plantation with different age is distributed around the Wulanaodu Region.

Experimental design and soil sampling

Soils were sampled in August 2020. Soil samples from 10-, 20, and 37-year-old *C. microphylla* plantation (designated as CM-10, CM-20, and CM-37, respectively), adjacent moving sand dunes (designated as

MS), and natural *C. microphylla* community (designated as NCM) were selected as experimental sites. The MS is considered as the original state before the plantation was established. In each site, three 30 m × 30 m plots at different sand dunes were set up for sampling. In each vegetation-covered plot, five *C. microphylla* clumps were selected, subsamples (0–10 cm) were collected from four directions around each clump, and then a total of 20 subsamples were pooled as a sample. Half of each sample was air-dried in the laboratory for soil property analysis, and the other half was immediately frozen at –80 °C for DNA extraction and enzymatic activity analysis.

Soil physicochemical property

Soil pH and electrical conductivity were measured in soil–water suspensions (1:2.5 and 1:5, respectively). Soil moisture was gravimetrically determined at 105 °C for 24 h. Soil organic matter and total nitrogen (N) were determined using the $K_2Cr_2O_7-H_2SO_4$ oxidation and semimicro-Kjeldahl digestion method, respectively. Soil TP and AP were measured using the Olsen and Dean method. Total and available potassium were measured using atomic absorption spectroscopy. NH_4-N was extracted using 1 M KCl solution and determined using an automated discrete analyzer (CleverChem 380, Germany). All the above-mentioned indexes were determined in accordance with the procedures described in Lin (2004) [33].

Potential of mineralization of OP and solubilization of IOP

The potential of soil P transformation was determined by culture method as described by Zheng and Zhang (1982) [34]. The basic culture medium included 100 g of glucose, 0.5 g of $(NH_4)_2SO_4$, 5.0 g of $CaCO_3$, 0.3 g of NaCl, 0.3 g of KCl, 0.3 g of $MgSO_4 \cdot 7H_2O$, minute amount of $MnSO_4$ and $FeSO_4$, and 1000 mL of distilled water. About 35 mg of phosphorite or lecithin was added in 35 mL of culture medium, and then high-pressure steam sterilization was performed. Next, 10 mL of soil–water suspension was inoculated in the medium and then cultured at 30 °C for 21 days in the dark. After incubation, the concentrations of AP in the media were measured. The proportions of AP to TP in the phosphorite- or lecithin-added media were calculated to estimate the potentials of OP mineralization and IOP solubilization, respectively.

Soil enzymatic activity

Soil urease activity was determined using urea as the substrate, and the released ammonium was assayed colorimetrically at 460 nm [35]. Dehydrogenase was measured following the method described in ISSCAS (1985) [36]. The activity of soil ALP was measured by using the original method of Tabatabai (1982) with some modification by Schinner et al. (1996) and Sardans and Peñuelas (2005) [37–39]. Protease activity was determined using the method of Ladd and Butler (1972) with some modifications described by Cao et al. (2008) [29, 40]. The activities of polyphenol oxidase and glucosidase were determined using the method described by Perucci et al. (2000) and phenol sulfonyl colorimetry, respectively [41–42].

Soil P fraction

Soil P fractions were determined by using the sequential extraction method of Hedley et al. (1982) with some modifications [43–44]. Briefly, 0.5 g of soil was extracted by 30 mL of distilled water and then centrifuged at 3500 rpm for 16 min at 4 °C. The supernatant was separated by using 0.45 µm filter membrane, and then AP was determined as H₂O-Pi (P in water). Fractioning was carried out following the same steps with the serial extracts: 0.5 mol L⁻¹ of NaHCO₃, 0.1 mol L⁻¹ of NaOH, 0.5 mol L⁻¹ of NaOH, and 1 mol L⁻¹ of HCl. The residual soil was air dried and digested for 3 h at 190 °C with HF and H₂SO₄ for residual-P determination. An aliquot of each extract was analyzed separately for TP. The OP of each extract was determined by the difference between TP and IOP. The general P is the sum of P from all fractions.

Amplification, quantification, and sequencing of *phoD* and *gcd* genes

Soil genomic DNA from different sites was extracted using the Soil DNA Quick Extraction Kit (Biotake, China). The *phoD* gene was amplified by PCR with the primer pair F1-TGGGAYGATCAYGARGT and R1-CTGSGCSAKSACRTTCCA [3]; *gcd* was amplified by PCR with the primer pair F2-CGGCGTCATCCGGGSITIYRAYRT and R2-GGGCATGTCCATGTCC [45]. The two genes were quantified through real-time quantitative PCR by using a Q5 Real-Time PCR System (Applied Biosystems, USA). The purified PCR products were sequenced on an Illumina MiSeq platform (Shanghai Personal Biotechnology Co., Ltd., Shanghai, China). The operational taxonomic unit (OTU) cutoffs were clustered with 75% similarity. OTU richness analysis was performed using the Mothur software (<https://www.mothur.org>, version 1.21.1), and α-diversity indexes including Shannon–Wiener index, Simpson diversity index, Pielou index, Chao's species richness estimator (Chao1), and observed species were calculated. Hierarchical clustering analysis was performed on the basis of the unweighted pair-group method with arithmetic means to compare the difference in β-diversity among different sites. The LEfSe method was used to detect the significantly different taxa of *phoD* or *gcd* communities among different sites, and a significance level of 0.05 and an effect size threshold of 3 were used for all biomarkers evaluated. All *phoD* and *gcd* gene sequences were submitted to the NCBI Sequence Read Archive under the accession number PRJNA739822.

Data analysis

The responses of soil factors, including physicochemical property, enzymatic activity, P fraction, and potential of P turnover, and the abundance of *phoD* and *gcd* genes to plantation age were fitted by using the linear regression model. All statistical analyses were performed using the SPSS software package (version 18.0), and statistical significance was considered at $P < 0.05$. Canonical correspondence analysis (CCA) was performed using CANOCO 4.5 to determine which of the soil factors have more significant effects on the *phoD* and *gcd* communities.

Results

Soil properties and P fractions along plantation development

The values of pH, soil moisture, electrical conductivity, organic matter, total N and K, $\text{NH}_4\text{-N}$, and available K and their variation tendencies are shown in Fig. 1. These indicators all significantly linearly increased with the plantation age ($P < 0.05$), indicating that the revegetation of *C. microphylla* on moving sand dunes can improve soil environment and facilitate the increase in soil nutrient.

The soil P fractions of different sites determined by the sequential extraction method (H_2O , 1.0 mol L^{-1} of HCl , 0.5 mol L^{-1} of NaHCO_3 , 0.5 mol L^{-1} of NaOH , and 0.1 mol L^{-1} of NaOH) are listed in Table 1. General P (total of different P fractions and residual P) increased with increasing plantation age. The IOP fractions extracted by H_2O , HCl , NaHCO_3 , and NaOH were all much lower than their respective OP, indicating that soil OP was the dominant P fraction along with plantation development. The most abundance OP fraction was NaOH-Po extracted by 0.5 and 0.1 mol L^{-1} solutions with averages of 50.17 and 49.09 mg kg^{-1} , respectively. Although the AP fractions including $\text{NaHCO}_3\text{-Pi}$ and $\text{H}_2\text{O-Pi}$ accounted for a small proportion, the values showed an increasing tendency along with plantation development. However, the concentration of $\text{NaHCO}_3\text{-Po}$, the stock of AP, ranged from 32.83 mg kg^{-1} to 44.31 mg kg^{-1} , and no significant difference was observed among different sites. At the MS site, except for 0.5 mol L^{-1} of $\text{NaHCO}_3\text{-Po}$, each P fraction concentration was lower than that of vegetation-covered sites. The regression analysis indicated that the concentrations of $\text{H}_2\text{O-Pi}$, 1.0 mol L^{-1} of HCl-Pi , 0.5 mol L^{-1} of $\text{NaHCO}_3\text{-Pi}$, 0.1 mol L^{-1} of NaOH-Pi , and 0.1 mol L^{-1} of NaOH-Po , residual-P, and general P all significantly linearly increased with plantation age ($P < 0.01$).

Table 1

Fractions of soil P under different *Caragana microphylla* plantations (mg kg⁻¹)

Fractions	MS	CM-10	CM-20	CM-37	NCM	ANOVA in response to plantation age		
						<i>R</i> ²	<i>F</i>	<i>P</i>
H ₂ O-Pi	1.345 ± 0.090	1.64 ± 0.462	2.175 ± 0.742	2.895 ± 0.773	4.24 ± 1.562	0.667	26.066	< 0.001
NaHCO ₃ -Pi (0.5 mol L ⁻¹)	5.820 ± 0.322	7.110 ± 0.773	10.69 ± 1.631	10.16 ± 1.022	15.17 ± 3.013	0.784	47.267	< 0.001
NaHCO ₃ -Po(0.5 mol L ⁻¹)	43.18 ± 4.510	37.89 ± 2.367	44.31 ± 3.445	41.34 ± 10.19	32.83 ± 8.193	0.169	2.651	0.127
HCl-Pi (1.0 mol L ⁻¹)	4.223 ± 0.983	6.657 ± 1.141	12.70 ± 1.281	15.05 ± 2.441	19.36 ± 0.671	0.918	145.00	< 0.001
HCl-Po (1.0 mol L ⁻¹)	11.44 ± 0.606	13.34 ± 2.717	12.64 ± 4.010	17.61 ± 6.416	13.97 ± 1.782	0.105	1.519	0.240
NaOH-Pi (0.5 mol L ⁻¹)	6.823 ± 1.773	7.559 ± 0.837	8.060 ± 1.978	7.496 ± 1.977	9.187 ± 2.063	0.168	2.624	0.129
NaOH-Po (0.5 mol L ⁻¹)	44.68 ± 2.07	49.44 ± 3.368	51.44 ± 5.448	52.50 ± 4.064	52.81 ± 4.176	0.169	2.641	0.128
NaOH-Pi (0.1 mol L ⁻¹)	9.211 ± 0.980	9.660 ± 0.766	11.68 ± 0.834	11.85 ± 2.001	14.13 ± 1.951	0.662	25.47	< 0.001
NaOH-Po (0.1 mol L ⁻¹)	40.29 ± 5.249	48.84 ± 9.903	40.32 ± 4.768	54.15 ± 8.846	61.87 ± 14.62	0.413	9.158	0.009
Residual-P	67.50 ± 26.15	91.00 ± 27.10	135.0 ± 15.80	258.5 ± 62.93	518.0 ± 32.32	0.897	113.1	< 0.001
General-P	234.5 ± 16.09	273.1 ± 24.94	329.0 ± 22.31	471.6 ± 70.48	741.6 ± 49.90	0.917	143.6	< 0.001
Values are means ± SD. MS: moving sand dune (0-yr); CM-10, CM-20, and CM-37: 10-, 20-, and 37-year plantation, respectively; NCM: natural <i>C. microphylla</i> community. <i>R</i> ² , <i>F</i> , and <i>P</i> values from regression analysis are given.								

Similar to soil physicochemical properties, the activities of soil phosphomonoesterase, urease, dehydrogenase, protease, glucosidase, and polyphenol oxidase in CM-10, CM-20, CM-37, and NCM sites increased by 7.71–69.90, 7.48–63.30, 1.06–5.54, 3.30–11.75, 4.03–9.90, and 1.38–2.35 times, respectively, compared with MS. The regression analysis showed that the activities of all selected enzymes had significantly linear relationships with plantation age ($P < 0.01$, Table 2). The activity of the transformation from soil insoluble phosphate or OP to AP was determined after 21 days of incubation.

The average rates of lecithin mineralization and phosphorite dissolution were 2.65% and 0.11%, respectively, and also linearly increased with the plantation age ($P < 0.05$, Fig. 2).

Table 2
Activities of soil enzyme under different *Caragana microphylla* plantations

Fractions	MS	CM-10	CM-20	CM-37	NCM	ANOVA in response to plantation age		
						R^2	F	P
DHA (mg TPF kg ⁻¹ 24h ⁻¹)	46.21 ± 1.328	48.86 ± 2.395	64.92 ± 11.35	97.65 ± 48.42	255.9 ± 71.29	0.711	31.97	< 0.001
Urease (mg 100 g ⁻¹ 24h ⁻¹)	0.510 ± 0.065	3.812 ± 0.052	9.809 ± 2.166	18.31 ± 11.33	32.28 ± 5.796	0.844	70.12	< 0.001
PHA (mg g ⁻¹ h ⁻¹)	2.276 ± 0.950	17.58 ± 8.111	45.99 ± 15.20	81.44 ± 10.40	159.4 ± 41.07	0.896	112.3	< 0.001
POA (μmol g ⁻¹ 10min ⁻¹)	1.985 ± 0.378	2.731 ± 0.681	3.246 ± 0.378	4.790 ± 1.001	4.662 ± 0.839	0.682	27.88	< 0.001
Protease (mg Tyr g ⁻¹ 2h ⁻¹)	8.169 ± 0.845	26.97 ± 11.02	55.54 ± 4.928	91.52 ± 14.89	96.03 ± 19.32	0.843	69.55	< 0.001
GLA (μg g ⁻¹ h ⁻¹)	0.139 ± 0.006	0.558 ± 0.035	0.580 ± 0.102	1.044 ± 0.031	1.372 ± 0.051	0.950	249.1	< 0.001
Values are means ± SD. MS: moving sand dune (0-yr); CM-10, CM-20, and CM-37: 10-, 20-, and 37-year plantation, respectively; NCM: natural <i>C. microphylla</i> community. DHA: dehydrogenase; POA: polyphenol oxidase; GLA: glucosidase; PHA: phosphomonoesterase. R^2 , F , and P values from regression analysis are given.								

Abundance of *phoD* and *gcd* genes

The abundance of *phoD* and *gcd* genes under MS, CM plantations, and NCM soils was determined by Q-PCR. The copies/g soil of *phoD* and *gcd* genes ranged from 7.44×10^5 in MS to 1.38×10^8 in NCM and 2.0×10^3 in MS to 2.16×10^6 in NCM, respectively, suggesting that the quantity of *phoD*-harboring microbes is greater than that of *gcd*-harboring microbes in sandy soil. The copies/g soil of *phoD* and *gcd* genes in CM-10, CM-20, CM-37, and NCM sites were 1.23 to 185.5 times and 23.68 to 1076.9 times greater compared with those in the MS site, indicating that the establishment of *C. microphylla* on moving sand dunes facilitated the restoration of soil *phoD* and *gcd* microbial communities. The abundance tendency of the two genes along with plantation development can be fitted by a quadratic regression model ($P < 0.001$, Fig. 3). Significant positive relationships were found among the abundance of *phoD* and *gcd* genes, soil AP ($\text{H}_2\text{O-Pi} + \text{NaHCO}_3\text{-Pi}$), and phosphomonoesterase activity ($r = 0.737$ to 0.901 , $P < 0.001$, data not shown). Overall, the variations in the abundance of soil *phoD* and *gcd* genes were consistent with those in soil nutrients, the activities of enzymes, P transformation function, and the concentrations of AP fractions.

Structures of soil *phoD* and *gcd* communities

A total of 489,065 and 1,031,717 *phoD* and *gcd* gene sequences were obtained from 15 soil samples, respectively, after quality filtering and removal of chimeras. OTUs with 75% similarity cutoff were clustered. The Simpson, Chao1, Shannon–Wiener, and Pielou indices and observed species were calculated on the basis of the result of OTU clustering (Table 3). The diversity indices (except for Simpson) of *phoD* community in vegetation-covered sites were all significantly higher than those in MS ($P < 0.05$). While no significant difference in α -diversity indices (except for Simpson) was observed in the *gcd* community between MS and CM-10, they were all significantly lower than those of the other three sites ($P < 0.05$). Overall, these indices of *phoD* community were higher than those of *gcd* community in all sites. Clustering analysis showed that most samples from the same sites can be clustered together, and the 15 samples can be divided into four or five groups (Fig. S1 and Fig. S2), suggesting the significant difference in the structure of soil *phoD* or *gcd* microbial community along with plantation development.

Table 3
Alpha diversity indices of soil *phoD* or *gcd* community under different plantations

Index		MS	CM-10	CM-20	CM-37	NCM	One-way ANOVA	
							<i>F</i>	<i>P</i>
Simpson	<i>phoD</i>	0.939 ± 0.006a	0.986 ± 0.004a	0.973 ± 0.018a	0.985 ± 0.006a	0.960 ± 0.038a	3.078	0.068
	<i>gcd</i>	0.940 ± 0.011a	0.878 ± 0.038b	0.948 ± 0.025b	0.967 ± 0.013b	0.941 ± 0.026b	5.550	0.013
Shannon-Wiener	<i>phoD</i>	6.068 ± 0.143a	7.916 ± 0.157b	7.592 ± 0.362b	7.836 ± 0.306b	7.356 ± 0.959b	7.111	0.006
	<i>gcd</i>	4.906 ± 0.315a	4.592 ± 0.404a	5.799 ± 0.497b	6.346 ± 0.414b	5.961 ± 0.471b	9.131	0.002
Pielou	<i>phoD</i>	0.667 ± 0.015a	0.791 ± 0.015b	0.760 ± 0.035b	0.783 ± 0.027b	0.733 ± 0.078a	4.465	0.025
	<i>gcd</i>	0.666 ± 0.041b	0.581 ± 0.057a	0.688 ± 0.050b	0.744 ± 0.017b	0.689 ± 0.039b	5.754	0.011
Chao1	<i>phoD</i>	765 ± 9a	1371 ± 78b	1382 ± 70b	1427 ± 67b	1450 ± 280b	13.35	0.001
	<i>gcd</i>	239 ± 59a	289 ± 58a	413 ± 27b	431 ± 124b	472 ± 73b	5.260	0.015
Observed species	<i>phoD</i>	548 ± 15a	1028 ± 51b	1014 ± 37b	1027 ± 48b	1048 ± 186b	17.08	< 0.001
	<i>gcd</i>	168 ± 33a	243 ± 40a	345 ± 37b	377 ± 90b	404 ± 58b	5.572	0.002
Values are means ± SD. MS: moving sand dune (0-yr); CM-10, CM-20, and CM-37: 10-, 20-, and 37-year plantation, respectively; NCM: natural <i>C. microphylla</i> community. <i>F</i> , and <i>P</i> values from One-way ANOVA are given.								
Means in row followed by different letters are significantly different (<i>P</i> < 0.05).								

phoD OTUs were classified into 5 different phyla, 20 orders, or 32 families. The *phoD* phyla included Actinobacteria (15.68–42.97%), Proteobacteria (9.03–15.79%), Planctomycetes (6.18–11.61%), Cyanobacteria (0.17–0.89%), and Firmicutes (0.44–1.56%), while 36.36–55.58% OTUs of *phoD* could not be classified at the phylum level within the GenBank database. Thirteen dominant *phoD* families (relative abundance > 1%) were detected, and their total relative abundance ranged from 37.17% in MS to 54.20% in CM-20 (Fig. 4). In the *phoD* community, Streptomycetaceae was the absolutely dominant family in all samples with a relative abundance from 10.63% in MS to 20.05% in NCM, followed by Pseudonocardiaceae (1.73–15.50%), Bradyrhizobiaceae (1.97–5.11%), Isosphaeraceae (1.99–4.24%), and Micromonosporaceae (0.22–5.37%). The relative abundance of most dominant *phoD* families in CM plantations and NCM was significantly higher than that in MS, suggesting that revegetation on moving

sand dunes was very helpful to restore the dominant taxa. However, some dominant families in MS, including Xanthomonadaceae, Burkholderiaceae, and Gemmataceae, decreased after the CM plantation was established.

On the basis of the GenBank database, *gcd* OTUs were classified into 6 phyla, 14 orders, 25 families, or 17 genera. The most dominant *gcd* phylum was Proteobacteria with a relative abundance from 26.9% in MS to 80.01% in CM-10, followed by Planctomycetes (8.17%) and Verrucomicrobia (1.46%). The relative abundance of Bacteroidetes, Actinobacteria, and Euryarchaeota was all less than 1%. Euryarchaeota (0.011%) was only observed in NCM samples (Fig. 4). Eight dominant *gcd* families (relative abundance > 1.0%) were found, namely, Rhizobiaceae (33.30%), Planctomycetaceae (7.22%), Pseudomonadaceae (6.68%), Opitutaceae (4.16%), Enterobacteriaceae (2.41%), Sphingomonadaceae (1.82%), Rhodospirillaceae (1.42%), and Bradyrhizobiaceae (1.29%). The sum of the relative abundance of these dominant families ranged from 45.27% in MS to 79.84% in CM-10 site. Revegetation on MS resulted in decreased abundance of Planctomycetaceae, Enterobacteriaceae, and Rhodospirillaceae and significantly increased abundance of Rhizobiaceae, showing waning and waxing variations. Although most OTUs could not be classified at the genus level in the GenBank database, several dominant genera including *Agrobacterium* (14.20%), *Rhizobium* (8.96%), *Pseudomonas* (5.12%), *Kluyvera* (1.78%), and *Bradyrhizobium* (1.26%) were detected.

LEfSe analysis was performed to determine differentiated taxa among MS, CM plantations, and NCM, and LDA distributions are shown in Fig. 5. At the MS site, differentiated *phoD* taxa included Caulobacteraceae, Xanthomonadaceae, Betaproteobacteria, Burkholderiaceae, *Cupriavidus*, and Caulobacterales; differentiated *gcd* taxa included *Kluyvera*, Opitutaceae, Opituta, Verrucomicrobia, Rhodospirillaceae, and Opituta. At the CM-10 site, differentiated *phoD* taxa were Nostocaceae and Aphanizomenonaceae; differentiated *gcd* taxa were Xanthomonadaceae, *Neorhizobium*, *Agrobacterium*, Rhizobiaceae, and Proteobacteria. At the CM-20 site, differentiated *phoD* taxa were *Bacillus*, Rubrobacteraceae, *Bacteria*, and Actinobacteria; differentiated *gcd* taxa included Betaproteobacteria, *Planctomyces*, Burkholderiaceae, *Acidovorax*, and Alphaproteobacteria. At the CM-37 site, differentiated *phoD* taxa included Acetobacteraceae, Rhodospirillales, and *Bradyrhizobium*; differentiated *gcd* taxa included Sphingobacteriaceae, Sphingobacteriia, Bacteroidetes, Methylocystaceae, *Bradyrhizobium*, and Sphingomonadaceae. At the NCM site, no differentiated *phoD* taxon was found, and only *gcd*-harboring *Enterobacter* was significantly different from other sites. These results showed the waxing and waning variations of the structures of soil *phoD* and *gcd* communities along with plantation development.

Dependence of *phoD* and *gcd* communities on soil properties

CCA was carried out to determine the influence of soil properties (including pH, electrical conductivity, organic matter, soil moisture, total N, total K, NH₄-N, available AK, NaHCO₃-Pi, and phosphomonoesterase). The results showed that the variations were explained by 57.5% for *phoD* community and 55.2% for *gcd* community along the first axis, and by 21.3% and 25.9% along the second axis, respectively (Fig. 6). The three samples of CM-10 and the three samples of MS can be clustered in

different groups, indicating the difference in the community structure among different sites. In general, the influence of the selected soil properties on *phoD* or *gcd* community was similar. Total N, $\text{NH}_4\text{-N}$, and available K were dominant factors influencing the *phoD* or *gcd* communities. In addition, soil $\text{NaHCO}_3\text{-Pi}$ and phosphomonoesterase activity were the main influencing factors on *gcd* community. However, the effects of soil pH, electrical conductivity, soil moisture, and total K on the structure of the two communities were relatively minor.

Discussion

Improvement of soil properties through the revegetation of *C. microphylla* on sand dunes

The improvement of soil environment induced by planting shrubs on moving sandy dunes is a comprehensive and continuous process that is affected by the interactions between biotic and abiotic factors [29]. The establishment of plantation can alter the microenvironment by reducing wind velocity and surface albedo, increasing ground roughness, and redistributing near-surface heat and water [26]. Simultaneously, high-density plantation facilitates the interception of dust, fine soil particles, and litter, which are rich in nutrients. The present study indicated that the establishment of *C. microphylla* plantation can significantly increase soil nutrients of moving sand dunes. Soil organic matter; total and available N, P (including $\text{H}_2\text{O-Pi}$ and $\text{NaHCO}_3\text{-Pi}$), and K; pH; and electronic conductivity all linearly increased with plantation age (Fig. 1). The N fixation, the mineralization of organic matter, and the decomposition of litter and dead roots via soil microbes resulted in increased soil N, P, and K. In addition, many annual and perennial herbs invaded and gradually increased with the development of *C. microphylla* plantation, which can provide important input of organic matter, N, and other nutrients by their rapid growth and death [32]. The improvement of soil nutrients can increase the diversity, total quantity, and activities of soil microorganisms including the ones involved in the cycles and transformations of C, N, P, and K elements [28, 30].

The distribution of different P fraction in soil is primarily affected by pH [46]. No significant difference in $\text{NaHCO}_3\text{-Po}$ (generally considered as easily mineralized OP) among the sites was observed. Moderate AP fractions including NaOH-Pi (binding with Fe and Al) and HCl-Pi (binding with Ca) linearly increased with the plantation age, possibly because of the slight increase in pH, which altered P solubility [44, 47]. The forms of most OP generally include phytate, esters, and microbial biomass [48–50]. Soil OP fractions (total $\text{NaHCO}_3\text{-Po}$, HCl-Po , and NaOH-Po) were positively correlated with organic matter (data not shown) and occupied 72.3% of total extractable P in NCM to 83.5% in MS. Moreover, the ratio decreased with the increase in plantation age, suggesting the improvement in the availability of soil P. The residual P, which was insoluble to chemical extractors, accounted for a larger proportion, suggesting that the availability of P in arid or semiarid soils is generally low.

Soil enzymes mainly originate from the physiological metabolism of microbes [42]. Therefore, the restoration of soil microbes after the establishment of *C. microphylla* plantation also induced the increase in activities of various soil enzymes [29]. In this study, the activities of soil

phosphomonoesterase, urease, dehydrogenase, protease, glucosidase, and polyphenol oxidase in vegetation-covered sites were all significantly higher than those in MS and linearly increased with plantation age (Table 2), which were the direct driving force for the mineralization of soil organic C, N, and P compounds. Similar to soil nutrient and enzymatic activity, the potential rates of OP mineralization and IOP dissolution also increased with plantation age (Fig. 2). However, the rate of soil OP mineralization was much higher than that of IOP dissolution either in MS or in vegetation-covered sites, suggesting that the accumulation of soil AP along with plantation development is largely dependent on the mineralization of OP, while IOP dissolution only contributed a small part. Therefore, the quantity of microbes involved in OP mineralization could be remarkably higher than those capable of IOP dissolution. This speculation was confirmed by comparing the abundance between *phoD* and *gcd* genes in soils. In vegetation-covered sites, the abundance of *phoD* gene was 20.6 to 80.9 times higher than that of *gcd* gene (Fig. 3). Thus, more phosphatase can be produced by *phoD*-harboring microbe to catalyze the reaction hydrolyzing OP to plant available phosphate [51]. On the whole, the availability of soil P mainly depends on the increase in the quantity of microbes related to OP mineralization, thereby resulting in the increases in soil phosphatase activity and mineralization rate.

Relationship of *phoD* gene abundance to phosphomonoesterase activity

Our results indicate a positive relationship among *phoD* gene abundance, phosphomonoesterase activity, and AP content in sandy soil. This result is similar to the results of some previous studies [3, 5]. Gradually increasing soil organic matter along with plantation development can raise more soil *phoD*-harboring microbes and increase the activity of soil phosphomonoesterase, which results in the improvement of the rate of OP mineralization [52]. The primary reason for this phenomenon is because the synthesis and production of phosphomonoesterase mainly depend on the quantity and expression of microbial *phoD* gene [8]. The activity of soil phosphomonoesterase is affected by pH, organic matter, and soil fraction [5, 53–54]. Soil organic matter can provide essential C and N resources for microbial survival. Therefore, it should be considered as the most important influencing factor. Different soil fractions can form specific microenvironments by containing different organic matter, adsorbing different compounds, and chelating different metal ions [54]. Cui et al. (2015) reported that the soil alkaline phosphatase activity is affected by OP fractions [51]. The present results also indicated that OP and IOP fractions are positively correlated to the activity of soil ALP. The activity of soil phosphomonoesterase was significantly associated with *phoD* gene abundance in this study, but not transcript copies. Fraser et al. (2015) hypothesized that high rates of P mineralization and high AP level would inhibit *phoD* transcription [5]. However, this phenomenon might not easily occur in such arid and sandy soil because the rate of P turnover and P status are relatively low. In addition, Ca^{2+} , considered as a cofactor for the production of *phoD* gene, was unlikely the limiting factor because the sandy soil was relatively rich in Ca [55].

Structures and variations of *phoD* and *gcd* microbial communities

phoD was spread across Actinobacteria, Proteobacteria, Planctomycetes, Cyanobacteria, and Firmicutes phyla and 20 families including Streptomyetaceae, Pseudonocardiaceae, Bradyrhizobiaceae,

Isosphaeraceae, and Micromonosporaceae, which contained at least one copy of the *phoD* gene. However, many OTUs cannot be classified due to the limitation of the database. Ragot et al. (2015) identified 20 bacterial phyla carrying the *phoD* gene using whole-genome and metagenome databases, and found that Actinobacteria, Proteobacteria, Gemmatimonadetes, Spirochaetes, and Verrucomicrobia are widely distributed in some Australian and Swiss grassland soils [3]. Some similar studies [53, 56] also observed that Actinobacteria, Planctomycetes, and Proteobacteria were the dominant *phoD*-harboring taxa in soils. Soil PSB also play an important role because of their metabolic activities to solubilize IOP for plant growth [22]. At present, most studies focused on the isolation and function of PSB strains with incubation method, while few have investigated the *gcd* gene diversity using culture-free method in natural environments. Yang et al. (2012) isolated 123 PSB from P-rich soil samples, and they were classified by phylogenetic analysis as Proteobacteria, Actinobacteria, and Firmicutes, including the genera of *Burkholderia*, *Pseudomonas*, *Acinetobacter*, *Enterobacter*, *Bacillus*, *Brevibacterium*, and *Arthrobacter* [57]. In this study, we found that the dominant *gcd*-harboring bacteria in this arid sandy soil were the phyla of Proteobacteria, Planctomycetes, and Verrucomicrobia, mainly including the families of Rhizobiaceae, Planctomycetaceae, Pseudomonadaceae, and Opitutaceae and the genera of *Agrobacterium*, *Rhizobium*, *Pseudomonas*, *Kluyvera*, and *Bradyrhizobium*. The results indicate that the diversity of soil PSB is relatively high even in nutrient-poor and arid sandy soil, and provide some useful information for further utilizing PSB to improve P solubilization. *Pseudomonas*, *Rhizobium*, and *Bradyrhizobium* were widely distributed in different sites in this study. Many strains of *Pseudomonas* have been isolated and confirmed to have the ability to facilitate plant growth by mineralizing OP and solubilizing insoluble phosphate [20–21, 57]. Many members of *Rhizobium* and *Bradyrhizobium* are free-living or symbiotic N₂-fixers [53], suggesting that they could be capable of coupling N and P pools via affecting the activities of phosphatase and nitrogenase.

Cluster analysis indicated a significant difference in the structures of soil *phoD* or *gcd* microbial community at MS, CM-10, CM-20, CM-37, and NCM sites (Fig. S1 and Fig. S2). Although the total quantity of soil microorganisms increased after revegetation on moving sandy dunes, the increase rates of different dominant taxa were asymmetric along with plantation development because of their different abilities of competition for limited nutrients and differences in utilization pattern of soil C and N resources [28, 31, 58]. Consequently, the successions of *phoD* and *gcd* communities along with vegetation development showed waxing and waning variations of dominant taxa. For example, Xanthomonadaceae, Burkholderiaceae, and Gemmataceae in the *phoD* community and Planctomycetaceae, Enterobacteriaceae, and Rhodospirillaceae in the *gcd* community all significantly decreased after the establishment of CM plantation. Figure 5 shows the significantly different taxa that were sensitive to plantation and soil development and can be considered as biological driving factors for the successions of *phoD* and *gcd* communities at different sites on the basis of LEfSe analysis. Although obvious successions of the two communities were observed during plantation development, the basic taxonomic composition of the communities among MS, CM plantations, and NCM soils was similar, indicating that the composition of dominant taxa was less affected by environmental variation in a specific area [3]. This is because many highly resilient and resistant microbial taxa, which are insensitive

to soil type and property, are widely distributed in soil microbial community. Therefore, the basic compositions of microbial community probably depend on land use history or other geographical/climatological factors within a continuous landscape [59].

Soil is the habitat of microorganisms; therefore, its properties can directly affect the structure of microbial communities by altering the abundance of dominant compositions. In this study, CCA indicated that total N, $\text{NH}_4\text{-N}$, and available K were the main factors influencing the *phoD* or *gcd* communities. Some studies have confirmed that pH is the primary driver affecting soil *phoD* community [18, 19]. However, in the present study, soil pH, electrical conductivity, and total K were not the main factors responsible for the successions of *phoD* and *gcd* communities because their values fluctuated among different sites at a small scale.

Conclusions

The results of this study indicated that revegetation by planting shrubs on semi-arid moving sand dunes can significantly improve soil physicochemical properties and microbiological activities, which facilitate the restoration of *phoD* and *gcd* microbial communities. The abundance of *phoD* and *gcd* genes linearly increased with the plantation age. However, the diversity of soil *phoD* microbes was more abundant than that of *gcd* microbes. The availability of soil P mainly depended on the mineralization of OP governed by *phoD* taxa. The *phoD* gene abundance and the fractions of total OP and IOP were all positively correlated to the activity of phosphomonoesterase.

Actinobacteria and Streptomycetaceae were absolutely dominant *phoD* taxa, while Proteobacteria and Rhizobiaceae were absolutely dominant *gcd* taxa. Plantation development facilitated the progressive successions of soil *phoD* and *gcd* communities resulting from the asymmetric increase in the abundance of dominant taxa. Soil total N, $\text{NH}_4\text{-N}$, and available K were the main factors affecting the structures of *phoD* or *gcd* communities, while pH was not the main influencing factor in such arid and nutrient-poor sandy soil. Many *phoD* or *gcd* OTUs were classified into *Rhizobium* and *Bradyrhizobium*, suggesting the coupling relationship between soil P turnover and N fixation. Synchronous surveys of involved soil N and P cycling microbes and the transcriptions or expressions of related functional genes should be investigated in future studies.

Declarations

Acknowledgement The authors are grateful for the technical assistance of the members of the Wulanaodu Experimental Station of Desertification Control under the Chinese Academy of Sciences. We thank the EnPapers (www.sci.vip) for English editing service.

Authors' Contributions Fei Wang: Investigation, Resources, and Data Curation. Ying Zhang: Methodology, Investigation, Resources. Yong Xia: Investigation, Resources, and Data Curation. Zhenbo Cui:

Investigation, Resources. Chengyou Cao: Writing- Original Draft, Reviewing and Editing, Conceptualization, Project administration, Data Curation.

Funding This work was supported by the National Natural Science Foundation of China (No. 41877536, No. 41371505, and 41403063).

Data Availability All sequences are publicly accessible on the NCBI Sequence Read Archive under the accession number PRJNA739822.

Compliance with Ethical Standards

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Competing Interests The authors declare no competing interests.

References

1. Westheimer FH (1987) Why nature chose phosphates. *Science* 235:1173–1178. <https://dx.doi.org/10.1126/science.2434996>
2. Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen phosphorus interactions. *Ecol Appl* 20:5–15. <https://dx.doi.org/10.1890/08-0127.1>
3. Ragot SA, Kertesz MA, Bünemann EK (2015) *phoD* alkaline phosphatase gene diversity in soil. *Appl Environ Microbiol* 81:7281–7289. <https://doi.org/10.1128/AEM.01823-15>
4. Chung H, Park M, Madhaiyan M, Seshadri S, Song J, Cho H, Sa T (2005) Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. *Soil Biol Biochem* 37:1970–1974. <https://doi.org/10.1016/j.soilbio.2005.02.025>
5. Fraser T, Lynch DH, Entz MH, Dunfield KE (2015) Linking alkaline phosphatase activity with bacterial *phoD* gene abundance in soil from a long-term management trial. *Geoderma* 257:115–122. <https://doi.org/10.1016/j.geoderma.2014.10.016>
6. Tanuwidjaja I, Vogel C, Pronk GJ, Scholer A, Kublik S, Vestergaard G, Kogel-Knabner I, Fuka MM, Schloter M, Schulz S (2021) Microbial key players involved in P turnover differ in artificial soil mixtures depending on clay mineral composition. *Microb Ecol* 81:897–907. <https://doi.org/10.1007/s00248-020-01635-1>
7. Chen WM, Yang F, Zhang L, Wang JM (2016) Organic acid secretion and phosphate solubilizing efficiency of *pseudomonas* sp. PSB12: effects of phosphorus forms and carbon sources. *Geomicrobiol J* 33:870–877. <https://doi.org/10.1080/01490451.2015.1123329>
8. Tan H, Barret M, Mooij MJ, Rice O, Morrissey JP, Dobson A, Griffiths B, O'Gara F (2013) Long-term phosphorus fertilization increased the diversity of the total bacterial community and the *phoD*

- phosphorus mineraliser group in pasture soils. *Biol Fertil Soils* 49:661–672.
<https://doi.org/10.1007/s00374-012-0755-5>
9. Vershinina OA, Znamenskaya LV (2002) The Pho regulons of bacteria. *Microbiology* 71:497–511.
 Valdespino-Castillo PM, Alcantara-Hernandez RJ, Merino-Ibarra M, Alcoce J, Macek M, Moreno-Guillen OA, Falcon LI (2017) Phylotype dynamics of bacterial P utilization genes in microbialites and bacterioplankton of a monomictic endorheic Lake. *Microb Ecol* 73:296–309.
<https://doi.org/10.1007/s00248-016-0862-1><https://dx.doi.org/10.1023/A:1020547616096>
 10. Wu JR, Shien JH, Shieh HK, Hu CC, Gong SR, Chen LY, Chang PC (2007) Cloning of the gene and characterization of the enzymatic properties of the monomeric alkaline phosphatase (PhoX) from *Pasteurella multocida* strain X-73. *FEMS Microbiol Lett* 267:113–120.
<https://doi.org/10.1111/j.1574-6968.2006.00542.x>
 11. Zappa B, Rolland J, Flament D, Gueguen Y, Boudrant J, Dietrich J (2001) Characterization of a highly thermostable alkaline phosphatase from the euryarchaeon *Pyrococcus abyssi*. *Appl Environ Microbiol* 67:4504–4511. <https://doi.org/10.1128/AEM.67.10.4504-4511.2001>
 12. Gomez PF, Ingram LO (1995) Cloning, sequencing and characterization of the alkaline-phosphatase gene (*phoD*) from *Zymomonas-mobilis*. *FEMS Microbiol Lett* 125: 237–245. <https://doi.org/10.1111/j.1574-6968.1995.tb07364.x>
 13. Zimmerman AE, Martiny A, Allison SD (2013) Microdiversity of extracellular enzyme genes among sequenced prokaryotic genomes. *ISME J* 7:1187–1199. <https://doi.org/10.1038/ismej.2012.176>
 14. Luo HW, Benner R, Long RA, Hu JJ (2009) Subcellular localization of marine bacterial alkaline phosphatases. *Proc Natl Acad Sci U S A* 106:21219–21223.
<https://doi.org/10.1073/pnas.0907586106>
 15. Kageyama H, Tripathi K, Rai AK, Cha-um S, Waditee-Sirisattha R, Takabe T (2011) An alkaline phosphatase/phosphodiesterase, *phoD*, induced by salt stress and secreted out of the cells of *aphanothece halophytica*, a halotolerant Cyanobacterium. *Appl Environ Microbiol* 77:5178–5183.
<https://dx.doi.org/10.1128/AEM.00667-11>
 16. Hu MJ, Penuelas J, Sardans J, Tong C, Chang CT, Cao WZ (2020) Dynamics of phosphorus speciation and the *phoD* phosphatase gene community in the rhizosphere and bulk soil along an estuarine freshwater-oligohaline gradient. *Geoderma* 365:114236.
<https://doi.org/10.1016/j.geoderma.2020.114236>
 17. Chen XD, Jiang N, Condron LM, Dunfield KE, Chen ZH, Wang JK, Chen LJ (2019) Impact of long-term phosphorus fertilizer inputs on bacterial *phoD* gene community in a maize field, Northeast China. *Sci Total Environ* 669:1011–1018. <https://doi.org/10.1016/j.scitotenv.2019.03.172>
 18. Hu YJ, Xia YH, Sun Q, Liu KP, Chen XB, Ge TD, Zhu BL, Zhu ZK, Zhang ZH, Su YR (2018) Effects of long-term fertilization on *phoD*-harboring bacterial community in Karst soils. *Sci Total Environ* 628–629:53–63. <https://doi.org/10.1016/j.scitotenv.2018.01.314>
 19. Ragot SA, Kertesz MA, Meszaros E, Frossard E, Bunemann EK (2017) Soil *phoD* and *phoX* alkaline phosphatase gene diversity responds to multiple environmental factors. *FEMS Microbiol Ecol*

20. Alori ET, Glick BR, Babalola OO (2017) Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Front Microbiol* 8:971. <https://doi.org/10.3389/fmicb.2017.00971>
21. Zeng QW, Wu XQ, Wen XY (2016) Effects of soluble phosphate on phosphate-solubilizing characteristics and expression of *gcd* gene in *Pseudomonas frederiksbergensis* JW-SD2. *Curr Microbiol* 72:198–206. <https://doi.org/10.1007/s00284-015-0938-z>
22. Rawat P, Das S, Shankhdhar D, Shankhdhar SC (2021) Phosphate-solubilizing microorganisms: mechanism and their role in phosphate solubilization and uptake. *J Soil Sci Plant Nutr* 21:49–68. <https://doi.org/10.1007/s42729-020-00342-7>
23. An R, Moe LA (2016) Regulation of pyrroloquinoline quinone-dependent glucose dehydrogenase activity in the model rhizosphere-dwelling bacterium *Pseudomonas putida* KT2440. *Appl Environ Microbiol* 82:4955–4964. <https://doi.org/10.1128/AEM.00813-16>
24. Rodríguez H, Rossolini GM, Gonzalez T, Li J, Glick BR (2000) Isolation of a gene from *Burkholderia cepacia* IS-16 encoding a protein that facilitates phosphatase activity. *Curr Microbiol* 40:362–366. <https://doi.org/10.1007/s002840010071>
25. Zhao HL, Zhou RL, Su YZ, Zhang H, Zhao LY, Drake S (2007) Shrub facilitation of desert land restoration in the Horqin Sand Land of Inner Mongolia. *Ecol Eng* 31:1–8. <https://doi.org/10.1016/j.ecoleng.2007.04.010>
26. Li SG, Harazono Y, Zhao HL, He ZY, Chang XL, Zhao XY, Zhang TH, Oikawa T (2002) Micrometeorological changes following establishment of artificially established *Artemisia* vegetation on desertified sandy land in the Horqin sandy land, China and their implication on regional environmental change. *J Arid Environ* 52:101–119. <https://doi.org/10.1006/jare.2001.0983>
27. Cao CY, Jiang DM, Alamsa Luo YM, Kou ZW (2000) Ecological processes of vegetation restoration of *Caragana microphylla* in the sand fixing area. *Chin J App Ecol* 11:349–354. <https://doi.org/10.13287/j.1001-9332.2000.0089>
28. Zhang Y, Cao CY, Cui ZB, Qian W, Liang CP, Wang CM (2019) Soil bacterial community restoration along a chronosequence of sand-fixing plantations on moving sand dunes in the Horqin sandy land in northeast China. *J Arid Environ* 165:81–87. <https://doi.org/10.1016/j.jaridenv.2019.04.003>
29. Cao CY, Jiang DM, Teng XH, JiangY, Liang WJ, Cui ZB (2008) Soil chemical and microbiological properties along a chronosequence of *Caragana microphylla* Lam. plantations in the Horqin Sandy Land of Northeast China. *Appl Soil Ecol* 40:78–85. <https://doi.org/10.1016/j.apsoil.2008.03.008>
30. Li HL, Zhang Y, Wang TT, Feng SW, Ren Q, Cui ZB, Cao CY (2019) Responses of soil denitrifying bacterial communities carrying *nirS*, *nirK*, and *nosZ* genes to revegetation of moving sand dunes. *Ecol Indic* 107:105541. <https://doi.org/10.1016/j.ecolind.2019.105541>
31. Cao CY, Zhang Y, Cui ZB, Feng SW, Wang TT, Ren Q (2017) Soil bacterial community responses to revegetation of moving sand dune in semi-arid grassland. *Appl Environ Microbiol* 101:6217–6228. <https://doi.org/10.1007/s00253-017-8336-z>

32. Zhang Y, Cao CY, Peng M, Xu XJ, Zhang P, Yu QJ, Sun T (2014) Diversity of nitrogen-fixing, ammonia-oxidizing, and denitrifying bacteria in biological soil crusts of a revegetation area in Horqin Sandy Land, Northeast China. *Ecol Eng* 71:71–79. <https://doi.org/10.1016/j.ecoleng.2014.07.032>
33. Lin DY (2004) *Guidance of Soil Science Experiment*. China Forestry Publishing House, Beijing, pp 45–70
34. Zheng HY, Zhang DS (1982) *Methods on Dynamics of Soil Biochemistry Study*. Science Press, Beijing, pp 167–169
35. Kandeler E, Gerber H (1988) Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biol Fertil Soils* 6:68–72. <https://doi.org/10.1007/BF00257924>
36. Institute of Soil Science, Chinese Academy of Sciences (ISSCAS) (1985) *Methods on Soil Microorganism Study*. Science Press, Beijing, pp 260–275
37. Tabatabai MA (1982) Soil enzymes. In: Page AL, Millar EM, Keeney DR (eds) *Methods of Soil Analysis*. ASA and SSSA, Madison, pp 501–538
38. Schinner F, Öhlinger R, Kandeler E, Margesin R (1996) *Methods in Soil Biology*, Springer Lab Manual. Springer, Berlin
39. Sardans J, Peñuelas J (2005) Drought decreases soil enzyme activity in a Mediterranean *Quercus ilex* L. forest. *Soil Biol Biochem* 37:455–461. <https://doi.org/10.1016/j.soilbio.2004.08.004>
40. Ladd JN, Butler JHA (1972) Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. *Soil Biol Biochem* 4:19–30. [https://doi.org/10.1016/0038-0717\(72\)90038-7](https://doi.org/10.1016/0038-0717(72)90038-7)
41. Perucci P, Casucc C, Dumontet S (2000) An improved method to evaluate the o-diphenol oxidase activity of soil. *Soil Biol Biochem* 32:1927–1933. [https://doi.org/10.1016/S0038-0717\(00\)00168-1](https://doi.org/10.1016/S0038-0717(00)00168-1)
42. Xu GH, Zheng HY (1986) *Manual of analytical methods of soil microorganism*. China Agriculture Press, Beijing, pp 266–269
43. Hedley MJ, Stewart JWB, Chauhan BS (1982) Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Sci Soc Am J* 46:970–976. <https://doi.org/10.2136/sssaj1982.03615995004600050017x>
44. da Silva VM, Antonioli ZI, Jacques RJS, Ott R, Rodrigues PED, Andrade FV, Passos RR, Mendonca ED (2017) Influence of the tropical millipede, *Glyphiulus granulatus* (Gervais, 1847), on aggregation, enzymatic activity, and phosphorus fractions in the soil. *Geoderma* 289:135–141. <https://doi.org/10.1016/j.geoderma.2016.11.031>
45. Bergkemper F, Kublik S, Lang F, Kruger J, Vestergaard G, Schlöter M, Schulz S (2016) Novel oligonucleotide primers reveal a high diversity of microbes which drive phosphorous turnover in soil. *J Microbiol Methods* 125:91–97. <https://doi.org/10.1016/j.mimet.2016.04.011>
46. Hinsinger P (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* 237:173–195. <https://doi.org/10.1023/A:1013351617532>

47. Wang RZ, Liu HY, Sardans J, Feng X, Xu ZW, Penuelas J (2021) Interacting effects of urea and water addition on soil mineral-bound phosphorus dynamics in semi-arid grasslands with different land-use history. *Eur J Soil Sci* 72:946–962. <https://doi.org/10.1111/ejss.13046>
48. Alamgir M, McNeill A, Tang CX, Marschner P (2012) Changes in soil P pools during legume residue decomposition. *Soil Biol Biochem* 49:70–77. <https://doi.org/10.1016/j.soilbio.2012.01.031>
49. Turner BL, McKelvie ID, Haygarth PM (2002) Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. *Soil Biol Biochem* 34:27–35. [https://doi.org/10.1016/S0038-0717\(01\)00144-4](https://doi.org/10.1016/S0038-0717(01)00144-4)
50. Brookes PC, Powlson DS, Jenkinson DS (1984) Phosphorus in the soil microbial biomass. *Soil Biol Biochem* 16:169–175. [https://doi.org/10.1016/0038-0717\(84\)90108-1](https://doi.org/10.1016/0038-0717(84)90108-1)
51. Cui H, Zhou Y, Gu ZH, Zhu HH, Fu SL, Yao Q (2015) The combined effects of cover crops and symbiotic microbes on phosphatase gene and organic phosphorus hydrolysis in subtropical orchard soils. *Soil Biol Biochem* 82:119–126. <https://doi.org/10.1016/j.soilbio.2015.01.003>
52. Turner BL, Haygarth PM (2005) Phosphatase activity in temperate pasture soils: potential regulation of labile organic phosphorus turnover by phosphodiesterase activity. *Sci Total Environ* 344:27–36. <https://doi.org/10.1016/j.scitotenv.2005.02.003>
53. Luo GW, Ling N, Nannipieri P, Chen H, Raza W, Wang M, Guo SW, Shen QR (2017) Long-term fertilisation regimes affect the composition of the alkaline phosphomonoesterase encoding microbial community of a vertisol and its derivative soil fractions. *Biol Fertil Soils* 53:375–388. <https://doi.org/10.1007/s00374-017-1183-3>
54. Hemkemeyer M, Christensen BT, Martens R, Tebbe CC (2015) Soil particle size fractions harbour distinct microbial communities and differ in potential for microbial mineralisation of organic pollutants. *Soil Biol Biochem* 90:255–265. <https://doi.org/10.1016/j.soilbio.2015.08.018>
55. Wang CY, Wang RY, Zhang SJ, Zhang XL, Tian LJ (1984) Soil type and soil forming process in Wulanaodu Area. In: Cao XS (ed) *Study on Integrated Control of Windy-sandy Arid Zone in Northeast Inner Mongolia* (I). Inner Mongolia People Publishing House, Huhehaote, pp 45–59
56. Lagos LM, Acuna JJ, Maruyama F, Ogram A, Mora MD, Jorquera MA (2016) Effect of phosphorus addition on total and alkaline phosphomonoesterase-harboring bacterial populations in ryegrass rhizosphere microsites. *Biol Fertil Soils* 52:1007–1019. <https://doi.org/10.1007/s00374-016-1137-1>
57. Yang PX, Ma L, Chen MH, Xi JQ, He F, Duan CQ, Mo MH, Fang DH, Duan YQ, Yang FX (2012) Phosphate solubilizing ability and phylogenetic diversity of bacteria from P-rich soils around Dianchi Lake drainage area of China. *Pedosphere* 22(12):707–716. [https://doi.org/10.1016/S1002-0160\(60056-3](https://doi.org/10.1016/S1002-0160(60056-3)
58. Jiang DM, Cao CY, Zhang Y, Cui ZB, Han XS (2014) Plantations of native shrub species restore soil microbial diversity in the Horqin Sandy Land, northeastern China. *J Arid Land* 6:445–453. <https://dx.doi.org/10.1007/s40333-013-0205-8>
59. Suleiman AKA, Manoeli L, Boldo JT, Pereira MG, Roesch LFW (2013) Shifts in soil bacterial community after eight years of land-use change. *Syst Appl Microbiol* 36:137–144.

Figures

Figure 1
Responses of soil properties to plantation development

Figure 2

Responses of potentials of organic P mineralization and inorganic P solubilization to plantation development.

Figure 3

Abundance of *phoD* and *gcd* genes and their responses to plantation development.

Figure 4

Relative abundance of dominant phyla and families of *phoD* and *gcd*-communities (average of three plots). MS: moving sanddune; CM-10: 10-year-old *Caragana microphylla* plantation; CM-20: 20-year-old *C. microphylla* plantation; CM-37: 37-year-old *C. microphylla* plantation; NCM: Natural *C. microphylla* community.

Figure 5

LefSe method identifies the significantly different abundant taxa of phoD and gcd communities. A: phoD; B: gcd. A significance level of 0.05 and an effect size threshold of 3 were used for all of the biomarkers evaluated. MS: moving sanddune; CM-10: 10-year-old Caragana microphylla plantation; CM-20: 20-year-old C. microphylla plantation; CM-37: 37-year-old C. microphylla plantation; NCM: Natural C. microphylla community.

Figure 6

Canonical correspondence analysis (CCA) of genus composition of phoD or gcd community and soil properties. A: phoD community; B: gcd community. MS: moving sanddune; CM10: 10-year-old Caragana microphylla plantation; CM20: 20-year-old C. microphylla plantation; CM37: 37-year-old C. microphylla plantation; NCM: Natural C. microphylla community. OM: Organic matter; TN: Total N; ALP: alkaline phosphomonoesterase; NP: $\text{NaHCO}_3\text{-Pi}$; TK: total K; AK: available K; EC: electrical conductivity; SM: soil moisture.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Fig.S1gcdclusteringupgma.pdf](#)
- [Fig.S2phoDclusteringupgma.pdf](#)