Soil Microbial Community Succession Based on PhoD and Gcd Genes along a Chronosequence of Sand-Fixation Forest

Fei Wang, Ying Zhang, Yong Xia, Zhenbo Cui and Chengyou Cao *

Abstract: Revegetation by planting shrubs on moving sand dunes is widely used to control desertification in arid/semi-arid areas. The soil including microbial community can gradually be improved 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) in the development of sand-fixating plantation and (2) to discuss the interactions between P turnover microbial communities and soil properties. We assessed the compositions of soil phoD gene (one of the Pho regulons encoding alkaline phosphomonoesterases) and gcd gene (encoding glucose dehydrogenase) in microbial community by using high-throughput Illumina MiSeq sequencing 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 the dominant phoD taxa, while Proteobacteria and Rhizobiaceae were the dominant gcd taxa. Plantation development facilitated the progressive successions of soil phoD and gcd communities resulting from the increase in the abundance of dominant taxa. Total soil N, NH$_4$-N, and available K were the main factors affecting the structures of phoD and gcd communities, while pH was not significantly 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.

Keywords: P turnover; PhoD gene; Gcd gene; Caragana microphylla; Sandy Land

1. 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 total soil P (TP), it must be transformed into available P (AP) form before it can be assimilated [8]. Under P limitation, 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 an enzyme (ALP) that can catalyze the hydrolysis reaction of phosphonoesters and phosphodiesters [10,15]. The phoD gene can be used as a key molecular marker to analyze the structure of microbial community involved in soil OP mineralization, and its abundance can 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 been carried out to investigate the response of soil P-transformation by microbial communities in 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 ecosystems, but also limits the prediction of the response of ecosystem processes to the improvement of soil environment.

Apart from the mineralization of OP, the solubilization of insoluble soil phosphate is another pathway that increases 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 function of various organic acids excreted by PSM. Organic acids can chelate Ca$^{2+}$, Fe$^{3+}$, and Al$^{3+}$ 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 can be used as a key molecular marker to detect the composition and the structure of soil PSM communities [22]. The ability of soil phosphate solubilization can 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 [22,23]. However, few studies have investigated the diversity of gcd-PSM based on culture-free methods in natural environments.

Horqin Sandy Land is the semi-arid steppe of northern China, with a total area of $5.18 \times 10^4$ 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. Some studies have confirmed that establishing plantations on moving sand dunes can alter the microclimate, reduce wind velocity and soil erosion, and improve 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 capability of microbial communities 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 sand land 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.

2. Materials and Methods

2.1. 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 (Figure 1). The average annual temperature, precipitation, and pan evaporation are 6.3 °C, 340.5, and 2500 mm, respectively. The soils were classified as Cambic Arenosols [29]. The original landscape is eim 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 × 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.

2.2. Experimental Design and Soil Sampling

Soil samples were collected from 10-, 20-, and 37-year-old *C. microphylla* plantation (designated as CM-10, CM-20, and CM-37, respectively), adjacent moving sand dune (designated as MS), and natural *C. microphylla* community (50 years old, designated as NCM) in August 2020 (Figure 1). The MS is considered as the original state before the plantation was established. Three sites of CM plantations, MS, and NCM were established in the Wulanaodu region, respectively. In each site, one plot (size 30 × 30 m) was 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.

2.3. Soil Physicochemical Properties

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$_2$Cr$_2$O$_7$–H$_2$SO$_4$ oxidation and semimicro-Kjeldahl digestion method [33], respectively. Soil TP and AP were measured using the Olsen and Dean method [33]. 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, Hamburg, Germany). All the above-mentioned soil properties were determined in accordance with the procedures described in Lin (2004) [33].
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2.4. Potential of Mineralization of OP and Solubilization of IOP

The potential of soil P transformation including the mineralization of OP and the solubilization of IOP were determined by culture method as described by Zheng and Zhang (1982) [34]. The detailed procedure was described in the Supplementary Materials and Methods.

2.5. 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].
2.6. Soil P Fraction

Soil P fractions were determined by using the sequential extraction method of Hedley et al. (1982) with some modifications [43,44]. The detailed procedure is described in the Supplementary Materials and Methods.

2.7. Amplification, Quantification, and Sequencing of PhoD and Gcd Genes

Soil genomic DNA from different sites was extracted using the Soil DNA Quick Extraction Kit (Bioteke, Wuxi, China). The \textit{phoD} and \textit{gcd} gene were amplified by PCR with the primer pairs reported by Ragot et al. (2015) [3] and Bergkemper et al. (2016) [45], respectively. The two genes were quantified through real-time quantitative PCR by using a Q5 real-time PCR System (Applied Biosystems, Waltham, MA, USA). The purified PCR products were sequenced on an Illumina MiSeq platform (Shanghai Personal Biotechnology Co., Ltd., Shanghai, China). The statistical analysis of obtained sequences, taxonomic classification, and community structures are described in detail in the Supplementary Materials and Methods. All \textit{phoD} and \textit{gcd} gene sequences were submitted to the NCBI Sequence Read Archive under the accession number PRJNA739822.

2.8. Data Analysis

Soil factors, including physicochemical properties, enzymatic activity, P fraction, potential of P turnover, and the abundance of \textit{phoD} and \textit{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 soil factors have significant effects on the \textit{phoD} and \textit{gcd} communities.

3. Results

3.1. Soil Properties and P Fractions along the Plantation Development

The values of pH, soil moisture, electrical conductivity, organic matter, total N and K, \( \text{NH}_4^+ \)-N, and available K and their variation tendencies are shown in Figure 2. All these values significantly increased with plantation age (\( p < 0.05 \)), indicating that the revegetation of \textit{C. microphylla} on moving sand dunes can improve soil environment and increase soil nutrients.

The soil P fractions of different sites determined by the sequential extraction method (\( \text{H}_2\text{O}, 1.0 \text{ of HCl, 0.5 of NaHCO}_3, 0.5 \text{ of NaOH, and 0.1 mol L}^{-1} \text{ of NaOH} \)) are listed in Table 1. Total P (total of different P fractions and residual P) increased with increasing plantation age. The IOP fractions extracted by \( \text{H}_2\text{O}, \text{HCl, NaHCO}_3, \text{and NaOH} \) 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 NaHCO\(_3\)-Pi and \( \text{H}_2\text{O-Pi} \) accounted for a small proportion, the values showed a linear increase along with plantation development. However, the concentration of NaHCO\(_3\)-Po, the stock of AP, ranged from 32.83 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 NaHCO\(_3\)-Po, each P fraction concentration was lower than that of vegetation-covered sites. The regression analysis indicated that the concentrations of \( \text{H}_2\text{-Pi, 1.0 of HCl-Pi, 0.5 of NaHCO}_3\text{-Pi, 0.1 of NaOH-Pi, and 0.1 mol L}^{-1} \text{ of NaOH-Po, residual-P, and general P all significantly linearly increased with plantation age (p < 0.01).} \)

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 a significantly linear relationship with plantation age (\( p < 0.01 \), Table 2). The activity of transformation of soil insoluble phosphate to available phosphate (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 linearly increased with the plantation age \( p < 0.05 \), Figure 3.

![Graphs showing soil properties over plantation age](image)

**Figure 2.** Responses of soil properties to plantation development.

| Table 1. Fractions of soil P at different *Caragana microphylla* plantations (mg kg\(^{-1}\)). |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|----------|
| Fractions                       | MS             | CM-10          | CM-20          | CM-37          | NCM            |
| H\(_2\)O-Pi                      | 1.35 ± 0.09    | 1.64 ± 0.46    | 2.18 ± 0.74    | 2.90 ± 0.77    | 4.24 ± 1.56    |
| NaHCO\(_3\)-Pi (0.5 mol L\(^{-1}\)) | 5.82 ± 0.32    | 7.11 ± 0.77    | 10.69 ± 1.63   | 10.16 ± 1.02   | 15.17 ± 3.01   |
| NaHCO\(_3\)-Pi (0.5 mol L\(^{-1}\)) | 43.18 ± 4.51   | 37.89 ± 2.37   | 44.31 ± 3.45   | 41.34 ± 10.19  | 32.83 ± 8.19   |
| NaOH-Pi (0.1 mol L\(^{-1}\))     | 4.22 ± 0.98    | 6.66 ± 1.14    | 12.70 ± 1.28   | 15.05 ± 2.44   | 19.36 ± 0.67   |
| NaOH-Pi (0.1 mol L\(^{-1}\))     | 11.44 ± 0.61   | 13.34 ± 2.72   | 12.64 ± 4.01   | 17.61 ± 6.42   | 13.97 ± 1.78   |
| NaOH-Pi (0.5 mol L\(^{-1}\))     | 6.82 ± 1.77    | 7.56 ± 0.84    | 8.06 ± 1.98    | 7.5 ± 1.98     | 9.19 ± 2.06    |
| NaOH-Po (0.5 mol L\(^{-1}\))     | 44.68 ± 2.07   | 49.44 ± 3.37   | 51.44 ± 5.45   | 52.50 ± 4.06   | 52.81 ± 4.18   |
| NaOH-Po (0.1 mol L\(^{-1}\))     | 9.21 ± 0.98    | 9.66 ± 0.77    | 11.68 ± 0.83   | 11.85 ± 2.00   | 14.13 ± 1.95   |
| NaOH-Po (0.1 mol L\(^{-1}\))     | 40.29 ± 5.25   | 48.84 ± 9.90   | 40.32 ± 4.77   | 54.15 ± 8.85   | 61.87 ± 14.62  |
| Residual-P                       | 67.50 ± 26.15  | 91.00 ± 27.10  | 135.0 ± 15.80  | 258.5 ± 62.93  | 518.0 ± 32.32  |
| Total-P                          | 234.5 ± 16.09  | 273.1 ± 24.94  | 329.0 ± 22.31  | 471.6 ± 70.48  | 741.6 ± 49.90  |

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 *C. microphylla* community. \( R^2 \), \( F \), and \( p \)-values from regression analysis are given.
Table 2. Activities of soil enzyme at different Caragana microphylla plantations.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>MS</th>
<th>CM-10</th>
<th>CM-20</th>
<th>CM-37</th>
<th>NCM</th>
<th>ANOVA in Response to Plantation Age</th>
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<tbody>
<tr>
<td></td>
<td>Enzyme</td>
<td>MS CM-10 CM-20 CM-37 NCM</td>
<td>R²</td>
<td>F</td>
<td>p</td>
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<tr>
<td>DHA (mg TPF kg⁻¹ 24 h⁻¹)</td>
<td>46.21 ± 1.33</td>
<td>48.86 ± 2.40</td>
<td>64.92 ± 11.35</td>
<td>97.65 ± 48.42</td>
<td>255.9 ± 71.29</td>
<td>0.711</td>
</tr>
<tr>
<td>Urease (mg 100 g⁻¹ 24 h⁻¹)</td>
<td>0.51 ± 0.07</td>
<td>3.81 ± 0.05</td>
<td>9.81 ± 2.17</td>
<td>18.31 ± 11.33</td>
<td>32.28 ± 5.80</td>
<td>0.844</td>
</tr>
<tr>
<td>PHA (mg g⁻¹ h⁻¹)</td>
<td>2.28 ± 0.95</td>
<td>17.58 ± 8.11</td>
<td>45.99 ± 15.20</td>
<td>81.44 ± 10.40</td>
<td>159.4 ± 41.07</td>
<td>0.896</td>
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<tr>
<td>POA (µmol g⁻¹ 10 min⁻¹)</td>
<td>1.99 ± 0.38</td>
<td>2.73 ± 0.68</td>
<td>3.25 ± 0.38</td>
<td>4.79 ± 1.00</td>
<td>4.66 ± 0.84</td>
<td>0.682</td>
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<tr>
<td>GLA (µg g⁻¹ h⁻¹)</td>
<td>0.14 ± 0.01</td>
<td>0.56 ± 0.04</td>
<td>0.58 ± 0.10</td>
<td>1.04 ± 0.03</td>
<td>1.372 ± 0.05</td>
<td>0.950</td>
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</table>

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 C. microphylla community. DHA: dehydrogenase; POA: polyphenol oxidase; GLA: glucosidase; PHA: phosphomonoesterase. R², F, and p-values from regression analysis are given.

Figure 3. Responses of potentials of organic P mineralization and inorganic P solubilization to plantation development.

3.2. Abundance of PhoD and Gcd Genes

The abundance of phoD and gcd genes in MS, CM plantations, and NCM soils was determined by Q-PCR. The copies/g soil of phoD and gcd genes ranged from 7.44 × 10⁵ in MS to 1.38 × 10⁶ in NCM and 2.0 × 10⁵ in MS to 2.16 × 10⁶ in NCM, respectively. This suggests that the quantity of phoD-harboring microbes is greater than that of gcd-harboring microbes in sandy soil. The soil copies/g 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 than 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 of the two genes can be fitted with a quadratic regression model to the sequence of plantation development (p < 0.001, Figure 4). Significant positive relationships were found between the abundance of phoD and gcd genes, soil AP (H₂O-Pi + NaHCO₃-P), and phosphomonoesterase activity, respectively (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.
3.2. Abundance of PhoD and gcd Genes

The abundance of phoD and gcd genes and plantation age (y).

3.3. 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 [19]. The Simpson, Chao1, Shannon–Wiener, and Pielou indices and observed species were calculated based on the results of OTU clustering (Table 3). The alpha diversity indices of phoD community in vegetation-covered sites were all significantly greater than those in MS (p < 0.05, except for Simpson’s). While no significant difference in alpha diversity indices (except for Simpson’s) was observed in the gcd community between MS and CM-10, they were significantly lower than those of the other three sites (p < 0.05). Overall, indices of phoD community diversity were greater than those of gcd community in all sites. Clustering analysis and PERMANOVA test showed that most samples from the same sites can be clustered together, and the 15 samples can be divided into four or five groups (Figures S1 and S2), suggesting the significant difference in the structure of soil phoD or gcd microbial community along the plantation development sequence.

Table 3. Alpha diversity indices of soil phoD or gcd community in different plantations.

<table>
<thead>
<tr>
<th>Index</th>
<th>phoD</th>
<th>gcd</th>
<th>phoD</th>
<th>gcd</th>
<th>phoD</th>
<th>gcd</th>
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<th>gcd</th>
<th>phoD</th>
<th>gcd</th>
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<tbody>
<tr>
<td>MS</td>
<td>0.934 ± 0.006 a</td>
<td>0.986 ± 0.004 a</td>
<td>0.973 ± 0.018 a</td>
<td>0.985 ± 0.006 a</td>
<td>0.960 ± 0.038 a</td>
<td>3.076 ± 0.0086</td>
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<td>CM-10</td>
<td>0.940 ± 0.011 a</td>
<td>0.878 ± 0.038 b</td>
<td>0.948 ± 0.025 b</td>
<td>0.967 ± 0.013 b</td>
<td>0.941 ± 0.026 b</td>
<td>5.550 ± 0.013</td>
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<td>CM-20</td>
<td>0.716 ± 0.041 a</td>
<td>0.791 ± 0.015 b</td>
<td>0.760 ± 0.055 b</td>
<td>0.783 ± 0.027 b</td>
<td>0.733 ± 0.078 a</td>
<td>4.465 ± 0.025</td>
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<td>CM-37</td>
<td>0.666 ± 0.041 b</td>
<td>0.581 ± 0.057 a</td>
<td>0.688 ± 0.050 a</td>
<td>0.744 ± 0.017 a</td>
<td>0.689 ± 0.039 a</td>
<td>5.754 ± 0.011</td>
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<tr>
<td>NCM</td>
<td>0.675 ± 0.09 a</td>
<td>1.371 ± 0.78 b</td>
<td>1.382 ± 0.70 b</td>
<td>1.427 ± 0.67 b</td>
<td>1.450 ± 0.28 b</td>
<td>13.35 ± 0.001</td>
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<tr>
<td>Chao1</td>
<td>168 ± 33 a</td>
<td>243 ± 40 a</td>
<td>345 ± 37 b</td>
<td>377 ± 90 b</td>
<td>404 ± 58 b</td>
<td>5.572 ± 0.002</td>
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<tr>
<td>Simpson</td>
<td>4.906 ± 0.143 a</td>
<td>4.592 ± 0.404 b</td>
<td>5.799 ± 0.497 b</td>
<td>6.346 ± 0.414 b</td>
<td>5.961 ± 0.471 b</td>
<td>9.131 ± 0.002</td>
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<tr>
<td>Shannon-Wiener</td>
<td>4.086 ± 0.031 a</td>
<td>4.592 ± 0.404 b</td>
<td>5.799 ± 0.497 b</td>
<td>6.346 ± 0.414 b</td>
<td>5.961 ± 0.471 b</td>
<td>9.131 ± 0.002</td>
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<td>Pielou</td>
<td>4.906 ± 0.143 a</td>
<td>4.592 ± 0.404 b</td>
<td>5.799 ± 0.497 b</td>
<td>6.346 ± 0.414 b</td>
<td>5.961 ± 0.471 b</td>
<td>9.131 ± 0.002</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Observed species</td>
<td>548 ± 15 a</td>
<td>1028 ± 51 b</td>
<td>1014 ± 37 b</td>
<td>1027 ± 48 b</td>
<td>1048 ± 186 b</td>
<td>17.08 &lt; 0.001</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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 C. microphylla community. F, and p-values from One-way ANOVA are given. Means in rows followed by different letters are significantly different (p < 0.05).

PhoD OTUs were classified into 5 different phyla, 20 orders, or 32 families. The phoD phyla included Actinobacteria (15.68% to 42.97%), Proteobacteria (9.03% to 15.79%), Planctomycetes (6.18% to 11.61%), Cyanobacteria (0.17% to 0.89%), and Firmicutes (0.44% to 1.56%), while 36.36% to 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 (Figure 5). In the phoD community, Streptomycetaceae was the dominant family in all samples with a relative abundance from 10.63% in MS to 20.05% in NCM, followed by
Pseudonocardiales (1.73% to 15.50%), Bradyrhizobiaceae (1.97% to 5.11%), Isosphaerae (1.99% to 4.24%), and Micromonosporaceae (0.22% to 5.37%). The relative abundance of the most dominant *phoD* families in CM plantations and NCM was significantly greater than that in MS, suggesting that revegetation on moving sand dunes was helpful in restoring the dominant taxa. However, some dominant families in MS, including Xanthomonadaceae, Burkholderiaceae, and Gemmataceae, decreased after the CM plantation was established.

![Figure 5. Relative abundance of dominant phyla and families of *phoD* and *gcd*-communities (average of three plots).](image_url)

Based on the GenBank database, *gcd* OTUs were classified into 6 phyla, 14 orders, 25 families, and 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 (Figure 5). 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 of MS resulted in decreased abundance of Planctomycetaceae, Enterobacteriaceae, and Rhodospirillaceae and a significantly increased abundance of Rhizobiaceae. Although most OTUs could not be classified at the genus level according to 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 differentially abundant taxa among MS, CM plantations, and NCM, and LDA scores are shown in Figure 6.
Figure 6. LEfSe analysis of microbial \textit{phoD} and \textit{gcd} communities shows taxa with significantly different abundance. (A) \textit{phoD}; (B) \textit{gcd}. A significance level of 0.05 and a threshold value of 3 were used. MS: moving sand dune; CM-10: 10-year-old \textit{Caragana microphylla} plantation; CM-20: 20-year-old \textit{C. microphylla} plantation; CM-37: 37-year-old \textit{C. microphylla} plantation; NCM: Natural \textit{C. microphylla} community.

3.4. Dependence of \textit{PhoD} and \textit{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, NH$_4$-N, available AK, NaHCO$_3$-Pi, and phosphomonoesterase). The results showed that 57.5\% and 55.2\% of variation was explained by the first axis for \textit{phoD} and \textit{gcd} communities, respectively; on the other hand, 21.3\% and 25.9\% of variation was explained by the second axis, respectively (Figure 7). For both \textit{phoD} and \textit{gcd}, MS and vegetation-covered samples cluster in different groups, while most samples of CM-20 and CM-37 cluster in a group. In general, the influence of the selected soil properties on \textit{phoD} or \textit{gcd} community was similar. Total N, NH$_4$-N, and available K were dominant factors influencing \textit{phoD} or \textit{gcd} communities. In addition, soil NaHCO$_3$-Pi and phosphomonoesterase activity were the main factors influencing on \textit{gcd} community. However, soil pH, electrical conductivity, soil moisture, and total K were not significant drivers for the community structure.
4. Discussion

4.1. Improvement of Soil Properties through the Revegetation of *Caragana 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 H$_2$O$_2$-Pi and NaHCO$_3$-Pi), and K, pH, and electrical conductivity linearly increased with plantation age (Figure 2). Soil microbes can play important roles for the improvement of soil nutrients via N fixation, the mineralization of organic matter and the decomposition of litter and dead roots. 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 fractions in soil is primarily affected by pH [46]. No significant difference in NaHCO$_3$-Po (generally considered as easily mineralized OP) among the sites was observed. A number of AP fractions including NaOH-Pi (binding with Fe$^{3+}$ and Al$^{3+}$) and HCl-Pi (binding with Ca$^{2+}$) linearly increased with the plantation age, possibly because of the slight increase in pH, which altered P solubility [44,47]. Most OP forms generally include phytate, esters, and microbial biomass [48–50]. Soil OP fractions (total NaHCO$_3$-Po, HCl-Po, and NaOH-Po) were positively correlated with organic matter (data not shown) and represented 72.3% and 83.5% of total extractable P in NCM and MS, respectively. The residual P, which was insoluble to chemical extractors, accounted for the largest 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 greater than those in MS and linearly increased with plantation age (Table 2). These enzymes are involved in the mineralization of soil organic C, N, and P compounds. Like soil nutrient and enzymatic activity, the potential rates of OP mineralization and IOP dissolution also increased with plantation age (Figure 3). However, the rate of soil OP mineralization was much greater than that of IOP dissolution in both MS and 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 amount. Therefore, the quantity of microbes involved in OP mineralization could be remarkably greater 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* genes was 20.6 to 80.9 times greater than that of *gcd* genes (Figure 4). More phosphatase can be produced by *phoD*-harboring microbes to catalyze the reaction hydrolyzing OP to plant available phosphate [51]. Overall, the availability of soil P mainly depends on the increase in the quantity of microbes capable of OP mineralization, thereby resulting in the increases in soil phosphatase activity and mineralization rate.

### 4.2. Relationship of PhoD Gene Abundance to Phosphomonoesterase Activity

Our results indicate a positive relationship between *phoD* gene abundance, phosphomonoesterase activity and AP content in sandy soil. This result is similar to the results of some previous studies [3,5]. The gradual increase in soil organic matter along with plantation development can result in a higher number of soil *phoD*-harboring microbes and increase in 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 one of the most important influencing factors. 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 soil alkaline phosphatase activity is affected by OP fractions [51]. The activity of soil phosphomonoesterase was significantly associated with *phoD* gene abundance in this study (r = 0.823, data not shown), 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 transcription of *phoD* gene, was unlikely the limiting factor because the sandy soil was relatively rich in Ca\(^{2+}\) [55].

### 4.3. Structures of PhoD and Gcd Microbial Communities

*PhoD* was spread across Actinobacteria, Proteobacteria, Planctomycetes, Cyanobacteria, and Firmicutes phyla and 20 families including Streptomycetaceae, 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 N\textsubscript{2}-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 (Figures S1 and 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 variations of dominant taxa. Figure 6 shows the significantly different taxa based on phoD and gcd gene analysis that responded to plantation and soil development. 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, suggesting 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 different taxa. In this study, CCA indicated that total N, NH\textsubscript{4}-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. Although plantation age is an indirect factor affecting soil microbial community, soil environment, especially nutrient status, can be improved with plantation development. The previous study of our team indicated that the biomass and plant diversity under the C. microphylla canopy all increased with the plantation age [27]. The improvement of microenvironment can facilitate the trapping of dust, soil C, N, and P level, and productivity, which promotes an increase in the quantity of microbial P-turnover, thereby increasing ALP activity, OP mineralization rate, and soil AP content. The recovery of microbial community can in turn accelerate soil nutrient cycling and increase plant diversity and productivity.

5. Conclusions

The results of this study indicated that revegetation by planting shrubs such as C. microphylla on semi-arid moving sand dunes can significantly improve soil physicochem-
lical 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* gene microbial community was higher than that of *gcd*. The *phoD* gene abundance was positively correlated to the activity of phosphomonoesterase.

Actinobacteria and Streptomycetaceae were the dominant *phoD* gene taxa, while Proteobacteria and Rhizobiaceae were the dominant *gcd* gene 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, NH$_4$-N, and available K were the main factors affecting the structures of *phoD* or *gcd* communities, while pH was not an 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 involving soil N and P cycling microbes and the transcriptions or expressions of related functional genes should be investigated in future studies.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/f12121707/s1, Figure S1: Clustering analysis of *gcd* communities based on UPGMA; Figure S2: Clustering analysis of *phoD* communities based on UPGMA; Supplementary Materials and Methods.

**Author Contributions:** F.W.: Investigation, Resources, and Data Curation. Y.Z.: Methodology, Investigation, Resources. Y.X.: Investigation, Resources, and Data Curation. Z.C.: Investigation, Resources. C.C.: Writing—Original Draft, Reviewing and Editing, Conceptualization, Project administration, Data Curation. All authors have read and agreed to the published version of the manuscript.

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